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LOS TUMORES GERMINALES. EVOLUCIÓN DEL SACO VITELINO SECUNDARIO HUMANO. ESTUDIO COMPARATIVO DEL FENOTIPO DEL SACO VITELINO NORMAL CON LOS TUMORES VITELINOS (ENDODERMICOS PRIMITIVOS)

Tesis Doctoral,

Presentada por:

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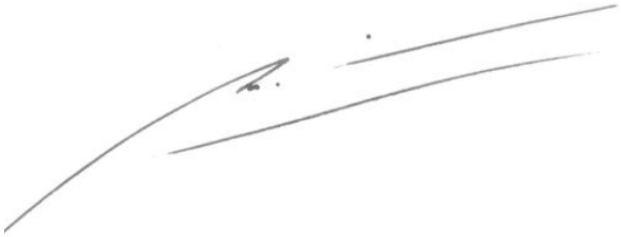

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Profesor Doctor Miguel Cecilio Botella López

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Índice

ABREVIATURAS UTILIZADAS EN ESTA TESIS

AFP	Alfafetoproteína
CGP	Células germinales primordiales
CK	Citoqueratina
GPC3	Glipican 3
hCG	Gonadotropina coriónica humana
HepPar1	Factor hepatocitario en parafina 1
NMDA-R	receptor N-metil-D- aspartato
PLAP	Fosfatasa alcalina placentaria
SALL4	Proteína 4 similar a SAL
SNPs	Polimorfismos de nucleótido simple
SV	Saco vitelino
SVH	Saco vitelino humano
SVSH	Saco vitelino secundario humano
TE	Túbulos endodérmicos
TCG	Tumores de células germinales
TCGM	Tumores de células germinales malignos
TTF1	Factor de transcripción tiroideo
TV(TEP)	Tumor vitelino tumor endodérmico primitivo
VIC	Vesículas intracelulares

Resumen

Introducción: Los tumores de células germinales (TCG) son desde el punto de vista morfológico, un grupo marcadamente heterogéneo de neoplasias que no hace sino remedar a modo de caricatura los distintos estadios de la embriogénesis reproduciendo tanto tejidos somáticos (teratomas) como extraembrionarios temporales tales como la placenta, con su contraparte tumoral el coriocarcinoma, y el saco vitelino con los *tumores vitelinos (tumores endodérmicos primitivos)* TV (TEP), término propuesto por nuestro grupo de investigación en 2012, motivado por la gran diversidad morfológica del tumor.

Propósito: el objetivo principal de esta tesis doctoral es establecer el fenotipo inmunohistoquímico del SVSH, haciendo énfasis en su actividad hepática, intestinal y pluripotencial, para aplicarlo posteriormente como panel inmunohistoquímico resultante a los TEP, para establecer la comparación con el SVSH y para el diagnóstico histopatológico. Adicionalmente, aplicar paneles diagnósticos tanto de marcadores de pluripotencialidad como de diferenciación, al estudio de tumores de células germinales comunes a todas las localizaciones, utilizando estos anticuerpos en situaciones neoplásicas características y problemáticas desde el punto de vista diagnóstico.

Materiales, métodos y resultados: el desarrollo de nuestros planteamientos nos permitió publicar 7 artículos:

1. *Nogales FF, **Dulcey I**. The secondary human yolk sac has an immunophenotype indicative of both hepatic and intestinal differentiation. Int*

J Dev Biol. 2012;56(9) : 755-60. doi: 10.1387/ijdb.120080fn. Factor de impacto: 2.823. http://www.citefactor.org/impact-factor-list-2012.html

2. *Nogales FF, Quiñonez E, López-Marin L, **Dulcey I**, Preda O. A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac. Histopathology. 2014 Jan 20. doi: 10.1111/his.12373.* Factor de impacto: 2.857. http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2559
3. *Preda O, Dema A, Iacob M, Goyenaga P, **Dulcey I**, Aneiros-Fernández J, Nogales FF. Urothelial carcinoma of the renal pelvis with simultaneous trophoblastic and malignant clear cell endodermal-type differentiation. Virchows Arch 2012 Mar;460(3):353-6. doi:10.1007/s00428-012-1211-5.* Factor de impacto: 2.491. http://www.citefactor.org/impact-factor-list-2012.html
4. *Preda O, Dulcey I, Nogales FF. Papel de los nuevos marcadores inmunohistoquímicos en los tumores de células germinales malignos gonadales. Rev Esp Patol. 2012;45(4):195-203.*
5. *Nogales FF, **Dulcey I**, Preda O. Germ Cell tumors of the ovary: an update. Arch Pathol Lab Med. 2014 Mar;138(3):351-62. Doi:18.5858/arpa.2012-0547-RA.* Factor de impacto: 2.78. http://www.researchgate.net/journal/0003-9985_Archives_of_pathology_laboratory_medicine
6. *Nogales FF, Preda O, Dulcey I. Gliomatosis peritonei as a natural experiment in tissue differentiation. Int J Dev Biol. 2012;56(10-12):969-74.*

doi:10.1387/ijdb.120172fn. Factor de impacto: 2.823. http://www.citefactor.org/impact-factor-list-2012.html

7. Dulcey I, Céspedes MU, Ballesteros JL, Preda O, Aneiros-Fernández J, Clavero PA, Nogales FF. Necrotic mature ovarian teratoma associated with anti-N-metyl-D-aspartate receptor encephalitis. Pathol Res Pract. 2012 Aug 15;208(8): 497-500. doi: 10.1016/j.prp.2012.05.018. Factor de impacto: 1.213. 2.491. 2.http://www.citefactor.org/impact-factor-list-2012.html

Conclusiones: El saco vitelino secundario humano (SVSH) tiene un inmunofenotipo híbrido de diferenciación intestinal y hepática. Estas funciones de síntesis y transferencia se reflejan en la expresión de proteínas características de función hepática como AFP, GPC3, SALL4 y HepPar-1, así como villina y CDX2, características de diferenciación intestinal. Este panel diagnostico inmunohistoquímico se aplicó a varios tipos de TV(TEP), encontrando que los patrones clásicos reproducen el inmunofenotipo del SVSH y del endodermo temprano con expresión variable de AFP y marcadores de diferenciación intestinal y hepática, mientras que los patrones somático glandulares con diferenciación intestinal tienen un inmunofenotipo incompleto. El panel de diagnóstico diferencial propuesto incluye los marcadores de pluripotencialidad SALL4 y LIN28, y los marcadores endodérmicos APF, GPC3 y villina. Este panel identifica la superposición de patrones de multidiferenciación en los inmunofenotipos primitivos y somáticos, lo cual apoya el término de tumor endodérmico primitivo. Adicionalmente, este panel hace posible la identificación de formas poco frecuentes de TV(TEP). Con estos datos de referencia aplicamos nuestro estudio

comparativo al análisis de nuevos marcadores de pluripotencialidad en el campo de tumores de células germinales malignos, marcadores que determinan una mayor sensibilidad diagnóstica.

Introducción

Las células germinales.

Las células germinales gonadales son el producto de la diferenciación de las células germinales primordiales (CGP), las cuales están originadas en el epiblasto proximal (1). Experimentalmente se constata que las proteínas morfogenéticas óseas (BMP) 2 y 4, secretadas por el ectodermo extraembrionario y por el endodermo visceral, inducen la expresión de marcadores de células germinales en grupos de aproximadamente 40 células (2). Estas también reciben estimulación con OCT4, BLIMP1, Stem Cell Factor (SCF) (factor estimulante de células madre), producidos por estructuras extraembrionarias como el saco vitelino, para la diferenciación y mantenimiento de su fenotipo (1).

Entre las semanas 4 y 5 post concepción, las CGP migran desde el mesenterio dorsal del intestino posterior por la pared corporal hasta la cresta gonadal, donde ingresan por la cara ventral del mesonefros (Figura 1) (1). La migración que inicia con el paso de las CGP a través del epitelio intestinal (3), se realiza inicialmente por un gradiente quimiotáctico mediado por altos niveles de SCF a lo largo de toda la ruta migratoria (1).

Se invocan algunos mecanismos reguladores: el SCF se une al C-kit (CD117), un receptor de tipo tirosin-quinasa presente en la membrana celular de las CGP. Al unirse SCF con C-kit se forma un dímero que activa el sistema tirosin-quinasa, desplegando una serie de señales intracelulares que permiten a las CGP

seguir la señal quimiotáctica hacia las gónadas (4), utilizando entre las semanas 7 a 14 las fibras nerviosas simpáticas del sistema nervioso autónomo en desarrollo, ruta con altos niveles de SFC, fibronectina y moléculas de adhesión (1).

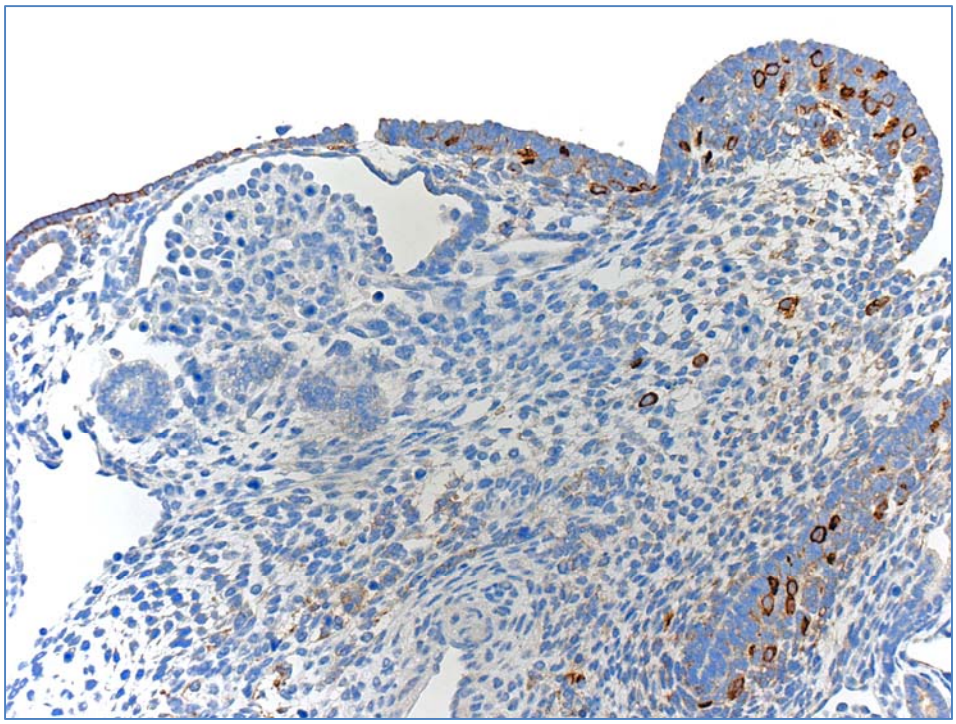

Figura 1. CGP positivas para LIN28 migrando a través de la cara ventral del mesonefros.

Para seguir la señal quimiotáctica, las CGP tienen motilidad intrínseca, que induce la formación de protrusiones citoplasmáticas (2, 5), con distintas velocidades y patrones migratorios (5), comprobado con la detección de CGP en diferentes tejidos de la vía migratoria: línea media y región paraaórtica (1). La migración de CGP constituye un fenómeno relevante a la localización ulterior de los tumores de células germinales. Por esta razón los tumores se distribuyen a lo largo de la línea media (desde la pineal al coxis) como expresión de esta

migración. La figura 1 ilustra excepcionalmente la migración temprana de CGP a una gónada todavía indiferente.

La inmunohistoquímica visualiza las CGP mediante la detección de proteínas de pluripotencialidad de dichas células tales como el LIN 28 y el OCT4. El OCT4 es un factor de transcripción producto del gen POU5F1. Sus funciones más importantes son mantener la pluripotencialidad y la viabilidad de la línea germinal en mamíferos. Las CGP que carecen de OCT4 sufren apoptosis (6). Las características del LIN28 serán expuestas posteriormente.

En el embrión tardío, las CGP que permanecen en la línea migratoria son eliminadas por apoptosis por medio de la proteína BAX (proteína X asociada al BCL2)(1). BAX forma parte de la familia de genes BCL2, cuyos miembros actúan como reguladores pro o anti apoptóticos (7). Algunas CPG fallan en el momento de dirigirse hacia las gónadas y continúan migrando por las fibras nerviosas autónomas. Cuando estas células no son eliminadas se pueden identificar en localizaciones ectópicas como pelvis, mediastino, tórax, SNC y en las glándulas adrenales (8). Ello sustancia la teoría de que las CGP ectópicas serían las responsables de los tumores germinales extragonadales, ya que se localizan en los sitios de mayor frecuencia de presentación de este tipo de tumores (1).

Una vez colonizadas las crestas gonadales, las CGP pierden la motilidad, y sufren cambios en el patrón de las histonas, desmetilación del genoma y reactivación de X inactivo en las células XX (2, 9). El medio ambiente gonadal (ovario o testículo) contribuye a iniciar la diferenciación hacia oogonia o

espermatogonia. Curiosamente, existen ejemplos en los que CGP ectópicas presentes en las glándulas adrenales del varón tienen la capacidad de diferenciarse hacia oogonias (1).

En el testículo la expresión del factor de transcripción SRY estimula al gen SOX9, que es el responsable de la diferenciación de las células de Sertoli, quienes a su vez crean el ambiente hormonal necesario para el desarrollo testicular (10). Las células germinales entran en la fase mitótica G1/G0, y justo antes de la pubertad inician la meiosis.

En el ovario, las células CGP son estimuladas por los productos de los genes foxl2, WNT4 y RSPO1, que las inducen a entrar en meiosis 1 (2).

Las células germinales van a constituir la célula madre totipotencial a partir de la cual se va a diferenciar la enorme variación histológica de los tumores de células germinales.

Tumores de células germinales

Epidemiología y factores de riesgo

Los tumores de células germinales (TCG) son desde el punto de vista morfológico, un grupo marcadamente heterogéneo de neoplasias que no hace sino diferenciar a modo de caricatura (11) los distintos estadios de la embriogénesis, desde remedar la célula germinal primitiva (seminomas/disgerminomas), hasta el desarrollo tisular (teratomas) a través de

estadios transitivos tales como el periodo morular y blastocisto (carcinoma embrionario). Los tejidos diferenciados por estos tumores pueden ser tanto somáticos (teratomas) como tejidos extraembrionarios temporales tales como el saco vitelino (tumores endodérmicos primitivos) y la placenta (coriocarcinoma).

La mayor incidencia de los TCG tiene lugar en niños y adultos jóvenes, en quienes representa respectivamente el 3,3% y el 13,8% de todos los cánceres (12). Su localización más frecuente es la gonadal con frecuencias en testículo y ovario de 92,5% y 3,9% respectivamente, y de modo menos frecuente en situación extragonadal donde ocurren con una frecuencia de 3,2% (12). En el testículo los tumores de células germinales malignos (TCGM) representan el tipo de tumor maligno más común (10), mientras que en el ovario apenas constituyen un 3% de todos los tumores malignos (13), afectando fundamentalmente a niñas y adolescentes (12).

Los factores de riesgo de los TCG son poco conocidos, ignorándose la génesis de las lesiones precursoras, que son bien conocidas en el testículo (neoplasia intratubular germinal) y en intersexos (gonadoblastomas), pero desconocidas en los TCGM ováricos. Los factores generales incluyen la raza, la predisposición genética, los aspectos medioambientales y las alteraciones en el desarrollo.

Grupos raciales. En los Estados Unidos se detecta una mayor incidencia en pacientes de raza blanca frente a los afroamericanos, africanos y asiáticos (14),

mientras que a nivel mundial, las tasas mayores de TCGM corresponden a los países de Europa Occidental (12).

Factores medio ambientales. La exposición intrauterina a altos niveles de estrógenos o a sustancias estrogenomiméticas, es uno de los factores de riesgo ambiental postulado como posible iniciador de la histogénesis (10, 14), ya que estas sustancias al parecer detienen el desarrollo de las CGP en un estadio gonocitario, lo que haría potencialmente vulnerables a los gonocitos a un daño genómico, lo que determinaría el posible desarrollo de una neoplasia intratubular germinal (10). Igualmente, se han barajado otros factores medioambientales, tales como el tabaquismo materno durante la gestación, el gemelismo dicigótico, las dietas ricas en queso, la talla definitiva en el adulto (14), la exposición a sustancias tóxicas, algunas de ellas estrogenomiméticas, como los pesticidas organoclorados, el dietilestilbestrol, el bifenil policlorinato y la ocratoxina A (15). Sin embargo, la relación no es clara en muchos casos, necesitándose estudios concluyentes para determinar una clara asociación con el desarrollo de TCG y el mecanismo biológico subyacente.

Alteraciones en el desarrollo genitourinario. El síndrome de disgenesia testicular que incluye la criptorquidia, las hipospadias y la hipoespermia, está asociada con el desarrollo de TCG. A su vez, el desarrollo del síndrome de disgenesia testicular está asociado con la exposición altos niveles de estrógenos durante la gestación, y con otros factores medioambientales con efecto antiandrogénico (14). Los pacientes con criptorquidia tienen un riesgo 4 veces

mayor, duplicado en los casos de bilateralidad del proceso, de desarrollar TCGM. Este riesgo, disminuye 2,2 veces con la realización de una orquidopexia (16).

Los síndromes intersexuales o desordenes del desarrollo sexual, están definidos como desarrollo incompleto de las gónadas en pacientes con discordancia entre el sexo fenotípico, gonadal y genético. Sin embargo, no todos los síndromes pertenecientes a este grupo tienen un aumento del riesgo de desarrollar TCGM. Los síndromes que presentan un aumento del riesgo son las disgenesias gonadales pura y mixta y los síndromes de subvirilización. Los síndromes de hipervirilización por exceso de secreción de testosterona no presentan un riesgo mayor que el de la población general (17).

Alteraciones cromosómicas. El síndrome de Klinefelter y el síndrome de Down están asociados con el desarrollo de TCGM. En el caso de los pacientes con síndrome de Klinefelter, el riesgo frente a la población general es 50 veces mayor con una localización característica de los tumores en el mediastino anterior. En los pacientes con síndrome de Down el aumento del riesgo está posiblemente relacionado con el aumento de la incidencia de la criptorquidia en este grupo poblacional (14).

Predisposición genética. En casos con historia familiar de TCGM el aumento del riesgo entre padres e hijos es de 4 veces mayor siendo 10 veces mayor entre hermanos. En los estudios genómicos realizados en familias afectadas de TCGM familiares, se postulan dos loci asociados con susceptibilidad

al desarrollo de TCG. El primero de ellos es el Xq27, asociado también con la criptorquidia, y el segundo el 12q (14).

Sin embargo, se cree que en el desarrollo de TCG existe una multicausalidad donde posiblemente, interactúan distintos eventos desencadenantes entre los genes alterados y el medio ambiente, así como con otros sucesos facilitadores del proceso neoplásico (14).

Histogénesis

La histogénesis de los TCG es sin duda, una de las mejores conocidas de todos los grupos tumorales debido a sus paralelismos, ya apuntados, con la embriogénesis. Los conceptos histogenéticos han evolucionado fundamentalmente al conocerse mejor los distintos estadios embriológicos del desarrollo y la capacidad pluripotencial de sus células. Los distintos acercamientos clasificatorios histogenéticos se han visto sustanciados hoy en día por el descubrimiento de marcadores histocitológicos que identifican la pluripotencialidad de las células y los estadios sucesivos del desarrollo. Dichos marcadores identifican los tipos tumorales y cristalizan una clasificación racional de los TCG (18).

El primer intento clasificatorio fue realizado en los años 50 del siglo pasado por el ya legendario fascículo de tumores testiculares de Dixon y Moore (19), donde propusieron un esquema ampliamente aceptado donde las células germinales figuran como origen de los TCG, a partir de las cuales se derivan dos rutas diferenciativas: la primera, la de los tumores seminomatosos, donde el

seminoma se considera en estado de diferenciación terminal incapaz de realizar diferenciaciones ulteriores. La segunda, conformada por los tumores no seminomatosos, en donde del carcinoma embrionario (CE) considerado como pluripotencial, sería el precursor de los teratomas y los coriocarcinomas (Figura 2). Este modelo inicial fue sustanciado ampliamente por las primeras investigaciones experimentales de teratomas murinos que demostraron las similitudes morfológicas entre los tumores y el estadio correspondiente del desarrollo (11, 20) y adicionalmente, por la introducción del concepto de tumores teratoides extraembrionarios por Teilum (21). Esta clasificación, con la modificación de Teilum (22) ha permanecido vigente durante más de 60 años (23), e incluso todavía está presente en un texto actual de referencia (24).

Debido a los progresos realizados en el estudio de los TCG, el modelo clásico no explicaba algunas cuestiones tales como la presencia de células del sincitiotrofoblasto o la expresión de marcadores inmunohistoquímicos epiteliales y endodérmicos en los germinomas puros, o bien la presencia de un componente seminomatoso en los tumores germinales mixtos y las metástasis no seminomatosas tras un seminoma puro.

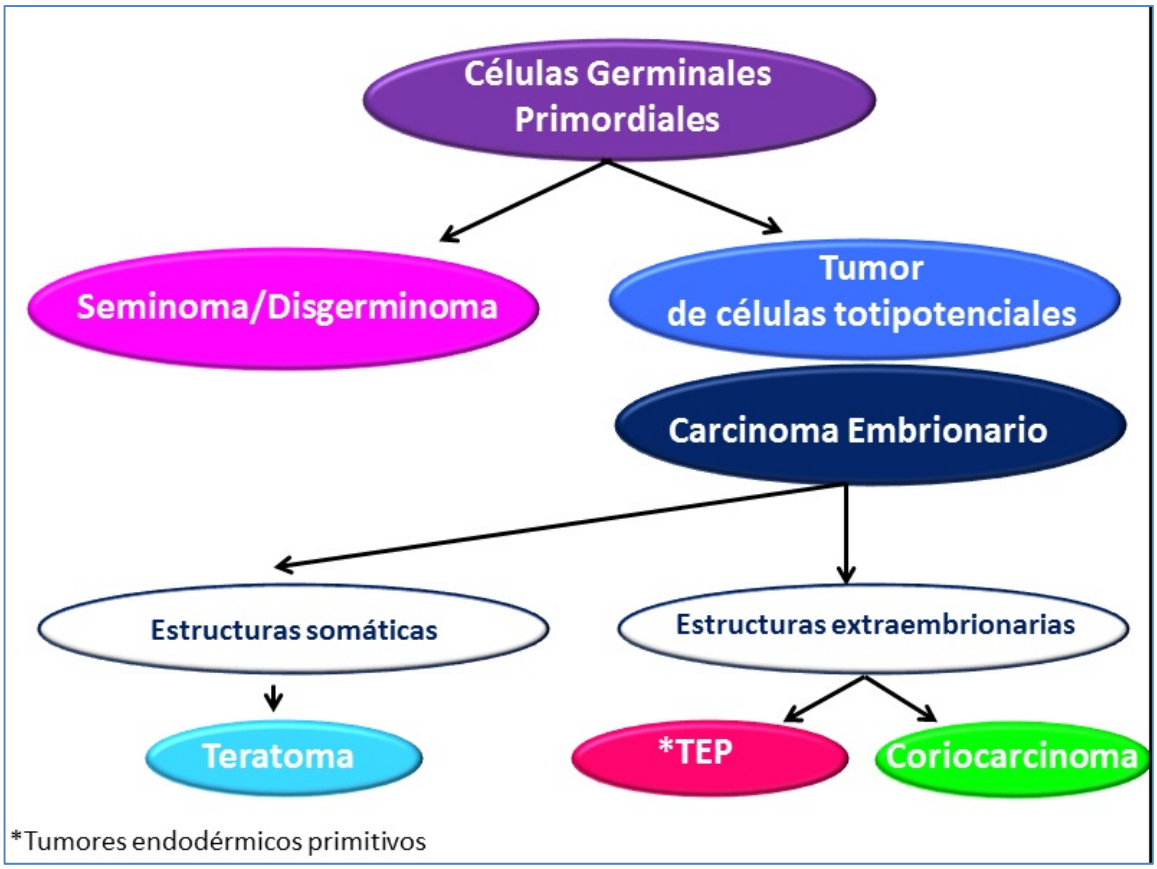

Figura 2. Modelo clásico de histogénesis propuesto por Dixon y Moore (19) y modificado por Teilum (22, 25).

Para responder a estos interrogantes se propuso un modelo fundamentado en la pluripotencialidad del seminoma/disgerminoma y la capacidad de diferenciación e interrelación entre los distintos tipos de tumores, fundamentalmente basado en la expresión inmunohistoquímica de cada entidad. Este esquema pierde la linealidad bidemensional del modelo clásico para convertirse en un tetraedro en el que las relaciones entre los distintos tipos tumorales son multidireccionales.

El modelo de Srigley muestra la expresión de marcadores inmunohistoquímicos tales como α-fetoproteína (AFP), citoqueratinas (CK) y gonadotropina coriónica humana (hCG) que identifican los componentes vitelino, trofoblástico y epitelial de los tumores TCG. (Figura 3). Sin embargo, este modelo es posiblemente cierto para los tumores testiculares, pero no totalmente aplicable para otras localizaciones de los tumores germinales. La ventaja principal del esquema es explicar la alta frecuencia de TCG mixtos.

Como este modelo tetraédrico no incluye la neoplasia intratubular germinal, Ulbright (26, 27) plantea una nueva hipótesis donde divide la histogénesis en dos: los tumores prepuberales donde las células germinales son las precursoras directas de los teratomas y los tumores endodérmicos primitivos; y los postpuberales, donde las células germinales dan origen a la neoplasia intratubular germinal, que se diferencia al seminoma, este al coriocarcinoma y a los tumores vitelinos endodérmicos primitivos y estos últimos a teratomas. El seminoma se puede diferenciar también al carcinoma embrionario y este al teratoma (Figura 4). Este modelo histogenético posee los mismos problemas que el anterior al estar basado en tumores testiculares, pero ignorando los de otras localizaciones.

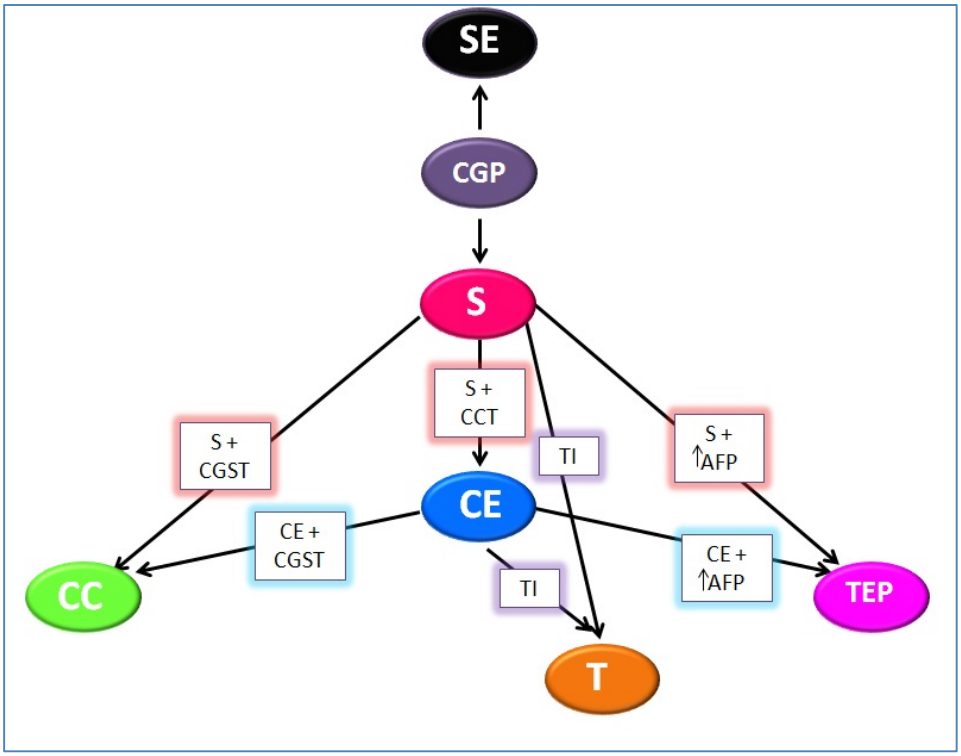

Figura 3. Modelo tetraédrico de los TCG propuesto por Srigley (28). SE: seminoma espermatocítico. CGT: Células germinales primordiales. S: seminoma. CE: carcinoma embrionario. CC: coriocarcinoma. T: teratoma. TEP: tumor endodérmico primitivo. TI: teratoma inmaduro. S+CGST: seminoma con células gigantes sincitiotrofoblasticas. S+CCT: seminoma con características carcinomatosas tempranas. S+AFP: seminoma con elevación de la AFP. CE+CGST: carcinoma embrionario con células gigantes del sincitiotrofoblasto. CE+AFP: carcinoma embrionario con elevación de la AFP (26).

Este esquema incluye la posibilidad de una cadena de eventos iniciada intraútero por un estímulo tumorigénico desconocido, el cual en los TCG masculinos llevaría al desarrollo de una neoplasia intratubular germinal que de modo postpuberal sería promovida por las hormonas sexuales hasta desarrollar tumores malignos. Ello plantearía una diferencia sustancial en la histogénesis de los tumores prepuberales y postpuberales (14) , al menos en el testículo.

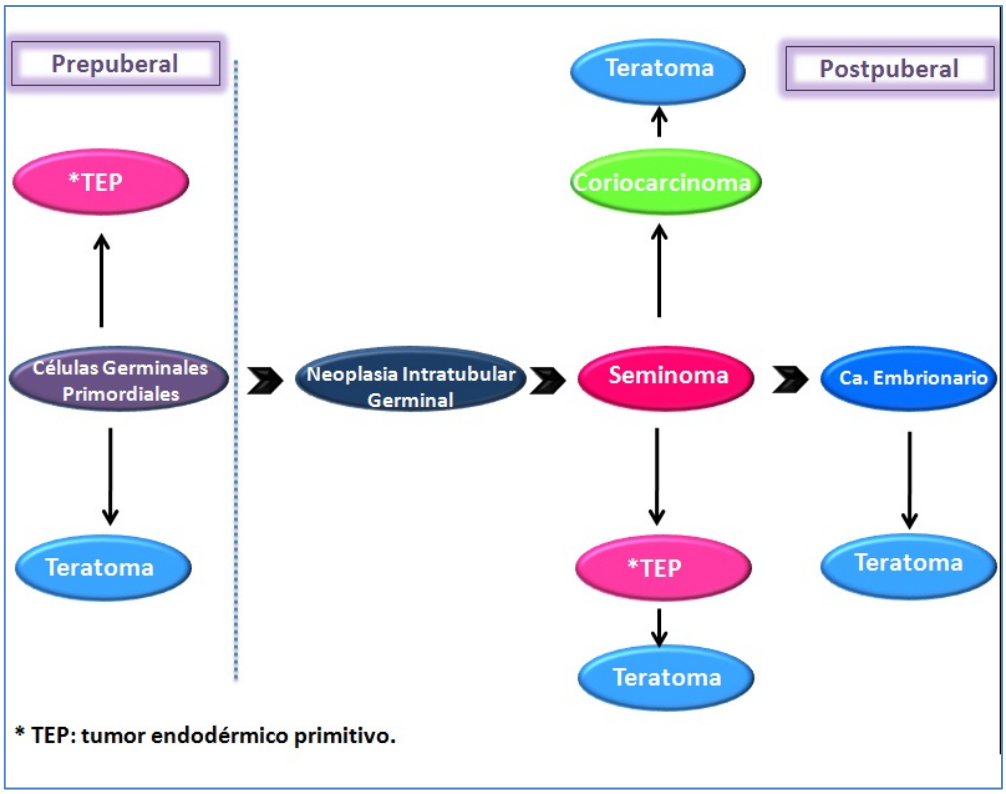

Figura 4. Modelo de histogénesis de los TCG propuesto por Ulbright (26, 27)

Recientemente, nuestro grupo de investigación ha propuesto un esquema clasificatorio basado en la expresión de marcadores de pluripotencialidad que contribuyen a la identificación diagnóstica de los distintos patrones de TCGM (figura 5) (29).

Si bien los modelos histogenéticos intentan explicar los pasos existentes entre una CGP normal y el desarrollo de un TCG, las alteraciones genéticas explicarían los eventos subyacentes a los cambios morfológicos. A continuación describiré las principales alteraciones genéticas encontradas en estos tumores.

Aunque aún no se ha podido establecer un perfil genético constante de las familias afectadas, en estudios de polimorfismo de nucleótido simple (SNPs) se han propuesto seis loci asociados con el desarrollo de TCG. Estos son: 5q31, 9p24, 12q21, que son relativamente constantes en varios estudios, y 5p15, 6p21 y 12p13. De este grupo, 3 loci 5q31, 6p21 y el 12q21, contienen genes relacionados con vías de señalización, que los relaciona con el desarrollo tumoral (10).

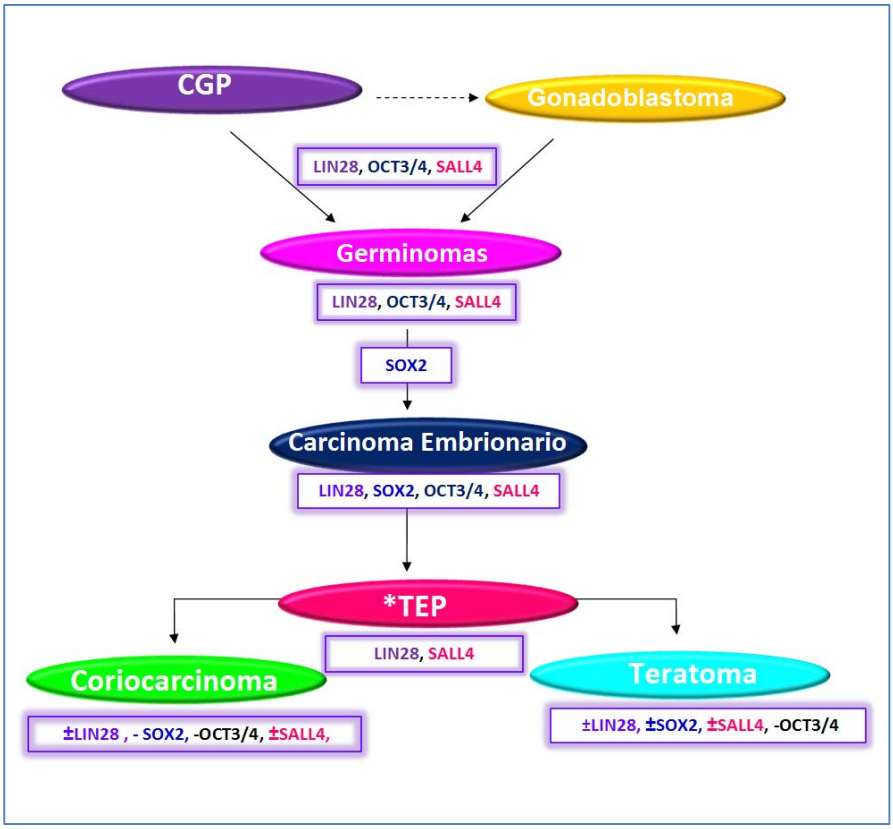

Figura 5. Modelo propuesto en base a marcadores pluripotenciales. en células germinales (29).

Genética.

Las alteraciones citogenéticas más frecuentemente encontradas son: ganancia de material genético en el brazo corto del cromosoma 12 (12p) y en los cromosomas

1, 5, 7, 8, 17 y X, así como pérdida de material genético en los cromosomas 4, 11, 13, 15, 18 y Y (10, 30).

En la n*eoplasia intratubular germinal* las alteraciones citogenéticas más comunes son la ganancia de material genético en los cromosomas 1, 5, 7 y X y en el brazo corto del cromosoma 12, así como pérdida de material en el cromosoma 18 (14) . También se observa una reexpresión de marcadores de pluripotencialidad como el CD117 y el OCT3/4, los que aparte de ser una herramienta diagnóstica, ayudarían a explicar la capacidad de esta lesión para diferenciarse a tumores invasivos seminomatosos y no seminomatosos (14, 31). El s*eminoma* está asociado citogenéticamente con ganancia de material genético en los cromosomas 12, 15q, 17q y 22q, y pérdida de material genético en el cromosoma 10q (10). Desde el punto de vista de los genes de pluripotencialidad, a parte del OCT3/4, el LIN28 (32) y la proteína 4 similar a SAL (SALL4), el seminoma también presenta sobreexpresión del c-Kit, a veces acompañado de sobre expresión del K-ras, con la activación de la cascada intracelular mediada por el fosfatidil inositol 3 quinasa. En experimentos in vitro la activación de esta ruta hace células tumorales más resistentes (14). El seminoma espermatocitico, a diferencia del seminoma clásico no muestra alteraciones en el cromosoma 12p pero si pérdida del material genético en el cromosoma 9 (33) . El c*arcinoma embrionario* a nivel citogenético tiene ganancia de material genético en 17p y pérdida en 2p (10). Adicionalmente, dentro del grupo de los no seminomatosos es el tumor que expresa más genes de pluripotencialidad tales como el NANOG, OCT3/4, SOX2 , LIN28 y SALL4, todos implicados en el mantenimiento de la

pluripotencialidad, en la diferenciación y crecimiento celular (14, 31, 32), lo que lo hace similar al perfil de las células embrionarias normales (por ejemplo las de la mórula embrionaria y macizo celular interno del blastocisto). Los otros tumores pertenecientes a este grupo al ser neoplasias más diferenciadas expresan menos genes de pluripotencialidad, el tumor vitelino (*tumor endodérmico primitivo*) -TV(TEP)- expresa SALL4 y LIN28 (34, 35), y el *teratoma* de forma variable SOX 2 y SALL4. El coriocarcinoma, al ser el más diferenciado, no expresa genes de este tipo (18).

El c*romosoma 12p*, isocromosoma 12p (i12p),es la alteración más frecuente encontrada en los TCG seminomatosos y no seminomatosos invasivos, y también en la neoplasia intratubular germinal (10, 14, 31) y en el disgerminoma (36). Se plantea que no es una condición necesaria para desarrollar neoplasia intratubular germinal porque se han encontrado neoplasias intratubulares germinales negativas sin tumores invasivos adyacentes negativos a i12p. Sin embargo, es siempre positivo en neoplasias intratubulares germinales adyacentes a tumores invasivos positivos, lo que permite plantear que la presencia de i12p sea necesaria para desarrollar tumores invasivos (10). Con respecto a los genes ubicados en el brazo corto del cromosoma 12 que podrían estar implicados en el desarrollo de TCG, el gen de la ciclina D2 ubicado en 12p está sobreexpresado en algunos TCG, pero se necesitan más estudios confirmatorios para vincular a este gen con el desarrollo de TCG y para detectar otros posibles genes candidatos (31). El isocromosoma 12p se utiliza como un marcador citogenético de TCGM (figura 6).

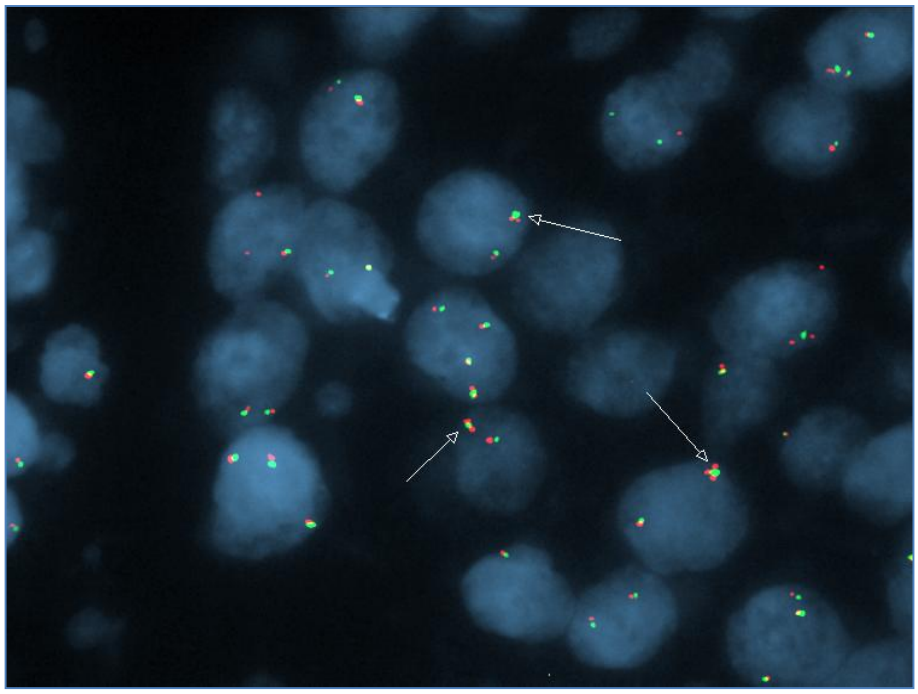

Figura 6. Isocromosoma 12q presente en un seminoma testicular.

