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Nanoparticles Bounded to Interfering RNAs as a Therapy for Pancreatic Cancer: A Systematic Review

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ABSTRACT

Pancreatic cancer is one of the tumors with poor prognosis and low survival due to late diagnosis, high resistance, and very limited effective therapeutic options. Thus, new pharmacological treatments are necessary to improve the prognosis of patients. In this context, nanoparticles represent an efficient system for transporting and administering therapeutic molecules. Furthermore, siRNA can be used in cancer treatment to selectively inhibit the expression of any target gene. Therefore, nanoparticles associated with siRNA have been tested as a new therapeutic strategy to solve the pancreatic cancer treatment failure in the clinical setting. The current article presents a systematic revision of the literature of the last 10 years in which nanoparticles loading siRNA are used in pancreatic cancer. This research was carried out in three databases (PubMed, Scopus, and Web of Science) obtaining 164 articles from which 37 were selected. Our results show an overall view of the high effectiveness of this new therapy that combines nanoparticles with genetic therapy in pancreatic cancer suggesting that siRNA-based medicines will likely open up a new therapeutic era in the treatment of this type of tumors.

1 | Introduction

Pancreatic cancer (PC) is one of the leading causes of cancer-related deaths. It is expected to become the second leading cause of cancer-related death in the United States by 2040, overtaking colorectal cancer and falling behind lung cancer. The main problem causing these high mortality rates of PC is the difficulty in detecting the disease until it has reached an advanced stage of development, due to the lack of symptomatology of the disease when it is still localized, so it is often detected after it has already metastasized (Kleeff et al. 2016). The risk factors associated with this disease are diverse, including unhealthy habits such as smoking or alcohol or diseases with a predisposition to generate PC such as chronic pancreatitis, in addition a large number of cases of PC are attributed to activating mutations of oncogenes such as KRAS, mutations in tumor suppressor genes such as CDKN2A, TP53, and SMAD4 and genetic alterations in DNA damage repair pathways and cell cycle regulation (Wood, Yurgelun, and Goggins 2019).

The main therapeutic strategies for pancreatic cancer is based on chemotherapy and surgery, although only 15%–20% of patients are suitable for the latter at the time of diagnosis (Kleeff et al. 2016), this is because most patients are diagnosed at

The first two authors contributed equally to this article.

metastatic stages, where tumor resection is no longer possible. To allow a more effective resection, neoadjuvant therapy is carried out (Mavros et al. 2021), trying to reduce the tumor size and thus facilitating resection. Because of this, the patients who show a significant pathological response to neoadjuvant therapy have a longer overall survival (Cloyd et al. 2017). In terms of chemotherapy, two combination treatment options are normally used, the first being the combination of 5-fluorouracil (5-FU), leucovorin, irinotecan, and oxaliplatin, called FOLFIRINOX, while the second option is the combination of gemcitabine and nab-paclitaxel (Rhim et al. 2012). Unfortunately, three out of four patients chosen for surgical resection usually develop new disease after 2 years, so to eliminate any tumor cells that may have remained after surgery, patients are usually treated with adjuvant chemotherapy postoperatively (Neoptolemos et al. 2017). However, chemotherapy is not entirely effective in pancreatic cancer as it is a highly resistant cancer. One of the most challenging and resistance-promoting features of pancreatic cancer is the tumor microenvironment, comprising many fibroblasts that compose a dense matrix that favors the physiological properties characteristic of pancreatic cancer, as well as the presence of tumor stem cells (Halbrook et al. 2023).

In this context, overcoming resistance to chemotherapy creates the need to look for new alternatives that have greater efficacy in the treatment of pancreatic cancer. In recent years, nanomedicine has emerged as a promising therapy in cancer treatment and diagnosis. There are a wide range of nanoformulations with dimensions ranging from 1 to 100 nm. Their size and characteristics endow them with the ability to be an optimal vehicle for drug delivery, some of their advantages include minimization of side effects of tumor agents, better targeting of the affected area, and higher concentration of drugs in the tumor area (Garbayo et al. 2020). Both organic and inorganic nanoparticles have been used to improve cancer treatment (Aghebati-Maleki et al. 2020). In fact, lipid-based nanoparticles have led the way as the first therapeutic nanoplatform clinically approved by the FDA for the treatment of cancer (Chaudhuri et al. 2022).

Gene therapy has also played an important role in cancer treatment. In this regard, RNA interference (siRNA) can silence the expression of specific genes with complementary sequences. The sequence of a therapeutic siRNA can be obtained based on the sequence of the mRNA that would encode the target protein (Dong, Siegwart, and Anderson 2019). Synthetic siRNAs stand out as the most suitable RNAi for use as drugs in cancer treatment, as they have advantages over other chemotherapeutic drugs and anticancer agents (Saw and Song 2020). siRNAs of 20 nucleotides in length can recognize any target gene with high specificity and minimal off-target effects, this is due to their recognition mechanism based on complementary base pairing, even with few nucleotides, siRNAs can induce significant gene silencing effects in the cell. Moreover, siRNAs act exclusively in the cell cytoplasm, generating their gene-silencing effect without entering the nucleus or integrating into the genome, which significantly reduces the risk of causing genetic mutations in the host. Another advantage of siRNA therapy is the unlimited number of possible targets. Advances in molecular biology and whole genome sequencing have enabled the creation of extensive human genome databases, cDNA libraries, and cancer-associated gene databases. By designing siRNAs based on the mRNA sequence of the specific gene, it is possible to develop an siRNA that effectively silences any cancer-causing gene (B. Hu et al. 2020; Singh, Trivedi, and Jain 2018). Recent advances in nanotechnology have led to the development of novel systems for the delivery of siRNA to cells in the tumor microenvironment, reaching both tumor cells and infiltrating immune cells (Siegler, Kim, and Wang 2016). siRNAs can be incorporated into an NP formulation by covalent bonds with the NP components or by electrostatic interactions with its surface given its negative charge (Acharya 2019). Consequently, the aim of this systematic review is to compile the most recent information on nanoparticles using siRNA as a treatment for pancreatic cancer and to deepen our understanding of them.