Diagnóstico histopatológico

Histopatológicamente, los TCG tienen igual morfología e inmunohistoquímica en ovario y testículo así como en las localizaciones extragonadales. A continuación describiré las principales características histopatológicas e inmunohistoquímicas necesarias para establecer un diagnóstico en los TCG.

Neoplasia intratubular germinal. La neoplasia intratubular germinal no clasificada es la lesión precursora de los tumores invasivos postpuberales con excepción del seminoma espermatocítico. Histopatológicamente está caracterizada por túbulos seminíferos con engrosamiento de la membrana basal y

espermatogénesis reducida o ausente, tapizados parcial o totalmente por una sola capa de células germinales aumentadas de tamaño (2 a 3 veces el tamaño de las células germinales normales). Citológicamente las células tienen núcleos agrandados con 1 o varios nucleolos prominentes y citoplasma claro (33). La lesión puede diseminarse de modo intraepitelial por la rete testis (33).

Aunque morfológicamente la lesión sea fácil de identificar, de un modo sencillo la tinción de PAS puede ayudar a hacer el diagnóstico ya que las células germinales de los túbulos normales son PAS negativas (33).

Inmunohistoquímicamente, las células germinales anormales son positivas para fosfatasa alcalina placentaria (PLAP), c-Kit, D-240, OCT3/4 y SALL4, este último estudiado por nuestro grupo de investigación (29); y negativas para AFP, hCG, CK y p53 (33). Aunque el diagnóstico es relativamente fácil, debe tenerse en cuenta que en pacientes con síndromes de baja virilización existe una expresión prolongada de marcadores fetales de las células germinales: c-Kit, D2-40, y OCT3/4. Así pues, en la valoración de estas muestras se debe tener en cuenta la edad del paciente en el momento de la biopsia, y la distribución de las células OCT3/4 positivas, que en la neoplasia intratubular germinal deben estar intratubulares y basales a diferencia de la maduración tardía donde se distribuyen a lo largo de todo el testículo (17).

También hay neoplasias intratubulares diferenciadas que tienen las mismas características del tumor invasor al que dan origen: seminoma intratubular, carcinoma embrionario intratubular y tumor vitelino intratubular (33).

Germinoma: Seminoma/Disgerminoma. El germinoma es el tumor testicular más común y el segundo TCGM ovárico. El seminoma se presenta en hombres con una edad de 35 a 45 años, mientras que el disgerminoma se presenta en mujeres de 15 a 30años. Tiene una citología característica con células redondas o poligonales de bordes citoplasmáticos eosinófilos bien definidos, citoplasma claro y núcleo central con uno o más nucléolos prominentes, dispuestas en sábanas atravesadas por septos fibrovasculares. Adicionalmente hay infiltrado inflamatorio de linfocitos T y en algunos casos reacción granulomatosa, que puede ser tan intensa que enmascara el tumor (18, 29, 33, 37).

Las células pueden disponerse ocasionalmente de forma cribiforme o formando microquistes, patrón que pueden llevar a confusión con TV (TEP)s. Histológicamente la diferencia se puede establecer porque en el germinoma los microquistes y los pseudotúbulos están tapizados por las típicas células germinales malignas, algunas de ellas esfaceladas en la luz, y no por células endodérmicas. Además los germinomas tienen infiltrado inflamatorio característico (18, 29, 33, 37). También pueden formar túbulos donde podría confundirse con un tumor de células de Sertoli, en el testículo, pero la presencia de neoplasia intratubular germinal adyacente habitualmente esclarecerá el diagnóstico. La disposición intertubular, donde las células neoplásicas se distribuyen entre los túbulos seminíferos que permanecen intactos, puede confundirse con una neoplasia hematolinfoide. Aquí se deben buscar áreas, si las hay, de expansión de los espacios intertubulares, aumento en el número de células de Leydig, y neoplasia intratubular adyacente. También se pueden encontrar células del

sincitiotrofoblasto, que explican el aumento de hCG y sus consecuencias clínicas, pero sin repercusión pronostica. Cuando hay pleomorfismo celular y abundantes mitosis (más de 3 en un campo de alto aumento) se puede hablar de un germinoma anaplásico. Un último fenómeno es la regresión espontánea del tumor que puede debutar como un proceso metastásico (29, 33, 37).

Aunque los germinomas son PAS positivos por su glucógeno citoplasmático, la utilización de inmunohistoquímica a veces es necesaria para apoyar el diagnóstico. Los germinomas son positivos para OCT3/4, SALL4, LIN28, c-Kit, PLAP y D-240, siendo negativos para AFP y hCG. La tinción con CK es positiva hasta en un 40%, con un patrón puntiforme (18, 29, 32). Algunas veces el citoplasma es eosinófilo o anfofílico acompañado de escaso infiltrado inflamatorio debiéndose diferenciar del carcinoma embrionario por la expresión de CD30, que es negativo en el germinoma. Cuando hay necrosis extensa donde no hay viabilidad tumoral se debe utilizar c-Kit, OCT3/4, SALL4 que mantienen su positividad a pesar de la necrosis (18, 29, 33, 37).

Seminoma espermatocítico. Varias características diferencian este tumor de los otros TCG, la primera es que se presenta exclusivamente en el testículo sin contraparte ovárica ni extragonadal, la segunda que no muestra neoplasia germinal intratubular adyacente, la tercera que no forma parte de tumores germinales mixtos, la cuarta que la edad de presentación está por encima de los 50 años y la quinta que no metastatiza (33, 37) . Microscópicamente está conformado por tres tipos de células: células pequeñas del tamaño de un linfocito (<10μm) con núcleos de cromatina densa, células medianas, entre 10-30 μm,

población tumoral predominante, y células gigantes entre 50 y 100 µm, las dos últimas tienen núcleos agrandados con cromatina filamentosa que recuerda los espermatocitos normales (de ahí su nombre), el citoplasma es eosinófilo o anfofílico sin glicógeno (células PAS negativas) (33, 37). Las células tumorales se disponen en cordones, islas y áreas sólidas en medio de un estroma edematoso, con frecuente diseminación intratubular. Además, se pueden encontrar componentes sarcomatosos caracterizados por células ahusadas indiferenciadas. También, hay una variante anaplásica caracterizada por marcado pleomorfismo celular con nucléolos prominentes, necrosis e invasión a la túnicas y linfovascular, pero sin elementos sarcomatosos (33, 37). Tanto en la variante anaplásica como en tumores con elementos sarcomatosos hay áreas habituales de seminoma espermatocítico que ayuda a establecer el diagnóstico. El diagnóstico diferencial de debe establecer con el seminoma clásico, donde el infiltrado inflamatorio y la neoplasia intratubular germinal adyacente descartan el diagnóstico tanto del seminoma espermatocítico como del linfoma de célula grande (33, 37).

Desde el punto de vista inmunohistoquímico, el seminoma espermatocítico es positivo para c-Kit y VASA, este último anticuerpo detecta la proteína codificada por el gen VASA expresado únicamente en las células de línea germinal: oogonias y espermatogonias; también es positivo para CK de forma variable. Es negativo sin embargo para marcadores de células germinales como OCT3/4, SALL4, LIN28 y PLAP, lo que ayudaría a establecer el diagnóstico diferencial con el seminoma. También es negativo para AFP, CD30 y hCG (32, 33, 37).

Carcinoma embrionario. Es un tumor de células pluripotenciales común en el testículo y muy infrecuente en el ovario, fenómeno explicado por la histogénesis: mientras que en el testículo se origina en células germinales malignas, en el ovario en células meióticas o postmeióticas (18). La edad promedio de presentación está entre los 15 a 25 años y no se presenta en niños. Histopatológicamente, alrededor de un 10% se presenta en forma pura, pero la gran mayoría forma parte de TCG mixtos (33). Microscópicamente, está caracterizado por células poligonales o indiferenciadas, con núcleos de cromatina vesicular u ópticamente vacíos, con citoplasmas granulares que a diferencia del germinoma tienen bordes citoplasmáticos indistintos. Estas células pueden disponerse de forma sincitial, papilar, sólida y glandular (29, 33, 37). Cuando se presentan estos últimos patrones se debe establecer un diagnóstico diferencial con germinomas y TV(TEP) (18). Durante el estudio microscópico se debe tener especial atención con las invasiones linfovasculares, ya que en muchos casos se trata de un “efecto de arrastre” durante el proceso de examen macroscópico. Dese el punto de vista inmunohistoquímico el carcinoma embrionario tiene dos marcadores que permiten establecer la diferencia con otros TCGM: el CD30, con una expresión membranosa (18), exclusivo del carcinoma embrionario dentro del grupo de los TCG (18, 29, 33, 37), y el SOX2, este último un marcador de pluripotencialidad asociado a OCT4, altamente expresado junto con NANOG en el blastocisto (38), que aunque tiene una positividad variable en el neuroepitelio de los teratomas inmaduros, permite establecer la diferencia con el seminoma clásico y con los tumores endodérmicos primitivos (29). El carcinoma embrionario es también positivo para LIN28, SALL4, y, OCT3/4 negativizado en las mestástasis post

quimioterapia, siendo también positivo para CK, PLAP y D2-40, este último con una positividad apical (18, 29, 32). Los carcinomas embrionarios pueden tener áreas positivas a AFP, lo que indicaría una diferenciación endodérmica (33), áreas también positivas al glipican 3 (GPC3), como en los cuerpos embrioides, delineando la cavidad vitelina primaria (18).Este tumor es negativo para hCG y c-Kit (29, 33, 37).

Coriocarcinoma. Su presentación como tumor puro en testículo y en el ovario es rara, con una frecuencia menor del 1% (18, 33, 37), siendo más habitual la forma testicular como componente de tumores mixtos (18, 33) . Se presenta en pacientes en la segunda o tercera décadas de la vida, generalmente como enfermedad metastatizante con síntomas relacionados con hemorragia en la localización de la metástasis, (hemoptisis, hemorragia del tracto digestivo o del sistema nervioso central), así como síntomas endocrinológicos relacionados con la elevación sérica de la hCG, tales como el hipertiroidismo y la ginecomastia (33). Microscópicamente, sobre un fondo de hemorragia y necrosis se distinguen dos poblaciones celulares, la primera conformada por células grandes con múltiples núcleos hipercromáticos dispuestos sobre un citoplasma eosinófilo, que representan al sincitiotrofoblasto; la segunda, conformada por células medianas, uniformes, con bordes citoplasmáticos claramente distinguibles y núcleo único, que representan al citotrofoblasto. Estas células se disponen entremezcladas en nidos y raramente en pseudovellosidades, estas últimas conformadas por las células del citotrofoblasto (33). Existen tres situaciones morfológicas que requieren especial atención, la primera ocurre en seminomas con células del

sincitiotrofoblasto, áreas que no deben ser confundidas con TCGM mixtos compuestos solo por seminoma y coriocarcinoma, ya que se debe recordar que esta combinación (sin un carcinoma embrionario acompañante) es extremadamente rara (33) y difícil de explicar con los modelos histogenéticos. Igual situación se presenta en los disgerminomas, donde menos del 10% tienen células del sincitiotrofoblasto acompañantes (18). La segunda situación ocurre en tumores ováricos donde se debe establecer el diagnóstico diferencial entre en TCGM con coriocarcinoma versus una metástasis ovárica de un coriocarcinoma gestacional. Para establecer el origen del tumor se deben tener datos clínicos de la paciente, en donde una historia obstétrica reciente inclina la balanza hacia un proceso metastatizante, así como, hacer un muestreo tumoral amplio para identificar (o descartar) áreas con otros tipos de TCGM (37). Y la tercera situación ocurre en estados postquimioterapia, en donde es posible encontrar tumores quísticos retroperitoneales tapizados por células trofoblásticas mononucleares (33). En los coriocarcinomas los marcadores hCG, CK, inhibina, CD10 (29) y GPC3, son fuertemente positivos (18, 29, 33). La citoqueratina es positiva en los tres tipos de trofoblasto (cito, sincitio y trofoblasto intermedio), la hCG en el sincitio y en el trofoblasto intermedio, la inhibina en el cito y en el trofoblasto intermedio, y el GPC3 aunque predomínate en el sincitio, se expresa también en el citotrofoblasto. El trofoblasto intermedio, el cual forma la rara contraparte masculina del tumor trofoblástico de sitio placentario, es también positivo a lactógeno placentario humano (18, 29, 33).

Teratoma. Al contrario de lo que ocurre con los germinomas y el coriocarcinoma, el teratoma puro es un tumor predominantemente ovárico con una frecuencia del 95% dentro del grupo de TCG frente a un 4% de los teratomas testiculares puros (37). Microscópicamente, los teratomas están conformados por tejidos derivados de las tres capas germinales: ectodermo, mesodermo y endodermo, lo que les confiere la capacidad de reproducir cualquier tipo de tejido, incluso en los teratomas inducidos experimentalmente a partir de células embrionarias humanas (39). Las principales características diferenciales entre los teratomas ováricos y testiculares pre y postpuberales se exponen en la tabla 1.

Quiste epidermoide. El quiste epidermoide es una entidad controvertida sobre la que se han formulado muchas teorías, entre ellas que sea un tipo de teratoma monodérmico, que, al igual que el quiste dermoide, tiene origen y curso clínico benigno. Está conformado exclusivamente por epitelio estratificado plano queratinizado, que en el quiste dermoide va acompañado de unidades pilosebáceas (13, 33).

Características	Teratoma maduro de ovario	Teratoma testicular postpuberal	Teratoma testicular prepuberal
Edad de presentación	Cualquier grupo de edad, más frecuentes en edad reproductiva	20-40 años	Niños 30% de tumores, en forma pura.
Macro	Quístico	Sólido	Quístico
Micro	Sin atipia citológica, arquitecturalmente organoide. Mitosis escasas	Con atipia citológica. Arquitecturalmente desordenado. Mitosis frecuentes.	Sin atipia citológica Arquitecturalmente organoide
Citogenética	Cariotipo normal 46XX. Homocigótico, explicado por un origen partenogenético. La heterocigosidad ocurre en un locus lejos del centrómero, sitio susceptible al crossing over durante la meiosis	Asociado a hiperdiplodía o hipotriploidía, puede tener i12p Como parte de TCG mixtos, los elementos teratomatosos muestran iguales alteraciones citogenéticas que los componentes no teratomatosos.	Cariotipo normal 46XY.
Origen	Células en meiosis I pero no en la II	Originado a partir de otros tumores de células germinales	-
Precursores	-	Neoplasia intratubular germinal	Ocasionalmente neoplasia intratubular germinal
Potencial maligno	El teratoma maduro es siempre benigno, porque la malignidad en un teratoma ovárico es conferida por la presencia y cantidad relativa de neuroepitelio inmaduro	Fenómenos metastatizantes de elementos no teratomatosos en 20 a 40% de los casos. Excepción quiste dermoide y epidermoide que se comportan de forma benigna.	Siempre benigno
Transformación maligna	Posterior al desarrollo del teratoma (transformación maligna post-teratomatosa)	Anterior al desarrollo teratomatoso (transformación maligna preteratomatosa)	-

Tabla 1. Principales características diferenciales de los teratomas ováricos y testiculares pre y postpuberales (18, 29, 37).

Teratoma inmaduro. La inmadurez en los teratomas está dada por la presencia de tejido neural inmaduro: neurotúbulos y blastema neural, y la graduación que al mismo tiempo sirve como factor pronóstico, por el porcentaje de tejido inmaduro dentro del tumor (18, 29, 33, 37). La estadificación tiene tres grados el uno, donde el tejido inmaduro se limita a un campo de bajo aumento, el dos donde el tejido inmaduro no excede 3 campos a bajo aumento, y el grado 3 donde escasean los elementos maduros y la inmadurez excede 4 campos (40). En el caso de encontrar elementos inmaduros se debe tener precaución con las siguientes situaciones: 1. Un sobrecrecimiento de tumores neuroectodérmicos primitivos (PNET), donde se debe intentar diferenciar de un teratoma inmaduro grado III, y 2. La presencia de ocasionales elementos de neuroepitelio o epéndimo en un teratoma maduro, situación que carece de significado pronóstico (18).

En los teratomas la inmunohistoquímica es necesaria para diferenciar los elementos inmaduros en áreas donde el diagnóstico con H&E no es claro. El tejido neural inmaduro es positivo para SOX2, SALL4 y GPC3 hallazgos reportados por nuestro laboratorio (18, 29), este último con una positividad heterogénea. Estos marcadores también son útiles en los casos de *gliomatosis peritoneal*, que es un fenómeno acompañante de los teratomas inmaduros, menos frecuentemente en los maduros, donde el tumor está acompañado por múltiples nódulos compuestos por tejido neural plenamente diferenciado. Aquí los marcadores inmunohistoquímicos permiten descartar la inmadurez en el tejido glial acompañante (41). El panel de anticuerpos debe complementarse con proteína ácidica glial fibrilar (GFAP), nestina y otros marcadores neurales. El panel de

anticuerpos (tanto para madurez como inmadurez) fue sugerido por nuestro grupo de investigación en 2012 (42).

Una situación clínica que se debe tener en cuenta es la encefalitis mediada por anticuerpos contra el receptor N-metil-D- aspartato, denominada encefalitis anti NMDAR (43-45) . El receptor N-metil-D-aspartato (NMDA-R) normalmente está presente en las neuronas de todo el cerebro pero es más común en las neuronas del lóbulo temporal y del hipocampo donde está protegido del sistema inmunológico. Sin embargo en los teratomas con elementos neuronales, la exposición antigénica da lugar a la producción de anticuerpos, que al atacar los receptores cerebrales origina las manifestaciones clínicas entre las que tenemos crisis convulsivas, movimientos estereotipados, hipoventilación, pérdida del conocimiento y alteraciones psiquiátricas (42, 43, 45), el tratamiento es la escisión temprana del tumor. En el diagnóstico histopatológico, la inmunohistoquímica nos ayuda a detectar las áreas neuronales dentro del teratoma, posibles causantes de la reacción inmunológica, tal como planteamos en el 2012 (18).

Tumores vitelinos (tumores endodérmicos primitivos). Desde su descripción inicial, este tumor ha tenido diferentes nombres como carcinoma mixomatode propuesto por White en 1910, adenocarcinoma del testículo infantil (46, 47), y orquioblastoma por un supuesto origen en los túbulos testiculares embrionarios, propuesto primero por Teoh (1960) y luego por Willis (1962), para la peculiar morfología de estos tumores testiculares en la edad infantil (48) . En el ovario Schiller reportó la similitud de este tumor con estructuras mesonéfricas, por lo que fue llamado mesonefroma ovarii (47), término que Kazancigil, al demostrar el

improbable origen mesonéfrico, propuso cambiar por el de papiloendotelioma ovarii (47). Aunque se comprobó que los tumores endodérmicos primitivos no están originados en el mesonefros, del trabajo de Schiller quedó como herencia la descripción de los cuerpos de Schiller-Duval, nombre dado a las estructuras glomeruloides formadas por un core fibrovascular rodeado por endodermo tumoral con distribución papilar (47). Otros términos utilizados fueron arquenteronoma, ya que el arquénteron es la estructura primitiva endodérmica embrionaria formada durante la gastrulación (49). Igualmente, el termino mesoblastoma vitelino es un término descriptivo manejado por Teilum (50), mientras que Teter en Polonia sugiere un término, gonocitoma (21), que tuvo cierto éxito en la bibliografía alemana de los años 50. En tumores extragonadales se utilizaron nombres como papiloendotelioma atribuyendo su génesis a estructuras vasculares (51), y ependimoma extraxial, cuando se localizaba en la región sacra señalando un posible origen en estructuras ependimarias (51). El término de yolk sac tumor (YST) fue propuesto por G.Barry Pierce, por el parecido histológico con el saco vitelino murino, cuando en los años 50 reprodujo experimentalmente *por primera vez* un tumor vitelino (tumor endodérmico primitivo), al implantar células madre derivadas de teratocarcinomas en el tejido celular subcutáneo de ratones, obteniendo una neoplasia constituida por células *primitivas* en medio de una abundante matriz hialina, que recordaba la membrana que separa el trofoblasto del endodermo en el saco vitelino parietal murino, llamada membrana de Reichert (52, 53). Mientras tanto, Gunnar Teilum en Dinamarca, planteó el término de tumor del seno endodérmico, por la similitud de las invaginaciones vasculo-endodérmicas neoplásicas con las de la placenta de los roedores, denominadas

senos endodérmicos (21, 25). Medio siglo después, en 2012 nuestro grupo propuso el término de **tumores endodérmicos primitivos**, porque la gran diversidad morfológica del tumor reproduce las múltiples caras de los derivados endodérmicos: patrones somáticos y extraembrionarios (51). Los tumores vitelinos (tumores endodérmicos primitivos) ocupan el tercer puesto dentro de los TCGM en el ovario, donde se presentan de forma pura más frecuentemente que en el testículo, donde dicho tumor representa un componente habitual de los tumores mixtos (37).

Los tipos tumorales pueden analizarse morfológicamente en 2 grandes grupos: patrones endodérmicos extraembrionarios y somáticos (51).

Patrones endodérmicos extraembrionarios. Dentro de este grupo se encuentran las formas “típicas” diagnósticas de los tumores vitelinos (tumores endodérmicos primitivos).

- *Patrón microquístico reticular*, es el más común ya que se presenta en aproximadamente el 80% de los casos (33). Se caracteriza por células cuboidales, algunas de ellas vacuoladas, o planas, las que tapizan una red de microquistes anastomosantes (33). En este patrón se conjugan dos características del tumor del saco vitelino del ratón: la abundancia de membrana basal (del saco vitelino parietal murino) PAS positiva y AFP negativa, y los glóbulos hialinos (del saco vitelino visceral murino), AFP positivos (Figura 7)(51).

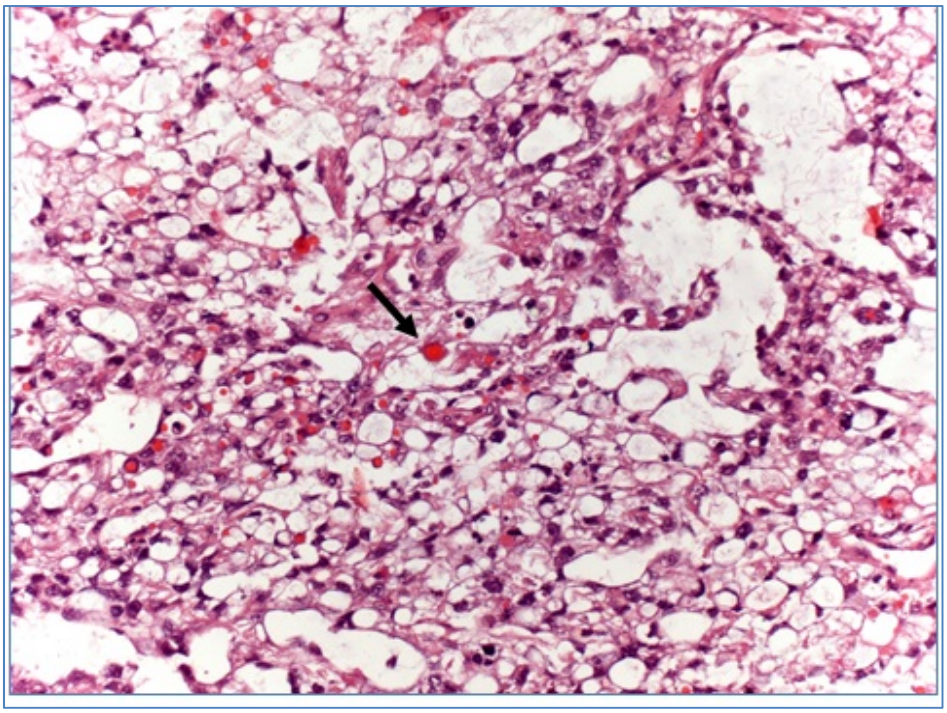

Figura 7. Tumor vitelino (tumor endodérmico primitivo) con patrón habitual de tipo microsquístico reticular, con abundantes glóbulos hialinos (flecha).

- *Seno endodérmico.* El término del seno endodérmico, se refiere a la reproducción por parte del tumor de una estructura papilar presente en la placenta coriovitelina de los roedores (seno de Duval). Fueron descritos por Schiller(47), como glomérulos mesonéfricos. En los últimos años esa estructura ha sido llamada cuerpos de Schiller-Duval. Consisten en ejes fibrovasculares papilares que crecen hacia un espacio quístico y que están recubiertos por células endodérmicas malignas, cilíndricas o cúbicas, (18, 29, 33). Aunque es una característica que se ha citado como típica de los tumores vitelinos (tumores endodérmicos primitivos), si bien solo es preponderante en un 20% de los tumores (29). Por asemejarse a estructuras papilares encontradas en los carcinomas embrionarios

(51), debe establecerse la diferencia utilizando marcadores inmunohistoquímicos explicados más adelante.

- *Polivesicular vitelino*. Esta rara variante es más frecuente en los tumores ováricos, donde se encuentra de forma pura (54). Está caracterizada por un estroma mixoide sobre el que se distribuyen múltiples espacios quísticos tapizados por dos tipos celulares que hacen la transición entre uno y otro. El primero caracterizado por células columnares altas, con atipia moderada y glóbulos hialinos intracitoplasmáticos, que son positivos a la tinción de PAS, algunas con vacuolización subnuclear (55); el segundo por células aplanadas similares a las mesoteliales las cuales carecen de glóbulos hialinos intracelulares (55). Las vesículas recuerdan a la alantoides, aunque en el pasado se creyó erróneamente que eran el reflejo de la conversión del saco vitelino primario al secundario (Figura 8) (51, 56).

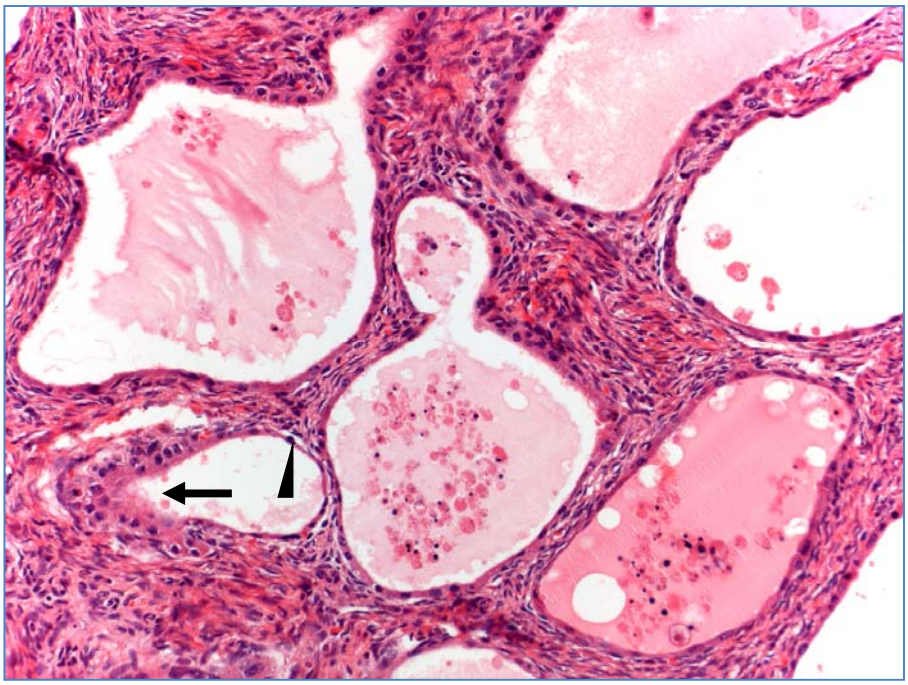

Figura 8. Patrón vitelino polivesicular donde se observan formaciones quísticas tapizadas por dos tipos celulares: células columnares (flecha larga) y células aplanadas (cabeza de flecha).

- *Cribiforme-tubular*. Es un patrón poco frecuente, **descrito por nuestro grupo (57)** que puede encontrarse de forma pura, sobre todo en tumores ováricos, situación que ocasiona problemas en el momento del diagnóstico. Histopatológicamente está conformado por túbulos angulados y nidos con pseudotúbulos, ambos, tapizados por células cuboidales y poligonales con citoplasma eosinófilo y núcleos con cromatina granular y nucléolo visible. Adicionalmente se pueden observar túbulos intra e intercelulares los cuales forman una red canalicular similar a la observada en el saco vitelino humano entre las semanas 7 a 8 (Figura 9) (51).

  Hemos demostrado, como desarrollaremos posteriormente en las conclusiones que esta histopatología es aquella que reproduce de

modo fidedigno la estructura del saco vitelino humano tanto desde el punto de vista inmunohistoquímico como de los finos detalles histológicos del saco vitelino humano en el periodo de estado.

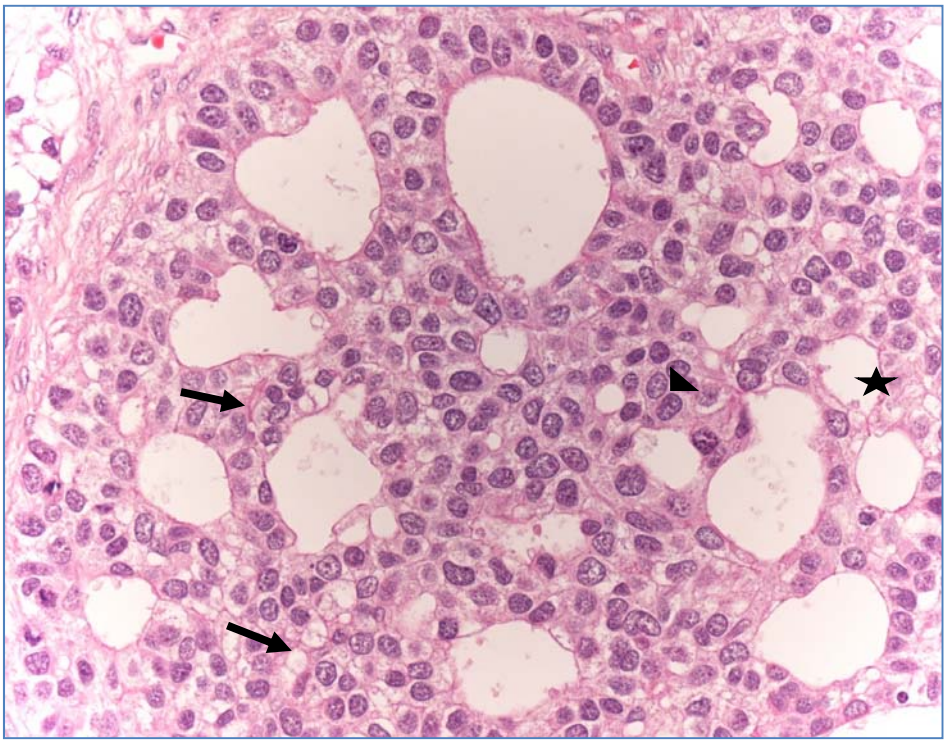

Figura 9. Patrón cribiforme tubular. Se observan numeroso túbulos intra (flecha larga) e intercelulares (cabeza de flecha), algunos de ellos formando una red canalicular (asterisco).

- *Hematopoyesis*. En algunos tumores se pueden observar células hematopoyéticas, postuladas como el origen de neoplasias hematolinfoides asociadas a los tumores vitelinos (tumores endodérmicos primitivos) mediastinales (Figura 10) (51). Para confirmar que las células sospechosas son realmente hematopoyéticas se deben solicitar anticuerpos como la glicoforina y el receptor de transferrina (CD71) que son positivas en los precursores eritroides.

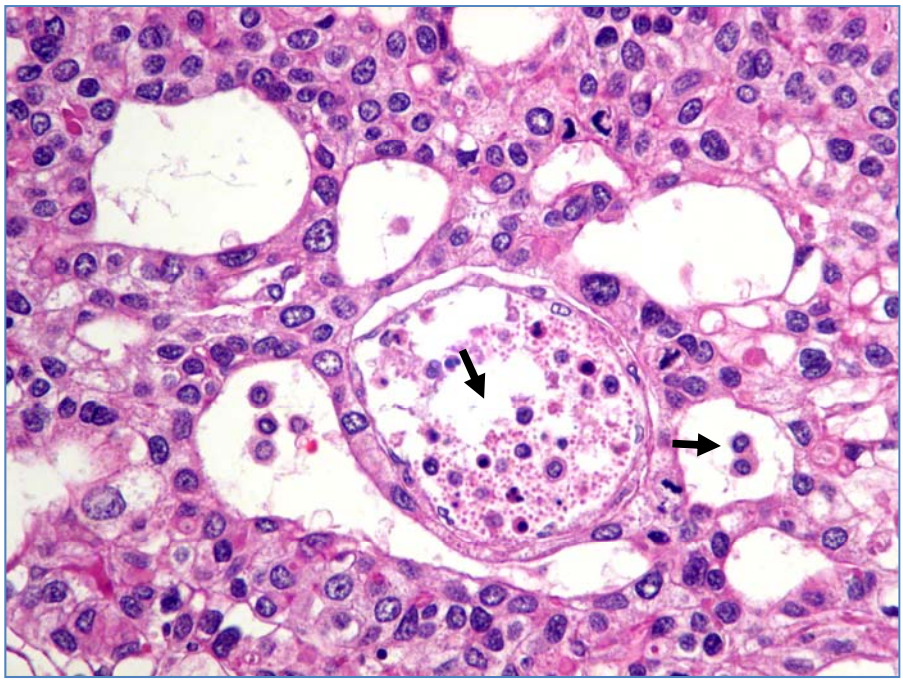

Figura 10. Escasas células eritropoyéticas (flechas) en medio de células endodérmicas descamadas).

- *Parietal*. Este patrón es infrecuente, pudiéndose encontrar en tumores postquimioterapia, caracterizado por una matriz de membrana basal hialina y amorfa en medio del cual se distribuyen células endodérmicas tumorales (51). Este patrón recuerda la producción de membrana basal por el saco vitelino murino (54).

Patrones endodérmicos somáticos. Estos patrones al ser una diferenciación somática del endodermo reproducen estructuras tales como hígado e intestino, lo que pueden llevar al patólogo a un error diagnóstico.

- *Diferenciación glandular*. Este patrón puede exhibir epitelios de tipo intestinal, pulmonar y endometrioide (51, 58-60). En estas diferenciaciones, la característica común es la presencia de formaciones tubulares de tamaño variable. En el caso de

diferenciación intestinal, similitud histológica con el intestino primitivo radica en la presencia de vacuolas intracitoplasmáticas en las células tumorales, y en la microscopía electrónica por la presencia de raicillas subvillositarias. Adicionalmente no se observan túbulos intra ni intercelulares (58, 59). En la rara diferenciación de tipo pulmonar (intestino anterior) la utilización del TTF1 (factor de transcripción tiroideo) es la mejor forma, junto con el resto del panel explicado más adelante, de confirmar el este tipo de diferenciación (51) (Figura 11). Debido a la vacuolización de estos tumores remotamente similar a las vacuolas del endometrio secretor, se ha utilizado el término de tumor vitelino endometrioide (60), que es incorrecto ya que confunde al clínico con un tumor de distinta estirpe y biología (61).

- *Diferenciación hepática*. Esta variante reproduce plenamente la diferenciación hepática con células endodérmicas tumorales plenamente diferenciadas, dispuestas en trabéculas. Adicionalmente se puede encontrar secreción biliar, y hematopoyesis (51, 54), también se observan globulos hialinos así como otros patrones de tumores vitelinos (Figura 12).

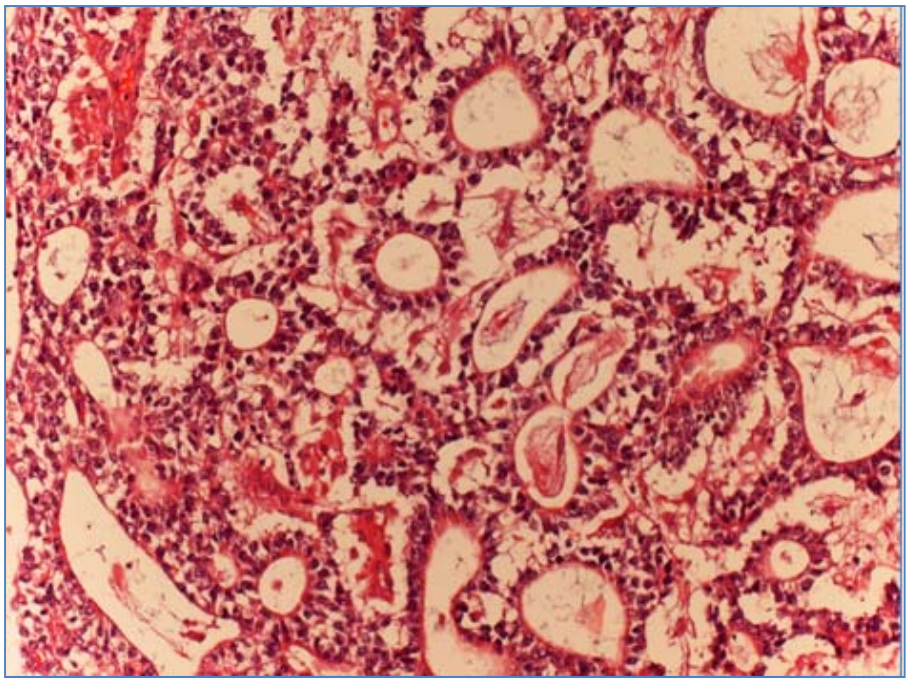

Figura 11. Patrón glandular con diferenciación intestinal. Se observan túbulos tapizados por células endodérmicas

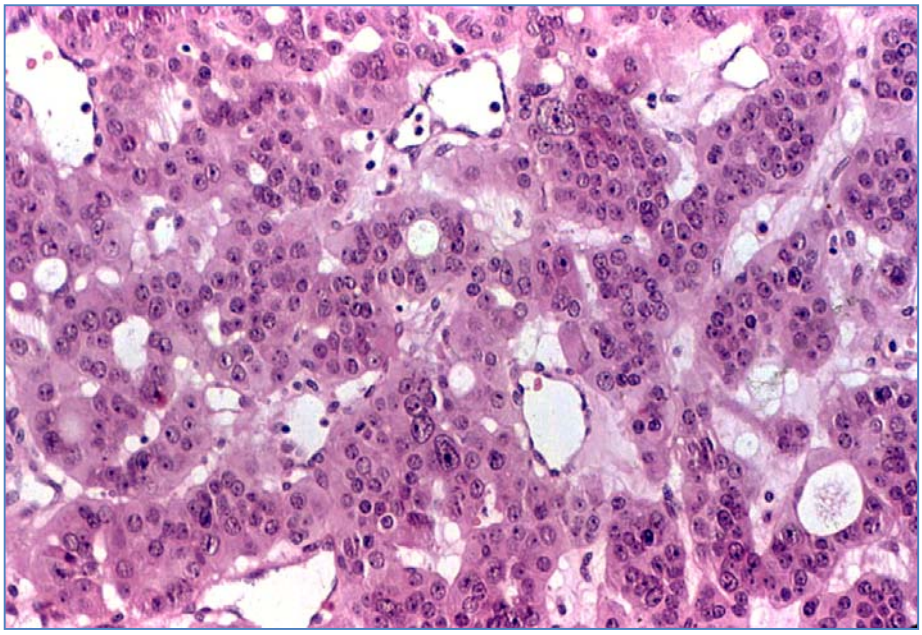

Figura 12. Tumor vitelino (tumor endodérmico primitivo) con diferenciación hepatoide.

Formas sólidas. Las formas sólidas cobran especial importancia en el momento del diagnóstico diferencial ya que pueden ser malinterpretadas como germinomas, carcinomas embrionarios o tumores hepáticos. Están caracterizadas por láminas de células endodérmicas malignas con núcleos redondeados con nucléolo prominente, y citoplasma eosinófilo o claro. Adicionalmente hay glóbulos hialinos, y presencia de otros patrones de TV(TEP), lo que ayudaría a establecer el diagnóstico diferencial (51, 62). Cuando nos enfrentamos a TV(TEP) puros (en menos del 4% de los casos) es necesario recurrir a la inmunohistoquímica para establecer el diagnóstico diferencial (51, 62) (Figura 13).

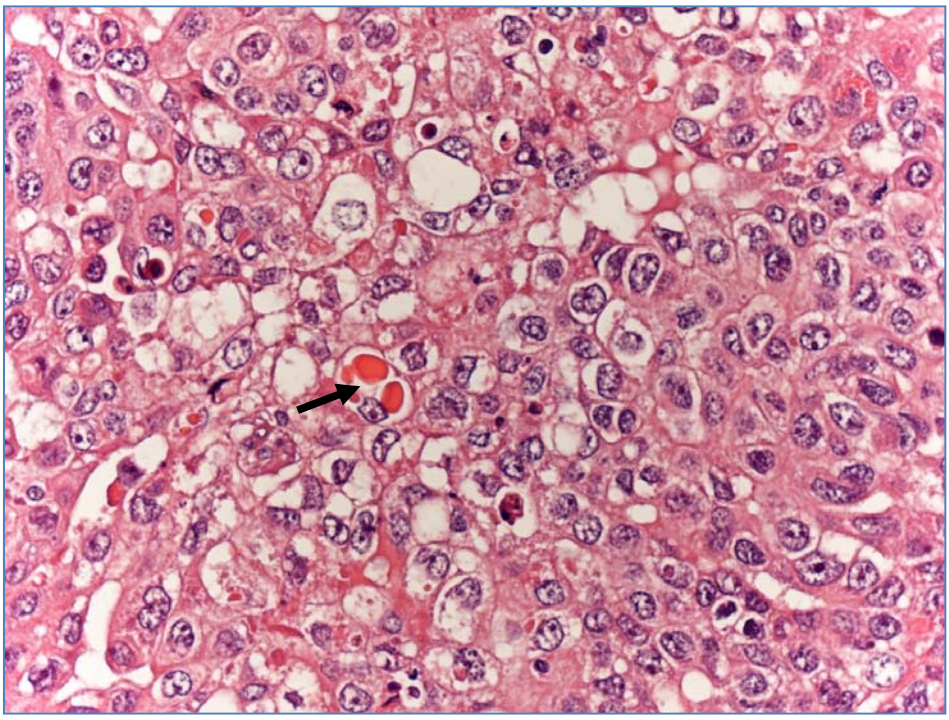

Figura 13. Patrón sólido, láminas de células con citoplasma eosinófilo y citoplasma claro y presencia de glóbulos hialinos (flecha).

Tumores vitelinos (tumores endodérmicos primitivos) con sobrecrecimiento mesenquimal. Algunos TV(TEP) presentan elementos mesenquimales heterólogos

como músculo liso o estriado. Se postula que es debió a un estímulo endodérmico imitando la embriogénesis temprana (51).

En la inmunohistoquímica no se observan diferencias en cuanto a la localización -ovárica o testicular- de los TV(TEP). Invariablemente e independiente del patrón, los TV(TEP) son positivos para AFP, un marcador clásico, con alta especificidad (54), expresado por el endodermo neoplásico (57). El patrón de tinción es citoplasmático granular, heterogéneo, con un fondo sucio debido a la reactividad con las proteínas séricas (29, 57). Si bien, todos los TV(TEP) son positivos para este marcador, en algunos casos con diferenciación glandular, la reactividad es focal, a veces limitada al borde apical de células aisladas (57). Aunque los glóbulo hialinos son positivos para AFP (63), en nuestro grupo de TV(TEP) la expresión fue poco frecuente (57). GPC3, tiene un patrón de tinción similar al de AFP, con fondo limpio y una positividad extensa, a excepción de los patrones glandulares donde es heterogénea (57). A diferencia de la AFP, la especificidad es menor, ya que también se expresa en el hígado y sus neoplasias, en el neuroepitelio y en el sincitiotrofoblasto (18, 51, 57, 64).

Marcadores hepáticos e intestinales como HepPar-1, CDX2 y villina son expresados por este grupo tumoral. HepPar-1 tiene una tinción citoplasmática granular, focal en patrones clásicos, extensa en patrones hepatoides (51, 65, 66). En casos de TV(TEP) con diferenciación glandular, la positividad de HepPar-1 es variable: en algunos casos focal, y en otros difusa (57). CDX2, muestra una tinción nuclear, extensa o heterogénea en patrones glandulares, y focal en otros tipos de TV(TEP) (51, 57, 65, 66), mientras que la villina, con inmunotinción citoplasmática

y de membrana, es extensamente positiva tanto en patrones clásicos como en glandulares (57).

Los TV (TEP) también son positivos para los marcadores de pluripotencialidad SALL4 (18, 29, 51) y LIN28, que es 100% sensible en tumores gonadales y en extragonadales (34, 35, 67). Ambos-SALL4 y LIN28- tienen expresión nuclear extensa, tanto en patrones clásicos como en glandulares, en estos últimos SALL4 puede ser focal, y en algunos casos, y LIN28 negativo en otros (57). Los TV(TEP) son negativos para CD30, OCT3/4, PLAP y c-Kit, lo que ayuda a establecer el diagnóstico diferencial con el carcinoma embrionario y el germinoma.

Clasificación de los tumores de células germinales

En 2005 *Oosterhuis y Looijenga* propusieron una clasificación para los TCG que los divide en 5 grupos de acuerdo a sus características morfológicas, epidemiológicas y biológicas (68, 69). El tipo I, conformado por el TV(TEP) y los teratomas, tiene su origen en células germinales más indiferenciadas (CGP) que las de los tipos II y III, las cuales tienen alteraciones en la maduración y la apoptosis. La mayoría de los teratomas tienen un cariotipo normal, a excepción de teratomas inmaduros presentados en hombres adultos, que tienen una traslocación entre los cromosomas 6 y 11. El TV(TEP) es aneupliode y tiene varias alteraciones cromosómicas que incluyen pérdida o ganancia de material genético en los cromosomas 1p, 4 y 6q, y, 1q, 12p, 20q y 22 respectivamente. La localización es gonadal (ovario y testículo) y extragonadal en la región sacroccígea, cabeza y cuello y regiones cerebrales hipotalámica-hipofisiaria y pineal (68). El tipo II, está conformado por seminoma/disgerminoma. Las células

de origen son las CGP y los gonocitos. Las lesiones precursoras son la neoplasia intratubular germinal, para los tumores testiculares, y el gonadoblastoma para los germinomas de las gónadas disgenéticas. Citogeneticamente tiene poliploidía y sobreexpresan isocromosoma 12p. La localización es gonadal y extragonadal en la línea media del cuerpo: mediastino anterior y regiones cerebrales hipofisiaria, hipotalámica y pineal (68). El tipo III, está conformados por el seminoma espermatocítico, de localización testicular. Está originado en una célula germinal más madura, y la lesión precursora es el seminoma espermatocítico intratubular. Citogenéticamente hay ganancia de material genético en el cromosoma 9 (68). El tipo IV, está conformado por el quiste dermoide, de localización ovárica, originado en las oogonias, mientras que el tipo V, lo conforma la mola hidatidifome (68) (tabla 2).

Tipo	Fenotipo	Edad	Localización	Célula de origen	Genotipo
I	Teratoma maduro/inmaduro TV(TEP)	Neonatos Niños	Testículo Ovario Región sacra Retroperitoneo Mediastino Línea media cerebral	CGP temprana Gonocito	Teratoma: diploide TV(TEP): aneupliode
II	Seminoma	>15 años	Testículo	CGP Gonocito	Aneuploide
	Disgerminoma	>4 años	Ovario	CGP Gonocito	Aneuploide
	Disgerminoma	Congénito	Gónada disgenética	CGP Gonocito	Diploide Tetraploide
	Seminoma	Adolescentes	Mediastino anterior	CGP Gonocito	Diploide Tetraploide
	Germinoma	Niños	Hipotálamo Glándula pineal	CGP Gonocito	Diploide Tetraploide
III	Seminoma espermatocítico	>50 años	Testículo	Espermatogonia Espermatocito	Aneuploide
IV	Quiste dermoide	Niños y adultos	Ovario	Oogonia Oocito	Diploide Tetraploide
V	Mola hidatidiforme	Edad fértil	Placenta	Óvulo vacío espermatozoide	Diploide

Tabla 2. Clasificación de tumores de células germinales (68).

Saco vitelino humano normal

El saco vitelino (SV) es una estructura imprescindible que acompaña una amplia variedad de especies animales desde el inicio de la concepción, interviniendo en el intercambio de nutrientes, la producción proteica y la hematopoyesis inicial, mientras se forman las estructuras maternas y fetales definitivas encargadas de tales propósitos.

En aves y reptiles (70, 71) el saco vitelino es el órgano inicial encargado del intercambio de nutrientes; en las aves además es el principal productor de células hematopoyéticas (72), así como un importante proveedor de lípidos y proteínas encargados de mantener la integridad vascular en los embriones (73).

En los mamíferos, el desarrollo, estructura y funciones del saco vitelino varían dependiendo de la especie, manteniendo las actividades básicas de intercambio de nutrientes y síntesis de proteínas (74). En cuanto a la estructura, en la etapa de blastocito es bilaminar (trofoectodermo mas mesodermo extraembriónico), volviéndose trilaminar tras la proliferación de mesénquima entre el endodermo y el ectodermo. Como excepción a esta regla tenemos los marsupiales, cuyo SV tiene sólo dos capas durante toda la gestación. Esta conformación es útil en etapas iniciales cuando existe un intercambio directo de nutrientes desde el endometrio, intercambio que disminuye en la gestación tardía, momento en el que hay una ruptura de la cápsula, para tomar los nutrientes derivados de la sangre materna (74). En todas las especies, las funciones básicas del SV son tres: intercambio, síntesis y transferencia. Dentro de las sustancias que atraviesan el SV en mamíferos tenemos aminoácidos, iones, transferrina e

inmunoglobulinas, estas últimas encargadas de la inmunidad pasiva en roedores. La síntesis incluye sustancias como la AFP y el colesterol, las cuales cumplen funciones vitales durante la etapa implantativa y de la organogénesis (74). Si bien, hay controversia sobre el origen de las células precursoras sanguíneas -SV vs. piso de la aorta embrionaria- (74-76), el SV, brinda el medio para su maduración y supervivencia mientras el hígado fetal está suficientemente maduro para ejercer la hematopoyesis (74).

En los humanos el SV tiene dos estadios: el saco vitelino primario y el secundario. En el noveno día, saco vitelino primario se forma por células provenientes del hipoblasto las cuales recubren el blastocisto, delimitando la cavidad exocelómica. Posteriormente, ocurre una nueva migración celular, también originada en el hipoblasto, que forma una nueva cavidad, el saco vitelino secundario (77).

El saco vitelino secundario humano (SVSH) es un órgano pequeño (4,5 a 5mm) y transitorio (4ª a 12ª semana de gestación), que permite la sobrevivencia del embrión en etapas tempranas del desarrollo. Es por esto que se han hecho estudios encaminados a correlacionar sus variaciones estructurales con alteraciones en el desarrollo de la gestación, inicialmente en muestras provenientes de abortos espontáneos y recientemente por ultrasonografía, encontrando cambios degenerativos inespecíficos en los primeros (77, 78), y alteraciones en la forma y tamaño en los segundos (79-81), cambios postulados como predictores de cromosomopatías (82).

Dentro de sus funciones, al igual que en otras especies, están la síntesis de proteínas (83-85), la transferencia de sustancias (86-88) y la hematopoyesis (86, 89-92). Algunas de las proteínas sintetizadas son AFP, transferrina, prealbúmina, albúmin, α1-antitripsina (83-85, 93), glicanos (94) y apolipoproteínas (93). La transferencia de sustancias ha sido demostrada en numerosos estudios, e incluyen hierro y sus proteínas fijadoras, hepcidina, ácido fólico, antioxidantes, oxígeno, sustancias de nutrición histiotrofica, entre otras (87, 88, 95-99).

Para cumplir con sus funciones el SVSH está conformado por tres capas, el endodermo en la parte interna, el mesénquima en la parte media, y el mesotelio, recubriendo las dos anteriores (100-102). El endodermo está caracterizado por células que inicialmente son cúbicas -sacos tempranos-, y luego cilíndricas -periodo de estado-, las cuales están en contacto con la luz del SV (superficie apical) y con el mesénquima (superficie basal). El núcleo es central, con uno a dos nucléolos visibles, y un amplio citoplasma eosinófilo, que tiene múltiples vacuolas picnóticas en el área apical, así como múltiples luces denominadas vesículas intracelulares (VIC), las cuales confluyen para formar túbulos endodérmicos (TE) (100-102). Gracias a la microscopia electrónica se sabe que la superficie apical está revestida por abundantes microvellosidades, que también tapizan los VIC y los TE (100, 101). Las células endodérmicas a lo largo de toda la evolución del SV son positivas para AFP, GLP3, villina y HepPar-1, y desde la 7ª semana para CDX2, SALL4 marcadores que declinan en sacos involutivos (102). LIN28, marcador de pluripotencialidad, es positivo nuclear en sacos tempranos (Figura 15) (57).

Tanto AFP, GLP3 como la villina tienen una fuerte tinción citoplasmática, adicionalmente, AFP y GLP3 son fuertemente positivos en la membrana, delineando las VIC y TE (Figuras 14 B, C y F). La expresión de HepPar-1 es fuerte, especialmente concretada en gránulos citoplasmáticos gruesos (Figura 14D). CDX2 (Figura 14E), SALL4 y LIN 28, son marcadores nucleares, con disminución de su intensidad durante la involución (57). A excepción de la AFP, la cual puede encontrarse expresada en las células hematopoyéticas intravasculares de la capa mesenquimal, estos anticuerpos tienen una expresión limitada al endodermo (102).

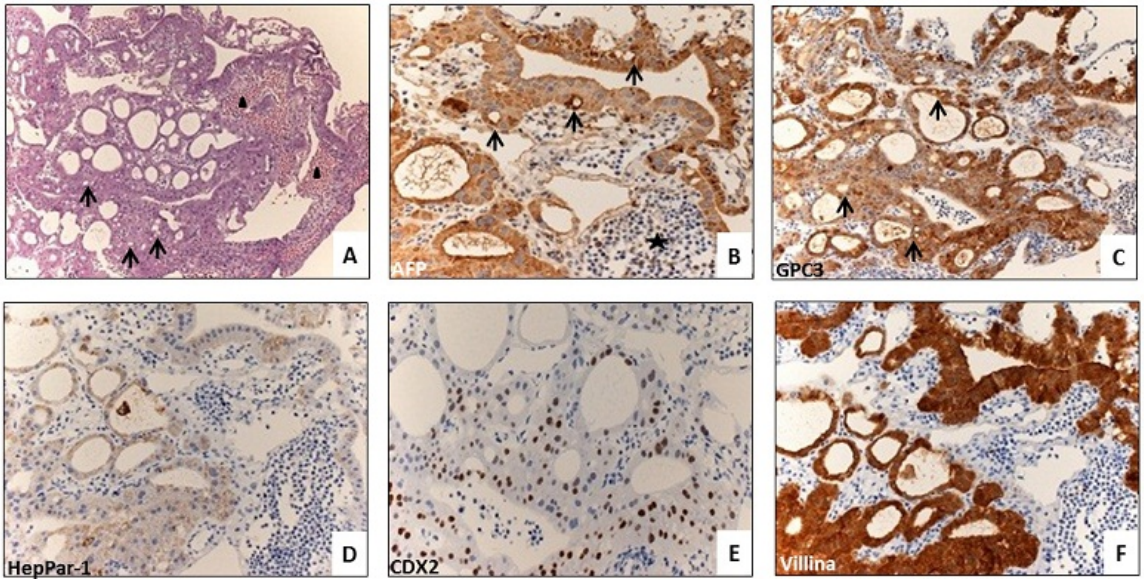

Figura 14. SVSH en periodo de estado, con abundantes túbulos intra e intercelulares, flechas, e islas hematopoyéticas cabeza de flecha(A). Inmunotinción citoplasmática de AFP y GPC3, delineando VIC yTE, flecha (B y C). AFP, es positiva en las células hematopoyéticas de las islas eritroides, estrella. Positividad citoplasmática granular para HepPar-1 (C), nuclear para CDX2 (D) y ciroplasmática intensa para villina (E).

La capa mesenquimal, que está en contacto estrecho con el endodermo y el mesotelio, tiene células esteladas y ahusadas (fibroblasto-like) que miden entre 12 y 20µm de diámetro, las cuales se disponen alrededor de vasos sanguíneos todo el conjunto descansa sobre una matriz extracelular. Los vasos sanguíneos son diferentes tamaños, están tapizados por endotelio sin membrana basal circundante, y contienen células hematopoyéticas inmaduras (92, 100, 101).

Las células mesoteliales son planas u ovales y al igual que las células endodérmicas, presentan microvellosidas regulares y cortas, recubiertas por una capa mucosa (92, 100, 101). Esta capa expresa podoplanina de forma constante y exclusiva, siendo negativa para otros marcadores mesoteliales como HBME-1 y calretinina, así como para marcadores endodérmicos y de diferenciación intestinal (102). El papel del mesotelio es discutido: algunos autores postulan un rol exclusivamente protector (101), mientras que otros un rol absortivo (86, 88).

Las características de estas tres capas varían dependiendo de la semana gestacional en la que se encuentren: sacos tempranos, semanas 4ª y 5ª; periodo de estado, semanas 7ª y 8ª; y sacos involutivos, semanas 9ª y 12ª (102). Durante todas las etapas, el mesotelio permanece inalterado. En los sacos tempranos, tanto las capas endodérmica como la mesenquimal son delgadas, las células endodérmicas forman una monocapa, pero presentan VIC, así como anisocariosis y abundantes mitosis, mientras que en el mesénquima se observan algunos vasos sanguíneos (102). En el periodo de estado, el endodermo está proliferado con abundantes cordones y columnas que se expanden hacia el mesénquima, llegando a estar en contacto con el mesotelio. En su citoplasma hay abundantes

VIC y TE, los cuales entran en contacto con los vasos sanguíneos mesenquimales, también abundantes. En los sacos involutivos, el endodermo es atrófico, adelgazado, con aplanamiento de las VE, y desaparición de las VIC, así como descamación de células endodérmicas hacia la luz. En el mesénquima, hay disminución de las islas hematopoyéticas. En esta etapa, el mesotelio puede presentar vacuolización. Algunos sacos atróficos, tienen fibrosis y calcificación distrófica (102).

Estas características permiten correlacionar los hallazgos morfológicos e inmunohistoquímicos con las funciones del SVSH. En el endodermo, las abundantes microvellosidades de la membrana celular recuerdan las presentes en las células intestinales, que sirven para aumentar la superficie de absorción de agua y nutrientes, funciones que también cumple el SVSH (102). Adicionalmente, este fenotipo intestinal se correlaciona con la positividad para villina (102), un anticuerpo presente en el endodermo de ratones, y en el intestino de roedores y humanos (103-105), y CDX2, el cual también está presente en el intestino desde etapas embrionarias. Este fenotipo intestinal, coexiste con un fenotipo hepático caracterizado por la red canalicular conformada por VIC y TE, implicados en el transporte de sustancias. También como parte del fenotipo hepático, está la fuerte positividad a AFP y GLP3, dos proteínas producidas por el SVSH (93) y el hígado en desarrollo, expresión que no continua durante la vida extrauterina en condiciones normales (102, 106, 107), y que ratifica el papel del SVSH como órgano sintetizador de proteínas, mientras el hígado embrionario está en capacidad de ejercerla. Por último, para completar el fenotipo hepático, está la

expresión en el SVSH del HepPar-1, un marcador presente casi de forma exclusiva en hepatocitos normales y neoplásicos (102, 108, 109). La expresión de SALL4, también puede estar vinculada con el fenotipo hepático, ya que al igual que AFP y GLP3 se expresa solo en hepatocitos en desarrollo, con el objetivo de controlar la diferenciación hepática (110).

En cuanto al rol hematopoyético, el SVSH provee al embrión precursores hematopoyéticos, incluyendo eritrocitos nucleados, quienes son los primeros transportadores de oxígeno (89, 91, 92, 111-113). La hematopoyesis inicia en el endodermo (114), posteriormente el mesénquima prolifera alrededor formando islas hematoppyéticas, para finalizar con la diferenciación de células endoteliales vasculares que rodean las células hematopoyéticas (89, 92). Las hematopoyéticas colonizan el hígado, lugar donde se iniciará la hematopoyesis embrionaria (89, 92).

En resumen, el SVSH es un importante órgano que cumple funciones intestinales, hepáticas y hematopoyéticas, mientras el embrión está suficientemente maduro para ejercerlas.

Inmunohistoquímica

A continuación describiré los anticuerpos utilizados para los estudios inmunohistoquímicos desarrollados en esta tesis.

Alfa-feto proteína. La AFP es una glicoproteína sialilada miembro de la superfamilia de los genes albuminoides, compuesta por 580 aminoácidos,

descubierta en 1956, tras experimentos de electroforesis de proteínas plasmáticas de neonatos (51, 115). Es producida por el SVSH, y después de las 11 semanas de gestación por el hígado fetal. Posterior al nacimiento sus niveles séricos caen hasta ser indetectables tras el primer año (116), ahí radica la importancia clínica, ya que sus niveles elevados en la vida adulta significan enfermedad hepática inflamatoria, o presencia de procesos neoplásicos hepáticos, gástricos, pancreáticos, TV(TEP), pulmonares, urológicos y ginecológicos (117-121) . Tiene varias isoformas, L1, presente en enfermedades hepáticas no neoplásicas, L2, presente en TV(TEP) y L3, presente en neoplasias hepáticas (122). La AFP actúa como ligando y proteína transportadora de numerosas sustancias como ácidos grasos, metales pesados, bilirrubina y esteroides; como regulador del crecimiento y de la tolerancia inmunológica (107). El patrón de tinción inmunohistoquímica es citoplasmático granular con fondo sucio (51).

Glipican 3. El GLP3 es un miembro de la familia de los proteoglicanos de tipo heparán sulfato, cuyo locus se encuentra en el cromosoma X. Guarda varias similitudes con la AFP, como que está compuesto por 580 amino ácidos (123), y que es producido por el SVSH y el hígado fetal, con niveles postnatales indetectables, elevados en procesos neoplásicos como tumores hepáticos, embrionarios, TV(TEP) , neoplasias con diferenciación hepatoide, melanoma, y carcinoma de células claras de origen ovárico (29, 51, 106, 124-127). Al ser una proteína de anclaje, está localizado en la membrana celular y en el citoplasma. Por su localización, una de sus funciones es la regulación de señales intracelulares promotoras o inhibidoras del crecimiento, mediante la regulación de vías de

señalización como Wnts y hedgehog, esta última vinculada con el desarrollo embrionario, y del factor de crecimiento de fibroblastos (123, 128, 129), también cumple un papel en la morfogénesis, ya que la pérdida de su función lleva al desarrollo del síndrome de Simpson-Golabi-Behmel, caracterizado por malformaciones severas (123, 130) con alteraciones experimentales en el desarrollo cardiaco y coronario (131). El patrón de inmunotinción es citoplasmático en grumos gruesos, y en ocasiones de membrana, parecido en distribución al de AFP, pero con un fondo limpio (51, 102).

Antígeno hepatocitario en parafina 1. El HepPar-1 es un anticuerpo monoclonal descubierto en 1993 en tejido hepático fijado en formol e incluido en parafina. Inicialmente se desconocía el antígeno sobre el cual reaccionaba, postulándose posteriormente la carbamoil fosfato sintetasa 1 (CPS1), enzima encargada del metabolismo de la urea localizada en las mitocondrias, como posible proteína blanco (108, 132). El patrón de inmunotinción es citoplasmático granular (correlacionandose con la localización mitocondrial de la CPS1) con realce del patrón canalicular (108). Normalmente es expresado en hepatocitos maduros, hepatocitos embrionarios y fetales (102, 108), SHYS (102), intestino delgado, glándulas fúndicas (109) y en metaplasia intestinal (133, 134), y negativo en ductos biliares (108). En procesos neoplásicos es positivo en carcinoma hepatocelular, carcinoma gástrico (con o sin diferenciación hepatoide), carcinoma de intestino delgado, de vejiga y de glándulas adrenales, estos últimos en menor frecuencia (109, 133).

CDX2. Es un gen de tipo homeodominio de tipo caudal, que codifica para el factor de transcripción 2 (proteína de tipo homeobox), involucrada en el desarrollo intestinal, ya que permite la diferenciación de las células madre, en los diferentes tipos de células epiteliales intestinales (135). En etapas tempranas del desarrollo sus funciones incluyen la determinación de la polaridad del blastocisto y el desarrollo del trofoectodermo (136), así como la diferenciación del trofoblasto (135). Normalmente su expresión es nuclear, con una tinción fuerte y difusa en el epitelio del intestino delgado y grueso, apéndice cecal y recto, así como en los ductos pancreáticos y en las células centroacinares (137). En procesos neoplásicos es difusamente positivo en adenocarcinoma colorrectal, adenocarcinoma gastroesofágico (aproximadamente 20%), y en tumores neuroendocrinos de tracto gastrointestinal (137). Adicionalmente, el CDX2 es positivo en el esófago de Barrett (138), en la metaplasia intestinal endometrial (139) y la metaplasia morular en diferentes órganos (140).

Villina. La villina es un polipéptido ácido, localizado en el centro de las microvellosidades del borde en cepillo del epitelio intestinal (141, 142). Tiene 3 sitios unidores de calcio de alta afinidad, que le permiten interactuar con la actina, ejerciendo control sobre su polimerización de una forma calcio-dependiente (141). En etapas embrionarias se expresa tempranamente (sexto día post implantación) en células endodérmicas de ratón, en el endodermo visceral del saco vitelino (103), en el intestino embrionario y en el saco vitelino humano (102). Es positiva en células absortivas intestinales y en los túbulos contorneados proximales del riñón, y en menor concentración, en células epiteliales ductales de conductos

biliares y pancreáticos. Estas últimas aunque carecen de borde en cepillo, tienen actividades absortivas y comparten el origen endodérmico de las células intestinales (103). En procesos neoplásicos, es positiva en tumores derivados del endodermo. Como característica durante el proceso de diferenciación intestinal, la expresión es citoplasmática en células inmaduras, para luego polarizarse a la superficie apical en las células maduras (141), por ende el patrón de tinción es citoplasmático en células inmaduras y de membrana apical en las diferenciadas.

*Proteína 4 similar a SAL. S*ALL4 es una proteína codificada por el gen que lleva su mismo nombre. Una de sus funciones es mantener la pluripotencialidad de las células madre embrionarias en estados preimplantativos, a través de la promoción de la expresión del factor de transcripción Pouf5f1, también conocido como OCT4 (143, 144). También está asociado con la regulación de la organogénesis: junto con SALL1 y SALL2, coordina el desarrollo adecuado de la neurogénesis y el cierre del tubo neural en ratones, y se postula que podría correlacionarse con al neurogénesis en humanos (145), Adicionalmente regula la diferenciación hepática (110). Por último, y vinculado con la organogénesis en humanos, las mutaciones heterocigotas del SALL4 causan el síndrome de Okihiro, caracterizado por malformaciones en la cara radial del antebrazo asociado al síndrome de Duane (malformaciones oculares) (146). La expresión normal se da en el blastómero de la mórula, el blastocisto, tejidos embrionarios, SVSH (102, 110), placenta (147) y espermatogonias indiferenciadas en testículos adultos (148, 149). Al ser un marcador de pluripotencialidad es positivo en tumores germinales: neoplasia intratubular germinal, seminoma/disgerminoma, CE, TV(TEP) (29, 57,

150-154), en carcinomas gástricos con diferenciación hepatoide (155), y en carcinoma pulmonar de célula grande (156). Al ser una proteína que regula procesos transcripcionales está ubicada en el núcleo lugar donde es detectada por la inmunohistoquímica (102).

LIN 28. Es una proteína oncogénica que tiene sitios específicos de unión para el micro ARN, por lo que se convierte en un inhibidor post-transcripcional de estos, específicamente del micro ARN let-7. Mientras el LIN28 promueve la pluripotencialidad, el let-7 favorece la diferenciación celular, por lo que el equilibrio entre LIN28 y let- 7 cumple funciones vitales durante la diferenciación embrionaria, la maduración de las células madre y la carcinogénesis (157). La expresión normal de LIN28 tiene su pico máximo durante el desarrollo embrionario (figura 15) pero disminuye progresivamente hasta ser indetectable durante el primer año de edad (en las células diferenciadas) (157, 158). Se encuentra en células germinales primordiales, pre-espermatogonias, gonocitos (159), células germinales ováricas premeióticas (160), SVSH –alrededor de las 5 semanas-(57). También se expresa en la placenta, pero es negativa en el amnios (161). En procesos neoplásicos es positivo en TCGM: neoplasia intratubular germinal, seminoma/disgerminoma, carcinoma embrionario, TV(TEP) y positivo heterogéneo en coriocarcinoma (35, 57, 67, 159). El patrón de expresión es citoplasmático con excepciones: es nuclear y citoplasmático en los gonocitos, en el seminoma y la neoplasia intratubular germinal. La causa de este patrón de tinción es desconocida, pero puede ser debida a la maduración del ARN micro (159).

Podoplanina (D2-40). La podoplanina es una proteína transmembranosa tipo mucina, detectada, por el anticuerpo monoclonal D2-40 (162). La podoplanina se expresa normalmente en podocitos renales (163), endotelio de los vasos linfáticos, células mesoteliales, osteocitos, ependima (162), testículos en desarrollo –negativa en testículos adultos-(164), y en la capa mesotelial del SVSH (102). En tumores es positiva en TCGM: seminoma/disgerminoma, neoplasia intratubular germinal, áreas glandulares y papilares de los CE con patrón apical (29), mesotelioma, tumores vasculares con diferenciación linfática, sarcoma de Kaposi, entre otros (162). El patrón de tinción es de membrana, lo cual correlaciona con su localización en la membrana celular.

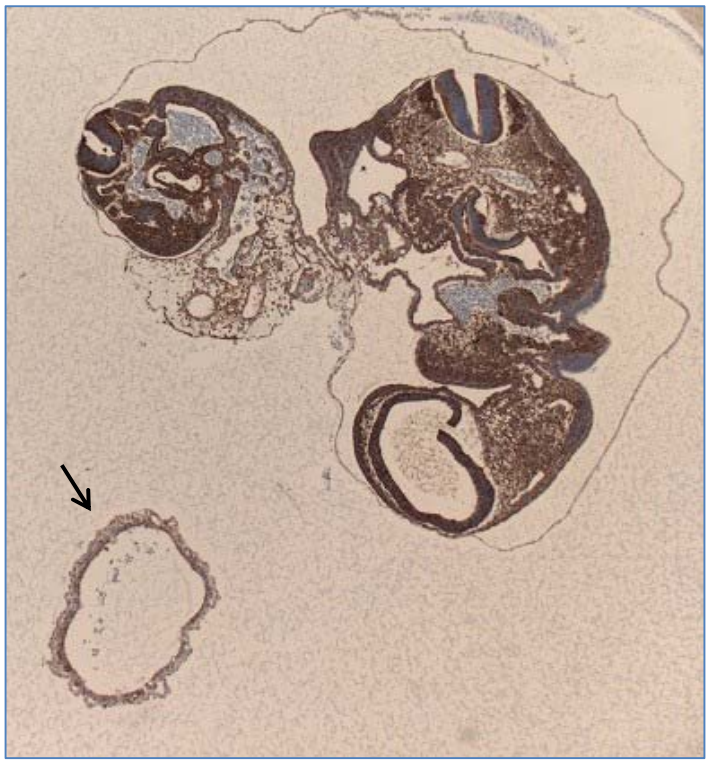

Figura 15. LIN28 con extensa positividad nuclear en tejido embrionario y en SVSH temprano (flecha).

Un último anticuerpo utilizado en esta tesis, es el factor de transcripción tiroideo (TTF-1). El TTF-1 es un factor de transcripción expresado en el tiroides, el pulmón y el diencéfalo (165). El patrón de inmunotinción es nuclear, y ayuda a establecer el diagnóstico en adenocarcinomas metastásicos (pulmonar vs otra localización) (165). Sin embargo, al aplicarlo a los SHYS, estos expresaron un patrón citoplasmático, patrón expresado en otros tejidos neoplásicos y no neoplásicos: hígado, carcinoma hepatocelular, adenocarcinoma de pulmón y cólon, carcinoma ductal de mama y carcinoma escamocelular laríngeo (166, 167). La causa de este fenómeno es desconocida -carece de importancia clínica- atribuida a una reacción cruzada con antígenos citoplasmáticos en los hepatocitos (167). Ya que el SVSH expresa un patrón hepático, es posible que esta sea la explicación para para este peculiar hallazgo. En TV(TEP) el TTF-1 es positivo en un caso de patrón clásico, y focalmente positivo en tres casos de patrón somático glandular (57).

Hipótesis

El saco vitelino secundario humano cumple un papel vital en el desarrollo embrionario, demostrando características morfológicas absortivas y de transferencia en numerosos estudios en microscopía electrónica y óptica.
Mediante la aplicación de marcadores inmunohistoquímicos al saco vitelino secundario humano podremos definir su fenotipo inmunohistoquímico haciendo énfasis en las función hepática, intestinal y posible pluripotencialidad, así como la replicación dicho perfil en tumores de células germinales, específicamente en los tumores endodérmicos primitivos, lo cual nos permitirá comparar la expresión de estos marcadores entre el fenotipo del tejido normal con su contrapartida tumoral y sus variaciones correspondientes.
Debido a la multidiferenciación presente en tumores germinales es necesario configurar un panel diagnóstico capaz de analizar la pluripotencialidad tisular y diferenciaciones específicas tanto de tipo neural como endodérmico, al ser estos los componentes relevantes desde el punto de vista diagnóstico y pronóstico en este grupo tumoral.

Objetivos

Objetivo general

1. Establecer la comparación del fenotipo inmunohistoquímico del saco vitelino secundario humano con el de los tumores endodérmicos primitivos (tumores vitelinos)

Objetivos específicos

1. Describir del fenotipo inmunohistoquímico del saco vitelino humano secundario normal, haciendo énfasis en su actividad hepática, intestinal y pluripotencial.
2. Establecer una comparación entre la estructura normal (saco vitelino) y su contraparte evolutiva tumoral.
3. Aplicar el perfil inmunohistoquímico del saco vitelino normal para el diagnóstico histopatológico de los tumores endodérmicos primitivos (tumores vitelinos).
4. Identificar nuevas variedades histológicas de tumores endodérmicos primitivos
5. Aplicar paneles diagnósticos tanto de marcadores de pluripotencialidad como de diferenciación al estudio de tumores de células germinales comunes a todas las localizaciones, gonadales y extragonadales.
6. Analizar problemas diagnósticos mediante la utilización de estos paneles a situaciones neoplásicas características y problemáticas desde el punto de vista diagnóstico.

Materiales métodos y resultados

E l desarrollo de nuestros planteamientos nos permitió publicar los siguientes 7 artículos:

1. *Nogales FF, **Dulcey I**. The secondary human yolk sac has an immunophenotype indicative of both hepatic and intestinal differentiation. Int J Dev Biol. 2012;56(9) : 755-60. doi: 10.1387/ijdb.120080fn.*

Factor de impacto: 2.823.
http://www.citefactor.org/impact-factor-list-2012.html

2. *Nogales FF, Quiñonez E, López-Marin L, **Dulcey I**, Preda O. A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac. Histopathology. 2014 Jan 20. doi: 10.1111/his.12373.*

Factor de impacto: 2.857.
http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2559

3. *Preda O, Dema A, Iacob M, Goyenaga P, **Dulcey I**, Aneiros-Fernández J, Nogales FF. Urothelial carcinoma of the renal pelvis with simultaneous trophoblastic and malignant clear cell endodermal-type differentiation. Virchows Arch 2012 Mar;460(3):353-6. doi:10.1007/s00428-012-1211-5.*

Factor de impacto: 2.491.
http://www.citefactor.org/impact-factor-list-2012.html

4. *Preda O, Dulcey I, Nogales FF. Papel de los nuevos marcadores inmunohistoquímicos en los tumores de células germinales malignos gonadales. Rev Esp Patol. 2012;45(4):195-203.*
5. *Nogales FF, **Dulcey I**, Preda O. Germ Cell tumors of the ovary: an update. Arch Pathol Lab Med. 2014 Mar;138(3):351-62. Doi:18.5858/arpa.2012-0547-RA.*

Factor de impacto: 2.78.
http://www.researchgate.net/journal/0003-9985_Archives_of_pathology_laboratory_medicine

6. *Nogales FF, Preda O, Dulcey I. Gliomatosis peritonei as a natural experiment in tissue differentiation. Int J Dev Biol. 2012;56(10-12):969-74. doi:10.1387/ijdb.120172fn.*

Factor de impacto: 2.823.
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7. Dulcey I, Céspedes MU, Ballesteros JL, Preda O, Aneiros-Fernández J, Clavero PA, Nogales FF. Necrotic mature ovarian teratoma associated

with anti-N-metyl-D-aspartate receptor encephalitis. Pathol Res Pract. 2012 Aug 15;208(8): 497-500. doi: 10.1016/j.prp.2012.05.018.

Factor de impacto: 1.213.

http://www.citefactor.org/impact-factor-list-2012.html

Int. J. Dev. Biol. 56: 755-760 (2012)
doi: 10.1387/ijdb.120080fn

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The secondary human yolk sac has an immunophenotype indicative of both hepatic and intestinal differentiation

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ABSTRACT Although the microscopy of the secondary human yolk sac (SHYS) is well known, few studies have addressed its immunohistochemical profile. The SHYS is involved in the synthesis, absorption and transfer of various proteins and behaves as a temporary liver and intestine. The objective of this study was to evaluate the presence of immunohistochemical markers of hepatic and intestinal function in the SHYS. We performed a retrospective histological and immunohistochemical study of 26 SHYS from spontaneous abortions and tubal pregnancies, 15 of which were from the 7th to 8th week. The antibodies used were against α-foetoprotein (AFP), glypican 3 (GLP3), hepatocyte-paraffin-1 (HepPar-1), villin, CDX2, SALL4 and podoplanin (D2-40). Early SHYS from the 5th to the 8th week revealed a network of intracellular vesicles communicating with the lumen of endodermal tubules that were highlighted by intense membrane AFP expression. Endodermal cells consistently expressed AFP, GLP3, SALL4, hep-par-1, villin and CDX2, while mesothelial cells only expressed D2-40. The endodermal layer of the SHYS from the 5th to the 8th week revealed a transient canalicular network which was highlighted by strong membranous AFP expression; this may represent the substrate of a SHYS transport system during its period of maximal activity. The synthetic and transfer functions of the yolk sac endoderm were reflected in a hybrid immunophenotype in which proteins characteristic of hepatic function such as AFP, GLP3, SALL4 and hep-par-1 were coexpressed simultaneously with others such as villin and CDX2, indicative of an intestinal role.

KEY WORDS: *secondary human yolk sac, hepatic function, intestinal differentiation marker, AFP, HepPar-1*

The secondary human yolk sac (SHYS) is an organ which plays a crucial role in early development. Although it has not attracted the attention it deserves in the literature, its optic microscopy and ultrastructure are well known (Hesseldahl *et al.,* 1969; Jones *et al.,* 1995a; Nogales-Fernandez *et al.,* 1977; Pereda *et al.,* 1999; Takashina *et al.,* 1993). Its clinical relevance in relationship with early pregnancy loss has been studied both ultrasonographically (Ferrazzi *et al.,* 1988; Hustin *et al.,* 1987; Jauniaux *et al.,* 2005; Jauniaux *et al.,* 1991; Kucuk *et al.,* 1999) and histologically (Nogales *et al.,* 1992; Nogales *et al.,* 1993; Nogales *et al.,* 1995).

This essential structure is vital for protein synthesis, as can be seen from its production of a wide range of substances (Gitlin *et al.,* 1969; Gitlin *et al.,* 1970; Gitlin *et al.,* 1972; Jones *et al.,* 1995b; Shi *et al.,* 1985), especially those contributing to blood formation (Gitlin *et al.,* 1969; Gulbis *et al.,* 1994). Furthermore, the types of proteins it synthesizes are evidence that, for a short period of time, the SHYS is involved in absorptive and transfer roles, thus behaving as a temporary liver and intestine (Gulbis *et al.,* 1998; Gulbis *et al.,* 1994; Gulbis *et al.,* 1992; Shi *et al.,* 1985). Indeed, it is the principal route of entry of many proteins and iron to the embryo. However, in the last decade, little information about the immunohistochemistry of the SHYS has been provided, with only few studies dealing with the shared immunohistochemical expression of some proteins (Preda *et al.,* 2011) by the SHYS and human yolk sac tumours in order to prove the vitelline identity of these neoplasms (Nogales *et al.,* 2012; Preda *et al.,* 2011).

The present work reports, for the first time, the demonstration and location in the SHYS of highly characteristic immunohistochemical markers that are associated with hepatic (glypican 3 and hepatocyte-paraffin-1) and intestinal (villin and CDX2) functions, thus providing a morphological basis for its temporary physiological role as an active interface between the exocoelomic cavity and the developing embryo. Additionally, SALL4, a pluripotency marker and podoplanin, a mesothelial marker, were also analyzed.

This hypothesis is supported further by the expression, distribution and location of transport proteins such as α-foetoprotein

Abbreviations used in this paper: AFP, alpha-foetoprotein; GLP, glypican; H&E, Hematoxylin and eosin stain; HepPar, hepatocyte-paraffin; SHYS, secondary human yolk sac.

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(AFP) in a network of intracellular channels connected with the endodermal tubules that may represent the substrate of a transfer system between embryo and the exocoelomic cavity.