2 | Materials and Methods

2.1 | Study Eligibility

The purpose of this systematic review was to examine the most up-to-date and representative information on research examining the therapeutic effectiveness of nanoparticles using RNA interference as a treatment for pancreatic cancer. This review has been carried out following the guidelines indicated in the PRISMA 2020 statement (Page et al. 2021). Moreover, it was previously registered in OSF database on 24 January 2024 with the DOI: 10.17605/OSF.IO/UF7Y3.

2.2 | Inclusion Criteria

This systematic review included scientific research published between January 2013 and December 2023, which had full text available, written in English or Spanish, and passed the established quality criteria.

2.3 | Exclusion Criteria

Studies that did not incorporate RNA interference inside the nanoparticle, using RNA interference as a separate treatment from the nanoparticle, were excluded. Articles were also excluded if they did not pass the established quality criteria, such as if the nanoparticle synthesis methodology was not properly defined. In addition, systematic reviews, literature reviews, meta-analyses, and books were excluded.

2.4 | Data Sources and Search Strategy

This systematic review has been carried out by means of an exhaustive literature search in three different databases: PubMed, SCOPUS, and Web of Science. For the PubMed search, the following MeSH terms were used: "nanoparticles," "RNA, Small Interfering," and "pancreatic neoplasms," resulting in the following search equation: (nanoparticles[MeSH Terms]) OR (Material, Nanocrystalline[Title/Abstract]) OR (Materials, Nanocrystalline[Title/Abstract]) OR (Nanocrystal[title/abstract]) OR (Nanocrystalline Material[title/abstract]) OR (Nanocrystalline Materials[title/abstract]) OR (Nanocrystals[title/abstract]) OR (nanoparticles[title/abstract]))

AND ((RNA, Small Interfering [MeSH Terms]) OR (Hairpin RNA, Short [title/abstract]) OR (Hairpin RNA, Small [title/abstract]) OR (Interfering RNA, Short [title/abstract]) OR (Interfering RNA, Small [title/abstract]) OR (RNA, Scan [title/abstract]) OR (RNA, Short Hairpin[title/abstract] OR (RNA, Short Interfering[title/abstract]) OR (RNA, Small Hairpin[title/abstract]) OR (RNA, Small Scan[title/abstract]) OR (Repeat Associated siRNA[title/abstract]) OR [Repeat-Associated siRNA[title/abstract]) OR (Scan RNA [title/abstract]) OR (Scan RNA, Small[title/abstract]) OR (Short Hairpin RNA[title/abstract]) OR (Short Interfering RNA[title/abstract]) OR (Small Hairpin RNA[title/abstract]) OR (Small Interfering RNA[title/abstract]) OR (Small Scan RNA[title/abstract]) OR (Trans Acting siRNA[title/abstract]) OR (Trans Acting siRNA[title/abstract]) OR (Trans- Acting siRNA[title/abstract]) OR (scnRNA[title/abstract]) OR (shRNA[title/abstract]) OR (siRNA[title/abstract]) OR (tasiRNA[title/abstract])) AND ((pancreatic neoplasms [MeSH Terms]) OR (pancrea* cancer*[title/abstract]) OR (pancrea* neoplasm*[title/abstract])))))) NOT (review [Publication Type]) NOT (systematic review [Publication Type]). The equation designed for the SCOPUS search was: (TITLE-ABS (pancreatic AND neoplasms) OR TITLE-ABS (pancrea* AND neoplasm*) OR TITLE-ABS (pancrea* AND cancer*)) AND (TITLE-ABS (rna, AND small AND inteTITLE-ABS (sirna) OR TITLE-ABS (shrna)) AND rfering) OR TITLE-ABS (nanoparticle*) AND (LIMIT-TO (DOCTYPE, "ar")). Finally, the following equation was used to perform the search in Web of Science: (TI=("pancrea* neoplasm*" OR "pancrea* cancer*" OR "pancrea* tumor*") OR AB=("pancrea* neoplasm*" OR "pancrea* cancer*" OR "pancrea* tumor*")) AND (TI=("RNA small interfering" OR "siRNA" or "shRNA") OR AB=("RNA small interfering" OR "siRNA" or "shRNA")) AND ((TI=(nanoparticle*) OR AB=(nanoparticle*)).

2.5 | Selection of Studies

The authors F.Q. and P.L. carried out the first bibliographic search, which yielded 164 articles. First, the articles that were duplicated in the different databases were eliminated, leaving 83 articles. Then, after checking whether they met the inclusion and exclusion criteria, the articles were selected by title and abstract. Authors P.L. and F.Q. carried out a thorough review of the full texts using predefined criteria to assess quality (Table 1).

Scoring was assigned by considering aspects essential to the quality of the article, 3 points, important elements to ensure quality, 2 points, and finally 1 point was assigned to complementary factors. Those articles scoring below 13 points were discarded due to their low quality, with articles scoring 12–16 points being considered medium quality, and those scoring above 17 points high quality. In case of discrepancy in the score between the two authors, a third reviewer was included to determine the final score.

2.6 | Data Extraction

After the selection of studies, authors P.L. and F.Q. carried out independent analysis of the selected articles for data extraction. The information extracted covered the cell lines in which the experiments were performed, the type of nanoparticle, the cell internalization capacity of the nanoparticle, the IC_{50} value of the nanoparticles in the cell lines studied, whether or not the nanoparticle is loaded with another drug, whether it is evaluated to decrease RNA expression by PCR, the main results obtained in experiments performed in vitro and the findings obtained in in vivo experiments, if performed.

TABLE 1 | Factors valued for developing quality assessment.

3 points	The article is peer-reviewed
	IC50 values are specified
	The type of nanoparticle is mentioned
2 points	The internalization capacity is analyzed
	Synthesis methodology well explained
	The results shown have an error bar (SD)
1 point	A well-detailed methodology
	At least one non-tumor line is included
	2 or more cell lines are tested
	Expression is studied by QPCR
	An in vivo test has been carried out

3 | Results

3.1 | Study Description

Initially, 164 articles were found in the different databases consulted. After discarding 81 articles that were duplicates and those that were not related by title and abstract, a total of 61 articles were selected for detailed analysis. Of these, 24 were discarded because they did not meet the established inclusion criteria and 21 due to the low score obtained in the quality test. Finally, a total of 37 articles were added to the systematic review. Figure 1 shows the flow chart showing the search process and the selection of articles.