Results

Hematoxylin and eosin stain (H&E)

The SHYS histology was assessed according to its developmental stage: the only available *early sac from the 5-6th week* showed a trilaminar wall structure with an inner linear endoderm and an external mesothelial layer with haemopoietic islands present in its intervening mesenchymal layer. The endodermal cells had rounded large nuclei with macronucleoli and an eosinophilic, granular cytoplasm populated by numerous intracytoplasmic vesicles. Frequent mitoses were present.

In sacs from the 7-8th week, the thickness of the endodermal layer was substantially increased and proliferated to form downwards columns of cells that eventually became hollow, empty tubules (Fig. 1A). Their cytoplasm exhibited abundant intracellular lumina (Fig. 1B) often in close apposition with the endodermal tubules and the haemopoietic islands present in the mesenchyme. Some anisokaryosis was often present (Fig. 1B). The mesothelium was unremarkable.

Involuting sacs from the 9-12th week revealed a flattened or coarsely vacuolated endoderm with collapse and flattening of the endodermal tubules and disappearance of the intracellular vesicles. Blood islands progressively disappeared while the mesothelial layer became prominent and vacuolated. At the end of this period, endodermal cells had become atrophic and been desquamated into the endodermal cavity as amorphous eosinophilic granular debris. Parietal fibrosis and calcification were present.

TABLE 1

ANTIBODIES USED IN THIS STUDY

Antibody	Clone	Dilution	Vendor
Glypican 3 (GLP3)	SP86	Prediluted	MasterDiagnostica, Spain
α-foetoprotein (AFP)	Polyclonal	Prediluted	DAKO, Denmark
Hepatocyte paraffin 1 (HepPar-1)	OCH1E5	Prediluted	DAKO, Denmark
CDX2	DAK-CDX2	Prediluted	DAKO, Denmark
Villin	1D2 C3	Prediluted	DAKO, Denmark
Podoplanin (D2-40)	D2-40	Prediluted	DAKO, Denmark
Calretinin	DAK-Calret 1	Prediluted	DAKO, Denmark
Anti-Mesothelioma antibody	HBME-1	Prediluted	DAKO, Denmark

Immunohistochemistry

α-*foetoprotein* (AFP) secretion was seen as early as the 5th week and remained positive throughout the evolution of the SHYS, even in the atrophic or shed endodermal cells of the involuting sacs of 10-11 weeks. Strong cytoplasmic stain was exclusively found in the endodermal layer. It is worth noting that in SHYS from 5-8 weeks, the numerous intracellular vesicles were highlighted by a strong membrane AFP expression (Figs. 1 C,D). Vesicles were seen to communicate with the endodermal tubules, which also expressed a strong luminal stain (Fig. 1D). Some hematopoietic cells were also AFP positive (Fig. 1C). Mesothelial layer cells were negative, although some diffusion and background stain occurred. In the accompanying embryonal tissues, a strong expression was identified in both the liver trabeculae and some blood cells.

Glypican 3 (GLP3) staining pattern was similar in strength and location to that of AFP, albeit with a minimal background stain, remaining positive throughout the evolution of endoderm. Although the endodermal cytoplasm was well stained, it had a stronger membranous intensity than AFP (Fig. 2A). The endodermal intracellular vesicles and tubules also showed a prominent apical and luminal stain. In the available embryonal tissues, liver cells showed a constantly strong positivity. This antibody was also expressed in developing mesenchymal cells and the neuroepithelial structures of embryos.

Hepatocyte Paraffin-1 (HepPar-1) expression was reduced to cells of the endodermal layer and was also constantly present throughout the evolution of the SHYS. It had a characteristically strong, coarsely granular cytoplasmic (mitochondrial-type) staining pattern (Fig. 2B). Even atrophied or shed endodermal cells of the involutive period also showed similar reactivity. Among the embryonal tissues, only liver was specifically stained with a similar expression.

Villin showed a strong cytoplasmic and membranous expression in the endoderm throughout the evolution of the

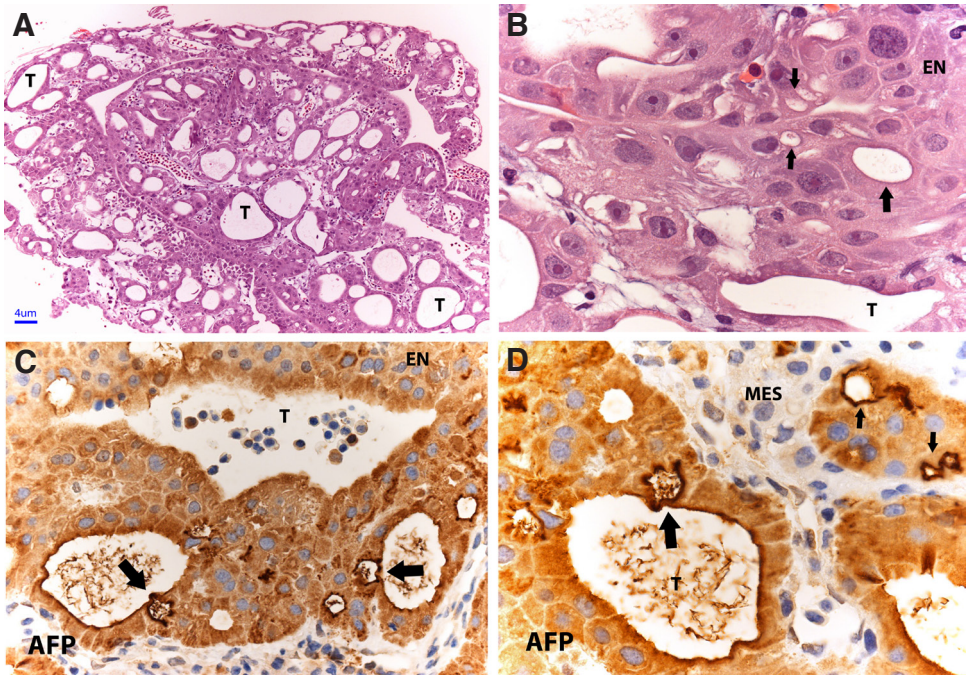

Fig. 1. A partly collapsed secondary human yolk sac from the 8th week. (A) *Note the numerous endodermal tubules(T).* **(B)** *Higher magnification of the endodermal epithelium (EN). Cells have large, irregular nuclei with prominent nucleoli.* **(C,D)** *Their ample cytoplasm reveals lumina (arrows) whose membranes are intensely AFP immunoreactive (arrows).* **(D)** *AFP immunoreactivity is absent in the mesenchyme (MES).* **(C,D)** *Intracellular lumina communicate with the large endodermal tubules; see arrows in (C,D).*

TABLE 2

SHYS IMMUNOPHENOTYPE

		ANTIBODIES						
Week	**#**	**AFP**	**GLP3**	**HepPar-1**	**Villin**	**CDX2**	**SALL4**	**D2-40**
5-6	1	1/1	-	-	-	-	-	-
7-8	15	15/15	15/15	12/14*	11/12*	10/14*	10/13*	15/15
9-11	10	10/10	10/10	9/10	5/9*	9/10	3/8*	8/9*

All antibodies, except for podoplanin D2-40, were expressed in the endodermal layer. Only podoplanin was positive in the mesothelium.

(*) In some cases, step sections failed to produce a sufficient number of slides to complete the study of some antibodies

TABLE 3

IMMUNOHISTOCHEMICAL EXPRESSION OF CONCOMITANT EMBRYONAL TISSUES

		ANTIBODIES					
Weeks	**#**	**AFP**	**GLP3**	**HepPar-1**	**Villin**	**CDX2**	**SALL4**
7-8	6	L 6/6	L 6/6 Mes 6/6 Neu 6/6	L 6/6	L 6/6 * Gut 3/3	* Gut 2/6	* L 1/4 Neu 6/6
9-11	4	L 4/4	L 4/4 Mes 4/4 Neu 4/4	L 4/4	L 4/4	-	Neu 4/4

(*) Not all embryonal organs were analyzed, due either to poor section orientation or absence of material in successive slides. KEY: L, liver; Mes, mesenchyme; Neu, neuroepithelium.

endodermal layer (Fig. 2C). In the embryonal tissues it was markedly positive in the cytoplasm of liver cells and in the apical membranes and cytoplasm of the intestinal lining.

CDX2 was strong and diffusely expressed in the nuclei of early and sacs from the 7th to 8th week, becoming weaker and more focal in distribution during the involution period (Fig. 2D). In embryos showing gut structures, lining cells had a strong nuclear positivity which was, however, absent in liver and other tissues.

SALL4 broadly expressed a similar nuclear staining pattern to CDX2 in both chronology and intensity (Fig. 2E). In the embryonal tissues it was strongly expressed in neuroepithelium.

Podoplanin (D2-40) expression was reduced to the mesothelial layer (Fig. 2F), which failed to stain for other markers. In the 3 instances where sections were still available, mesothelium was negative for other characteristic mesothelial antibodies such as calretinin and HBME-1.

The immunohistochemical findings of both SHYS and embryos are shown in Tables 2 and 3. As a summary, Fig. 3 A-E shows the comparative expression of the antibodies in a 7th week SHYS.

Discussion

Clinical interest in SHYS morphology has been focused mainly on its ultrasound appearance during the first trimester. Although it has been proposed that SHYS changes may act as markers for some chromosomal abnormalities (Schmidt *et al.,* 2011), it seems that the predictive value of SHYS measurements in determining the outcome of an early pregnancy is limited, as the alterations in SHYS size are the consequence of poor embryonic development or embryonic death rather than being the primary cause of early pregnancy failure (Jauniaux *et al.,* 2005). This clinical perception agrees with morphological studies of the SHYS in spontaneous pregnancy loss, which revealed only non-specific, degenerative features related to embryonal death and retention (Nogales *et al.,* 1992; Nogales *et al.,* 1993; Nogales *et al.,* 1995).

In the present paper, we analyze SHYS material originating from spontaneous pregnancy loss. Sacs from all evolutive periods were included and there were only minimal degenerative changes. Due to the limits imposed by such scanty material, we focused on the demonstration of a short series of readily available antibodies characteristic of the presumed secretory, synthetic and absorptive

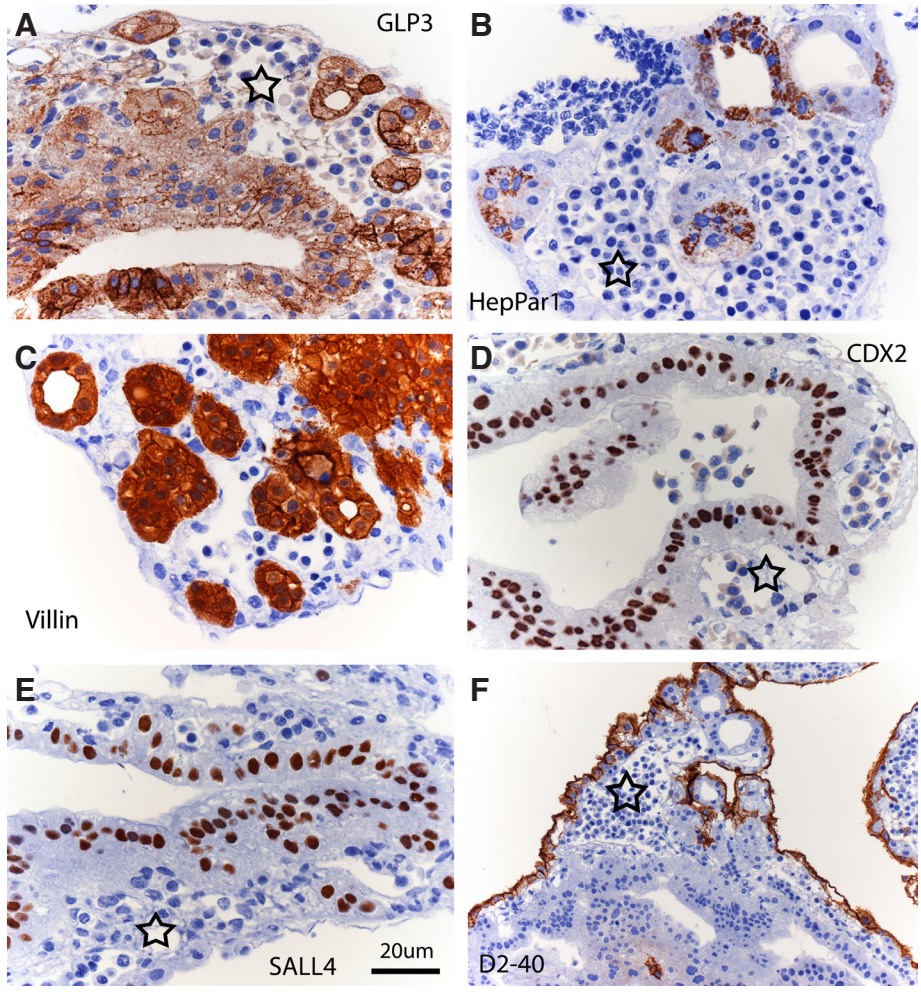

Fig. 2. Immunophenotype of another secondary human yolk sac from the 8th week, during which haematopoiesis (*) is prominent. (A) *GLP3 delineates endodermal membranes.* **(B)** *Granular cytoplasmic (mitochondrial) positivity for HepPar1 is prominent.* **(C)** *Villin shows strong membrane and cytoplasm expression, while CDX2* **(D)** *and SALL4* **(E)** *reveal nuclear positivity.* **(F)** *D240 podoplanin is only expressed in mesothelial cells.*

functions of the SHYS as a temporary liver and intestine and also used in the diagnosis of yolk sac tumours (Nogales *et al.,* 2012).

SHYS has been considered a transfer organ between the embryo and the exocoelomic cavity (Gulbis *et al.,* 1998), implying a role of active synthesis, absorption and transference of proteins during a short but crucial period of ontogenesis.

Optic microscopy shows the endoderm lining the yolk sac cavity to have a progressively complex structure, developing short columns of cells that contain an abundant network of intracellular vesicles. These structures are present in the earliest SHYS of this study at the 5th week, but they eventually collapse and disappear in involutive sacs. Indeed, they seem to be present only during its period of maximum activity.

Ultrastructurally, SHYS endodermal cells share many common features with hepatic ones, having a glycogen-rich cytoplasm, well-developed Golgi complex, and abundant rough endoplasmic reticulum profiles (Jones *et al.,* 1995a; Pereda *et al.,* 1999; Takashina *et al.,* 1993). Well-developed apical microvilli are present on the tubular surface and also line the numerous intracellular vesicles (Takashina *et al.,* 1993) that will eventually coalesce to form a complex system of endodermal tubules. They are likely to have a role in transport of various substances due to their close relationship with blood islands, mesenchymal capillaries and mesothelium.

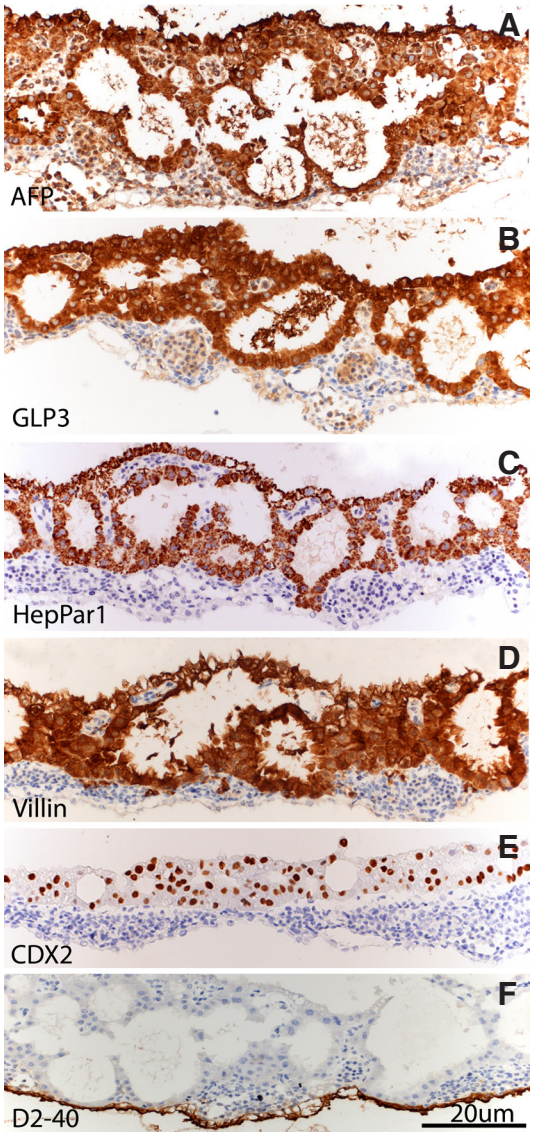

Fig. 3. Immunophenotype of a secondary human yolk sac from the 7th week. *The endoderm is strongly positive in both cytoplasm and membrane for* **(A)** *AFP and* **(B)** *GLP3.* **(C)** *HepPar1 exhibited a coarse granular expression.* **(D)** *Villin immunoreactivity is intense in the cytoplasm and membrane.* **(E)** *CDX2 labeling is intense in the nucleus.* **(F)** *Only the mesothelial layer presented podoplanin immunoreactivity.*

In this paper we demonstrate that this transient canaliculotubular complex displays a strong apical membrane staining for both AFP and GLP3, possibly indicating an active transport of these important proteins.

The functional similarities of the SHYS endodermal cells with hepatic and intestinal ones lie in their shared synthesis of proteins such as AFP, prealbumin, albumin, caeruloplasmin, fibrinogen, plasminogen, lipoproteins, α1-protease inhibitor, transferrin, GLP3, etc. (Gitlin *et al.,* 1969; Gitlin *et al.,* 1970; Gitlin *et al.,* 1972; Gulbis *et al.,* 1998; Gulbis *et al.,* 1992; Preda *et al.,* 2011; Shi *et al.,* 1985). Among these, AFP and GLP3 are only expressed by developing hepatocytes (Kandil *et al.,* 2007; Mizejewski *et al.,* 2001) and have a similar distribution in both SHYS and liver. AFP function resides in the binding and transport of various ligands and recently an additional role as a growth regulator has been recognized (Mizejewski *et al.,* 2001). Glypican 3 also acts as a modulator, activating intracellular signaling proteins and growth factors (Filmus *et al.,* 2008). Their similar location and staining patterns in SHYS would reflect a synergic relationship, possibly related to cell growth control.

HepPar-1 is an empirically obtained monoclonal antibody (Wennerberg *et al.,* 1993) raised against formalin-fixed, paraffin embedded hepatic tissue that is highly specific of both adult and embryonal liver cells, both normal and neoplastic. Outside the liver, it is only focally present in the glands of the small intestine and shows a weak expression in gastric glands (Lugli *et al.,* 2004). Recently, the antigen for this antibody has been shown to be carbamoyl phosphate synthetase 1, an enzyme in the urea cycle located in mitochondria (Butler *et al.,* 2008). This hepatic antigen has not been previously studied in the SHYS. Here, it was consistently present throughout the development of the SHYS from the 7th week onwards and was also exclusively expressed by the liver cells of the accompanying embryos.

Villin is a Ca^{2+} regulated actin-binding protein that is expressed early during embryogenesis, being present in the mouse yolk sac in early stage visceral endodermal cells (Maunoury *et al.,* 1988). In the human embryo, it is found at the 8th week in the early intestinal tube. It is considered an early marker of endodermal cell lineage and it is identified in gastrointestinal, renal and urogenital epithelial cells (Robine *et al.,* 1985). Villin is regarded as an early marker of committed intestinal absorptive cells (Khurana *et al.,* 2008), being expressed also by liver ducts. The expression of villin in the SHYS and embryonal liver cells is consistent with both an intestinal and hepatic phenotype for the SHYS, where it is present in both the free membrane surfaces delineating endodermal tubules and the intracellular vesicles. A diffuse cytoplasmic stain was also present in both the SHYS and embryonal liver cells.

CDX2, a caudal-like homeodomain-containing transcription factor, is expressed in intestinal endoderm posterior to the stomach throughout gestation and adult tissues, where its strong expres-

sion is mostly found in the nuclei of small and large intestine and pancreatic ducts (Moskaluk *et al.,* 2003). Metaplastic conditions that reproduce intestine are also CDX2 positive (Nicolae *et al.,* 2011) as well as some unusual metaplasias such as morules in various organs (Houghton *et al.,* 2008). Its early functions include promotion of trophoblast differentiation (Stringer *et al.,* 2012) and determination of blastocyst polarity (Jedrusik *et al.,* 2008). Later on, it is involved in the differentiation and development of the intestine, but is not expressed by liver, even at an early stage of differentiation, as confirmed in this study by its absence in the hepatic tissue of all our accompanying embryos. It is worth noting that its expression was strong and diffuse in sacs from the 7-8th week but diminished to a weak and focal stain in the older involuting ones.

SALL4 is a transcriptional activator of Pou5f1 and has a critical role in the maintenance of cell pluripotency by modulating Oct4 expression (Zhang *et al.,* 2006). In the liver, SALL4 plays a decisive role in controlling the lineage commitment of hepatoblasts not only by inhibiting their differentiation into hepatocytes but also driving their differentiation toward cholangiocytes (Oikawa *et al.,* 2009). So, it would seem that SALL4 is crucial in liver cell differentiation and its strong expression in early SHYS could be related to its early hepatic function.

Bile secretion only takes place at the 12th week (Crawford *et al.,* 2002) and is not needed at this developmental stage; consequently it is neither present in the SHYS nor in the embryonal liver, only appearing in the foetal stage.

The role of SHYS mesothelium has been assessed as being active in protein transfer (Gulbis *et al.,* 1998; Jauniaux *et al.,* 2000), although others have proposed a merely protective role (Pereda *et al.,* 1999). The only marker that was positive in the mesothelium of the SHYS was D2-40, a monoclonal antibody against podoplanin (Kalof *et al.,* 2009). However, other frequently used mesothelial markers such as calretinin and HBME-1 were negative, although they were not performed in all sacs due to the depletion of tissue in the paraffin blocks.

The above results reveal that the SHYS endodermal cells have a hybrid immunophenotype of both liver and intestinal cells that parallel their synthetic and transport functions. Their predominantly hepatic features of differentiation are present at a histological level (intracytoplasmic vesicles configuring a canalicular system) and immunohistochemically (expression of specific liver cell markers such as AFP, HepPar-1 and GLP3). Moreover, it seems that expression of SALL4 is crucial in liver cell differentiation. The intestinal phenotype is represented by the expression of villin, which is present in both intestine and early liver. Furthermore, CDX2 is characteristic of intestinal cells but absent in liver cells.

Materials and Methods

26 SHYS were obtained from the routine histopathological archives of our hospital between 1986 and 2007, corresponding to 24 products of conception from spontaneous abortions and 2 tubal ectopic pregnancies. Gestational ages were assessed by taking into account both clinical data and morphological milestones. The earliest sac corresponded to a 5 week tubal pregnancy, 15 were in the 7th to 8th week range and 10 corresponded to the involutive period of 9-11 weeks. All had a good histological preservation, with minor changes of maceration in only 3. Concomitant embryos were found in 11 cases, of these, 8 were fresh and in only 3 were there minor maceration changes.

The SHYS and embryos were formalin fixed and subsequently embedded in paraffin and stained with H&E. Step sections were performed.

Immunohistochemistry was done using the antibodies listed in Table 1. Functionally, these antibodies recognize the following functional proteins: *α-foetoprotein (AFP),* a protein is expressed in the early endoderm that binds and transports various ligands, being highly characteristic of yolk sac and immature liver; *Glypican 3 (GLP3),* a cell surface heparan sulphate proteoglycan is expressed by both yolk sac and liver that acts as a modulator, activating intracellular signaling proteins and growth factors; *Hepatocyte-paraffin-1 (HepPar-1)*, a mitochondrial urea cycle enzyme highly characteristic of embryonal and adult liver cells; *Villin*, a Ca^{2+} regulated actin-binding protein present in both embryonal and adult intestinal cells; *CDX2*, a protein from a ParaHox gene which interacts in trophoblast differentiation, axial development and particularly, in gut differentiation; *SALL*, a stem cell nuclear transcriptional factor, expressed in early development as part of a transcriptional core network that maintains the pluripotent properties and self-renewal capacities of embryonal stem cells and *Podoplanin (clone D2-40)*, a membrane glycoprotein with mucin-like characteristics that is expressed in the apical membrane of the mesothelium.

Their nuclear, cytoplasmic or membranous expression was analyzed in both the endodermal and mesothelial layers and in the accompanying embryonal tissues when these were available. In some cases, due to the small amounts of tissue, serial sections failed to produce a sufficient number of viable slides for immunohistochemistry. Additionally, in some embryos, the initial haphazard paraffin wax inclusion and block orientation precluded a detailed study of every embryonal organ.

Author's roles

Francisco F Nogales designed the study and participated in the analysis, execution and manuscript drafting and critical discussion. Isabel Dulcey retrieved archive and clinical material, performed the immunohistochemical and bibliographical analysis and participated in the manuscript drafting and critical discussion.

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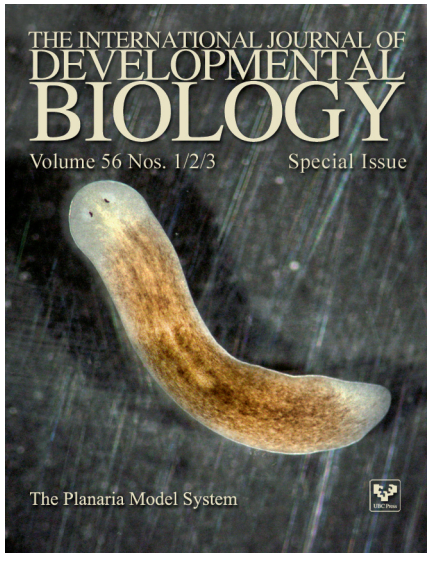

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A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac

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A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac

Aims: To establish a diagnostic immunohistochemical panel for various histotypes of yolk sac (primitive endodermal) tumours (YSTs) by comparison with the human yolk sac (HYS) immunophenotype.
Methods and results: Twenty-five YSTs showing either classical patterns (CPs) of histology (microcystic/reticular, $n = 14$; polyvesicular, $n = 1$; and hepatoid, $n = 1$) or somatic glandular patterns (SGPs; $n = 9$) were analysed for expression of α-fetoprotein (AFP), glypican-3 (GPC3), villin, hepatocyte paraffin-1 (HepPar-1), CDX2, SALL4 and LIN28. AFP expression was constantly heterogeneous in CPs but tended to be focal/absent in SGPs. GPC3 was diffuse in CPs but heterogeneous (seven cases) or focal/absent (two cases) in SGPs. HepPar-1 expression was focal in all but three cases (diffuse in one CP-hepatoid and two SGPs). CDX2 positivity was focal in CPs but heterogeneous (seven cases) or diffuse (two cases) in SGPs. Villin, SALL4 and LIN28 were diffusely positive in nearly all cases.
Conclusions: CPs reproduce the immunophenotype of HYS and early endoderm with variable expression of both AFP and markers of early gut or hepatic differentiation. SGPs with intestinal differentiation often have incomplete immunophenotypes. A differential diagnosis panel, including both markers of pluripotentiality (SALL4 and/or LIN28) and endoderm (AFP, GPC3 and villin), is proposed. It identifies overlapping multidifferentiation of primitive and somatic immunophenotypes, supporting the recently proposed term of primitive endodermal tumours.

Keywords: human yolk sac, immunohistochemistry, pluripotentiality, primitive endodermal tumour, yolk sac tumours

Introduction

Human yolk sac tumours (YSTs) are a heterogeneous group of tumours which reproduce various patterns of endodermal differentiation, including the yolk sac. In order to reflect their capacity to differentiate into various immature and mature endodermal cell types with both extraembryonal and somatic differentiation, it has been proposed recently that use of the term 'primitive endodermal tumours' would be more appropriate.[1,2]

The secondary human yolk sac is a vitally important developmental structure whose morphology[3–5] and immunophenotype have been analysed in few studies. Only recently has it been shown that this temporary organ has an immunophenotype with both intestinal and hepatic features.[6]

We feel that comparative analysis of the immunophenotypes of the human yolk sac and YSTs can be used as a key to understanding the various types of differentiation present in this tumour group, and assist in the differential diagnosis with other neoplasms.

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Consequently, we compared the immunophenotype of a representative sample of both usual and special histological variants of YSTs with the human yolk sac.[6]

We aim to demonstrate that the highly heterogeneous histology of the tumours has a correspondingly variable immunophenotype. Thus we analysed differentiation and pluripotentiality markers in both human yolk sac and YSTs in order to design a comprehensive immunohistochemical panel that may prove useful in the differential diagnosis with other germ cell and somatic tumours.

Materials and methods

TUMOURS

A sample of 25 YSTs from our routine and consultation files was analysed comparatively for the expression of proteins described in the normal human yolk sac.[6] Alpha-fetoprotein (AFP), glypican3 (GPC3), hepatocyte-paraffin-1 (HepPar-1), CDX2, villin and SALL4 were studied in all cases.LIN28 expression was studied in 17 cases and thyroid transcription factor (TTF-1) in six. Immunohistochemistry was performed on representative sections of paraffin-embedded tissue. Positive immunostaining was assessed as diffuse (occurring in most epithelial cells and some stromal ones), heterogeneous (defined as irregularly stained patches of tissue) and focal (positivity in isolated epithelial cells).

NORMAL HUMAN YOLK SACS

Immunoprofile data from our previous study of normal human yolk sacs[6] were complemented by the additional study of LIN28 expression in seven morphologically normal human yolk sacs the earliest one corresponding to the 5th week of gestation, and three each to the 7th and 8th weeks. The source, clones and dilutions of these antibodies are shown in Table 1.

Results

We studied 12 ovarian and 13 testicular YSTs from eight prepubertal (age range 6 months–11 years) and 17 postpubertal and adult (18–76 years) patients. Three postpubertal cases were associated with concurrent somatic tumours.

The tumours were divided into two groups based on their histological patterns. Sixteen cases corresponded to classical patterns with a characteristic histology, comprising the usual microcystic/reticular and the less frequent polyvesicular and hepatoid patterns. Nine had somatic glandular patterns, consisting of a complex network of cysts and glandular spaces that often had luminal papillary projections. Two of these cases were associated with somatic tumours, one with endometrioid adenocarcinoma and the other with a clear cell carcinoma. A further case coexisted with an insular-type carcinoid. The cell lining of the glandular spaces had a variable morphology: five cases had gland-like spaces with empty lumina lined by compact, closely packed columnar cells with scanty cytoplasm (Figure 1A); in four instances, the spaces were filled with dense eosinophilic material and lined by tall columnar epithelial cells with apical or subnuclear vacuolation, similar to the embryonal gut (Figure 1B), occasionally with isolated differentiated goblet cells.

Patient ages, and tumour sites and growth patterns with their relative percentages present in each case, are shown in Table 2.

Table 1. Antibodies used in this study

Antibody	Clone	Dilution	Vendor
α-fetoprotein (AFP)	Polyclonal	Prediluted	Dako, Denmark
Glypican- 3 (GLP3)	SP86	Prediluted	MasterDiagnostica, Spain
Hepatocyte paraffin 1 (HepPar-1)	OCH1E5	Prediluted	Dako, Denmark
CDX2	DAK-CDX2	Prediluted	Dako, Denmark
TTF-1	8G7G3/1	Prediluted	MasterDiagnostica, Spain
Villin	1D2 C3	Prediluted	Dako, Denmark
SALL4	6E3	Prediluted	MasterDiagnostica, Spain
LIN28	EP150	Prediluted	MasterDiagnostica, Spain

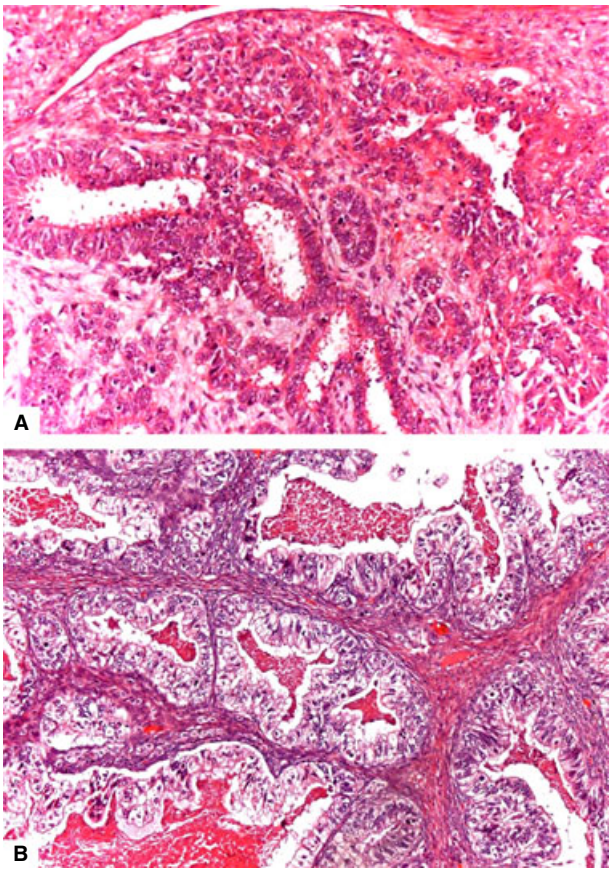

Figure 1. Somatic glandular patterns of yolk sac tumour. **A**, Tubular glands with compact cell lining in a testicular tumour. **B**, Tubular glands lined by cells with marked apical and basal vacuolation, similar to early gut, from an ovarian yolk sac tumour associated with an endometrioid adenocarcinoma.

IMMUNOHISTOCHEMISTRY

There were no noticeable differences in staining between ovarian and testicular neoplasms. To some degree all but one expressed AFP, which appeared as a granular cytoplasmic deposit, often delineating intra- or intercellular lumina (Figure 2A). There was usually excess background staining due to labelling of serum proteins. Expression was heterogeneous, patchy and restricted to the endodermal epithelium, although occasional stromal cells were also positive. Hyaline globules were often negative. Classical pattern tumours showed heterogeneous but constant AFP expression. However, in somatic glandular pattern tumours, staining was either only focal (Figure 2B,C) or absent, often restricted to the cytoplasm and apex of isolated columnar cells, and difficult to identify at low power.

Glypican-3 staining showed labelling of cytoplasm, cell membranes and intercellular lumina. In contrast to AFP, expression was usually diffuse in classical pattern tumours (Figure 2D), while expression in somatic glandular pattern tumours was heterogeneous in seven cases (Figure 2E), reduced to a single focus in one and absent in another (Figure 2F).

HepPar-1 expression appeared as strong dark granular cytoplasmic staining. It was expressed focally in isolated epithelial cells of most classical pattern (Figure 2G) and somatic glandular pattern (Figure 2H) tumours, but was present diffusely both in one case of hepatoid histology and in two ovarian cases exhibiting somatic glandular patterns, one of which was associated with a clear cell carcinoma and the other with insular carcinoid (Figure 2I).

In all cases displaying classical patterns, CDX2 showed nuclear expression in isolated epithelial cells (Figure 2J). In contrast, for those with a somatic glandular pattern, staining was heterogeneous in seven cases (Figure 2K) and diffuse in only two (Figure 2L).

TTF-1 was negative in all but one case of classical pattern, but showed focal expression in three of five cases of somatic glandular pattern.

Villin showed diffuse cytoplasmic and membrane expression, and was a universal marker of all epithelial cells in both classical (Figure 2M) and somatic glandular pattern (Figure 2N,O) tumours.

SALL4 showed diffuse nuclear expression in almost all cases with either classical (Figure 2P) or somatic glandular (Figure 2Q) patterns, with focal staining in only one case of the latter type (Figure 2R).

Human yolk sac immunophenotype data were obtained from a previously published paper.[6] Additionally, LIN28 expression was analysed in both the yolk sac at various developmental stages and in 17 YSTs, 12 with classical and five with somatic glandular patterns. Only the earliest (5th-week) yolk sacs showed cytoplasmic reactivity to LIN28 in endodermal cells (Figure 3A), older sacs from the 7th and 8th weeks being negative (Figure 3C,E). LIN28 was expressed by endodermal cells of tumours with both classical (Figure 3B,D) and somatic glandular (Figure 3F) patterns, the latter being negative in one instance.

Comparative immunophenotypes of tumours and the human yolk sacs are shown in Table 3.

Discussion

The terminology of this tumour group was defined originally by comparative studies between the histology of murine structures and murine experimental tumours.[7–9] The aim of this paper was to establish a

Table 2. Age, site and histopathology of yolk sac tumour (YST) cases

Age	Site	Predominant YST	%	Other patterns	Additional data
Classical patterns					
2	O	Microcystic/reticular	100	–	–
11	O	Microcystic/reticular	100	–	–
36	O	Microcystic/reticular	100	–	–
9	O	Microcystic/reticular	100	–	–
1	T	Microcystic/reticular	100	–	–
6 mo.	T	Microcystic/reticular	100	–	–
22	T	Microcystic/reticular	30	–	Associated with polyembryoma
67	T	Microcystic/reticular	50	–	Mixed GCT
6 mo.	T	Microcystic/reticular	20	–	Mixed GCT
21	T	Microcystic/reticular	70	–	Mixed GCT
24	T	Microcystic/reticular	40	–	Mixed GCT
32	T	Microcystic/reticular	40	–	Mixed GCT
25	T	Microcystic/reticular	80	–	Mixed GCT
26	T	Microcystic/reticular	60	–	Mixed GCT
9	O	Polyvesicular	70	Microcystic/reticular	–
18	O	Hepatoid	60	Microcystic/reticular	–
Somatic glandular patterns					
4	T	Glandular	50	Microcystic/reticular	–
18	T	Glandular	100	–	–
20	O	Glandular	100	–	–
26	T	Glandular	70	Microcystic/reticular	–
33	O	Glandular	60	Solid	–
40	O	Glandular	40	–	–
52	O	Glandular	100	–	Clear cell carcinoma
41	O	Glandular	100	–	Endometrioid adenocarcinoma
76	O	Glandular	80	–	Insular carcinoid

O, ovary; T, testis; GCT, germ cell tumours.

comparative immunophenotypical analysis between YSTs and the normal human yolk sac. Similar studies of human yolk sacs have been attempted in small numbers at electronmicroscopic level.[4,5,10] In this paper, we have compared the expression of different human yolk sac proteins associated with synthetic, absorptive, transferential and pluripotentiality functions[6] in a representative sample of 25 YSTs including various growth patterns, and occurring in diverse ages and organs as well as in association with somatic tumours. We believe that this analysis provides new insights into the various types of endoder-

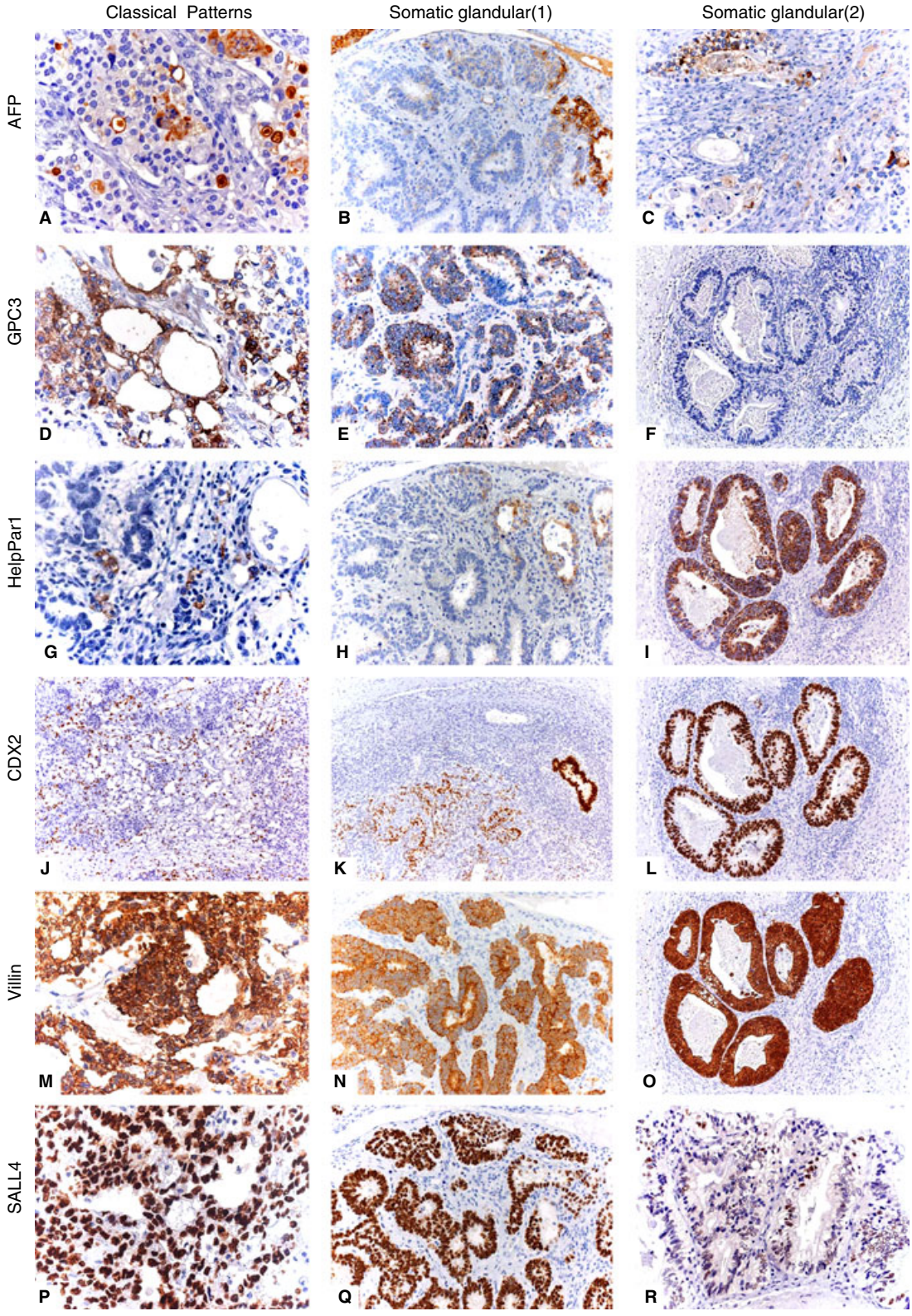

Figure 2. Comparative marker expression (antibodies shown in vertical column on the left-hand side) between classical patterns of yolk sac tumour and two somatic glandular patterns, both compact (1) (corresponding to Figure 1A) and vacuolated (2) (corresponding to Figure 1B).

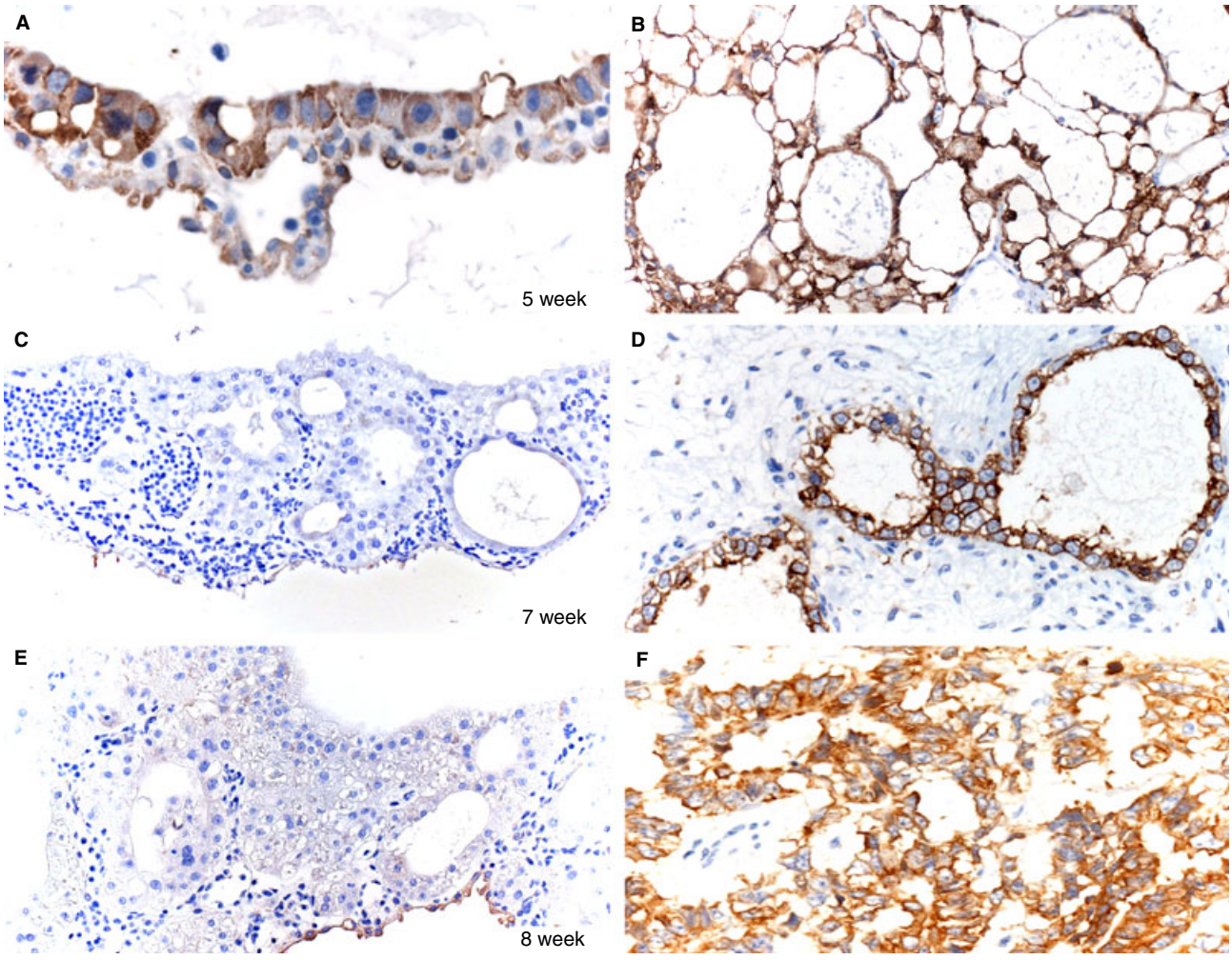

Figure 3. Comparative LIN28 expression between various developmental stages of the normal human yolk sac and patterns of yolk sac tumours. LIN28 is only expressed in an early yolk sac of the 5th week (**A**), eventually disappearing in sacs of the 7th (**C**) and 8th (**E**) weeks. All epithelial cells in tumours with classical (**B**,**D**) and somatic glandular (**F**) patterns show marked cytoplasmic positivity.

mal differentiation found in YSTs and configures a diagnostic antibody panel that will prove useful in identifying rare morphological variants, especially when YSTs are found outside their characteristic locations or constitute a predominant morphological pattern.

The human yolk sac displays consistent expression of immunohistochemical markers associated with hepatic (HepPar-1 and GPC3) and intestinal (villin and CDX2) endodermal functions,[6] thus providing a morphological basis for its temporary physiological role as an active transferential and synthetic interface between the exocoelomic cavity and the developing embryo.[11] In addition, it expresses SALL4.[6] Furthermore, in the present paper we have added new data on the expression of LIN28, a pluripotentiality protein, demonstrating that it is expressed only in early human yolk sacs in the 5th week, being however down-regulated at the 7th and 8th weeks; this is possibly a reflection of the early but terminal differentiation of endodermal cells, as the yolk sac has only a short functional lifespan.

YSTs present a spectrum of neoplastic endodermal differentiation with heterogeneous histology. Their overlapping immunophenotype reflects the various differentiation stages of endodermal cells, from primitive ones expressing pluripotentiality and early embryonal proteins, to differentiated ones showing characteristics of organs such as the intestine, liver, etc.

Since 1975,[12] AFP has been considered a gold standard for the diagnosis of YSTs, although it is known that this embryonal protein can be expressed in ovarian clear cell carcinoma[13] and in ovarian

Table 3. Comparative immunohistochemical findings between yolk sac tumours and human yolk sac

	Immunohistochemical staining results							
Histology	AFP	GPC3	HepPar–1	CDX2	Villin	TTF–1	SALL4	LIN28
Classical patterns								
Microcystic/reticular	14/14 H	14/14 D	14/14 F	14/14 F	14/14 D	–	13/13D	10/10 D
Polyvesicular	1/1 H	1/1 D	1/1 F	1/1 F	1/1 D	1/1	1/1 D	1/1 D
Hepatoid	1/1 H	1/1 D	1/1 D	1/1 H	1/1 D	–	1/1 D	1/1 H
Somatic glandular patterns	7/9 F	7/9 H	7/9 F	7/9 H	9/9D	3/5F	8/9 D	4/5 D
Normal human yolk sac[6]	D	D	D	D	D	ND	D	1/1 (5th week) 0/6 (7–8th weeks)

H, heterogeneous; D, diffuse; F, focal; ND, not done.

metastases of gastric carcinoma, among others. In the normal yolk sac, AFP is present as early as the 5th week, with granular cytoplasmic positivity on immunochemistry which is concentrated at the intra- and intercellular lumina and tubular surfaces that constitute a complex transfer system. In tumours with classical YST patterns, AFP expression was constant but heterogeneous in the epithelium, where it also delineated occasional cellular lumina. In cases with somatic glandular patterns, however, its distribution tended to be focal or absent, as reported in some extragonadal tumours.[14] Thus it can be said that AFP negativity does not necessarily rule out a diagnosis of YST.

In the normal yolk sac, GPC3 displays cytoplasmic or membranous expression that delineates inter- and intracellular tubules. GPC3 is also expressed extensively or patchily in YSTs.[15] We found that GPC3 was expressed diffusely in classical pattern tumours but had a heterogeneous distribution or was even negative in somatic glandular ones. Similar in distribution to AFP, it is a more sensitive marker although not as specific as AFP,[16] being expressed in the female genital tract, in tumours such as neural areas of immature teratoma, squamous cell carcinomas, carcinosarcomas and placental site trophoblastic tumours,[2] among many others. Some clear cell carcinomas may also express it and, consequently, its demonstration may not be useful in differential diagnosis,[17] especially in those YSTs originating from somatic tumours such as endometrioid or clear cell adenocarcinomas.

HepPar-1 is expressed throughout the lifespan of the human yolk sac, and thus it is not surprising that HepPar-1 can also represent a marker of human yolk sac differentiation in YSTs. HepPar-1 positivity has been reported in both the hepatoid areas of YST, as in one of our cases, and in hepatoid carcinomas.[18] Our study would suggest that the frequent, but focal, HepPar1 positivity in YSTs does not necessarily correspond to hepatic differentiation, but can also reflect human yolk sac-type differentiation. Furthermore, it is remarkable that both our cases with somatic glandular patterns that showed intense and diffuse HepPar-1 expression were associated with endometrioid and clear cell carcinomas of postmenopausal patients.

CDX2 expression is consistently present in the human yolk sac,[6] indicative of its intestinal role. In classical pattern cases CDX2 positivity was focal, as reported by previous studies,[19,20] but in somatic glandular pattern tumours it displayed stronger expression, with staining being heterogeneous or diffusely positive. Therefore, it would seem that CDX2 positivity will highlight areas of both yolk sac and intestinal differentiation, the latter being more evident in somatic glandular pattern tumours, especially those with vacuolated epithelia resembling the embryonal gut.

Villin is expressed consistently during early embryogenesis in both human yolk sac and early endoderm[6] and is a highly sensitive endodermal epithelial component marker in all cases of YSTs, both with classical and somatic glandular patterns, but it is absent in pure embryonal carcinoma and seminoma/dysgerminoma. In our experience, villin is negative in ovarian clear cell carcinomas. Its diffuse cytoplasmic expression parallels that of the human yolk sac[6] and intestinal adenocarcinomas, where both diffuse and apical staining patterns occur.[21] To our knowledge, villin has not been used previously as a marker for the epithelial components of YSTs.

SALL4 is a marker of high sensitivity but low specificity for YSTs, as it is also expressed consistently by all malignant primitive germ cell tumours of both gonadal and extragonadal locations.[22–24] It is also present throughout the life cycle of the human yolk sac, reflecting the degree of pluripotency retained by this temporary organ in its endodermal cell component. This could explain the eventual differentiation of liver cell tissue in the placenta, which may originate from displaced yolk sac remnants.[25] SALL4 proved a consistent marker in all cases of both classical and somatic glandular groups.

LIN28 is a micro-RNA binding protein that is expressed in human primordial and undifferentiated embryonal stem and germ cells and represents part of the pluripotency network.[26] At the end of the first year of life, LIN28-positive germ cells are no longer detected.[27] In the present paper we have observed that in a sample of seven normal yolk sacs, LIN28 was expressed in only a very early sac of the 5th week, being down-regulated in older ones, possibly as a result of their higher degree of differentiation. The absence of coexpression of SALL4 and LIN28 in older sacs could be explained by the fact that these different protein complexes are involved in regulating distinct biological functions.[28] In a similar fashion to SALL4, LIN28 is also a highly sensitive marker for malignant germ cell tumours.[13,29–31] However, its expression is not specific for YSTs, although it has been proposed that it might be useful for immunohistochemical detection of metastasized YSTs[13,30,31] and for differential diagnoses with clear cell carcinoma of the ovary, where both AFP and GPC3 can also be expressed. Other pluripotentiality markers, such as OCT4 and SOX2, are expressed in embryonal carcinoma but not in YSTs.[32]

YSTs arising in conjunction with somatic neoplasms[33] can present with both classical or glandular patterns and have a similar immunophenotype to that of YSTs of germ cell origin.

We can conclude that a diagnostic antibody panel, including markers both of pluripotentiality (SALL4 and LIN28) and endodermal identity (AFP, GPC3 and villin), is useful for recognizing the multiple patterns of differentiation present in YSTs. Their overlapping phenotypes of primitive and differentiated areas supports the newly proposed term of primitive endodermal tumours.[2] This diagnostic panel will also prove useful in the differential diagnosis of unusual histological variants of YSTs lacking the classical diagnostic patterns. Furthermore, it would help both to identify YSTs arising from somatic neoplasms and differentiate them from clear cell carcinomas, especially in elderly patients. However, some tumours with a glandular somatic pattern may show an incomplete immunophenotype, being negative for AFP or GPC3 and unexpectedly positive for HepPar-1 and CDX2, especially when they occur in association with somatic neoplasms. Consequently, AFP or GPC3 negativity in the presence of other markers such as villin, SALL4 or LIN28 should not preclude the diagnosis of YST.

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CASE REPORT

Urothelial carcinoma of the renal pelvis with simultaneous trophoblastic and malignant clear cell endodermal-type differentiation

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Embryonal-type differentiations in urothelial neoplasms are uncommon [1, 2]. These frequently underdiagnosed phenomena may include either the presence of trophoblastic areas or, even more rarely, admixed yolk sac tumour patterns (YST) [2]. We present for the first time the association of both trophoblastic and malignant endodermal-type elements within an aggressive high-grade urothelial carcinoma (HGUC). Due to the presence of the endodermal component, which had cells with a prominent clear cytoplasm, a differential diagnosis with other papillary and clear cell renal neoplasms was necessary.

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Clinical history

A 47-year-old male presented with intense pain in the lumbar region. Seventeen years previously, he had been treated for a non-seminomatous testicular tumour with lung and liver metastases. He eventually underwent a right orchidectomy followed by platinum-based chemotherapy. Follow-up showed only residual mature teratomatous tissue in the biopsy material from the remaining testicle.

On his recent admission, elevated levels of serum β-hCG of 9,897.06 mUI/ml (normal 0–2.6 mUI/ml) and a lactate dehydrogenase of 1,280 U/L (normal 0–480 U/l) were found. Serum α-foetoprotein (AFP) was negative. A CT scan showed a large, ill-defined retroperitoneal mass involving the left kidney and ureter. There was marked infiltration of the psoas, and lung and vertebral metastases were prominent. No lesions were detected in the left testicle after both clinical and ultrasonographical exploration. A left ureteronephrectomy with resection of the surrounding tissues was performed. Patient refused chemo- or radiotherapy and 18 months after diagnosis is alive but has a large residual mass and pulmonary metastases.

Materials and methods

The ureteronephrectomy specimen was bivalved and fixed overnight in 10% buffered formalin and routinely processed. Seven tissue blocks were taken, and H&E-stained sections were analysed. Immunohistochemistry was performed on representative sections with the antibodies shown in Table 1.

Table 1 Antibodies used in this study

Antibody	Clone	Vendor
AFP	Polyclonal	DAKO, Glostrup, Denmark
Glypican 3	1G12	Bio Mosaics, Burlington, VT
SALL4	Sc-101147	Santa Cruz Biotechnology, Inc
CDX2	AMT 28	MasterDiagnostica, Granada
HepPar-1	OCH1E5	DAKO, Glostrup, Denmark
β-hCG	polyclonal	DAKO, Glostrup, Denmark
hPL	polyclonal	MasterDiagnostica, Granada
α-Inhibin	R1	DAKO, Glostrup, Denmark
Cytokeratin 20	K_s20.8	DAKO, Glostrup, Denmark
Cytokeratin 7	Ovtl 12/30	DAKO, Glostrup, Denmark
p63	4A4	MasterDiagnostica, Granada

Results

The nephrectomy specimen showed a dilated pelvis harbouring a solid 6×4.7-cm mass which infiltrated the adjoining kidney parenchyma, perirenal fat and the psoas muscle. Microscopically, a solid HGUC originating from the pelvis had numerous scattered syncytiotrophoblasts, which were also present in the numerous tumour emboli (Fig. 1a). In close transition with the urothelial tumour (Fig. 1b), a complex tubulopapillary neoplasm lined by tall, vacuolated, clear, atypical cells (Fig. 1c) was seen protruding into the pelvic space.

Immunohistochemical markers differentiated the cytokeratin 7 (CK7) and p63-positive (Fig. 2a) urothelial component from the trophoblastic cells which were positive for β-hCG, α-inhibin (Fig. 2b) and human placental lactogen (hPL). In contrast, the clear cell tubulopapillary cells had a characteristic primitive endodermal immunophenotype positive for glypican 3 (GLY3), CDX-2 and SALL4 (Fig. 3a–c) which, however, failed to stain for AFP, a fact which precluded a diagnosis of glandular yolk sac tumour. Cytokeratin 20 and HepPar-1 were negative.

Discussion

The capacity of urothelial carcinomas to differentiate into various cell lineages is well recognized. Amongst others, glandular, squamous, neuroendocrine and lymphoepithelioma-like patterns are relatively frequent in the bladder [1].

The rare trophoblastic differentiation in transitional tumours represents a variant of carcinomas associated with a poor prognosis [1, 3, 4]. Immunohistochemistry performed in the present case demonstrated the coexistence of atypical transitional cells with a characteristic CK7 and p63 positivity showing both syncytio- and intermediate trophoblastic differentiations, the former positive for β-hCG and α-inhibin and the latter for hPL. This highly specific immunophenotype helped to differentiate this variant of urothelial carcinoma from other giant cell-containing tumours [1].

This case also showed the concomitant presence of a clear cell tubulopapillary growth that merged with the urothelial neoplasm. This aberrant component had a characteristic primitive endodermal immunophenotype which was positive to SALL4, a stem cell marker [5],

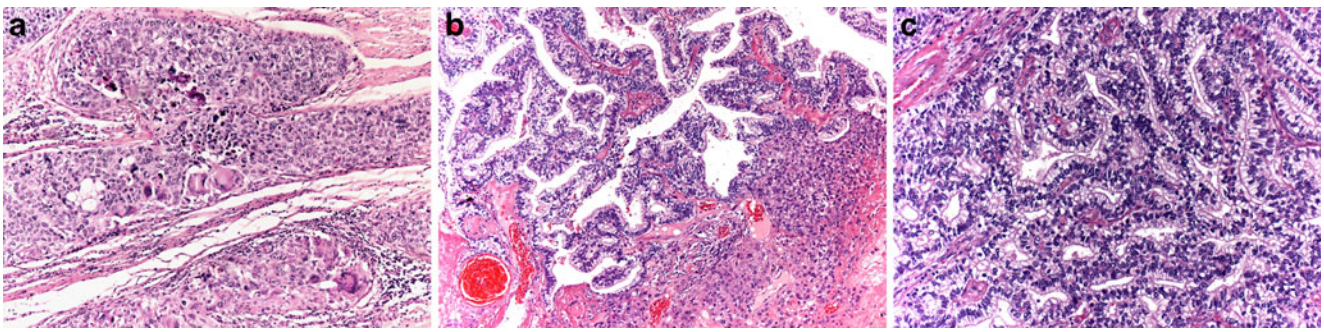

Fig. 1 Pelvic urothelial tumour with abundant syncytiotrophoblasts (**a**), merging with a tubulopapillary growth (**b**) lined by cells with prominent clear cytoplasm (**c**)

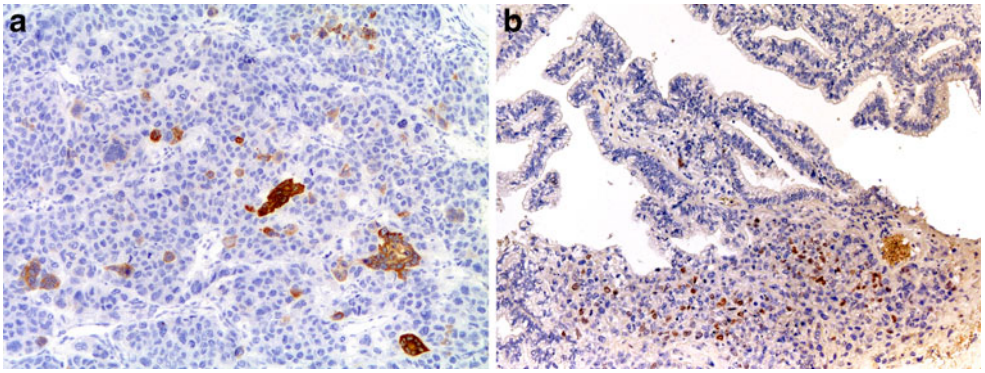

Fig. 2 Trophoblastic component of urothelial tumour is positive for α-inhibin (**a**). p63 positivity in urothelial neoplasm (**b**) contrasts with its negativity in the clear cell areas

and to GLY3, a marker for both yolk sac and liver cell tumours [6]. Both antibodies are always co-expressed in yolk sac tumours [7].

The primitive malignant endodermal or YST-type differentiation may be present in somatic, non-embryonal tumours of the female genital tract [8, 9], although it can also develop in the sinonasal area, stomach, colon, etc., where it is also associated with both high-grade tumours and a poor prognosis [7]. In the urinary tract, this association has been reported in rare, isolated cases of AFP-producing adeno- or transitional cell carcinomas and demonstrated by AFP stains only. Histologically, these cases consistently reveal the presence of a distinctive vacuolated epithelium with a characteristic AFP-positive immunophenotype [2]. The higher sensitivity of recently introduced antibodies such GLY3 [6] and SALL4 [5] has facilitated the demonstration of areas of primitive endodermal differentiation (YST) [7] present in various mixed tumours. In our case, tubulopapillary areas showed an incomplete primitive endodermal phenotype due to presence of a strong GLY3 and SALL4 positivity in the absence of AFP staining, a more specific but less sensitive marker of endodermal differentiation [6]. Furthermore, CDX2, an intestinal type differentiation marker [10], was also positive in our case, supporting an intestinal differentiation in the malignant primitive endodermal component [7, 11]. This immunophenotype helped to differentiate the endodermal tubulopapillary areas from both clear cell renal cell carcinoma (negative for CK7 and p63 but positive for renal cell carcinoma marker, vimentin and CD10) and from the newly introduced entity of tubulopapillary clear cell carcinoma (positive for CK7 and vimentin but negative for CD10, alpha-methyl CoA racemase and renal cell carcinoma marker) [12, 13].

Simultaneous placental and endodermal-type differentiations have not been previously reported in association with transitional cell carcinomas. The non-germ cell origin of some embryonal-type tumours, arising from somatic type neoplasms, has been recently reviewed [7], and an origin from pluripotent malignant stem cells present in the somatic tumour has been proposed [14]. We believe that the relationship of this complex renal tumour with the previous testicular tumour is coincidental, as there was a successful response to chemotherapy with no short-term recurrence; furthermore, the present lesion showed an intimate admixture of somatic and embryonal histological patterns reflecting tumour heterogeneity rather than a metastasis.

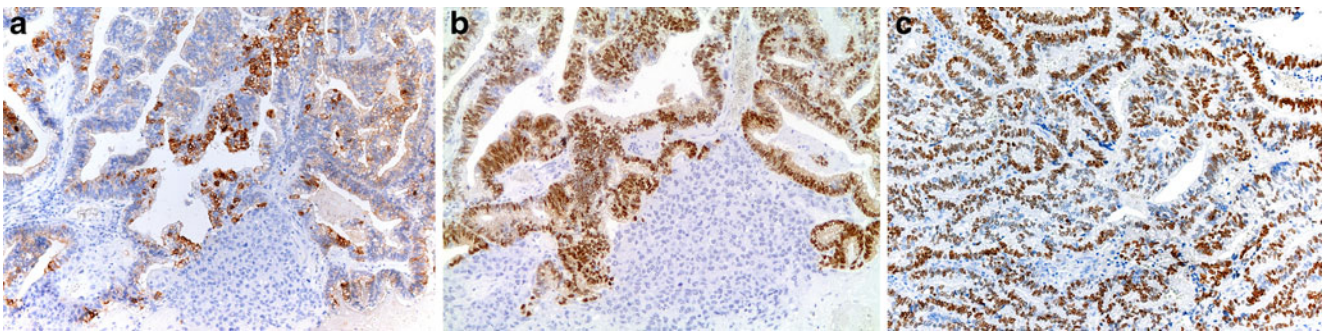

Fig. 3 Clear cell tubulopapillary areas with a primitive endodermal phenotype are positive for GLY3 (**a**), CDX2 (**b**) and SALL4 (**c**)

Conflict of interest None.

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Germ Cell Tumors of the Ovary

An Update

Francisco F. Nogales, MD, PhD; Isabel Dulcey, MD; Ovidiu Preda, MD, PhD

• ***Context.***—**The field of ovarian germ cell tumors (OGCTs) has remained relatively unchanged in the last 2 decades. However, the introduction of new stem cell pluripotency markers has provided a new understanding into the identification and taxonomy of OGCT types. New data have provided new insights into unusual teratoma-associated autoimmune disorders and the origin of gliomatosis peritonei.**

Objective.—**To review the impact of new pluripotency markers in the diagnosis of malignant OGCT (MOGCT) and analyze new nomenclature proposals and clinicopathologic entities.**

Data Sources.—**Ovarian germ cell tumors from routine material and expert consultation files at San Cecilio University Hospital, Granada, Spain, and the relevant literature were reviewed.**

Conclusions.—**Although a correct diagnosis of MOGCT can often be made with histologic and classic immunohistochemical studies, the new immunohistochemical pluripotency markers give higher diagnostic accuracy. Germ cell tumors represent a caricature of the phases of normal embryonic differentiation from primordial germ and stem cells to extraembryonal and somatic tissue differentiation. Since every stage of differentiation and its related tumor type exhibit characteristic markers, the analysis of their expression facilitates tumor typing, thus complementing the use of classic antibodies. They also allow a more precise evaluation of the degree of immaturity in teratoma. The new term, primitive endodermal tumors, simplifies the understanding of the complex histology of the yolk sac tumor group, as this terminology encompasses its multiple endodermal differentiations. Recently described autoimmune encephalitis due to antibodies against the N-methyl-D-aspartate receptor has become the most frequent autoimmune disorder associated with ovarian teratoma.**

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The aging of the population in Western countries has resulted in a decrease in the overall number of ovarian germ cell tumors (OGCTs), as they are usually found in younger patients. Nevertheless, they still represent a high percentage of ovarian neoplasms in many countries, most being mature teratomas. As the histologic features and clinical behavior of these benign neoplasms are well known, only rarely do they present diagnostic problems such as identification or degree of maturity of particular tissue components, presence of secondary malignancies, or some unusual clinical manifestations.

On the other hand, malignant ovarian germ cell tumors (MOGCTs) account for only a small fraction of ovarian germ cell neoplasms. Interest in their clinical and histologic features has somewhat diminished in the last 2 decades, with relatively few clinicopathologic series reported.[1,2] This low priority may be a consequence of their fortunately good response to platinum-based chemotherapy, regardless of histologic type, which would be reflected in a more relaxed attitude in grossing and sampling standards, histologic analysis, and possibly, less meticulous staging and surgery.

Some MOGCTs share a similar histologic profile with malignant testicular germ cell tumors (MTGCTs) of the adult, which occur far more frequently than their ovarian counterparts. The latter have, however, a different histogenesis, originating in the testis from malignant germ cells,[3] as opposed to ovarian germ cell neoplasms, which are mostly parthenogenetically conditioned.[4,5] Consequently, MTGCTs exhibit genetic markers, such as a 12p isochromosome and chromosome 12 overrepresentation,[6] that are less frequently observed in MOGCTs,[7] which often resemble testicular infantile teratomas/yolk sac tumours,[8] with the pure ovarian teratomas showing negativity for 12p.[9] For this reason, although equating ovarian and testicular germ cell tumors may not be totally correct biologically, their morphology and diagnostic immunohistochemistry are practically similar.

An unknown percentage of malignant germ cell tumors reported in phenotypic women may, in fact, represent MTGCTs (seminomas, embryonal carcinomas, mixed germ cell tumors), as they may have originated from the malignant germ cell component of gonadoblatomas present in dysgenetic gonads of patients with an unrecognized Y

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chromosome–containing genotype[8], often difficult to demonstrate.[10] The GBY locus, a portion of the centromeric region of the short arm of chromosome Y, contains the testis-specific protein 1 gene (*TSPY1*), which seems to play a critical role in the pathogenesis of gonadoblastoma.[11] Historically, this fact was exemplified in 1930 when Robert Meyer coined the term *dysgerminoma* as the characteristic tumor of the dysgenetic gonad.[12] Moreover, the recent recognition of neoplasms with a germ cell phenotype, but derived from normal and somatic tissue tumors, has provided yet another origin for rare, usually malignant, teratoid tumors, which may arise from a pluripotent malignant stem cell population of somatic neoplasms.[13–16]

The groundbreaking research of Kleinsmith and Pierce[17] in the early 1960s represents a paradigmatic model of progressive neoplastic differentiation from pluripotent stem cells, whereby germ cell tumors constitute a caricature of normal embryogenesis. This is worth noting, since recent stem cell research has provided various pluripotency markers that have been applied to the diagnostic immunopathology of malignant germ cell tumors.[18,19] Since these markers are sequentially expressed in tumors according to their differentiation stage (in a progressive flow from primordial germ cells to extraembryonal and somatic malignancies), they can be highly diagnostic of a particular tumor type. This is especially applicable to the MOGCTs, for which mixed forms are rare[9] and overlapping among various neoplastic phenotypes is minimal.

In this review, we will especially emphasize the impact of some of the newly reported and readily available pluripotency markers (SALL4, OCT3/4, and SOX2) on the diagnosis of MOGCTs. We have not included other nuclear (AP-2γ, NANOG, UTF1, TLC1, etc) and cytoplasmic (Lin28 and IMP-3) pluripotency markers, since they broadly provide similar data and are not as widely used for diagnostic purposes. We also propose integration of these markers with other specific markers, such as α-fetoprotein (AFP), and with characteristic but nonspecific ones such as cytokeratins, CD30, podoplanin (D2-40), placental alkaline phosphatase (PLAP), c-KIT (CD117), and glypican-3 (GLP3).

We will also review recent nomenclature proposals concerning the yolk sac tumor group and, in benign germ cell tumors, we will only consider new and relevant advances in paraneoplastic manifestations of mature cystic teratoma and on the origin of gliomatosis peritonei.

DYSGERMINOMA

The histologic profile of dysgerminoma is stereotypic and identical to that of testicular seminoma and midline germinomas. Large, clear, neoplastic, primitive-type germ cells are consistently associated with a variable cytotoxic T-cell lymphocytic response[20] that helps to identify the tumor, even in cases with complex histology. Adequate fixation is important for a prima facie diagnosis, but often poor fixation destroys highly labile cells owing to a minimal amount of cytoskeletal fibrils.[21] Cells are usually arranged in sheets or islands but they can grow in individual cords. Rarely, microcysts (Figure 1, A) or pseudoglandular spaces distort the usual architecture, creating differential diagnosis problems with the highly malignant small cell carcinoma of hypercalcemic type or even struma ovarii (Figure 1, B). Further complex diagnoses include dysgerminomas with minimal lymphocytic response or exhibiting cells with an epithelioid eosinophilic cytoplasm (Figure 2, A), which, as they occur in the testis,[22] should be differentiated from the rare solid varieties of embryonal carcinoma (EC) and yolk sac (primitive endodermal) tumors (YS[PE]Ts).[23]

Cases showing isolated cells, embedded in an extensive fibrous or chronic inflammatory (granulomatous) matrix (Figure 3, A), can also represent diagnostic pitfalls. Finally, solid clear cell carcinomas can also mimic dysgerminomas.

Immunophenotype

Diagnostic immunohistochemistry for dysgerminomas still relies, in many laboratories, on the classic membrane staining of PLAP. In our experience, however, PLAP is relatively unreliable, especially in poorly fixed material; furthermore, it may stain positively in a high percentage of cases of both EC and YS(PE)T, as it also occurs with CD117 (c-KIT).[24] c-KIT mutation has been shown to occur in a third of cases of dysgerminoma[25]; however, its expression does not imply a response to imatinib mesylate.[26] This, nevertheless, would be redundant owing to its already excellent response to cisplatin-based regimens, which remain the gold standard for treatment of these tumors.

Clone D2-40 of podoplanin is a glycoprotein initially identified in renal podocytes[27] that is also a good marker of both the endothelium of lymph vessels and mesothelium.[28] In dysgerminoma it behaves as a relatively specific and stable marker,[29] even in poorly fixed and necrotic material, where it strongly stains the cytoplasm and cell membranes. D2-40 only stains seminoma when antigen retrieval technique is not performed.[30] However, when this is performed in the testis, it can also show a characteristic apical expression in glandlike areas of EC.[31]

OCT3/4, also known as POU5F1, is possibly one of the most useful antibodies in the diagnosis of MOGCTs. OCT3/4 is a nuclear transcription factor interacting with other nuclear factors, such as NANOG, SOX2, SALL4, and KLF4,[32] that maintain pluripotency in primordial germ and stem cells. It is expressed very early during embryogenesis and has an essential role in blastocyst differentiation. After gastrulation, its expression is limited to primordial germ cells throughout their migration (Figure 4, A) until their eventual transformation into spermatogonia during the second trimester of gestation[33]; on the other hand, when female germ cells enter meiosis the expression of OCT3/4 is down-regulated.[34] It is constantly expressed in precursor lesions of malignant germ cell tumors, such as the germ cell component of gonadoblastoma and intratubular germ cell neoplasia. OCT3/4 regularly shows positivity in dysgerminoma but can also be expressed in EC[35] and in some immature neural elements of ovarian teratoma.[36] However, considering that EC is exceptionally rare in the ovary, OCT3/4 can be considered as a selective marker of ovarian dysgerminoma.

OCT3/4 is particularly useful in demonstrating the primitive germ cell identity of poorly fixed tissue or microcystic cases, helping to differentiate them from small cell tumors and even struma ovarii (Figure 1, C and D). It also identifies isolated dysgerminoma cells masked by fibrosis or inflammation (Figure 3, B). Furthermore, it is particularly useful in the identification of the primary tumor in distant metastases.[37]

SALL4 is a nuclear factor and a member of the family of *SALL* genes, which are also involved in totipotency and are expressed at an early stage of embryogenesis.[38] We have been able to identify SALL4 nuclear expression in retroper-

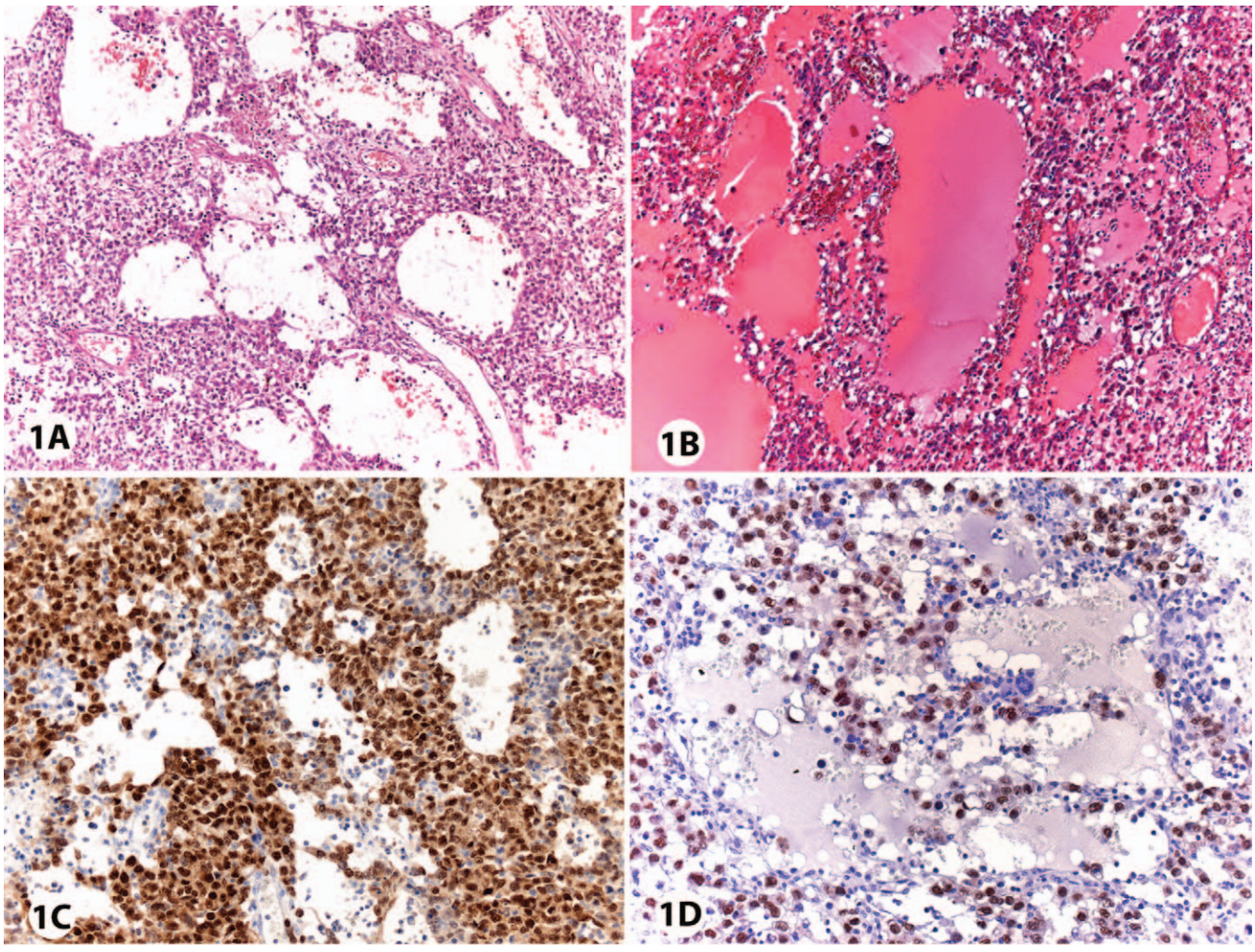

Figure 1. *Unusual arrangements in dysgerminoma: empty microcystic spaces (A) mimic small cell carcinoma with hypercalcemia, while colloid-filled spaces simulate struma ovarii (B). OCT3/4-positive nuclear staining (C and D) confirms the diagnosis of dysgerminoma (hematoxylin-eosin, original magnifications ×100 [A and B]; original magnifications ×100 [C and D]).*

itoneal primitive germ cells during their migration toward the gonadal crests in two 12-week-old embryos (Figure 4, B). SALL4 is strongly expressed by dysgerminomas. However, since it is a pluripotency marker, it can show positivity in EC, YS(PE)T, and primitive areas of immature teratoma; consequently, it represents a good, broad marker for MOGCTs.[39] Nevertheless, its expression has also been shown in myeloid leukemia and in some gastric carcinomas.[40,41]

Cytokeratin expression does not exclude the diagnosis of dysgerminoma, since positivity ranging from strongly diffuse to focal and dotlike (Figure 2, B) can be seen, in our experience, in up to a third of cases. Thus, it is not advisable to differentiate dysgerminoma from EC by using this antibody alone, as was initially thought.[42] Expression of cytokeratins has unknown significance but it may be related to an eventual differentiation of primitive germ cells from dysgerminoma into somatic-type cells. This assumption is partly supported by the focal positivity of blood group–related antigens in the cytoplasm of dysgerminomas,[43] which may reflect a somatic-type differentiation. Additionally, trophoblastic cell differentiation in dysgerminoma is always cytokeratin positive.

In summary, the diagnostic problems associated with dysgerminoma are frequently related to poor fixation and unusual growth patterns, and in both situations immunohistochemistry enables a correct identification of the proliferating germ cells. These data should be considered together with classic histologic dysgerminoma features such as lymphocytic infiltrates.