The number of articles published on NPs that incorporate siRNA has remained stable over time, with the last year, 2023, being when the most publications were found in this regard (five articles), while the lowest number of publications were found in the years 2013 and 2014 (one and two articles, respectively) (Figure 2A). After carrying out the analysis of the articles, it has been possible to observe the great diversity of nanoformulations recently used for pancreatic cancer therapy. Some types of NPs that have been used although less usually are micellar NPs (H. S. Min et al. 2018), perfluoroalkyl NPs (Yu et al. 2022). In addition, it has been observed that most of the articles do not encapsulate drugs in their nanoformulations, only 13 articles incorporate drugs in their studies (Figure 2B), of which gemcitabine seems to be the drug of choice, accounting for 46% of the total drugs analyzed in the review, followed by paclitaxel with 23%. To determine the internalization capacity of the NPs by the cells, different analytical techniques were used (Figure 2C). Among them, immunofluorescence stands out, which has been

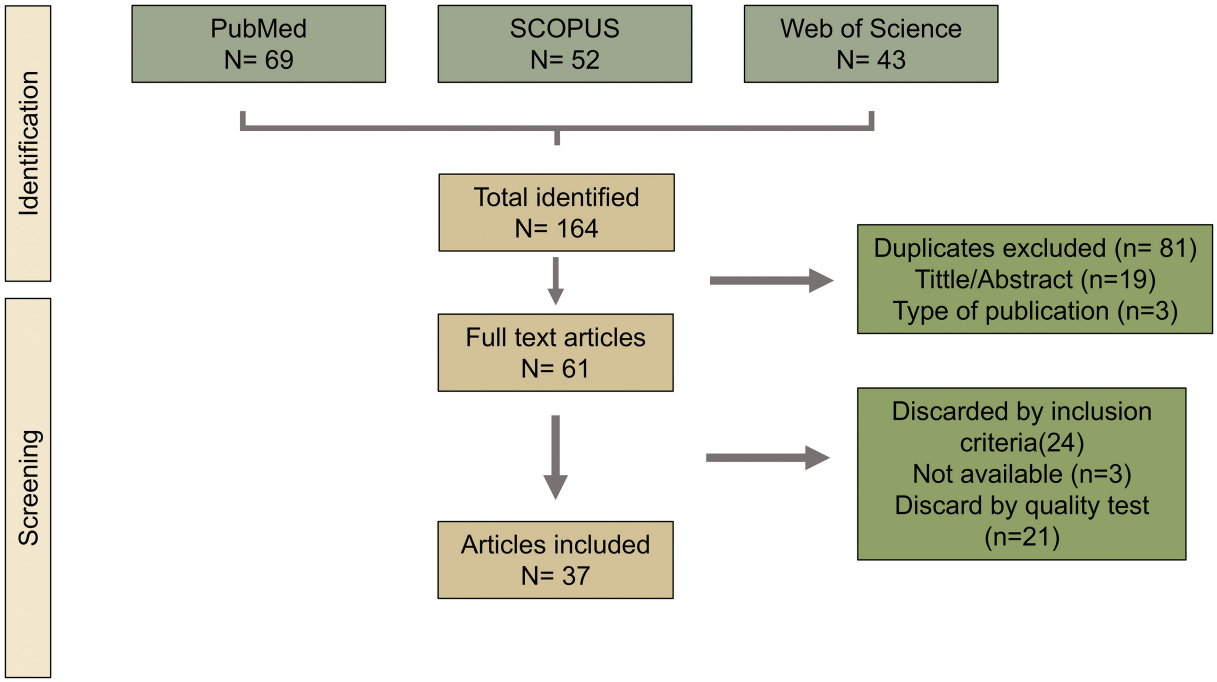

FIGURE 1 | Flow diagram that represents the articles included in the systematic review.

implemented in a total of 15 articles, allowing the internalization of NPs to be visualized and quantified by detecting specific markers or the NPs themselves labeled with fluorophores. In addition, other techniques such as confocal microscopy or flow cytometry have been used for the quantification of NP internalization.

After a detailed analysis of the articles, it has been possible to identify a wide diversity of target genes for siRNAs incorporated in NPs (Figure 2D). Among these genes, the KRAS gene stands out, which has been the subject of study in a total of 13 articles, representing approximately 36% of the investigated set. This high prevalence of studies on the KRAS gene suggests its importance in pancreatic cancer research. In addition to KRAS, other genes have also been of interest. For example, research related to the PLK-1 gene has been found in 4 articles. Similarly, the PD-L1 gene has been studied in two articles. However, the analysis reveals that there is a wide variety of other specific genes that have also been the subject of research, gaining importance in the field of gene therapy and nanomedicine.

The cell lines most frequently used in the articles analyzed are represented in Figure 2E. Prominent among these cell lines is PANC-1, identified as the preferred cell line to carry out the research. This cell line has been used in a total of 21 articles, which highlights its importance and its wide use as an experimental model. Following the PANC-1 cell line, we find the BxPC-3 cell line, present in nine articles that choose it as a study line. In addition, the MIA PaCa-2 cell line has also been identified as one of the most used cell lines in the studies analyzed, being mentioned in a total of seven articles. Although in a smaller proportion than the previous ones, their importance in pancreatic cancer research is also highlighted. These lines have been selected by researchers for various reasons, which may include their biological relevance, their availability, or their ability to replicate the pathological conditions studied.

In the analysis of the in vivo trials performed, covering a total of 31 articles, a wide variety of parameters were studied to evaluate the efficacy of the proposed treatments (Figure 2F). In this context, it is noteworthy that approximately 63% of the in vivo trials analyzed the decrease in tumor volume as a measure of response to treatment. This reflects the importance attributed to tumor size reduction as a key indicator of therapeutic efficacy and tumor progression. In addition, about 23% of the analyzed articles investigate metastasis, recognizing metastasis as a critical aspect in disease progression and prognosis. Finally, it is identified that 14% of the articles focus on analyzing median survival as a key indicator of treatment efficacy, providing crucial information on the clinical benefit of patients.