YOLK SAC (PRIMITIVE ENDODERMAL) TUMORS

We have recently reviewed[16] the current data on the histopathology and immunophenotype of these neoplasms and proposed the new term *primitive endodermal tumors*. This is a more apt definition of their complex, multifaceted histologic features, which comprise early endodermal differentiation into secondary yolk sac and primitive gut, and their derivatives, such as intestine, liver, and lung. This proposal parallels the widely accepted terminology for primitive neuroectodermal tumors (PNETs), which encompasses the multiple differentiation capacity into diverse neural cell lines present in PNET.[44] Other terms, such as *endodermal sinus tumors*, are arcane, as the structure it purports to represent is nonexistent in human embryogenesis. The YS(PE)T terminology also defines endodermal

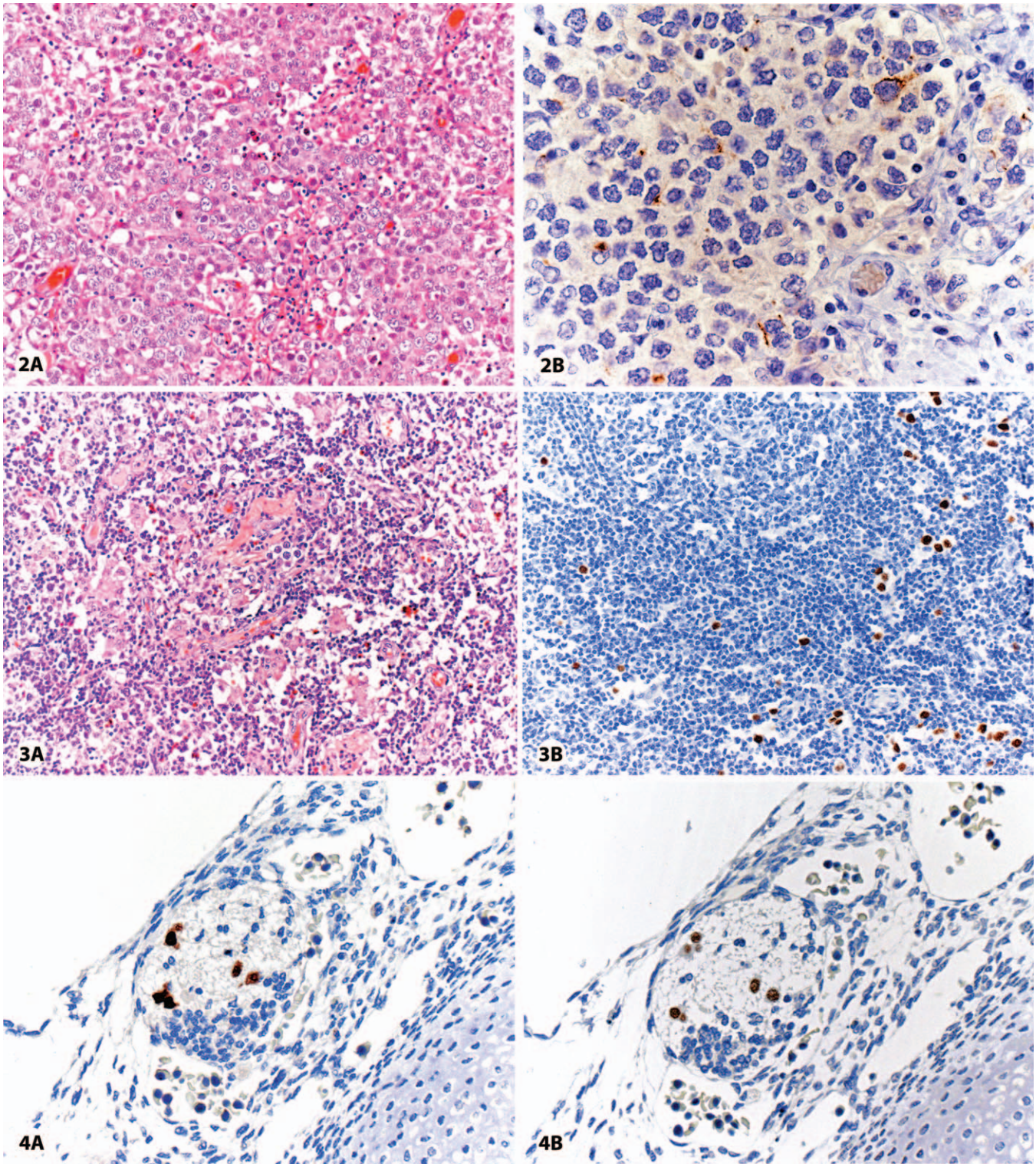

Figure 2. *Compact architecture of an epithelioid dysgerminoma with minimal lymphocytic infiltration (A) that simulates solid embryonal carcinoma. Cytokeratin (CAM 5.2) staining shows only a dotlike expression (B) (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×200 [B]).*

Figure 3. *Dysgerminoma with extensive inflammatory response: malignant germ cells are difficult to identify at medium power (A). OCT3/4-positive nuclear staining recognizes characteristic germ cells (B) (hematoxylin-eosin, original magnification ×100 [A]; original magnification ×100 [B]).*

Figure 4. *Migrating germ cells in the retroperitoneum of a 12-week fetus are positive for pluripotentiality markers OCT3/4 (A) and SALL4 (B) (original magnifications ×200 [A and B]).*

tumors developing from somatic neoplasms, such as endometrioid carcinoma and some unusual clear cell, AFP-secreting neoplasms of the stomach, lung, and bladder.

Classic histologic features in these tumors almost always include reticular microcystic areas with hyaline globules and amorphous acellular basement membrane material,[45,46] which often provide the diagnostic clue in tumors exhibiting complex histologic profiles. Pure patterns, such as the polyvesicular type,[47] hepatic,[48] and intestinal,[49] are extremely rare and often mimic other neoplasms such as hepatoid and endometrioid carcinoma. Rarely, the mature intestinal component of YS(PE)T may give rise to a mucinous carcinoid.[50]

Yolk sac tumors can originate from malignant stem cells present in somatic tumors of the ovary and uterus, usually endometrioid adenocarcinoma[51] (Figure 5, A) and carcinosarcoma.[15] The histology of these unusual YS(PE)Ts is identical to that of tumors of germ cell origin. Their characteristic immunophenotype helps to differentiate them from the somatic tumor from which they arise.

Immunophenotype

Presence of α-fetoprotein remains the gold standard for the diagnosis of YS(PE)Ts. This protein is expressed by the primary yolk sac before specialized tissue differentiation occurs and it is a marker of the secondary yolk sac until its involution at the 11th week. It is also expressed in early endodermal derivatives, such as allantois and liver, and focally, in the early intestine. In the normal secondary human yolk sac, AFP is secreted in the cytoplasm and is transferred to intercellular and intracellular vesicles and channels.[52] In YS(PE)Ts, AFP is strongly expressed in the labyrinthine network of microcystic reticular patterns where it has a granular cytoplasmic stain (Figure 6, A) and often, but not always, stains positively in hyaline globules. Its expression is generally patchy and often associated with a dirty background of serum proteins. Both endodermal hepatic and intestinal glandular patterns, displaying characteristic apical and basal vacuolation, often exhibit variable AFP staining.[16] α-fetoprotein staining can become negative in some recurrences occurring after treatment with chemotherapy.[53]

Glypican 3 is a useful marker in liver cell carcinoma[54] and is a complementary antibody in the diagnosis of YS(PE)Ts. Glypican 3 is also secreted by the early secondary yolk sac and liver. Glypican 3 cytoplasmic, and less often membranous, staining is almost, but not exactly, parallel to that of AFP, having the advantage of presenting a clean background[55,56] (Figure 6, B). There are AFP-negative tumors that are GLP3 positive. We believe that since AFP is such a specific marker, the coexpression of both markers is a sure diagnostic feature of YS(PE)Ts. However, GLP3 positivity alone does not allow a diagnosis of YS(PE)T unless it is associated with other characteristic YS(PE)T markers. Glypican 3 may also be focally present in EC, teratoid glands, neuroepithelium, and syncytiotrophoblasts.[56]

SALL4 has a consistently strong expression in the nuclei of YS(PE)Ts regardless of their germ cell or somatic origin (Figure 5, B) and of their diverse growth patterns, namely, microcystic, intestinal (Figure 6, C), and even hepatic.[16] Neoplastic stromal cells are also frequently stained, thus indicating their pluripotent character, which would be reflected in several mesenchymal differentiations from YS(PE)T.[57] Furthermore, its expression is evident in the secondary human yolk sac until its final involution.[52]

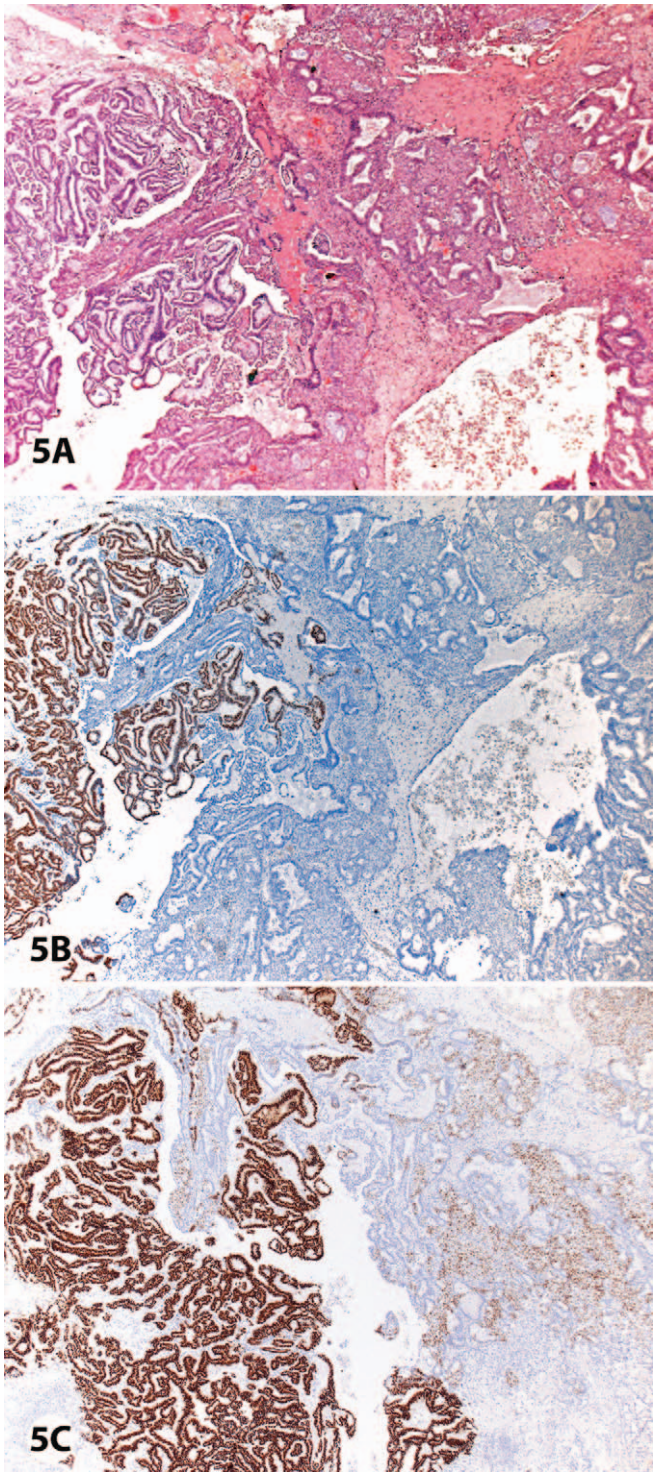

Figure 5. *Glandular-type yolk sac (endodermal primitive) tumor (YS(EP)T) (left) originating from an ovarian endometrioid adenocarcinoma with morular metaplasia (right) (A). SALL4 expression clearly differentiates YS(EP)T from endometrioid carcinoma (B). Glandular YS(EP)T shows a strong nuclear positivity for CDX2, which is also present in the nuclei of morular metaplasia cells in endometrioid carcinoma (C) (hematoxylin-eosin, original magnification ×25 [A]; original magnifications ×25 [B and C]).*

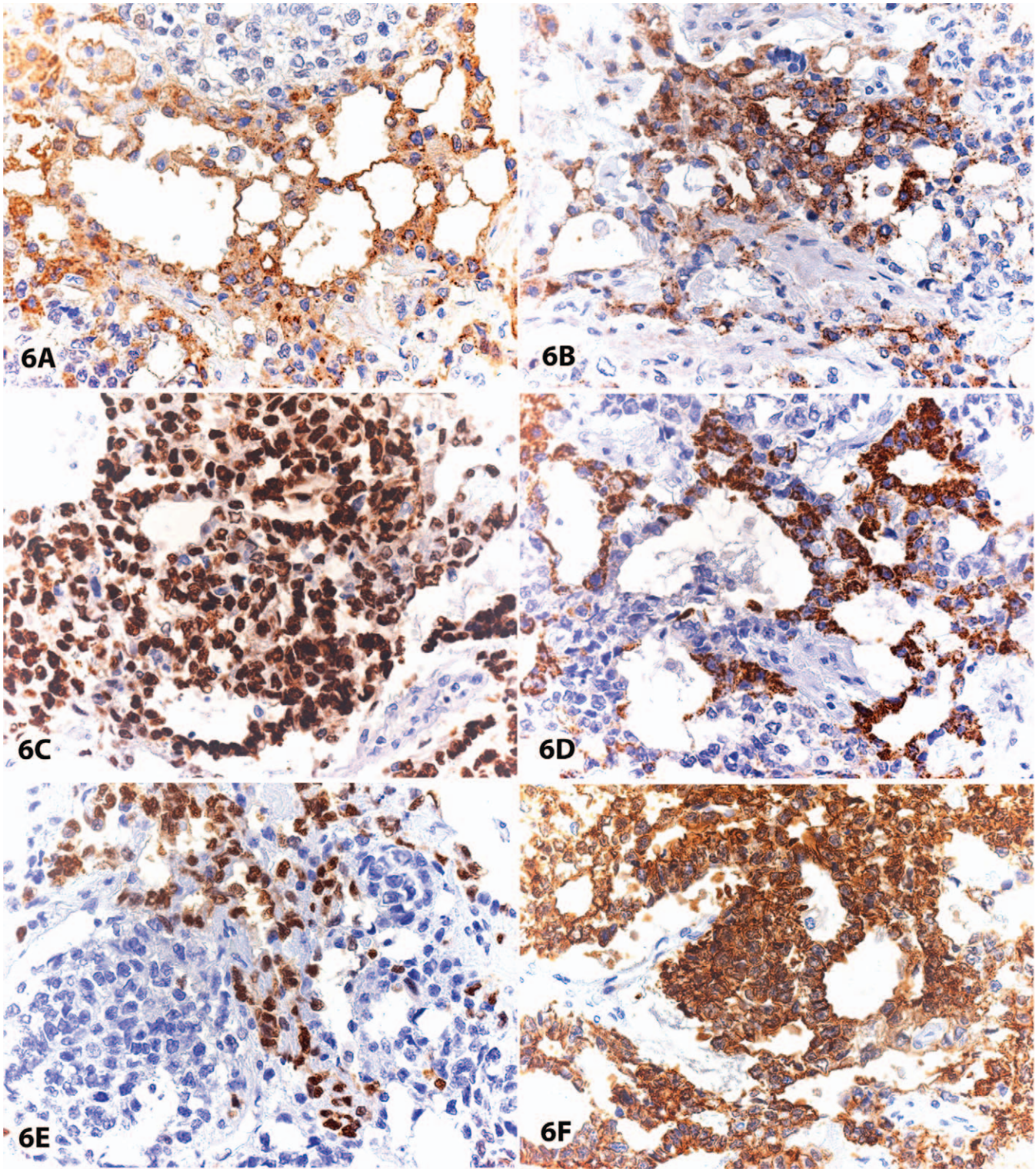

Figure 6. *Immunophenotype of yolk sac (endodermal primitive) tumor: α-fetoprotein staining in epithelial cells delineates intracellular vesicles (A). Glypican 3 reveals a similar expression (B). SALL4 stains positively in epithelial and in some mesenchymal cells (C). Cystic spaces are lined by an epithelium positive for hepatocyte paraffin 2 antibody (D). CDX2 stains epithelial cells focally (E). Villin shows a diffuse epithelial expression (F) (original magnifications ×200 [A through F]).*

Endodermal tissue specializations from YS(PE)T express their characteristic markers: hepatic areas are positive for hepatocyte paraffin antigen 1 (HepPar-1)[58] (Figure 6, D); and intestinal areas, for CDX2[59] (Figures 5, C, and 6, E) and villin (Figure 6, F). Glands differentiating into foregut express thyroid transcription factor 1.[14,15]

Other markers, such as Lin28,[60] CD117,[22] and IMP-3,[61] are also expressed in varying percentages in YS(PE)Ts.

Some metastases of unusual gastric tumors, such as the clear cell adenocarcinoma with hepatoid change, can mimic an ovarian YS(PE)T,[16] particularly when only 1 ovary is involved. They may show an identical immunophenotype expressing all characteristic markers such as AFP, GLP3, HepPar-1, and SALL4. It must be borne in mind, however, that unilateral ovarian involvement of Krukenberg tumors occurs in as many as 37% of cases, although in many instances both ovaries are not removed or rigorously examined microscopically.[62]

EMBRYONAL CARCINOMA

Embryonal carcinoma represents a malignant stem cell tumor with a totipotent differentiation capacity, as demonstrated by Kleinsmith and Pierce[17] as early as 1964 in an experimental murine model that provided the first demonstration of the stem cell origin of cancer. Embryonal carcinoma is, however, a characteristic testicular tumor that represents, together with seminoma, the pluripotent component of mixed germ cell tumors. This preference for a testicular location reflects the different histogenesis of testicular and ovarian germ cell tumors, the former originating from primitive germ cells with a malignant character,[3] while the latter mostly have a parthenogenetic origin from postmeiotic or meiotic cells.[4,5] For these reasons, the presence of EC in the ovary is extremely rare. In 40 years' experience of ovarian germ cell tumor consultations, the senior author of this article has only identified 3 bona fide cases. It is possible that many of the embryonal carcinomas reported in older series were misinterpretations of solid forms of YS(PE)T, or epithelioid dysgerminomas. It is also likely that some reports may have included cases of Y chromosome–containing gonadal dysgenesis. In these cases, the precursor lesion would be a gonadoblastoma. Therefore, any diagnosis of EC in a female patient should prompt a chromosomal study.

Solid and glandular patterns of EC can overlap and mimic both dysgerminoma and YS(PE)T,[22,23,63] which occur far more frequently in the ovary. For this reason, the putative immunophenotype of a rare EC should be excluded in the differential diagnoses of MOGCT.

Immunophenotype

Most EC immunophenotypic data arise from testicular tumors, but we have identified a similar staining pattern in the few available cases of ovarian EC.

All ECs are cytokeratin positive. CD30 membrane expression remains one of the most reliable and accessible markers for EC.[64] Anti-CD30 is an antibody against a surface glycoprotein corresponding to a cytokine receptor, and CD30 is a member of the superfamily of tumor necrosis factors. CD30 is expressed by many other tumors, including anaplastic lymphomas, and by Reed-Sternberg cells.[65] Some reactive inflammatory conditions may also show CD30-positive immunoblasts.[66]

SOX2 is another nuclear transcription factor also involved in totipotency. It is also responsible for neuronal differentiation[67] and useful, together with CD30, in the differentiation of solid areas of EC with dysgerminoma. SOX2 and OCT3/4 coexpression in the papillary areas of EC contrasts with these markers' negativity in Schiller-Duval sinuses of YS(PE)T (Figure 7, A and B).

Expression of PLAP, OCT3/4, and SALL4 in EC is shared with dysgerminoma. D2-40 has a particular apical membranous positivity in the glandular and papillary areas of EC.[31]

Glypican3 shows patchy positivity in EC, especially in areas of early endodermal differentiation, such as the organoid areas (primitive yolk sac endodermal cavities) of embryoid bodies in the rare polyembryoma.[56]

CHORIOCARCINOMA

Pure nongestational choriocarcinoma is exceptionally rare in the ovary. Similar to dysgerminoma and EC, it represents more a testicular-type tumor, and its presence should prompt an analysis of the patient's genotype. It should be differentiated from the also rare dysgerminoma with syncitiotrophoblastic giant cells, which accounts for fewer than 10% of dysgerminomas and has a behavior identical to classic dysgerminoma. Choriocarcinoma is positive to a host of antibodies. Trophoblast stains strongly for cytokeratin, human chorionic gonadotropin, α-inhibin, CD10, and GLP3.[56,68] Human placental lactogen can identify the intermediate (extravillous) trophoblastic component.[69]

TERATOMAS

Most ovarian teratomas show differentiation of either mature or, less frequently, immature tissues derived from the 3 germ layers. Stem cell scientists usually restrict the term *teratoma* to tumors differentiating tissues from the 3 germ layers.[70] In gynecologic pathology, however, this strict differentiation is not possible, since monodermal ovarian teratomas with 1-sided tissue differentiation (monophyletic teratomas), such as struma ovarii, often occur. Furthermore, the presence of ovarian tumors containing an uncommon tissue unrelated to normal embryogenesis (eg, ependymoma[71] and nephroblastoma[14]) does not always imply a germ cell origin, as they may originate from totipotent stem cells from the same tumor (so-called neometaplasia).[72]

Human reprogrammed/induced pluripotent stem cells[73] transplanted subcutaneously or by intratesticular injection into immunodeficient mice can grow, in a short period of time, tumors that are histologically identical to ovarian immature teratomas with characteristic neuroepithelial tubules (Figure 8, A) and AFP-positive embryonal endodermal areas resembling either Schiller-Duval sinuses[16] or gut elements of glandular YS(PE)Ts (Figure 8, B).

Immature teratoma is the most frequent MOGCT[74] and the histologic assessment of its degree of immaturity is a highly reliable prognostic factor and therapeutic indicator.[75] Grading is performed by a subjective, semiquantitative analysis of the relative number and atypicality of the foci of immature neural tissues (neuroepithelial tubules and neural blastema) present in the tumor. This is accomplished either by a 2-tier system (low grade and high grade)[76] or by assigning 4 grades ranging from fully mature (0) to highly immature (3).[77] Rarely, immature and atypical neural components may overgrow the original teratoma, displaying various patterns of PNETs.[78] This overgrowth is often difficult to separate from a high-grade (grade 3) immature teratoma. The difference usually lies in the mainly monomorphic appearances of the PNET overgrowth.

Mature cystic teratomas may show minute, isolated neuroepithelial/ependymal foci lacking any prognostic significance.[79] Their presence, however, may be worrisome and should not be reported as grade 1 immature teratoma but as prognostically irrelevant microscopic foci of immature tissue in mature cystic teratoma.

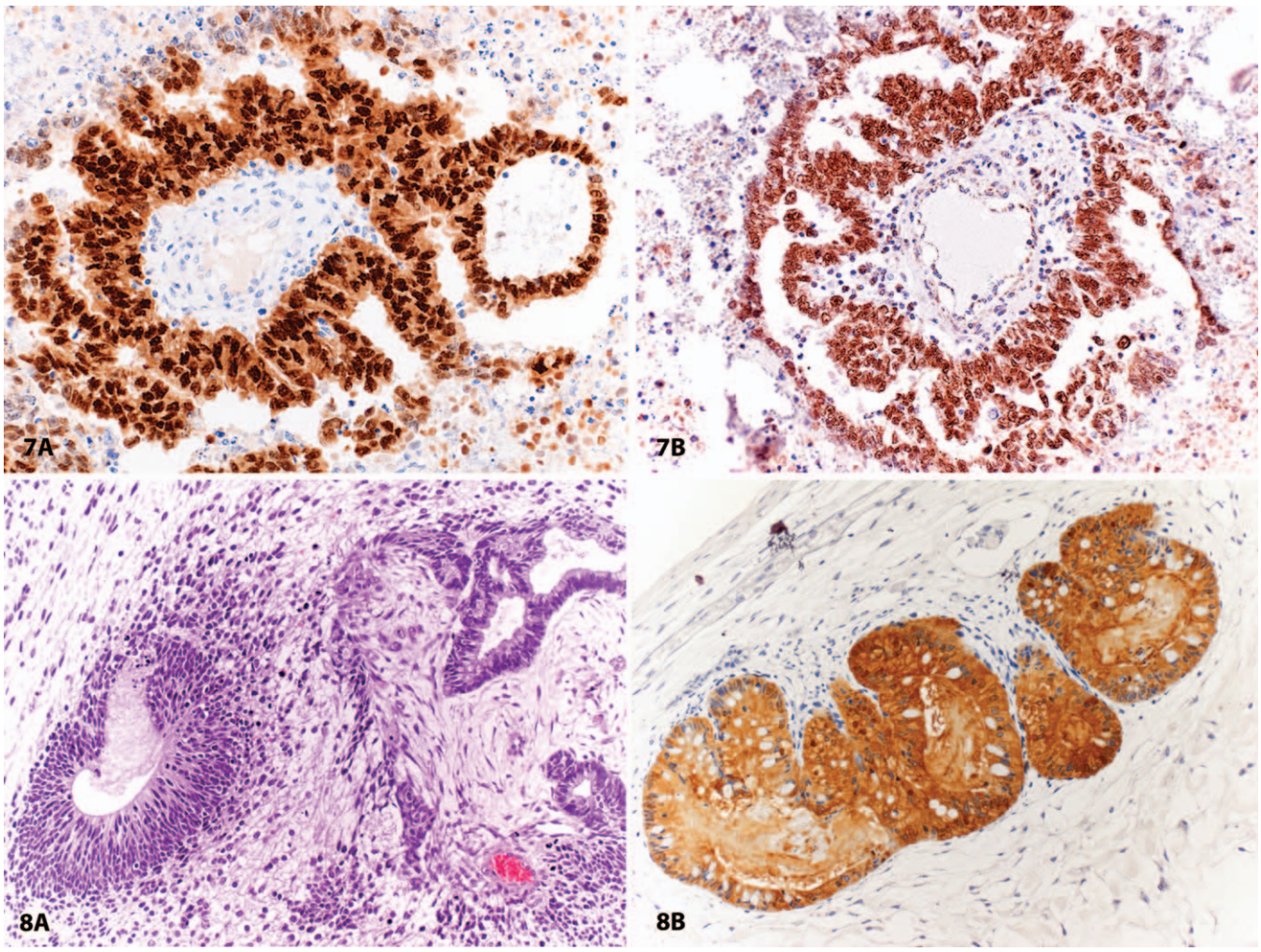

Figure 7. *Papillary structures in an embryonal carcinoma simulate Schiller-Duval sinuses. However, SOX2 (A) and OCT3/4 (B) positivity exclude a diagnosis of yolk sac (endodermal primitive) tumor, since the latter does not express these nuclear markers (original magnifications ×200 [A and B]).*

Figure 8. *Xenotransplanted tumor grown from induced pluripotent stem cells showing a histologic appearance identical to that of ovarian immature teratoma with both neuroepithelial tubules (A) and α-fetoprotein–positive endodermal glands (B) (hematoxylin-eosin, original magnification ×200 [A]; original magnification ×200 [B]).*

Gliomatosis peritonei (GP) is a fascinating condition whereby immature and, less often, mature teratomas become associated with a myriad of peritoneal nodular or miliary implants composed of mature glia. Despite its clinical stage III, its behavior is benign, since mature glial cells are not aggressive and remain stable for long periods of time. However, on rare occasions, GP can induce a florid vascular proliferation that may result in peritoneal hemorrhage and shock[80] (Figure 9, A) and can even develop a secondary malignant glial tumor.[81]

In the last decade, attention has been paid to the histogenesis of this rare phenomenon. Genetic studies of microdissected peritoneal implants that analyzed multiple microsatellite markers[82–84] revealed a heterozygosity pattern identical to that of normal tissue and different from ovarian teratoma, which showed homozygosity of the same loci. These findings proposed a different genetic identity for ovarian tumor and GP, the latter originating from peritoneal pluripotent cells stimulated by growth factors present in the primary tumor that would induce differentiation into glial cells. Although the genetic evidence is incontrovertible,[82–84] the traditional origin for GP as a peritoneal seeding via capsular rupture from the ovarian teratoma is also supported by the following facts: (1) GP nodules often show multiple tissue differentiation (skin, gut, cartilage); (2) neural tissue itself is polydifferentiated with several neurogenic lines including microglia (Figure 9, B); (3) immature neuroepithelial tubules coexist in some cases with mature glia, indicating maturation from embryonal precursors; (4) shed hair and keratin scales from teratoma (Figure 9, C) are often found associated with GP; and (5) lymph node involvement by mature glia may occur in the absence of GP.[85] These facts would support an origin from ovarian teratoma in most cases, although it may be possible that some cases of GP with a monomorphic cell population have a metaplastic identity.

Immunophenotype

The use of new pluripotency markers can enhance the identification of tissue components as well as their degree of immaturity, contributing to a better grading of immature teratoma. It is necessary to identify the immature character

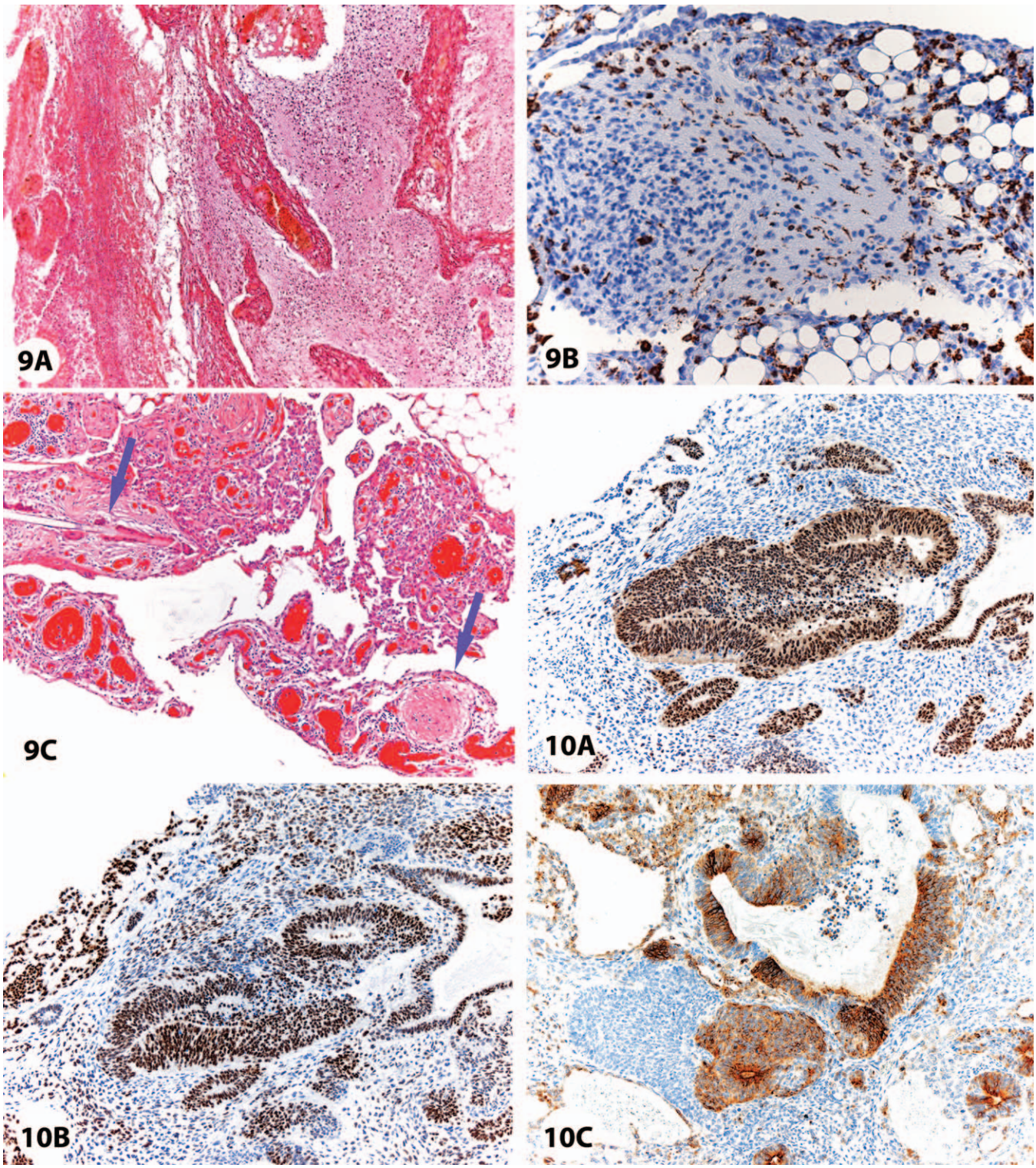

Figure 9. *Mature peritoneal glial implants can show a marked vascular proliferation leading to hemoperitoneum (A). Glial implants are rarely monomorphic, harboring multiple cell lines. Here, microglia-like cells are stained by CD68 (B). Hairs (left arrow) shed into the peritoneum from primary ovarian teratoma can be found next to glial nodules (arrow, right lower corner) (C) (hematoxylin-eosin, original magnifications ×25 [A and C]; original magnification ×100 [B]).*

Figure 10. *Immature ovarian teratoma showing neuroepithelial tubules with a strong SOX2 expression (A). Tubules express SALL4, which is also positive in other immature stromal and epithelial elements (B). Glypican 3 also shows a patchy expression in neuroepithelium (C) (original magnifications ×100 [A through C]).*

Comparative Immunohistochemical Expression in Malignant Ovarian Germ Cell Tumors of Classic, Pluripotency, and Somatic Differentiation Markers

	Immunohistochemical Markers											
	Classic					Pluripotency			Somatic Differentiation			
Tumor	PLAP	CD30	AFP	GLP3	D2-40	OCT3/4	SOX2	SALL4	Villin	CDX2	HepPar-1	TTF1
Dysgerminoma	+	−	−	−	+	+	−	+	−	−	−	−
Yolk sac tumor	+/−	−	+	+	+/−	−	−	+	+ INT	+ INT	+ HEP	+ FRG
Immature teratoma	−	−	−	−	−	−	−	+	−	−	−	−
	−		+ END	+ NEP	+ STR		+ NEP		+ INT	+ END		
Embryonal carcinoma	+	+	−	+ Focal	+/− Apical	+	+	+	NA	−	−	−
Choriocarcinoma	−	−	−	−	−	−	−	−	−	−	−	−
	+SYNC			+SYNC								

Abbreviations: AFP, α-fetoprotein; END, endodermal; FRG, foregut; GLP3, glypican3; HEP, hepatic; INT, intestinal; NA, not available; NEP, neuroepithelium; PLAP, placental alkaline phosphatase; STR, stroma; SYNC, syncytiotrophoblast; TTF1, thyroid transcription factor 1.

of some areas when hematoxylin-eosin images are not conclusive. This may occur in cases of mature teratoma containing both glandular structures and ependymal spaces that may resemble the standard diagnostic immature neuroepithelial tubules useful for grading. Markers such as SOX2 and SALL4 (Figure 10, A and B) are strongly expressed by immature neuroepithelium but are weaker or absent in well-differentiated neural areas. However, implanted GP astrocytes may still express SOX2, indicating that they are not terminally differentiated. Glypican 3 may also show a patchy neuroepithelium staining (Figure 10, C). In our experience, SOX2 behaves as the more specific antibody for immature neural areas, being particularly useful in PNET overgrowths of teratoma. Identification of neural areas can be complemented by more characteristic neural makers such as glial fibrillary acidic protein, nestin, and others. OCT3/4 has been reported to be focally positive in neural components.[36]

Benign and malignant ovarian mucinous tumors associated with mature cystic teratomas may show massive mucin secretion, goblet cells, carcinoid-like patterns, pseudomyxoma ovarii and peritonei, and signet ring cells characteristic of a gastrointestinal phenotype, with markers such as CDX2, HepPar-1 and villin, as well as a cytokeratin 7–negative/cytokeratin 20–positive profile.[86] All these features would point toward a teratoid origin for this mucinous component, which should be differentiated from a metastasis from a gastrointestinal primary tumor. Demonstration of teratoma foci may be difficult in rare cases when they are small and escape sampling or become overgrown by the mucinous neoplasm.

A recent interesting clinical breakthrough on ovarian teratomas has been their identification as a causative factor of severe neurologic disease. Since 2005 Dalmau et al[87,88] and Vitaliani et al[89] have reported more than 100 cases of autoimmune encephalitis due to antibodies against the N-methyl-D-aspartate receptor (anti-NMDAR), a condition that frequently involves temporal lobes and hippocampus. Its recognition is important, as removal of the ovarian tumor and early immunosuppressive therapy will often improve the outcome, with full recovery or only a residual mild neurologic deficit.[87] In this association, the presence of anti-NMDAR antibodies can be demonstrated in the neuronal-like cells of both mature and immature teratomas, although the latter have a proportionally much higher representation than the mature cystic teratomas, since they contain a much higher amount of neural tissues. Few articles dealing with this condition have reached pathology journals,[90] possibly owing to the absence of unusual histologic peculiarities in the concomitant teratomas. This association has now become the most frequent autoimmune disorder associated with ovarian teratoma.[90]

CONCLUSIONS

Even if careful histologic and classic immunohistochemical studies allow a relatively correct diagnosis of MOGTCs, the use of new immunohistochemical pluripotency stem cell markers results in a higher diagnostic accuracy. This is accomplished by the specific expression of these markers as they are switched on or off according to the stage of differentiation, thus permitting a better identification of the tumor, which in germ cell tumors is determined by a progressive flow from primordial germ cells to extraembryonal and somatic malignancies. This is especially applicable to ovarian MOGCTs for which mixed forms of malignant germ cell tumors are rare and overlapping among various neoplastic phenotypes is minimal. The new antibodies also permit a more precise evaluation of immature, diagnostic areas in teratoma. The Table shows the comparative expression of diagnostic immunohistochemical markers analyzed in this review.

Recently described autoimmune encephalitis due to antibodies against the N-methyl-D-aspartate receptor has become the most frequent autoimmune disorder associated with ovarian teratoma. New data have provided new insights into the origin of gliomatosis peritonei.

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REVISIÓN

Papel de los nuevos marcadores inmunohistoquímicos en los tumores de células germinales malignos gonadales

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PALABRAS CLAVE
Ovario;
Testículo;
Células madre;
Tumores de células germinales;
Disgerminoma/seminoma;
Carcinoma embrionario;
Tumor vitelino

Resumen Los tumores de células germinales malignos (TCGM), debido a su tratamiento individualizado, se presentan como un reto diagnóstico y terapéutico donde el estudio inmunohistoquímico reproducible es de suma importancia. Revisamos el valor diagnóstico de nuevos anticuerpos provenientes de investigaciones de células madre tales como OCT3/4, SOX2 y SALL4. Su expresión en los TCGM confirma una vez más el carácter pluripotencial de estas neoplasias. El SALL4 puede ser considerado un marcador general de los TCGM. La expresión de OCT3/4 en disgerminoma/seminoma lo confirma como precursor de otros TCGM. La expresión simultánea de SALL4, SOX2 y OCT3/4 confirman al carcinoma embrionario como el tumor de células madre pluripotenciales, con SOX2 y CD30 como marcadores altamente característicos. El D2-40 es útil para diferenciar el disgerminoma/seminoma del carcinoma embrionario.

La alfa-fetoproteína es diagnóstica de tumores vitelinos, pero en casos de escasa expresión, la reevaluación positiva con GLP3 y SALL4 y la negatividad frente al OCT3/4 confirman el diagnóstico.

Un panel mínimo de anticuerpos con cobertura de los tipos más frecuentes de los tumores de células germinales debería incluir alfa-fetoproteína, CD30, D2-40, OCT3/4, GLP3 y SALL4.

KEYWORDS
Ovary;
Testis;
Stem cells;
Germ cell tumours;
Dysgerminoma/seminoma;

The role of new immunohistochemical markers in malignant germ cell tumours of the gonads

Abstract Malignant germ cell tumours (MGCT) of the ovary and testis often represent a diagnostic challenge due to their frequent overlap and primitive histology. New antibodies, mostly originating from the stem cell research field, provide accurate tools for the identification of different tumour types. The expression of antibodies such as OCT3/4, SOX2 and SALL4 indicates the pluripotent character of these neoplasms. SALL4 represents a good screening

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Embryonal carcinoma;
Yolk sac tumours

antibody for the diagnosis of MGCT while OCT3/4 indicates a totipotential role for dysgerminoma/seminoma. OCT3/4, SOX2 and SALL4 are coexpressed in embryonal carcinoma, where SOX2 and CD30 represent highly specific markers. D2-40 podoplanin is useful to differentiate dysgerminoma/seminoma from embryonal carcinoma.

AFP is highly diagnostic of yolk sac (endodermal primitive) tumours but in cases with low expression, diagnosis is facilitated by a concurrent positivity of Glypican3 and SALL4 and OCT3/4 negativity.

An antibody panel that includes alpha-foetoprotein, CD30, D2-40, OCT3/4, Glypican3 and SALL4 is useful in the identification and taxonomy of MGCT.

Introducción

Los tumores de células germinales representan un alto porcentaje de la patología tumoral del ovario de pacientes jóvenes en el periodo reproductivo[1]. Los tumores de células germinales malignos (TCGM) son relativamente raros pero ocasionan frecuentemente problemas de interpretación diagnóstica. El desarrollo de técnicas inmunohistoquímicas representa un avance real en la valoración de estas neoplasias, especialmente en los TCGM. Su utilidad se complementa con su alta sensibilidad incluso en tumores con fijación defectuosa. Además, al tener un tratamiento individualizado y basado en quimioterapia, la aplicación reproducible y bien orientada de las técnicas inmunohistoquímicas es de suma importancia.

El presente trabajo expone una breve revisión de los principales marcadores útiles en el diagnóstico de los TCGM malignos, incluyendo anticuerpos clásicos y los más recientes propuestos en la literatura, procedentes en su mayor parte de la investigación con células madre[2,3].

Esta actualización refleja nuestra experiencia personal en un extenso material de TCGM del ovario y testículo.

Disgerminoma/seminoma

Histopatología

El disgerminoma y el seminoma, su correspondiente testicular, se caracterizan por una arquitectura sólida y están constituidos por células con grandes cantidades de glucógeno citoplasmático responsable de su aspecto claro. Las células presentan monotonía nuclear, mientras que a nivel del estroma tumoral siempre se observan, en mayor o menor cantidad, linfocitos de tipo T citotóxicos[4] (fig. 1). Aunque su aspecto clásico en general no implica problemas de diagnóstico diferencial, ocasionalmente la variante sólida del carcinoma embrionario (CE) y del tumor vitelino (TV) pueden remedar al disgerminoma/seminoma (D/S)[5]. Otros aspectos arquitecturales, tales como su disposición trabecular, seudoglandular, microquística o bien en cordones o células aisladas, representan situaciones en las que el estudio inmunohistoquímico es diagnóstico.

Inmunofenotipo

De los marcadores clásicos, la fosfatasa alcalina placentaria (PLAP) con tinción membranosa, y más raramente

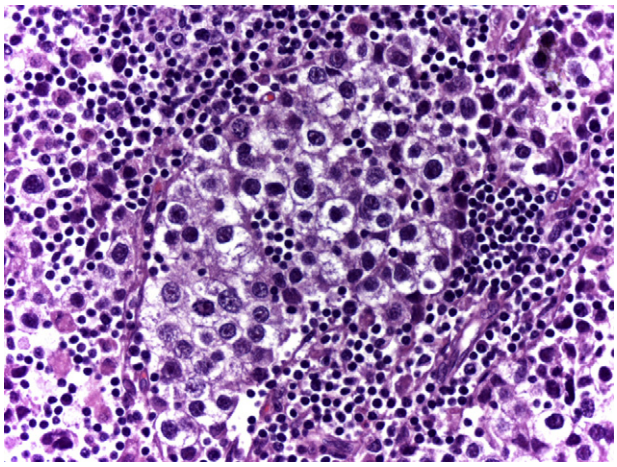

Figura 1 Disgerminoma: patrón clásico con células claras e infiltrado linfocítico.

citoplasmática, ha sido el marcador clásico en el diagnóstico de los D/S[6]. Sin embargo, este anticuerpo carece de sensibilidad en material mal fijado y tiene poca especificidad, al poder expresarse también en un gran porcentaje de CE y TV. Menos frecuentemente es expresado por tumores de pulmón, mama, riñón y colon[7], neoplasias que potencialmente pueden metastatizar al ovario, causando problemas de diagnóstico diferencial.

La expresión del *CD117 (c-kit)* es habitual en el D/S, pero también es positiva en el CE y en algunos casos de TV[7]. En nuestra experiencia, los D/S presentan intensa positividad citoplasmática y de membrana, mientras que en los CE la tinción es más frecuente de membrana y con menor intensidad. En los tumores estromales gastrointestinales la expresión del anticuerpo tiene carácter diagnóstico y terapéutico por su respuesta al tratamiento con Gleevec® en los casos asociados a mutación de gen c-KIT. En los D/S, dicha mutación se ha identificado mediante FISH en aproximadamente un tercio de los casos. Sin embargo, la utilidad de dicho tratamiento no ha sido demostrada[8], hecho en parte lógico debido a su excelente respuesta quimioterápica a su tratamiento establecido con cisplatino y carboplatino, que no justificaría otras terapias no suficientemente probadas.

Otro marcador recientemente considerado como útil en el diagnóstico de los TCGM es la clona *D2-40* de la podoplanina que inicialmente identificó una glucoproteína de los podocitos renales[9]. Es también un marcador de

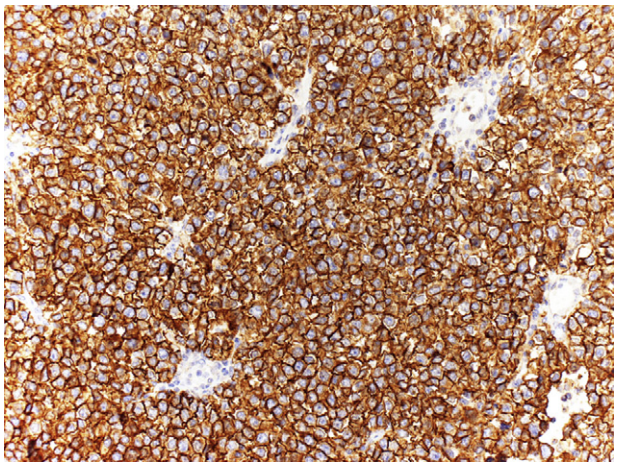

Figura 2 Disgerminoma: expresión de D2-40 podoplanina.

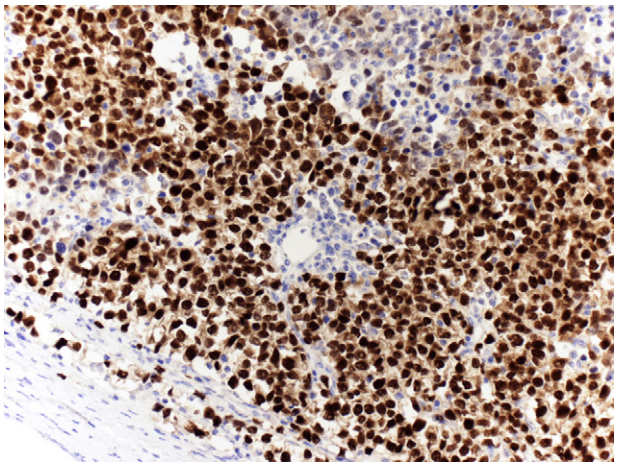

Figura 4 Disgerminoma: expresión nuclear de OCT3/4.

los vasos linfáticos y del mesotelio, siendo muy inespecífico al ser positivo en diversos tejidos[8]. En el grupo de los TCGM, sin embargo, puede comportarse como un marcador específico del D/S, aunque se han comunicado positividades en casos de CE, TV y teratomas. Nuestra experiencia con este marcador, utilizando la recuperación antigénica con calor húmedo, confirma la positividad intensa y difusa, citoplásmica y de membrana de los D/S (fig. 2), pero también hemos observado una tinción apical en las áreas glandulares y papilares de los CE (fig. 3). También se expresa aisladamente en TV en transición con CE y, frecuentemente, en los elementos estromales de los teratomas.

Probablemente uno de los mas útiles anticuerpos en el diagnóstico de los TCGM es el *OCT3/4*, un factor de transcripción nuclear que interacciona con otros factores nucleares como NANOG, SOX2 y SALL4, hallándose implicado en el mantenimiento de la totipotencialidad de las células madre y células germinales primordiales[10]. Se expresa tempranamente en la embriogénesis y presenta un papel esencial en la diferenciación del blastocito. En el embrión, tras la gastrulación, su expresión se halla limitada a las células germinales primordiales hasta su transformación en las espermatogonias durante el segundo trimestre del embarazo[11]. En las lesiones precursoras de los TCGM se reexpresa en el gonadoblastoma y en la neoplasia germinal testicular intratubular[10]. Nuestra experiencia con este marcador confirma su positividad en todos los casos de D/S (fig. 4) y CE, pero también su negatividad en otros tipos de TCGM. Es por consiguiente un marcador selectivo, especialmente en el ovario, donde el CE es mucho menos frecuente que en el testículo. Ha sido confirmado también como un marcador útil en la identificación de las metástasis de los D/S y CE debido a su excepcional especificidad, reducida a estos 2 tipos tumorales.

SALL4 es un miembro de la familia de genes SALL, también expresados tempranamente en la embriogénesis. También forman parte de la red de genes implicados en la totipotencialidad con un papel preponderante en la diferenciación del endodermo[12]. Su expresión en las células germinales primordiales no ha sido mencionada; sin embargo, la hemos identificado en dichas células durante su migración hacia las crestas gonadales en el retroperitoneo en 2 embriones de 12 semanas de gestación. En ambos, SALL4 se coexpresó junto con OCT3/4 y D2-40 pero no con PLAP. En los pocos artículos publicados en la literatura y corroborados por nuestra experiencia, este anticuerpo debe ser considerado como un marcador general, si bien inespecífico, de los TCGM con resultados variables en coriocarcinomas y teratomas[13]. Sin embargo, este anticuerpo ha sido también descrito en la leucemia mieloide en fase blástica[14] y en variantes raras de adenocarcinomas gástricos[15].

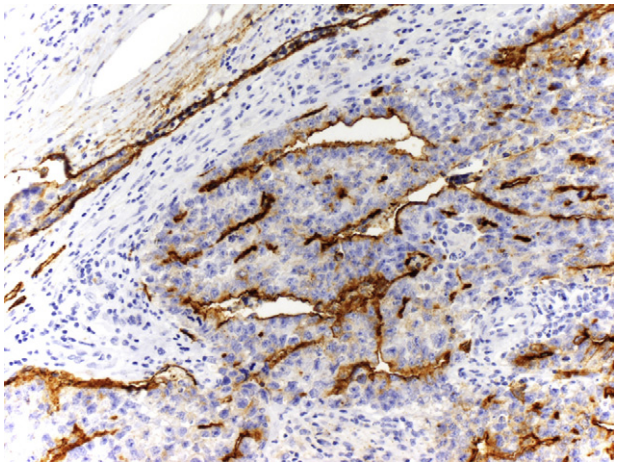

Figura 3 Carcinoma embrionario: la D2-40 podoplanina se expresa exclusivamente en zonas apicales.

Tumor vitelino o tumor endodérmico primitivo

Histopatología

Recientemente hemos revisado exhaustivamente los TV desde distintos puntos de vista: morfológicos, inmunohistoquímicos y de nomenclatura[13]. Debido a su compleja morfología que reproduce numerosas diferenciaciones endodérmicas tales como intestino, pulmón, hígado e incluso

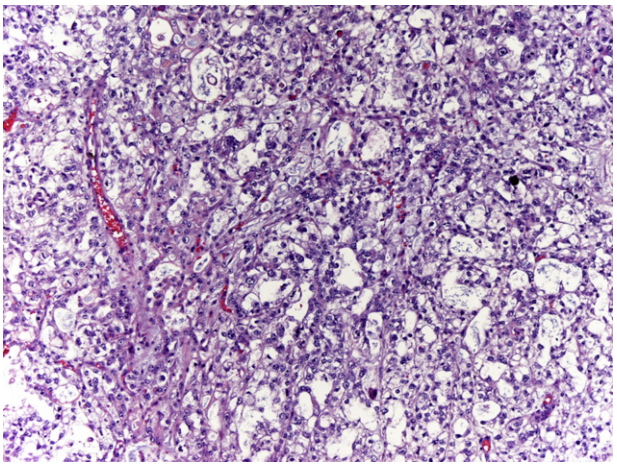

Figura 5 Patron clásico microquistico, fácilmente reconocible, de tumor vitelino (tumor endodérmico primitivo).

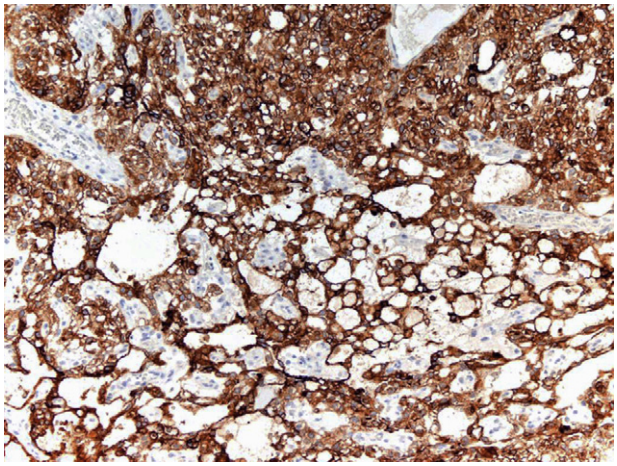

Figura 6 Tumor vitelino (tumor endodérmico primitivo), expresión limpia sin fondo del GLP3.

patrones morfológicos de neoplasias de otras especies, hemos propuesto para estos tumores el término de «tumor endodérmico primitivo», por analogía con el término de tumor neuroectodérmico primitivo (PNET), concepto que incluye la presencia de todas las posibles diferenciaciones a expensas del neuroectodermo primitivo[16]. Esta nueva terminología, más amplia, abarca también áreas de diferenciación endodérmica presentes en tumores de tipo somático (más frecuente en el tracto genital femenino) y algunos tumores de células claras secretantes de alfa-fetoproteína (AFP) en estómago, pulmón y vejiga[13]. Estas neoplasias poseen una apariencia glandular endodérmica y son totalmente indistinguibles del TV, e incluso muestran un comportamiento muy agresivo.

Los patrones histológicos de tipo primitivo del TV tales como los de arquitectura reticular microquística (fig. 5) son los más característicos para realizar el diagnóstico, que se complementa con la presencia de glóbulos hialinos y de masas amorfas de material de membrana basal extracelular. Los corpúsculos de Schiller-Duval se hallan en menos del 20% de estos tumores[17]. Aun menos frecuentes son las áreas de tipo parietal, polivesicular, tubular, hepática o las asociadas a hematopoyesis. En el diagnóstico diferencial tienen particular importancia los patrones endodérmicos de tipo somático de arquitectura glandular y hepática, que pueden simular otros patrones de tumores de origen no germinal. Se han comunicado tipos histológicos aun más raros, tales como los de sobrecrecimiento mesenquimal[18] y los de diferenciación hacia el carcinoide mucinoso[19]. Algunas metástasis ováricas de tumores gástricos, como el de células claras con cambio hepatoide, pueden simular un TV primitivo, sobre todo cuando la afectación es unilateral.

Inmunofenotipo

El marcador clásico y todavía de mayor especificidad de los TV es la AFP[20], una proteína segregada tempranamente en la embriogénesis por el saco vitelino secundario donde posee una tinción característica, delimitando espacios tubulares intracelulares e intercelulares. Su tinción es de tipo granular citoplasmática, a veces positiva en los glóbulos hialinos[21]. Su expresión es generalmente parcial y frecuentemente acompañada de un fondo sucio[13]. Ocasionalmente se negativiza en las recidivas post-quimioterapia. Solamente algunos elementos glandulares teratoides de diferenciación tipo intestinal expresan la AFP, al igual que las áreas de diferenciación hepática.

El *GLP3* es también un marcador útil en hepatopatología y viene a completar el perfil inmunohistoquímico de los TV con una tinción más intensa y extensa, generalmente con fondo limpio. Pone de manifiesto áreas endodérmicas que habitualmente pasan desapercibidas (fig. 6). Sin embargo, el GLP3 no tiene la alta especificidad de la AFP, ya que también puede ser expresado en áreas de CE, glándulas teratoides, neuroepitelio y sincitiotrofoblasto[20].

Ya hemos mencionado el *SALL4* como marcador general de los TCGM; así, en los TV, independientemente de sus diferenciaciones histológicas (primitivas o somáticas), se expresa con una intensa tinción nuclear, reflejo de la retención de un carácter pluripotente del tumor (fig. 7). Es necesario recordar que el saco vitelino normal conserva dicha expresión en mayor o menor medida hasta su involución. Otros marcadores con resultados parecidos al SALL4 son el Lin28 y el IMP-3, proteínas conectadas al ARNm e implicadas también en los fenómenos de pluripotencialidad[13].

Carcinoma embrionario

Histopatología

El CE es el verdadero tumor de células madre malignas con máxima totipotencialidad capaz de reproducir distintos tipos tisulares tanto benignos como malignos. Esta capacidad ya fue demostrada por Pierce[22] en estudios experimentales y representó, sin duda, la primera demostración del papel de las células madre en el cáncer y la confirmación de la teoría clonal de las neoplasias. Es un tumor típicamente testicular donde forma el componente maligno de muchos tumores mixtos[23]. Esta preferencia de

localización testicular es debida a la distinta histogénesis de los tumores testiculares y ováricos; los primeros, originados a expensas de células madre malignas, y los segundos, de origen partenogenético postmeióticos o meióticos[24,25]. Por ello, su incidencia real en el ovario es muy discutible, ya que es posible que muchos de los carcinomas embrionarios reportados en series antiguas carentes de estudios cromosómicos incluyan casos de pacientes con disgenesias gonadales portadores de cromosoma Y, y por tanto tumores de tipo testicular más que ovárico[26]. En estos, la lesión precursora sería el componente germinal maligno in situ del gonadoblastoma, idéntico al de la neoplasia germinal intratubular testicular[27]. Así pues, un diagnóstico de CE en ovario debe sugerir un estudio detallado del cariotipo. En nuestra extensa experiencia en tumores de células germinales, debemos decir que apenas hemos visto un par de casos en 40 años.

Su celularidad es característica, con elementos de aspecto epitelioide con citoplasma abundante basófilo y núcleo vesiculoso de cromatina pulverulenta (fig. 8). Su atipia es mayor que en los D/S y TV, mostrando superposición nuclear, áreas de necrosis tumoral y mitosis más frecuentes. Su arquitectura compleja puede mostrar áreas sólidas, glandulares y papilares que pueden simular patrones de D/S o TV[5], indicándose pues en muchas situaciones un estudio inmunohistoquímico diagnóstico.

Inmunofenotipo

El marcador característico del CE, y probablemente uno de los más específicos para este grupo de tumores, es el *CD30*[28], anticuerpo que detecta un antígeno de superficie celular correspondiente al receptor de las citoquinas, el cual forma parte de la superfamilia de los factores de necrosis tumoral[29]. Su expresión se halla restringida a pocas neoplasias, entre las que destacan los linfomas T anaplásicos CD30 positivos y las células de Reed-Sternberg[30]. También muestran positividad los inmunoblastos de algunas lesiones inflamatorias reactivas[31].

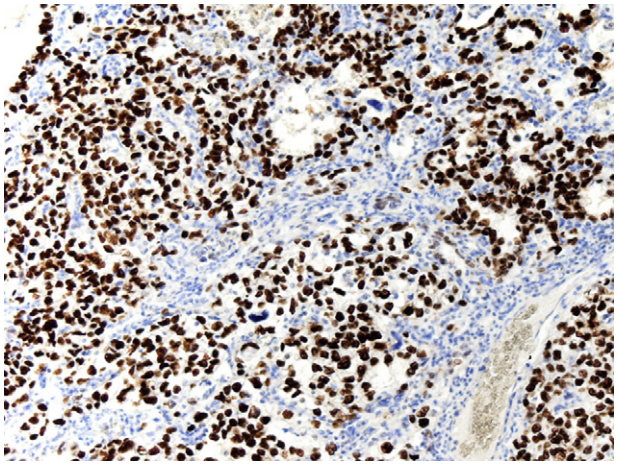

Figura 7 Tumor vitelino (tumor endodérmico primitivo), expresión nuclear de SALL4 que confirma retención de carácter pluripotente.

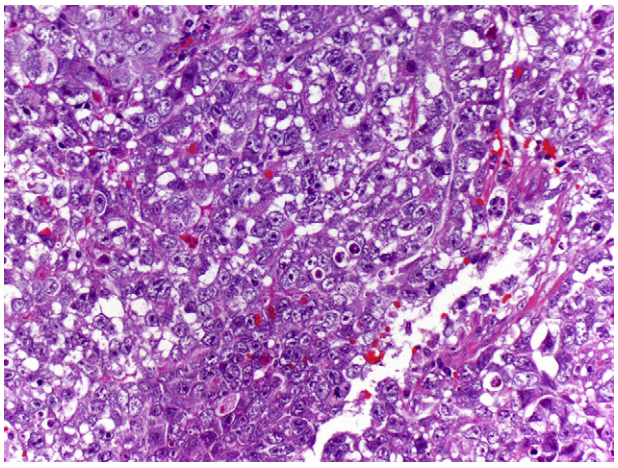

Figura 8 Carcinoma embrionario. En el diagnóstico diferencial con disgerminoma, obsérvese la celularidad epitelioide y la ausencia de infiltración linfocítica.

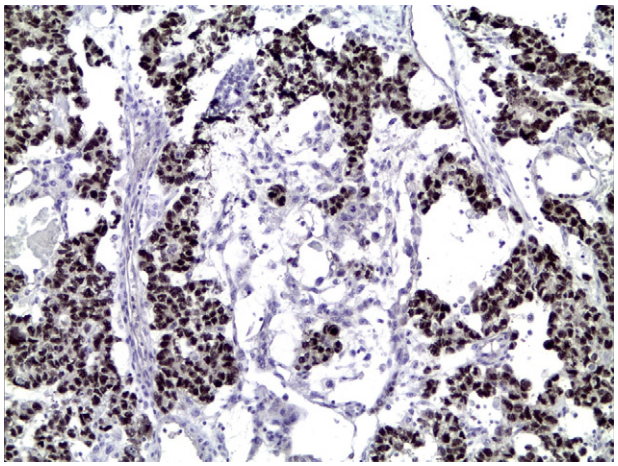

Figura 9 Carcinoma embrionario. Expresión diferencial de SOX2 en áreas papilares, ausente en la zona coexistente (centro) de tumor endodérmico primitivo.

La *PLAP* es poco específica, ya que es positiva en un gran porcentaje de varios tipos de TCGM[32]. Asimismo, hemos observado una expresión apical característica y difusa de la *podoplanina D2-40* (fig. 3). Su positividad concomitante en el endotelio linfático ayuda al diagnóstico de linfangioinvasión de CE. Tanto *OCT3/4* como *SALL4* no son específicos de CE, ya que también se expresan en los D/S.

El *SOX2* es un gen responsable de la diferenciación neuronal[33] y es otro factor de transcripción nuclear, parte de la red de genes implicados en totipotencialidad[2]. Su expresión proteica en los TCGM está restringida solamente a los CE y al neuroepitelio inmaduro[34]. Este anticuerpo puede complementar el panel de anticuerpos útil para diferenciar el D/S del CE o también el CE con áreas papilares del TV (fig. 9) que pueden remedar senos de Schiller-Duval. Con la expresión proteica simultánea de los 3 genes OCT3/4, SOX2 y SALL4 se confirman una vez más las bases de la totipotencialidad del CE.

EL *GLP3* puede mostrar positividad irregular en CE, posiblemente en áreas de diferenciación endodérmica. A veces, en tumores muy organoides tales como los poliembriomas,

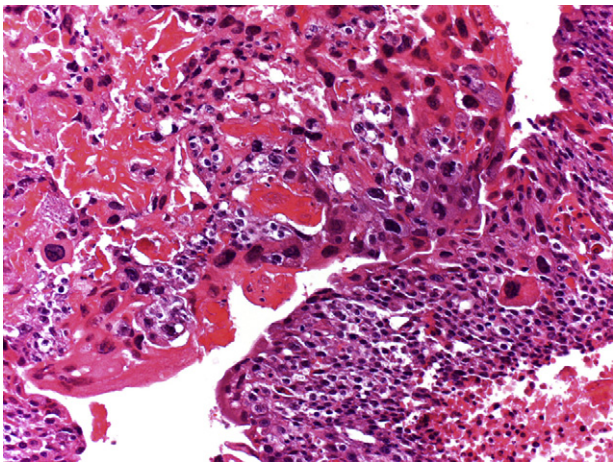

Figura 10 Infrecuente coriocarcinoma teratoide con patrón plexiforme diagnóstico.

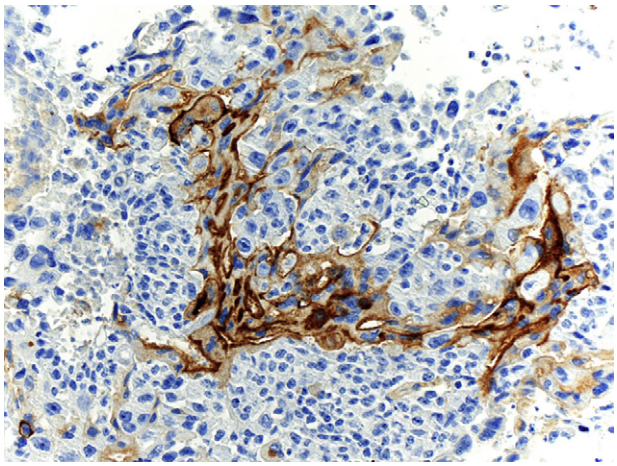

Figura 11 Coriocarcinoma teratoide: Expresión sincitiotrofoblástica de GLP3.

el GLP3 y la AFP delinean las cavidades vitelinas primarias de los embrioides[20].

Coriocarcinoma

Histopatología

Es muy poco habitual como un tumor puro y casi siempre se asocia con otros tipos de TCGM[23]. La presencia en un patrón plexiforme de células atípicas mononucleadas de tipo citotrofoblasto, trofoblasto intermedio y células sincitiotrofoblásticas son requisitos obligatorios para el diagnóstico (fig. 10). En general, las células se disponen en nidos centrados por citotrofoblasto con sincitiotrofoblasto en la periferia. Las áreas de hemorragia y necrosis son características, algunas veces tan extensas que hacen difícil la identificación de las células tumorales.

Inmunofenotipo

El perfil inmunohistoquímico del coriocarcinoma muestra una característica positividad de gonadotrofina coriónica humana (hCG) en las células sincitiotrofoblásticas[35]. Existe casi siempre un fondo sucio debido a la impregnación hormonal de suero y tejidos circundantes. De la misma manera, pero con un fondo más limpio, el sincitiotrofoblasto se puede identificar tras la tinción con la alfa-inhibina o con citoqueratinas[36]. Entre los nuevos anticuerpos, el GLP3 también marca fuertemente el sincitiotrofoblasto (fig. 11), si bien el citotrofoblasto muestra una tinción débil según algunos autores[37]. El lactógeno placentario y el CD10 identifican el citotrofoblasto y la frecuente diferenciación hacia trofoblasto intermedio[38].

Teratomas

Histopatología

Son tumores que reproducen de manera caricaturesca y ectópica tejidos inmaduros e maduros de origen ectodérmico, mesodérmico y endodérmico. También incluyen los casos con malignización de tipo somático, representadas por las áreas de transformación maligna de los varios componentes, especialmente los escamosos[39].

En el ovario, el aspecto diagnóstico de mayor importancia radica en la identificación de los tejidos inmaduros de tipo neural en el teratoma inmaduro, ya que su cuantificación subjetiva es base de los distintos sistemas de gradación de los mismos, sea en 2 o 3 niveles de inmadurez, dependiendo de las cantidades relativas de tejidos inmaduros[40,41]. La gradación histológica es moderadamente reproducible y tiene gran valor pronóstico, indicando la conducta terapéutica a seguir. Aunque en la mayoría de los casos la valoración puede ser fácil (fig. 12), en tumores con defectos de fijación o bien con áreas celulares o asociadas con hemorragia o necrosis pueden ocasionarse problemas de gradación y, consecuentemente, un tratamiento erróneo.

Raramente, los teratomas inmaduros exhiben un sobrecrecimiento neoplásico de alta malignidad de su componente neural a expensas de una neoplasia de tipo PNET[42].

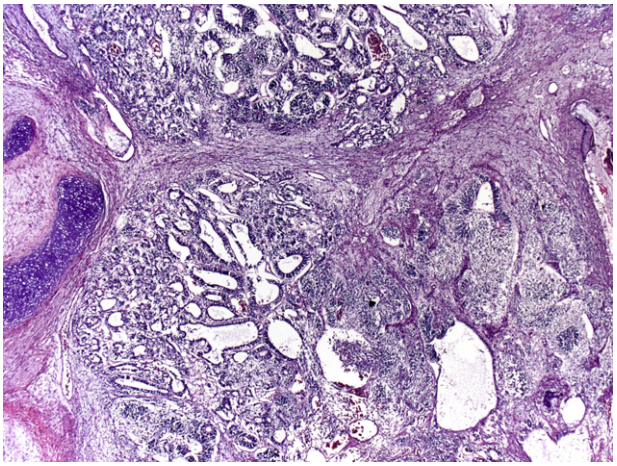

Figura 12 Teratoma inmaduro de alto grado con abundante tejido neuroectodermico primitivo.

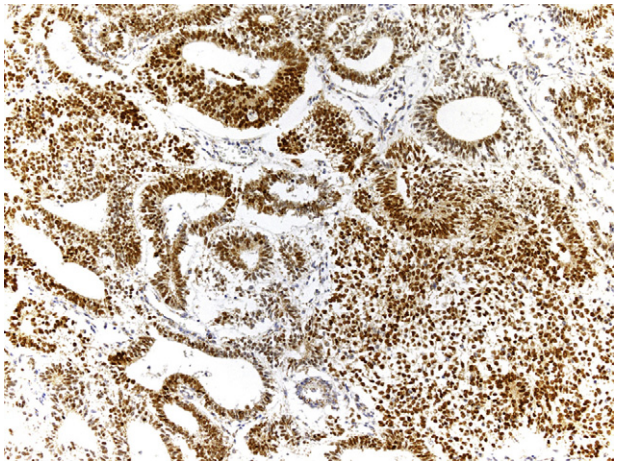

Figura 13 Teratoma inmaduro de alto grado. SOX2 es fuertemente positivo en las áreas de immadurez neuroectodermica.

Inmunofenotipo

Si la valoración de áreas neurales inmaduras en la tinción con H&E no es suficiente, la introducción de algunos de los nuevos marcadores puede ser útil en su identificación. El *GLP3* se expresa en el neuroepitelio embrionario, pero en los casos menos diferenciados puede presentar una menor sensibilidad[20]. El *SALL4* también los identifica, pero de forma parcial y con una tinción débil. Probablemente el anticuerpo de mayor especificidad, útil incluso para identificar áreas de sobrecrecimiento tipo PNET, es el *SOX2*[43] (fig. 13). Su tinción nuclear facilita la interpretación, pero su positividad en los casos de CE, aunque rara en ovario, implicaría la valoración conjunta con otros marcadores específicos, tales como los neurales (proteína acídica glial fibrilar, nestina, enolasa, sinaptofisina, etc.), así como el *OCT3/4*, positivo en CE pero negativo en áreas neurales de teratoma.

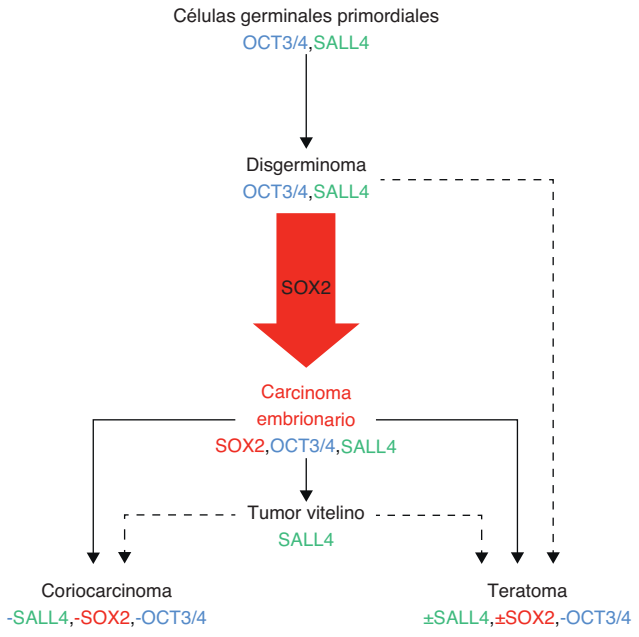

Figura 14 Flujo diferenciativo que muestra la expresión alternante de marcadores *(switch on/off)* en tumores de células germinales.

Aunque similares desde punto de vista morfológico, los estudios genéticos de los tumores germinales gonadales han demostrado la presencia del isocromosoma 12p y la sobreexpresión del cromosoma 12p en los tumores testiculares del adulto, mientras que en el ovario, bien sea por el escaso número de estudios comunicados o bien por su diferente histogénesis también, estos cambios son pocos frecuentes y menos constantes[44]. Sin embargo, la integración diagnóstica de la expresión de los marcadores en relación con el flujo de diferenciación celular (paralelo al desarrollo embrionario) puede considerarse común para los tumores germinales de las ambas gónadas (fig. 14).

Conclusiones

El estudio inmunohistoquímico comparativo y la revisión de los nuevos anticuerpos muestra el valor diagnóstico de los marcadores clásicos, pudiéndose sin embargo obtener una mayor especificidad y sensibilidad diagnóstica con la incorporación de nuevos marcadores, muchos de ellos procedentes de resultados de investigación en el campo de las células madre.

Anticuerpos como el OCT3/4, el SOX2 y el SALL4 son marcadores de células madre y representan la expresión inmunohistoquímica de factores intrínsecos que regulan la autorrenovación y la pluripotencialidad de las células germinales. Su expresión en los TCGM confirma una vez más la capacidad pluripotente de estas neoplasias.

El OCT3/4 se comporta como un marcador de los tumores no diferenciados de células germinales, demostrando que el disgerminoma/seminoma no es un tumor con diferenciación de tipo terminal sino un precursor de otros TCGM. La expresión simultánea de SALL4, SOX2 y OCT3/4 confirma al carcinoma embrionario como el tumor de células madre pluripotentes, con el SOX2 y el CD30 como marcadores altamente característicos. La ausencia de marcadores de células madre en el coriocarcinoma y los teratomas, los identifica como tumores con una diferenciación de tipo terminal.

El SALL4 puede ser considerado un marcador general de los tumores germinales, pero sin embargo puede estar presente en raros tumores somáticos con áreas de sobrecrecimiento de tipo germinal.

La mayoría de los carcinomas embrionarios se diagnostican mediante una combinación de CD30 y SOX2, mientras que OCT3/4 es otro marcador útil para confirmar los casos dudosos de carcinomas embrionarios con un patrón papilar o adenoide, simulador de adenocarcinomas.

El D2-40 es útil para diferenciar el disgerminoma/seminoma del carcinoma embrionario y tiene la ventaja adicional de poder identificar endotelios linfáticos y, en consecuencia, la presencia de émbolos tumorales.

La tinción positiva con AFP es diagnóstica de tumores vitelinos, pero en los raros casos de la expresión incompleta de la misma, la reevaluación positiva con el GLP3 y SALL4 y la negatividad frente al OCT3/4 es diagnóstica.

El GLP3 tiene una sensibilidad superior, aunque menor especificidad que la AFP en la identificación de áreas de tumores vitelinos puros y mixtos, ya que el anticuerpo tiñe más cantidad de tejido y es más limpio de fondo. Sin

Tabla 1 Resumen de la expresión inmunohistoquímica de los anticuerpos evaluados en los tumores de células germinales malignos (TCGM)

	Disgerminoma/ seminoma	Carcinoma embrionario	Tumor vitelino	Coriocarcinoma	Teratoma inmaduro
SALL4	+	+	+	—	+
OCT3/4	+	+	—	—	—
SOX2	—	+	—	—	—/+ neuroepitelio
GLP3	—	—/+	+	—/+ sincitiotrofoblasto	+/— neuroepitelio
D2-40	+	+	—/+	—	—/+ sincitiotrofoblasto
PLAP	+	+	—/+	—/+ sincitiotrofoblasto	—/+ sincitiotrofoblasto
CD30	—	+	—	—	—
AFP	—	—	+	—	—/+ elementos glandulares

embargo, es menos específico que la AFP, ya que puede también teñir áreas de carcinoma embrionario, el sincitiotrofoblasto y elementos glandulares y neuroepiteliales de los teratomas.

Un panel mínimo de anticuerpos con cobertura de los tipos más frecuentes de los tumores de células germinales deberá incluir AFP, CD30, D2-40, OCT3/4, GLP3 y SALL4. La expresión inmunohistoquímica de los anticuerpos evaluados en los TCGM se resume en la tabla 1.

Responsabilidades éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad de los datos. Los autores declaran que en este artículo no aparecen datos de pacientes.

Derecho a la privacidad y consentimiento informado. Los autores han obtenido el consentimiento informado de los pacientes y/o sujetos referidos en el artículo. Este documento obra en poder del autor para correspondencia.

Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

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Gliomatosis peritonei as a natural experiment in tissue differentiation

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ABSTRACT ***Gliomatosis peritonei*** **(GP) is an unusual condition in which nodules of mature astroglia, often miliary and microscopic in size, are widespread in the peritoneum and abdominal lymph nodes. Its behaviour is benign and it is usually found in association with ovarian teratoma and rarely with teratomas of other organs. Implants grow rapidly and can remain unchanged for life. Astroglia is the main component, but other neural lineage elements and many other tissues can be found. Cells are mature but not terminal, since they express SOX2. Secondary associated lesions include: a) degenerative astrocytic changes, b) granulomatous and follicular chronic inflammatory changes, c) association with hormonally related changes, such as decidual peritoneal metaplasia and endometriosis and d) endothelial and adventitial vascular hyperplasia leading to haemoperitoneum. Two pathogenetic mechanisms are considered: direct seeding of immature neural cells from a primary tumour with subsequent differentiation and metaplasia from peritoneal stem cells.The former proposal is supported by clinicopathologic data such as ample cellular heterogeneity, coexistence of mature astroglia with neural blastema, as well as the shed keratin and hairs from the ovarian neoplasm. However, metaplasia is sustained by a heterozygosity pattern of GP nodules, identical to the normal tissue and different from the coexistent ovarian teratoma. GP would constitute a response to growth factors from teratoma or macrophages. While an implantative origin from ovarian teratoma remains in most cases a more probable mechanism, metaplasia from peritoneal stem cells would explain cases of GP which present a monomorphic astrocytic cell population.**

KEY WORDS: *gliomatosis peritonei, ovary, teratoma, ectopic glia, SOX2*

Introduction

Gliomatosis peritonei (GP) is an unusual condition consisting in the presence of multiple nodules of mature astrocytes in the serosal peritoneal surface of the abdominal cavity. Although its clinical picture of peritoneal spread is that of advanced stage neoplasia, its behaviour is almost invariably benign, since its differentiated cells lack proliferative activity (Fortt *et al.,* 1969; Nogales *et al.,* 1974; Robboy *et al.,* 1970).

The majority of cases of GP are associated with an immature ovarian teratoma and only rarely with mature teratomas (Dhingra *et al.,* 2007; Gocht *et al.,* 1995); albeit a rare phenomenon, it can occur in up to a fourth of all cases of ovarian immature teratoma. Although GP has been reported in association with isolated cases of teratomas of the stomach, liver and bladder (Karlo *et al.,* 2009; Torikai *et al.,* 2007; Yeo *et al.,* 2010), such exceptional cases do not really conform to the classic picture of miliary peritoneal spread of GP that occurs with ovarian teratoma; instead they are large, circumscribed masses more suggestive of usual-type metastases.

Traditionally, the association with a concomitant neoplasm has favoured the hypothesis of GP as a phenomenon of implantation and subsequent maturation of neural precursor cells detached from the primary tumour. However, in the female peritoneum, the presence of ectopic benign tissues such as serous tubal epithelium is extremely frequent. Exceptionally, numerous nodules of highly differentiated thyroid tissue may be found in the peritoneum; the so called benign strumosis (Karseladze *et al.,* 1994), which may occur in association with struma ovarii. Also, well differentiated Sertoli cell tumours can implant foci of benign immature Sertoli-cell tubules in the peritoneum (Onida *et al.,* 2010). The pathogenesis of these mature ectopic tissues is not clear and both mechanisms of direct seeding/differentiation from a primary tumour (Robboy *et al.,* 1970) and peritoneal metaplasia (Ferguson *et al.,* 2001) from stem cells have been proposed.

Abbreviations used in this paper: GP, gliomatosis peritonei.

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In this review we will focus on the clinical and histological features of GP, with special consideration of its histogenesis, which may include two alternative mechanisms.

Clinical features

GP affects a broad age range, from childhood to postmenopausal patients, with a peak in the second and third decades of life and only few instances above the age of sixty. Often occurring as an incidental finding during surgery for ovarian tumour or in second look operations after a diagnosis of teratoma, it is consistently associated with a unilateral solid tumour. GP may occur after capsular rupture during surgery or spontaneously. When oophorectomy alone is performed without accompanying salpingectomy, there is a high chance of rupturing the capsule at the ovarian pedicle at hilar level.