3.2 | Lipidic Nanoparticles

Current FDA-approved lipid nanoparticle formulations are composed of four lipids: an ionizable cationic lipid, ancillary lipids that include 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and a polyethylene glycol (PEG)-lipid conjugate (Figure 3). These constituents have advantages in nanoparticles, facilitating the formation of monodisperse nanoparticles, enabling efficient encapsulation of nucleic acids, aiding cellular uptake, and improving nanoparticle stability (Hald Albertsen et al. 2022).

In this perspective, Anthiya et al. developed a therapeutic strategy based on anti-KRAS siRNA encapsulated in lipid NPs functionalized with the tLyp-1 peptide that recognizes the neuropilin-1 receptor overexpressed in some tumor types. They carried out trials in the CFPAC-1 line, where they also combined anti-KRAS siRNA treatment with gemcitabine, first, extensive in vitro studies were carried out to identify the siRNA constructs with the highest KRAS silencing efficiency. Once the appropriate siRNA was selected and tested for proper internalization

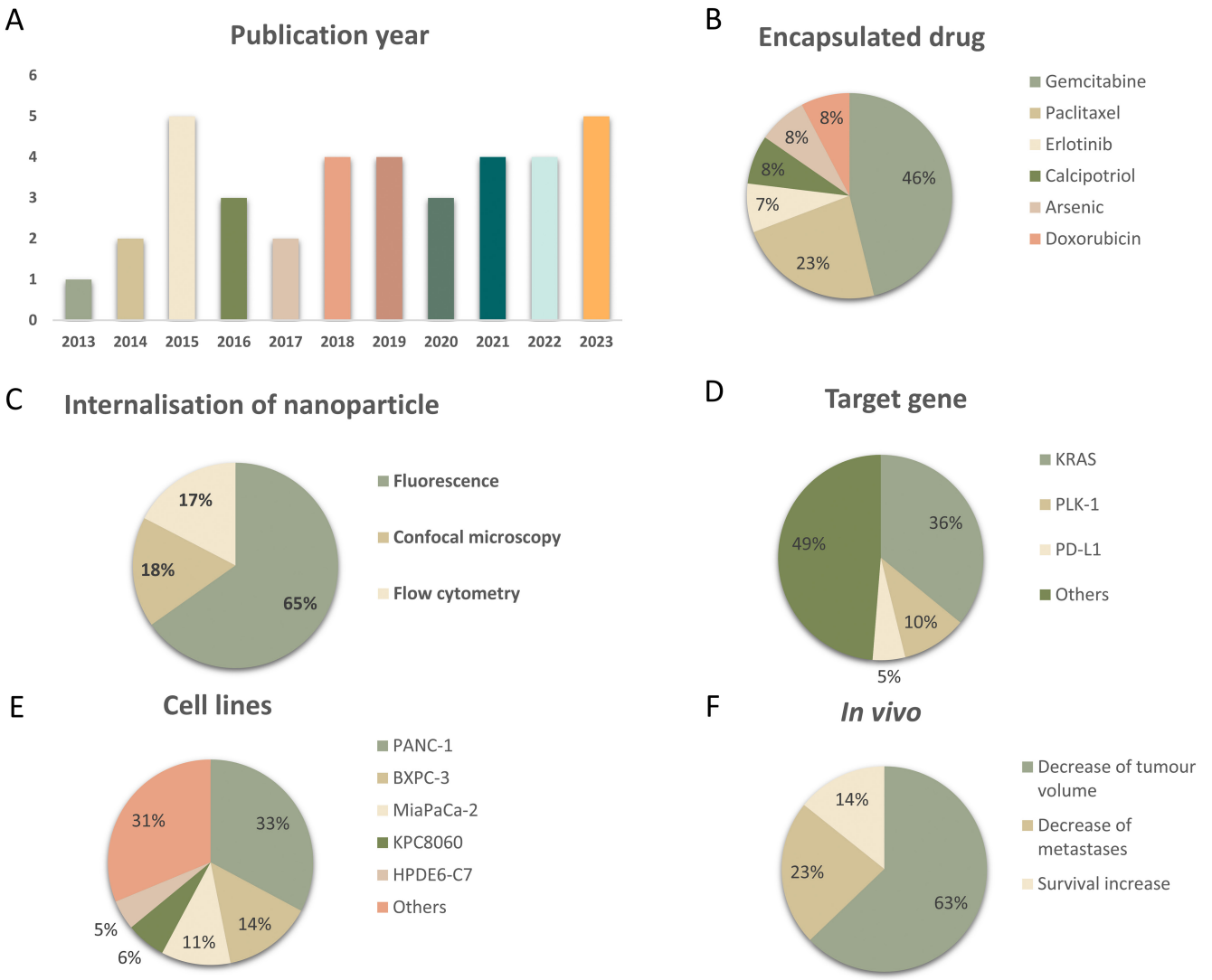

FIGURE 2 | Data of the of articles included in the manuscript. (A) Number of publications per year. (B) Percentage of articles that incorporate the mentioned drugs. (C) Percentage of articles that use the mentioned techniques to analyze internalization capacity. (D) Main genes studied. (E) Cell lines most used in the articles described. (F) Main parameters measured by the articles analyzed in the in vivo experiments.