The overall prognosis of GP is excellent and chemotherapeutic treatment is unnecessary. Long follow-up studies have demonstrated its benign course (Fortt *et al.,* 1969; Robboy *et al.,* 1970). A recent study comparing ovarian immature teratomas with and without GP demonstrated a similar overall good survival but with a higher incidence of early recurrences in the cases associated with GP (Mann *et al.,* 2008; Yoon *et al.,* 2012). Occasionally, GP may precede highly malignant neuroectodermal tumours (Dadmanesh *et al.,* 1997; Shefren *et al.,* 1991; Trabelsi *et al.,* 2002). Cases from the older literature reporting early malignant behaviour may correspond to recurrences of incompletely sampled peritoneal disease, with foci of immature tissue not identified at the time of surgery.

Implants can grow rapidly. In one instance, they were detected in a second look operation performed only one month after oophorectomy for immature teratoma (Nogales *et al.,* 1974). In rare instances, such as another of our cases, PG can be an incidental autopsy finding of asymptomatic residual disease in elderly patients who had had ovarian teratoma in their youth.

Among serum markers, CA125 levels are elevated in GP (Yoon *et al.,* 2012) and some authors (Bahari *et al.,* 1980; de Graaff *et al.,* 1980; Hokama *et al.,* 1991) have also reported elevated alpha-fetoprotein levels, possibly related to endodermal elements present in the immature teratoma deposits.

Pathology findings

Primary teratoma

GP occurs in association with a unilateral solid ovarian teratoma. Histologically the primary tumour shows tissues with a variable degree of immaturity. However, the predominant tissue is of neural type and comprises large amounts of well differentiated glial tissue with other neuroectodermal components. Other frequent non-neurological constituents are skin, developing teeth, gastrointestinal derivatives although, eventually, any imaginable tissue can be found (Nogales *et al.,* 2003).

Histological tumour grading of teratomas is a valuable tool for predicting their behaviour (Nogales *et al.,* 1976; Norris *et al.,* 1976; Thurlbeck *et al.,* 1960). It is performed by a subjective, semiquantitative analysis of the relative number and atypicality of immature neural tissues present in the neoplasm such as neuroepithelial tubules and neural blastema. This is accomplished either by the traditional approach of assigning 4 grades ranging from fully mature (grade 0) to highly immature (grade 3) or by establishing a two tier system into low grade and high grade tumours (O'Connor *et al.,* 1994). Ovarian neoplasms associated with GP are most frequently of grade 1 or 2 and only rarely may correspond to grades 0 (fully mature) or 3 (highly immature). When found in association with grade 0 solid teratomas, the tumour warrants a more extensive tissue sampling in order to exclude any immature foci. Evaluation of immaturity can be enhanced by the immunohistochemical analysis of pluripotency markers such as SALL4 and SOX2 which are highly sensitive in the identification of neural immature cells (Nogales *et al.,* 2012).

An interesting aspect of ovarian immature teratoma is the concomitant vascular response present in association with neural tissues. There is an extensive endothelial proliferation of vessels, similar to that occurring in tumours of the central nervous system, originating as a response to angiogenic factors secreted by immature neural elements (Baker *et al.,* 2002; Nogales *et al.,* 2002;

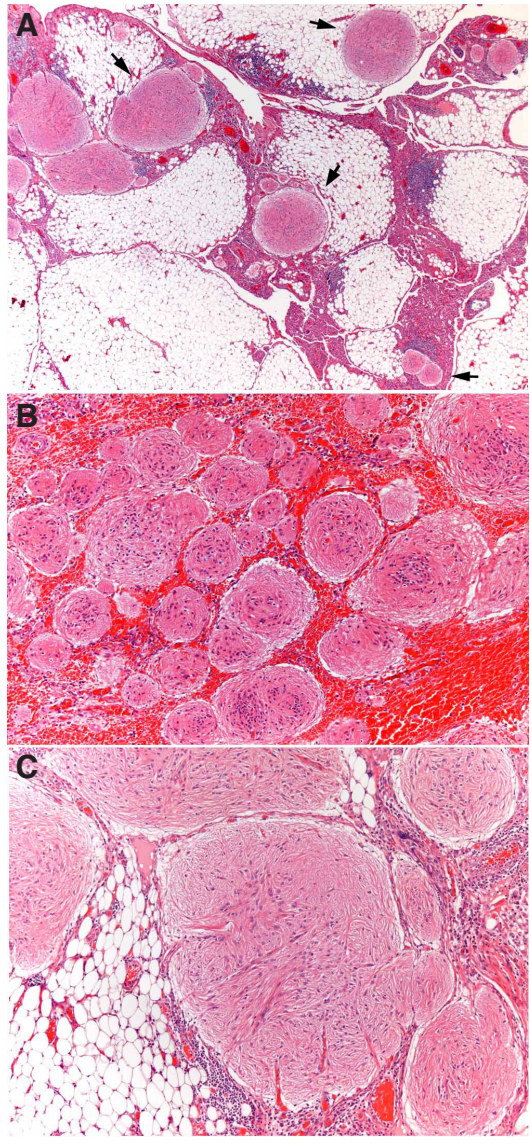

Fig. 1. Characteristic appearance of *Gliomatosis peritonei* (GP) at low power. *Multiple astrocytic nodules (arrows) are scattered throughout the omental surface and underlying fatty tissue* **(A)**. *Glial nodules are surrounded by haemorrhage* **(B)**. *Higher magnification* **(C)** *reveals uniform, mature glial cells set in a fibrillary matrix.*

Nogales *et al.,* 1983; Nogales *et al.,* 1974). Only on rare occasions a secondary, highly malignant neural tumour, such as primitive neuroectodermal tumour (PNET) may develop from the stem cells present in ovarian immature teratoma. In these cases, metastases are always of high grade (Morovic *et al.,* 2008).

Gliomatosis peritonei

Appears as miliary deposits scattered throughout the peritoneum involving every serosal surface including recesses, cul-de-sac, intestinal surface, etc. In a few cases the coexistence of adhesions due to endometriosis may give it a more complex appearance. Haemorrhage can be present in the nodules. Surgical sampling should be as extensive as possible in order to evaluate fully the immaturity of the peritoneal deposits. Chemotherapy will depend on grading of GP, being indicated for high grades and not administered in grade 0 implants.

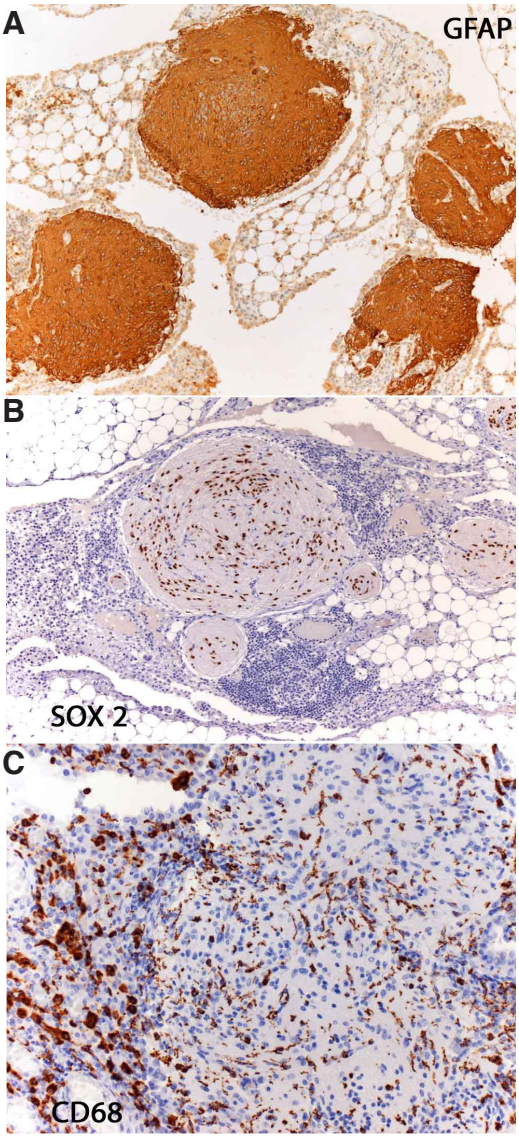

Fig. 2. Immunohistochemistry of *Gliomatosis peritonei* (GP). *Nodules are intensely positive for glial fibrillary acidic protein* **(A)**. *Nuclei, despite a mature appearance, express SOX2* **(B)**. *CD68 stains macrophages and intranodular stellate cells identical to microglia* **(C)**.

Macroscopically, GP appears as white or yellow nodules of variable size ranging from 1mm to 1cm and can be difficult to visualize. Indeed, they are often only microscopic and sometimes are an incidental finding in specimens from an omentectomy performed at the time of oophorectomy (Nogales *et al.,* 1974). True GP should be differentiated from large, discrete nodules of peritoneal metastases.

Microscopically, nodules are scattered through the peritoneum (Figs 1A,B) and composed of a glial cell population with mature features, minimal atypia and only rare mitoses. The predominant cell types are fibrous astrocytes (Fig 1C) staining for glial fibrillary acidic protein (Fig 2A). Their nuclei do not express pluripotency gene SALL4 protein, which is present in immature neural tissue (Ma Y.2012, Nogales *et al.,* 2012) but are positive for SOX2 (Fig 2B), a pluripotency transcription factor involved in neurogenesis (Noisa *et al.,* 2012), indicating that cells are mature but not terminally differentiated cells. Ultrastructurally, presence of other neural lineages such as oligodendroglia, ependymal, melanocytic and even neurons is demonstrated (Gonzalez-Campora *et al.,* 1979). Immunohistochemically, the NeuN neuronal nuclear antibody often shows scattered positive neuronal cells. Additionally, we have been able to detect the presence of CD68 positive microglia-like cells in the GP nodules (Fig 2C).

There are instances where the coexistence of foci of immature neuroepithelium with a mature glia is indicative of its differentiation from immature precursors (Fig 3A). Other non-neural tissue

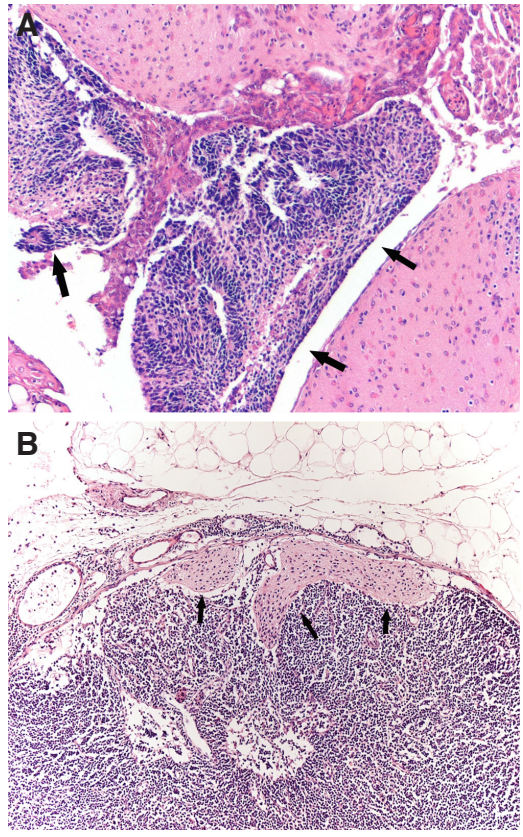

Fig. 3. Mature glial nodules *(gliomatosis peritonei) may coexist with foci of immature neural tissue exhibiting neuroepithelial tubules (arrows)* **(A)**. *Gliomatosis peritonei (GP) (arrow) is present in the marginal sinus of an abdominal lymph node* **(B)**.

components such as epidermis, cartilage, respiratory and digestive tract epithelia may also be present in the nodules.

GP involvement of lymph nodes (Heifetz *et al.,* 1998; Mann *et al.,* 2008; Muller *et al.,* 2002) is often an incidental finding in abdominal lymphadenectomy specimens, occurring in the marginal sinus (Fig 3B).

Secondary associated lesions in GP may include the following:

1. Degenerative changes in the GP astrocytes such as Rosenthal fibres, granular gemistocytes (Fig 4A), corpora amylacea etc.
2. Inflammatory changes: Chronic inflammation, frequently with follicular formation, is a common phenomenon around GP nodules (Fig 4B). Granulomatous reaction of foreign-body type occurs in relationship with keratin-rich desquamated epidermis or hairs (Fig 4C,D). In cases of long standing GP, a chronic macrophagic reaction can practically overgrow and erase the glial component.
3. Hormonal changes such as decidual transformation of mesothelium can also arise in the neighbouring peritoneum in cases of GP occurring during pregnancy (Fig 4E).
4. Vascular hyperplasia occurs in the vicinity of the nodules exhibiting a complex glomeruloid appeareance due to the proliferation of endothelial and adventitial vascular cells (Nogales *et al.,* 2002) (Fig 4F). These fragile, irregular vessels can be the source of hemoperitoneum.
5. Post-chemotherapy changes reveal degenerative nuclei in astrocytic cells (Fig 4G).
6. Endometriosis. Foci of endometrial tissue displaying both glands and stroma can coexist in the ovarian surface and peritoneum, where isolated glands are surrounded by GP. However, no cases of coexistence of leiomyomatosis peritonealis disseminata or endosalpingiosis/endocervicosis have been reported in GP (Fig 4H).

Pathogenesis

It is by no means clear. Two alternative mechanisms of differentiation have been proposed:

Peritoneal implantation

The aetiology of GP has been related, since its initial description (Robboy *et al.,* 1970), to implantation of immature neural tissue into the peritoneum subsequent to capsular rupture, either spontaneous or surgical. Thus there would be seeding of immature precursors that eventually differentiate into benign, terminally differentiated cells, including glia.

Data supporting this possibility include the following:

a) GP nodules do not only contain glia, but several other neurogenic lines (Gonzalez-Campora *et al.,* 1979) and other tissues such as skin, gut, cartilage etc. This ample range of differentiation is characteristic of teratoma. Furthermore, immature neuroepithelial tubules may coexist with other neural cell lines

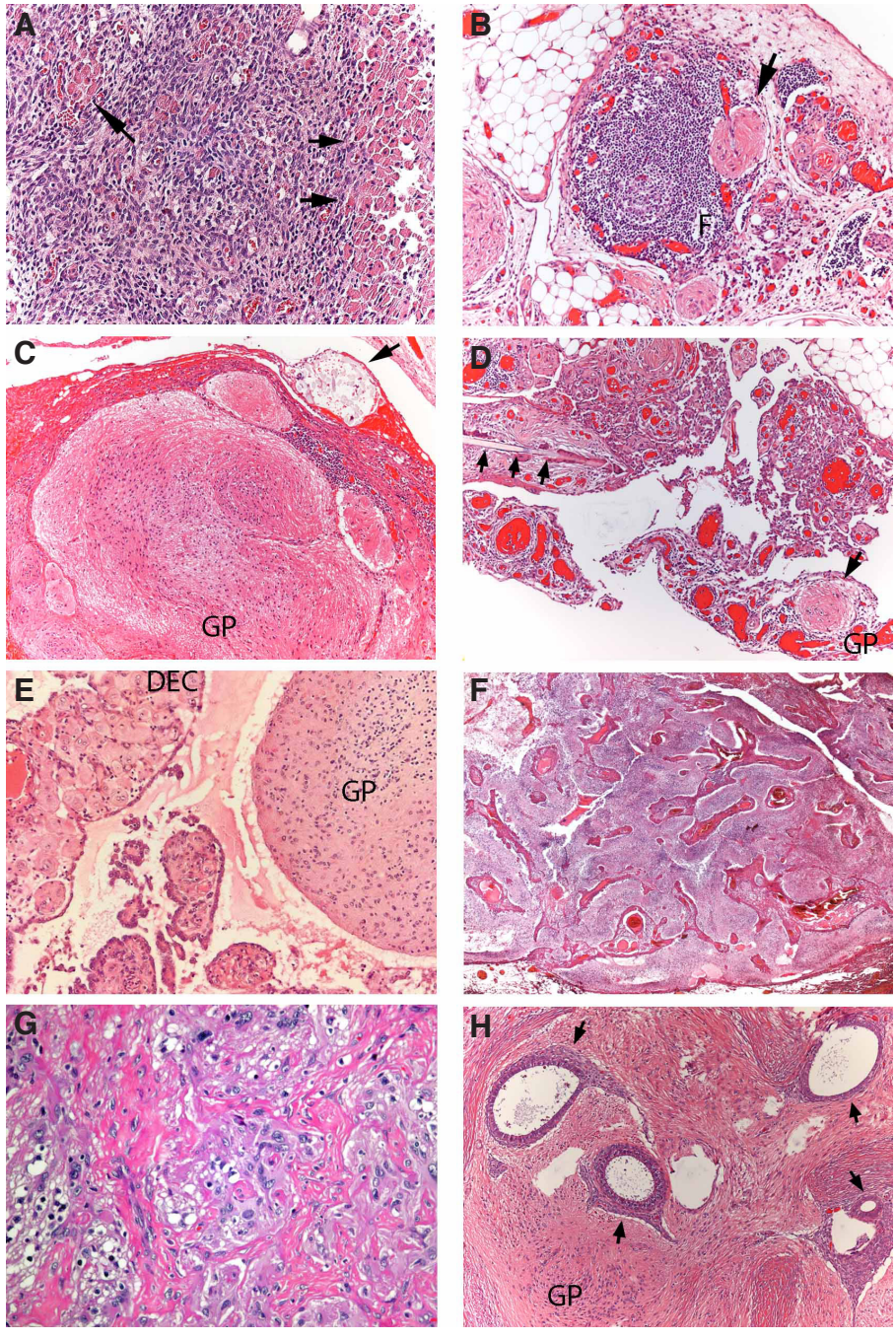

Fig. 4. Secondary changes in *Gliomatosis peritonei* (GP). *Presence of granular gemistocytes* **(A)**. *Chronic lymphocytic, follicular (F) infiltration around a GP nodule (arrow)* **(B)**. *Coexistence of GP with keratin peritoneal deposits (arrow)* **(C)**. *A GP nodule coexists with teratomatous hairs from ovarian primary (arrows)* **(D)**. *Decidual peritoneal change (DEC) in a pregnant patient coexists with GP* **(E)**. *Florid vascular hyperplasia in a case associated with haemoperitoneum* **(F)**. *Postchemotherapy glial atypia* **(G)**. *Coexistence of GP with numerous embedded foci of ectopic endometrium (arrows)* **(H)**.

(Fig. 3A). All those features would indicate a teratoid maturation from embryonal, immature precursors from the ovarian tumour. Local differentiation would occur either spontaneously or induced by platin-based chemotherapy (Gibas *et al.,* 1993; Kane *et al.,* 2009; Yoon *et al.,* 2012). Although most cases of implanted glial tissue mature spontaneously, chemotherapeutic conversion of neural immature cells into benign ones is the proposed mechanism for cases of growing teratoma syndrome associated with GP (Hsieh *et al.,* 2009; Umekawa *et al.,* 2005).

b) Shed keratin scales and hairs from the primary ovarian teratoma (Figs 4C,D) are often associated with GP, representing a gross but evident marker of its origin in the ovary and its subsequent transport into the peritoneum through the capsule.

c) Cases showing lymph node involvement (Figs 3B) by foci mature glial, even in the absence of a peritoneal lesion (El Shafie *et al.,* 1984; Heifetz *et al.,* 1998; Perrone *et al.,* 1986), would indicate a lymphatic transport of neural immature precursors that would eventually undergo full differentiation in the lymph nodes.

d) There are rare cases of GP associated with ventriculo-peritoneal shunts which would constitute a natural experiment of the implantative capacity into the peritoneum of glial cells present in the cerebrospinal fluid (Hill *et al.,* 2000; Lobotesis *et al.,* 2009; Lovell *et al.,* 1989).

e) Some ovarian tumours such as struma ovarii (Karseladze *et al.,* 1994) and well differentiated Sertoli cell neoplasms (Onida *et al.,* 2010), are capable of producing highly differentiated nodular and miliary implantations in the peritoneum after tumour rupture or manipulation.

Multifocal peritoneal metaplasia induced by growth factors

In the last decade, genetic studies analysing multiple microsatellite markers in microdissected GP implants (Best *et al.,* 2004; Ferguson *et al.,* 2001; Kwan *et al.,* 2004) have demonstrated that they have a heterozygosity pattern identical to the normal tissue and different from the coexistent ovarian teratoma, which is homozygous at the same loci. Although performed in only a few cases, these findings would imply a different genetic identity for the ovarian tumour and GP and thus challenge the classical implantative mechanism. This would favour the possibility of a metaplastic phenomenon from pluripotent subperitoneal cells, which would be a response to growth factors originating either from the coexistent teratoma (Ferguson *et al.,* 2001), local macrophages (Gocht *et al.,* 1995) or in the cerebrospinal fluid of ventriculoperitoneal shunts (Ferguson *et al.,* 2001).

This would parallel a mechanism analogous to that giving rise to monoclonal peritoneal proliferations of such diverse tissues as smooth muscle (Guarch *et al.,* 2001; Nogales *et al.,* 1978) and epithelia such as endometrial (Clement *et al.,* 2007), tubal- (Dallenbach-Hellweg *et al.,* 1995; Donne *et al.,* 1998) and endocervical (Liu *et al.,* 2009), which would originate in stem cells with a capacity to develop into Müllerian cell lines under the influence of hormonal growth factors. This has been aptly called the secondary Müllerian system (Lauchlan *et al.,* 1994), which is not restricted to the peritoneum, but also present in the urinary bladder (Donne *et al.,* 1998), ureter (Nogales *et al.,* 1999), pleura and even in the abdominal and axillary lymph nodes (Stolnicu *et al.,* 2011).

The proposed local metaplastic peritoneal origin of GP would imply that stem cells would also be endowed with a further capacity to develop into non Müllerian lineages such as astroglia. The occasional association of PG and endometriosis (Fig. 4H) (Albukerk *et al.,* 1979; Alexander *et al.,* 2011; Bassler *et al.,* 1982; Calder *et al.,* 1994; Dworak *et al.,* 1988; Killeen *et al.,* 1997) would give partial support to this assumption. Endometriosis is a common condition currently considered to be of metaplastic origin in most cases (Clement *et al.,* 2007). However, since pathogenetically related leiomyomatosis peritonealis disseminata, endosalpingiosis or endocervicosis have not been reported in association with GP, the association of endometriosis with GP may be coincidental, as endometriosis is a very common condition.

Taking into account both possibilities it would seem that an implantative origin from ovarian teratoma pluripotent precursors remains in most cases the more probable mechanism, although a metaplastic transformation from peritoneal stem cells under adequate growth factor stimulation is conceivable. We believe that this latter pathway would be restricted, however, to cases of GP that have a monotonous, monomorphic astroglial cell population, which would represent a selective cell lineage differentiation.

Author's roles

Francisco F. Nogales designed the study and participated in the analysis, execution and manuscript drafting and critical discussion. Isabel Dulcey retrieved archive material, performed the immunohistochemical and bibliographical analysis and participated in the manuscript drafting and critical discussion. Ovidiu Preda was responsible for the illustrations and immunohistochemistry.

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Teaching cases

Necrotic mature ovarian teratoma associated with anti-N-methyl-D-aspartate receptor encephalitis

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ABSTRACT

A 20-year-old female with a diagnosis of autoimmune encephalitis against N-methyl-D-aspartate receptor was found to have a 13 mm teratoma in the left ovary. The tumor had undergone massive coagulative necrosis within a normal ovary, a previously unreported feature. Necrosis of a mature cystic teratoma is very rare in the absence of ovarian torsion. It is proposed that necrosis may have induced a massive liberation of neuronal antigens.

The vast majority of the tumors associated with this newly described condition are ovarian teratomas containing neural tissues. In this paper, we review their different histopathological aspects that may explain the relative incidence of various tumor types associated to this form of encephalitis. Anti N-methyl-D-aspartate receptor encephalitis has now become the most frequent autoimmune disorder associated with ovarian teratoma.

Introduction

Albeit only rarely, ovarian teratomas can be associated with autoimmune disorders; autoimmune hemolytic anemia [1] and hyperthyroidism in both benign [2] and malignant struma ovarii [3] have been reported.

Since 2005, a neurosciences research group led by Dalmau [4–6] has identified over a hundred cases of autoimmune encephalitis due to antibodies against the N-methyl-D-Aspartate receptor (anti-NMDAR), and which most frequently involves the temporal lobes and hippocampus. This clinically severe form of encephalitis is often associated with an ovarian teratoma, although in a high proportion of cases, ranging from 41 to 80%, the tumor is not detected [6,7]. Removal of the ovarian tumor and early immunotherapy often improves the outcome with full recovery or only a residual mild neurological deficit [6,8,9]. We believe that this association has now become the most frequent autoimmune disorder associated with ovarian teratoma.

The histopathology of these tumors has not been analyzed in detail, although it has been reported that the majority contain neural tissue [5,10]. We report a case of a small ovarian mature cystic teratoma with the unusual histology of a massive coagulative necrosis and which was associated with clinical anti-NMDAR encephalitis.

Clinical data

A 20-year-old female with a previous history of a presently inactive Crohn's disease and minor bronchial asthma presented with headache, hyperthermia, diarrhea and vomiting. Four days later she developed tonic-clonic seizures, agitation and hypoventilation and required intubation. An initial computerized axial tomography (CAT) scan of the head performed 48 h after the initiation of symptoms was unremarkable. The cerebrospinal fluid (CSF) showed leukocytosis with mononuclear predominance, slightly elevated proteins and a normal glucose. Microbiological stains and specific tests for mycobacteria and cryptococcus were negative. Viral serology for herpes, varicella zoster, parotiditis and enterovirus was also negative.

She was referred to San Cecilio University Hospital with a diagnosis of encephalitis. On admission, she was unconscious but with open eyes, marked dystonia and frequent seizures. Under sedation,

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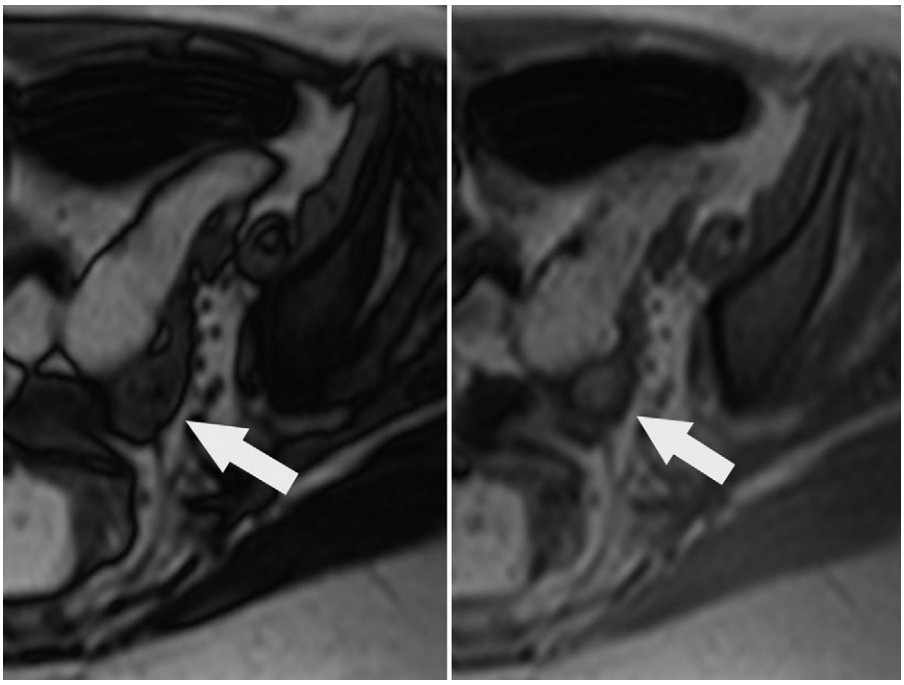

Fig. 1. Magnetic resonance imaging of left adnexum. Tumor (arrow) can be seen in both T1-weighted (T1W) *out-phase* (left) and T1W *in-phase* images (right).

both repetitive flexion of extremities and stereotyped movements in face and mouth were present. Further CAT and magnetic resonance (MRI) head scans were unremarkable. Once both neoplastic and viral aetiologies were discarded, a possible autoimmune disorder was considered and subsequently supported by the presence of positive Anti-NMDAR antibodies in the CSF, which established a diagnosis of anti-NMDAR encephalitis. Immunosuppressant treatment with corticoids and immunoglobulin followed by Rituximab and Cyclophosphamide was started but with little improvement.

Initially, further imaging studies, including abdominal ultrasonograms and a CAT scan, failed to demonstrate any pelvic tumor, however, a subsequent pelvic MRI revealed a rounded intraparenchymatous mass of 20 mm × 18 mm in the left ovary which was diagnosed as a fat-containing tumor, possibly a teratoma (Fig. 1). The right ovary was unremarkable. An abdominal laparotomy with left oophorectomy was performed.

Materials and methods

The surgical specimen was fixed in 10% buffered formalin, and the entire ovarian tissue (5 blocks) was processed for light microscopy. Immunohistochemical techniques for glial fibrillary acid protein (GFAP), (DAKO, Glostrup, Denmark, prediluted), neuron-specific enolase (NSE), (DAKO, prediluted), microtubules-associated protein 2 (MAP2), (DAKO, prediluted) and neuronal specific nuclear protein (NeuN), (Master Diagnostica, Granada, Spain, prediluted) were done.

Results

Macroscopically the ovary had an unremarkable external appearance, but a sagittal section revealed a 13 mm round, well delineated cyst filled by a mass of red, necrotic, friable tissue admixed with few hairs (Fig. 2A).

Microscopically, the cyst contained a teratomatous protuberance that had undergone massive coagulative necrosis (Fig. 2B) that only allowed identification of ghost tissues such as skin, appendages and fat (Fig. 3). Hairs and desquamated epithelium

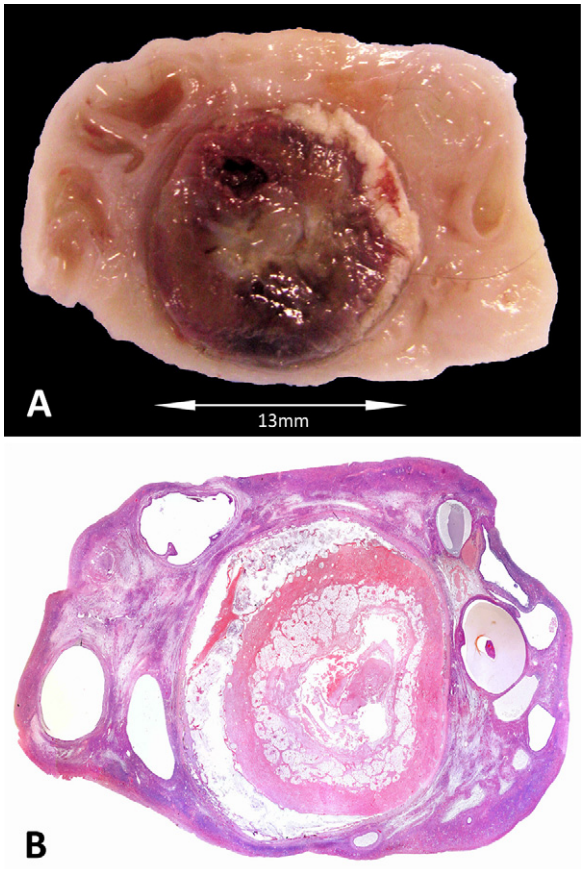

Fig. 2. Sagittal section of intraparenchymatous 13 mm necrotic teratoma (A). (B) A low power H&E stain (40×) shows a cavity containing hairs and a protuberance displaying massive infarction, where only "shadow" tissues are identifiable. The surrounding ovarian tissue is unremarkable, lacking signs of torsion.

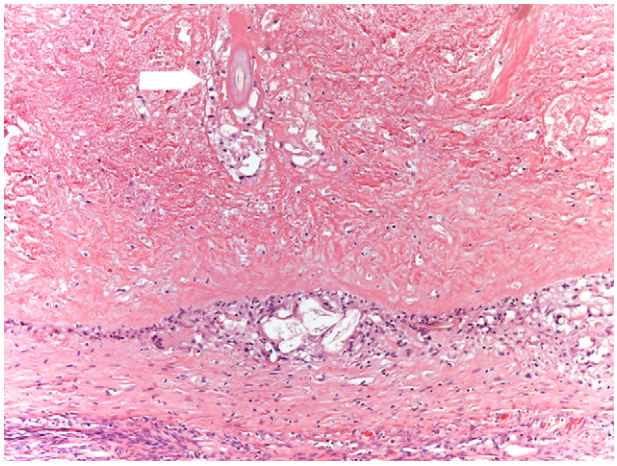

Fig. 3. A higher magnification H&E stain (400×) of the protuberance's edge showing necrotic tissue with a remaining hair (arrow).

filled the remaining cystic space. The central part was cystic and surrounded by a band of homogeneous liquefied tissue. Neither inflammatory infiltrates nor torsion-related histological features such as congestion or hemorrhage were present. The surrounding ovarian tissue was normal. Immunohistochemical attempts to demonstrate neural tissues by staining for GFAP, NSE and neuronal markers such as MAP2 and NeuN proved negative, probably due to the absence of immunoreactivity of the necrotic tissue.

Clinical follow up: There was a slight clinical improvement after surgery; dystonia was slightly reduced and the seizures stopped. The NMDAR antibody title was 1/10. Six months after the onset of symptoms and 3 months postoperatively, the patient has no more seizures, has regained consciousness, partly recovered her speech and voluntary movements and is now able to recognize family members. She is still undergoing immunosuppressant treatment.

Discussion

Most articles dealing with this new entity focus on the clinical and immunological features of the patients [5–7,11], while the histopathology of associated teratomas deals either with immunohistochemical findings of neural tissues expressing antibodies to N-methyl-D-aspartate receptors or the immunophenotype of their inflammatory infiltrates [10].

The majority of associated tumors correspond to ovarian teratomas containing neural tissues, where anti-NMDA receptor antibodies may be demonstrated in neuronal-like cells [5,10]. Only two isolated cases of testicular and mediastinal locations have been reported [6]. Unusually, this entity may take place in association with non-teratoid tumors exhibiting neuroendocrine differentiation [6,11,12]. There is only one report on a non-teratoid sex-cord stromal tumor, presumably lacking neural tissues, in the ovary [6].

63% of the ovarian teratomas reported in association with Anti-NMDAR encephalitis were mature cystic types and 27% were immature ones [6]. However, proportionally, the infrequent immature teratomas have a much higher representation in this condition than the mature cystic ones when compared with their relative overall incidence; mature cystic teratomas are the most frequent ovarian tumors in young females. The higher ratio of immature teratomas associated with this entity can be explained by the large mass of neural tissues present in them which often differentiate neurons and their precursors [13] and that are likely to be related to the formation of Anti NMDAR antibodies [5,10]. In mature cystic teratomas, neurons are present in only about 7–9% of tumors [14] which often have organoid arrangements reminiscent of central ganglia, cortex and cerebellum [15]. Furthermore, the lower frequency and smaller amounts of neural tissues present in testicular and mediastinal teratomas would partly explain their rarity in this condition.

None of the reported cases have described any particular histological features such as necrosis. The teratoma in our case had a massive coagulative necrosis, a previously unreported feature. Necrosis of a mature cystic teratoma is indeed a most unusual event in the absence of ovarian torsion, which had not occurred here as the characteristic features of venous infarction were absent. Thus, there are no objective reasons to explain the presence of necrosis in this small tumor. We do not believe that cyclophosphamide can cause necrosis of mature tissues which are identical to other adult tissues; nor is there a vasculitis phenomenon or inflammatory response that may be considered the source of ischemia. Although the advanced stage of necrosis precluded immunohistochemical identification of neural tissues that were likely to be present, since they occur in over half of cystic mature teratomas, it can be speculated that necrosis may have induced a massive liberation of neuronal antigens. Moreover, it can be hypothesized that this small necrotic tumor may eventually have been reabsorbed, leaving little imagenological evidence which could explain the poor detection rate of associated tumors in many instances. In the present case, the small size of the tumor made image diagnosis difficult, causing a delay in the final diagnosis. However, there has been a substantial improvement 3 months after surgery, consciousness has been regained and seizures have ceased. This positive response to surgery and immunosuppressant therapy stresses the fact that early diagnosis is of paramount importance, as it may result in almost complete recovery [8,9,16].

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Conclusiones

1. El saco vitelino secundario humano (SVSH) tiene un inmunofenotipo híbrido de diferenciación intestinal y hepática.

   Estas funciones de síntesis y transferencia se reflejan en la expresión de proteínas características tanto de función hepática (AFP, GPC3, SALL4 y HepPar-1), como de diferenciación intestinal (villina y CDX2). Adicionalmente, los SVSH de 5 a 8 semanas poseen un sistema de vesículas intracelulares que se comunican con las luces de los túbulos endodérmicos, sistema puesto en evidencia por la expresión de membrana de la AFP.

   Trabajo publicado:
   *Nogales FF, **Dulcey I**. The secondary human yolk sac has an immunophenotype indicative of both hepatic and intestinal differentiation. Int J Dev Biol. 2012;56(9) : 755-60. doi: 10.1387/ijdb.120080fn.*

2. El panel diagnostico inmunohistoquímico para varios tipos histológicos de tumor vitelino (tumor endodérmico primitivo) (TV(TEP) se estableció mediante comparación con el inmunofenotipo del SVSH referido en la conclusión anterior. Los patrones clásicos reproducen el inmunofenotipo del SVSH y del endodermo temprano con expresión variable de AFP y marcadores de diferenciación intestinal y hepática. Los patrones somático glandulares con diferenciación intestinal tienen un inmunofenotipo incompleto. El panel de diagnóstico diferencial propuesto incluye los marcadores de pluripotencialidad SALL4 y LIN28, y los marcadores endodérmicos AFP, GPC3 y villina. Este panel identifica la superposición de patrones de multidiferenciación en los inmunofenotipos primitivos y somáticos, lo cual apoya el término de tumor endodérmico primitivo.

   Este panel hace posible la identificación de formas poco frecuentes de TV(TEP).

   Trabajos publicados:

*Nogales FF, Quiñonez E, López-Marin L, **Dulcey I**, Preda O. A diagnostic immunohistochemical panel for yolk sac (primitive endodermal) tumours based on an immunohistochemical comparison with the human yolk sac. Histopathology. 2014 Jan 20. doi: 10.1111/his.12373*
*Preda O, Dema A, Iacob M, Goyenaga P, **Dulcey I**, Aneiros-Fernández J, Nogales FF. Urothelial carcinoma of the renal pelvis with simultaneous trophoblastic and malignant clear cell endodermal-type differentiation. Virchows Arch 2012 Mar;460(3):353-6. doi:10.1007/s00428-012-1211-5.*

3. Con estos datos de referencia aplicamos nuestro estudio comparativo al análisis de nuevos marcadores de pluripotencialidad en el campo de tumores de células germinales malignos, que implican una nueva visión de la identificación y taxonomía de estos tumores. Basados en un amplio material, analizamos el impacto de los nuevos marcadores de pluripotencialidad en el diagnóstico de los TCGM.

   Los nuevos marcadores de pluripotencialidad determinan una mayor sensibilidad diagnóstica. Los tumores de células germinales son una caricatura de la diferenciación embriológica anormal, desde las células madre y las células germinales primordiales hasta la diferenciación tejidos somáticos y extraembrionarios. Cada etapa de diferenciación exhibe marcadores característicos, cuyo análisis facilita la taxonomía tumoral. Estos nuevos marcadores también ayudan a identificar de forma precisa el grado de inmadurez de los teratomas inmaduros. El nuevo término de tumores endodérmicos primitivos, simplifica el entendimiento de la compleja histología del grupo de los tumores primitivos, así como su terminología, la cual acompaña sus diferenciaciones endodérmicas múltiples.

   Trabajos publicados:
   Preda O, Dulcey I, Nogales FF. Papel de los nuevos marcadores inmunohistoquímicos en los tumores de células germinales malignos gonadales Rev Esp Patol. 2012;45(4):195-203.
   *Nogales FF, **Dulcey I**, Preda O. Germ Cell tumors of the ovary: an update. Arch Pathol Lab Med. 2014 Mar;138(3):351-62. Doi:18.5858/arpa.2012-0547-RA*

4. Como aplicación de este análisis de marcadores a otros tipos de tumores teratoides de ovario y testículo, hemos publicado durante el periodo de realización de esta tesis, dos trabajos relacionados con teratoma maduro e inmaduro.

    a. *Nogales FF, Preda O, Dulcey I. Gliomatosis peritonei as a natural experiment in tissue differentiation. Int J Dev Biol. 2012;56(10-12):969-74. doi:10.1387/ijdb.120172fn.*
    b. *Dulcey I, Céspedes MU, Ballesteros JL, Preda O, Aneiros-Fernández J, Clavero PA, Nogales FF. Necrotic mature ovarian teratoma associated with anti-N-metyl-D-aspartate receptor encephalitis.. Pathol Res Pract. 2012 Aug 15;208(8): 497-500. doi: 10.1016/j.prp.2012.05.018*

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