into the cell, treatment was combined with gemcitabine, and observed that NP therapy enhanced the effect of the drug. In addition, these authors demonstrated the safety of repeated dosing of the treatment in healthy mice at higher doses than previously administered, so that, this treatment claims to have a better safety profile (Anthiya et al. 2023). On the other hand, Xie et al. conducted an anti-miR-210 and siKRAS^{G12D} siRNA nanotherapy based on the polymeric antagonist CXCR4 using pancreatic cancer lines KPC8060 and COLO357. The triple-action nanotherapy favorably modulated the tumor microenvironment by inactivating pancreatic stellate cells (PSCs), inhibiting cancer cells and blocking cancer-stroma interaction. The combination therapy showed an improved therapeutic effect compared to individual therapies, resulting in delayed tumor growth, reduced fibrosis and prolonged survival (Xie et al. 2020). Following the therapy using anti-KRAS siRNA, a liposome-coated gold NP (LPAR) sensitive to near-infrared (NIR) light was developed. The authors of this study demonstrated that LPAR exhibited high photothermal conversion efficiency and NIR-controlled siRNA delivery capability and could inhibit the mutant KRAS gene in PANC-1 pancreatic cancer cells. The results showed that the internalization of the NPs in the presence of NIR was successful, furthermore, the inhibition of KRAS by treatment with encapsulated siRNA showed an inhibition of cell viability of 92.5%, following these promising results, in vivo assays were carried out in which a tumor reduction of 96.6% was observed, thus achieving a photothermal synergistic therapy with a high efficiency for the treatment of pancreatic cancer (Jia et al. 2022). Zeng et al. demonstrated that the combination of siRNA targeting silencing of the KRAS oncogene and induction of apoptosis by arsenic using a nanomedicine strategy exerted a more potent effect on the study cells, PANC-1, and BxPC-3, than the use of siRNA or arsenic alone both in vitro and in vivo. Following combination treatment, there was a 34.32% inhibition of cell proliferation and a 41.43% apoptosis rate, which was reflected in in vivo assays showing a clear reduction in tumor growth, the tumor volume of the As-NP treated mice was $174.89 \pm 16.63\,mm^3$ and for the si + As-NP mice $151.01 \pm 6.94\,mm^3$, in addition, the combination therapy led to cell cycle arrest in G_0/G_1 phase (Zeng et al. 2014). Wang et al. also carried out a combined treatment,

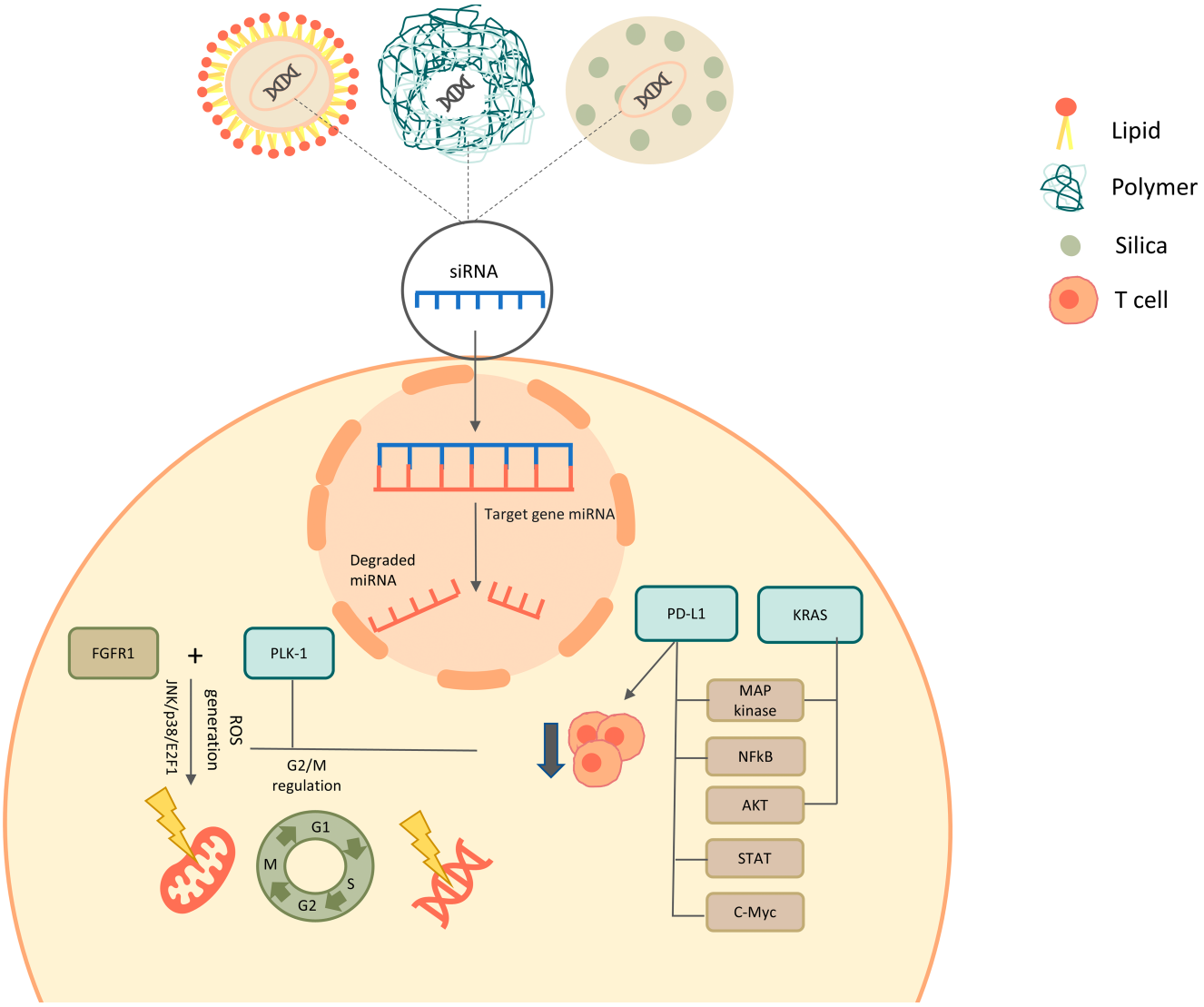

FIGURE 3 | Graphic representation of action mechanisms of the NPs. Nanoparticles include in their interior siRNAs, molecules capable of binding to a target miRNA, producing its degradation through internal processes in the cell. The NPs mostly described in the article inhibit the gene expression of three targets involved in the development of pancreatic cancer such as KRAS, PL-D1, and PLK-1. These genes are responsible for regulating certain pathways such as MAP-kinase (MAPK), PI-kinase (PI3K)/AKT/mTOR, MAPK, STAT, or c-Myc, reducing cellular malignancy processes such as cell proliferation, migration, and metastasis after their inhibition.

in this case, the authors demonstrated that paclitaxel enhances the release of the pegylated cationic NP complex-siRNA anti-survivin, an inducible chemoresistance gene, in the Hs766T cell line. The results obtained showed a decrease in the IC_{50} level in the combined treatment with respect to paclitaxel and siSurvivin separately, with a value of 145 nM at 22 h of treatment. As for the results of the in vivo studies, co-administration of PCat-siSurvivin and paclitaxel significantly enhanced the efficacy of the treatment, reporting tumor regression of 29% and a delay in tumor growth. In addition, in the group receiving both treatments, median overall survival increased to 21 days, while the control groups showed a median overall survival of 7–10 days. Finally, the toxicity of the treatment did not result in a greater loss of body weight than in the control groups (J. Wang, Lu, et al. 2015). Continuing with the combined treatments, Tang et al. designed a biomimetic drug delivery system, consisting of membrane-coated NPs, for the co-delivery of gemcitabine (GEM), erlotinib (Er), and anti-IRAK4 siRNA. The study was conducted in pancreatic cancer lines PANC-1 and SW-1990. Silencing of IRAK4 by siRNA leads to suppression of tumor fibrosis and improved penetration and efficacy of the drugs into the cells, with a cell apoptosis rate of 60% after treatment. In vivo trials also show encouraging results for this treatment, prolonging the average survival of mice and decreasing tumor cell proliferation (H. Tang et al. 2022). The genes ARHGEF4, CCDC88A, LAMTOR2, mTOR, NUP85, and WASF2 related to pancreatic cancer invasion and metastasis were the focus of the study of Taniuchi et al. who used folic acid (FA)-modified polyethylene glycol (PEG)-chitosan oligosaccharide lactate (COL) nanoparticles (siRNA-FA-PEG-COL NPs) to inhibit them. All of them showed a significant reduction of their expression both in vitro in S2-013, PANC-1, and HPNE lines and in vivo. Inhibition of all six genes led to reduced cell motility and invasion in vitro and retroperitoneal invasion and distant metastasis in vivo in an orthotopic mouse model of pancreatic cancer. NPs against LAMTOR2, mTOR, and NUP85 showed a greater

inhibitory effect on peritoneal dissemination and mice survival, suggesting that these genes could be promising therapeutic targets for pancreatic cancer. Furthermore, these NPs showed no systemic toxicity in vitro or in vivo, making them safe and biocompatible (Taniuchi et al. 2019) (Table 2).

3.3 | Polymeric Nanoparticles

Over the past few years, polymeric nanoparticles (Figure 3) have generated great interest due to their small size (Cano et al. 2020). The advantages of these nanoparticles as drug vehicles include their potential for controlled release, the ability to protect drugs and other biologically active molecules, and to improve their bioavailability and therapeutic index (Owens and Peppas 2006).

In this context, Jung et al. developed a PLGA-based NP carrying siRNA targeting programmed death ligand 1 (PD-1/PD-L1). According to the results obtained, treatment with siPD-L1@PLGA increased the susceptibility of pancreatic cancer cells to ovalbumin-specific T cell-mediated lysis, determining that the optimal NP concentration for in vitro assays was 2 mg/mL. In vivo assays, NPs significantly reduced tumor size and increased CD8 T cells for IFN-gamma as well as the number of apoptotic cells (Jung et al. 2021). In this sense, PD-L1 is the subject of studies carried out by Wang et al. The researchers synthesized NPs capable of releasing TGF-β receptor inhibitors (LY2157299) and PD-L1-targeted siRNA in response to the acidic pH of the tumor microenvironment. Following assays, it was determined that these NPs could reduce type I collagen production, which facilitates T-cell infiltration into the tumor, and may also increase CD8+ T-cell cytotoxicity (Y. Wang et al. 2020). Agbaria et al. carried out the synthesis of PLGA NPs encapsulating siRNA against the VAV1 protein and the anti-tumor peptide LL37, using Dan-G and PANC-1 cell lines in which there was a 50% and 35% inhibition of proliferation, respectively, after treatment with siVAV1-LL37. Furthermore, after treatment with these NPs, tumor volume in in vivo assays was 1.6-fold lower than with treatment alone (Agbaria et al. 2023).

On the other hand, Li et al. carried out a combined treatment of paclitaxel-encapsulating NPs and siRNA against the nuclear receptor TR3. For in vitro assays, they used the pancreatic cancer cell line PANC-1. The authors demonstrated that co-delivery of PTX and siTR3 by the PSPGP nanovehicle had a synergistic effect both in vitro and in vivo by reducing the expression of anti-apoptotic proteins Nur77, Bcl-2, and Survivin, which led to decreased proliferation, induced apoptosis and reduced expression of anti-apoptotic genes. TR3/Nur77 activation promoted apoptosis and decreased tumor growth. In addition, NP biodistribution studies were performed in which mice treated with PSPGP-siRNA showed a higher intensity fluorescent signal at the tumor site compared to mice given the drug and free siRNA (Y. Li et al. 2017). EPAS1, on the other hand, is a hypoxia-inducible transcription factor that contributes to pancreatic cancer progression, in this context, PLGA/poloxamer NPs loaded with anti EPAS1 siRNA have been developed. These nanoparticles could inhibit cell proliferation and induce apoptosis in the BxPC-3 cell line under hypoxic conditions in vitro, and in vivo assays significantly reduced tumor volume and microvascular density in the mouse (Pan et al. 2015). In this context of the development of polymeric NPs, Zhao et al. synthesized lipid-polymer hybrid NPs (LENPs) for the co-delivery of siRNA against HIFα and gemcitabine. These NPs showed a synergistic antitumor effect both in vitro in the PANC-1 cell line, where a 91% down-regulation of HIF1α mRNA expression was observed in the LENP-si-HIF1α-treated groups, and the expression of the HIF1α downstream gene VEGF decreased by 70%. The in vivo results when combined LENP-si-HIF1α with gemcitabine, showed a good biocompatibility and a low immune response (Zhao et al. 2015).

Wang et al. studied the effect of a nanodrug incorporating calcipotriol and anti-CXCL12 siRNA in inactivating pancreatic stellate cells (PSCs) and remodeling the immunosuppressive microenvironment of pancreatic cancer. The nanodrug showed pH-sensitive release of calcipotriol and siRNA into PSC lysosomes, leading to a decrease in collagen I and CXCL12, markers of PSC activation and fibrosis. In addition, regulatory T cells were also reduced and cytotoxic T cell infiltration into pancreatic tumors increased, which enhanced the response to immune checkpoint blockade with anti-PD-L1 antibodies (R. Wang et al. 2023). Within this classification of polymeric NPs, there are also less common nanocarriers such as star polymers (Teo et al. 2016), the authors of this study carried out the synthesis of three-star polymers (star 1, star 2, star 3), for siRNA delivery to MIA PaCa-2, HPAF-II, and HPDE cell lines. These polymers were designed to contain different lengths of cationic poly (dimethyl aminoethyl methacrylate) (PDMAEMA) side arms and different amounts of poly [oligo (ethylene glycol) methyl ether methacrylate] (POEGMA). The star-POEGMA polymers were shown to be non-toxic to cells and were highly effective in delivering siRNA to silence the TUBB3/βIII-tubulin gene, which is involved in regulating tumor growth and metastasis. In vitro results showed that the star polymer 2-siRNA decreased cell viability by 15%, while for the polymers without POEGMA, the decrease was 90%. In addition, the star polymer 3-siRNA reduced TUBB3/βIII-tubulin protein expression by 80%. In contrast, Tang et al. developed an siRNA delivery system based on the polymeric CXCR4 antagonist modified with α-tocopherol for silencing STAT3 expression. In vitro studies were performed on KPC8060 and PANC-1 cell lines and demonstrated an apoptosis rate of 45% in the KPC8060 line and 24% in PANC-1, after performing a colony formation assay, an inhibition of colony-forming ability of 69% in KPC8060 and 75% in PANC-1 was observed. Migration rate was reduced by 89% in the KPC line and 77% in PANC-1. Both lines showed cell cycle arrest in G_2/M phase. Treatment with these NPs also led to a decrease in Bcl-2 protein expression by 31% in KPC8060 and 77% in PANC-1 (S. Tang et al. 2023). Burks et al. developed a polyplex NP that selectively binds to the cholecystokinin-B receptor (CCK-B) overexpressed in pancreatic cancer for the delivery of anti-gastrin siRNA to block tumor growth and metastasis. A correct internalization of siRNA was determined in AsPC-1, BxPC-3, and PANC-1 cell lines, leading to a decrease in cancer processes such as proliferation and migration, achieving the highest rate of inhibition of proliferation at a concentration of 480 nmol/L of siRNA (Burks et al. 2018). The chemokine receptor CXCR4 and polo-like kinase 1 (PLK1) play crucial roles in pancreatic cancer progression, metastasis, and chemoresistance, in this sense, the construction of polyionic complex (PIC) micelles with anti-tissue factor (TF) antibody fragments (Fab´) for the delivery of

TABLE 2 | Summary of the most relevant characteristics of the lipid nanoparticles.

Reference	Cell lines	Type of nanoparticle	Drug loaded	Target gene	In vitro results	In vivo results
Anthiya et al. 2023	CFPAC-1	Lipidic NPs functionalized with tLyp-1	GEM	KRAS	Decrease of ERK expression and inhibition of cell proliferation	Reduction in tumor size, decreased tumor KRAS expression, and pERK activation in CFPAC-1 xenograft model
Xie et al. 2020	KPC8060, COLO357	Polymeric CXCR4 antagonist NPs	PCX	miR-21 and KRAS	Induction of apoptosis, decreased migration, and colony formation	Decreased metastasis, decreased tumor size, and increased survival in KPC8060 xenograft model
Jia et al. 2022	PANC-1	LPAR NPs	—	KRAS	Decreased KRAS protein expression with LPAR + siRNA and increased apoptosis induction	High NPs arrival in the tumor, decreased tumor volume, and decreased KRAS protein expression in PANC-1 xenograft model
Zeng et al. 2014	PANC-1, BxPC-3	PEG-PLL NPs	Arsenic	KRAS	Decreased KRAS protein expression, cell proliferation inhibition, induction of cell cycle arrest, and decreased colony formation and migration	Decreased tumor volume in si + Asp-NP mice, decreased Bcl-2 protein expression and apoptosis induction in PANC-1 xenograft model
J. Wang, Lu, et al. 2015	Hs766T	PEGylated cationic lipoplexes (PCat)	PCX	Survivin	PCX + PCat-siSurvivin increased the presence of siSurvivin in tumor cells	PCat-siSurvivin + PCX delayed tumor growth and increased mice survival, PCat-siSurvivin did not showed toxicity and reversed survivin induction by PCX, enhancing PCX-induced apoptosis in Hs766T xenograft model
H. Tang et al. 2022	PANC-1, SW1990	Membrane-coated NPs	GEM, ER	IRAK4	siIRAK4/Er@GEM-SS-PC-M decreased colony formation and migration in both cell lines	Tumor growth and metastasis inhibition, apoptosis induction and increased mice survival in SW1990 xenograft model
Taniuchi et al. 2019	S2-013, PANC-1, HPNE	FA-modified COL-PEG lactate NPs	—	ARHGEF4, CCDC88A, LAMTOR2, mTOR, NUP85, WASF2	Decreased CCDC88A and WASF2 protein expression, inhibition of cell motility and suppression of ARHGEF4, LAMTOR2, mTOR, and NUP85 genetic expression	Decreased protein expression of CCDC18 and WASF2, metastasis inhibition, and increased mice survival in S2-013 xenograft model

Abbreviations: COL: oligosaccharide lactate; ER: erlotinib; GEM: gemcitabine; LPAR: Lipo-PBA-Au-Crgd; NP: nanoparticle; PCX: paclitaxel; PEG: polyethylene glycol; PEG-PLL: poly(ethylene glycol)-block-poly-l-lysine.

anti-PLK1 siRNA was carried out by Min et al. Micelles with three Fab´ molecules (3(Fab´)-micelles) showed the highest binding affinity to BxPC-3 cells expressing TF on their surface. The results concluded that PIC micelles with Fab´ have a high anti-cancer potential (H. S. Min et al. 2018). In this regard, Tang et al. carried out the combination of a polymeric CXCR4 antagonist (PAMD-CHOL) with anti-PLK1 siRNA which has been shown to enhance the therapeutic effect of gemcitabine. PAMD-CHOL/siPLK1 NPs were developed and tested in KPC8060 and S2-013 cell lines. These NPs showed a high antitumor capacity supported by the IC_{50} values obtained, PAMD-CHOL for KP8060 had an IC_{50} of 85.6 μg/mL and for S2-013 33.2 μg/mL, while PAMD-CHOL/siPLK1 + GEM NPs for KPC8060 had an IC_{50} of 4.9 μM and for S2-013 58 μM (S. Tang et al. 2021). On the other hand, Yan et al. carried out the synthesis of NPs composed of quantum dots, graphene (GQD), and biodegradable polyester vectors (BCPV) to perform a synergistic activated light therapy for pancreatic cancer therapy. The NPs were able to load doxorubicin (DOX) and KRAS-targeted siRNA, following promising results in in vitro assays and improved release of the NPs in PANC-1 and MIA PaCa-2 cell lines after laser light irradiation, mouse trials showed that GQD/DOX/BCPV/siRNA NPs led to an apoptosis of tumor cells of 40.5%, while when these NPs were irradiated with laser light, the apoptosis rate increased to 74.1% (C. Yang et al. 2019). Authors such as Wang et al. observed a similar effect when developing nanocomplexes of manganese and zinc selenide doped quantum dots (d-dots) and siRNA for the delivery of siRNA molecules to PANC-1 pancreatic cancer cells by inducing KRAS silencing. A decrease in KRAS gene expression was observed, furthermore, cell viability studies showed that the d-dots/PAH nanocomplexes were biocompatible and non-toxic even at high concentrations such as 160 μg/mL (Y. Wang, Yang, et al. 2015). In turn, Lo et al. developed nanocomplexes incorporating anti-KRAS siRNA using the iRGD peptide as a tumor penetration domain. The results affirmed significant silencing of KRAS at both the gene and protein level. Specifically, they found that tumor doubling time was extended to 13.3 days in the siKRAS-treated mice compared to 10.1 days in the siRNA-targeted mice, demonstrating that NP-siKRAS is highly distributed in tumor tissue and delays tumor growth (Lo et al. 2018).

Hu et al. developed aggregation-induced emission (AIE) polymeric NPs for delivery of anti-KRAS siRNA to MIA PaCa-2 cells. The authors used two FDA-approved surfactant polymers, Pluronics F127, and pegylated phospholipid, for the synthesis of the AIE NPs. After verifying the successful internalization of the NPs into cells and reduction of KRAS expression, both in vitro and in vivo assays were performed, proving the hemocompatibility and stability of the NPs (R. Hu et al. 2015). The biodegradable cationic poly (lactic acid) (CPLA) NPs are shown to be a good nanocarrier for the delivery of anti-KRAS siRNA to the PANC-1 cell line (Lin et al. 2013). The authors of this study claimed 90% cell transfection efficiency of the NPs. In addition, the use of white NPs that were non-toxic to cells was tested, resulting in 90% cell viability. In this regard, Yang et al. developed a NP based on a new biodegradable polyester-based loaded biodegradable vector (BCPV) also for the delivery of anti-KRAS. The article claims a significant decrease in tumor malignancy processes after studies in the MIA PaCa-2 line following KRAS inhibition by BCPV NPs (C. Yang et al. 2015). In line with previous articles, Strand et al. developed a peptide-based NP system loaded with anti-KRAS siRNA. To carry out the study, these NPs were tested in KPC-1, BxPC-3, KCKO, Capan-1 cell lines, and the results showed a cell death rate of 53%–55% in the KPC-1 line after treatment with the NPs. In addition, an in vivo study showed a 4.6-fold reduction in tumor volume in NP-treated mice compared to saline-treated mice (Strand et al. 2019) (Table 3).

3.4 | Inorganics Nanoparticles

Of all the types of NPs that have been studied, inorganic NPs (Figure 3) are one of the most interesting because of their characteristics that allow for drug protection and bioavailability, targeted action, limited adverse reactions in the body, and greater efficiency due to increased drug penetration (Ahlawat et al. 2020). Inorganic NPs include metal and metal oxide NPs such as silver, gold, iron oxide, zinc oxide, and silica (Ijaz et al. 2020).

In this context, Luo et al. developed polyethylenimine-modified hydroxyapatite NPs (HAp-PEI) for the delivery of anti-KRAS siRNA for pancreatic cancer therapy. The PANC-1, BxPC-3, CFPAC-1, and HPDE6-C7 lines were chosen to carry out the studies to test the NPs. The results evidenced an efficient silencing of KRAS in all cell lines, moreover, after HAp-PEI/siRNA treatment the cell viability of PANC-1, BxPC-3, and CFPAC-1 was 40%, 65%, and 40%, respectively, while it did not cause toxicity in normal pancreatic cells (Luo et al. 2021). In the same way, Pei et al. detailed a novel strategy to simultaneously target TGF-β and KRAS signaling pathways using fraxinellone-modified NPs and siRNA. Fraxinellone is an antifibrotic compound that inhibits TGF-β signaling, reverses cancer-associated fibroblast activation, and attenuates the dense stromal barrier. The rate of cell apoptosis in the PANC-1 cell line was 60% after treatment with siKRAS-LCP-AppoE3, however, in BxPC-3 there was no difference in the rate of apoptosis between NP-treated and control cells. The combination of fraxinellone and siKRAS showed greater antitumor efficacy than cells treated with gemcitabine alone (Pei et al. 2019). In this sense, Xu et al. chose lamellar double hydroxide (LDH) NPs as a treatment for pancreatic cancer. The authors aimed to simultaneously inhibit glucose and glutamine metabolism in this tumor type by silencing the KRAS gene and glutaminase 1. The results showed a 40% decrease in cell viability. In addition, treatment with the NPs decreased ATP levels by 80% and reduced the concentration of glucose, glutamine, glutamate, and aspartic acid in both in vitro assays and in the tumors generated in the mice (Xu et al. 2022). CircFRASA is a novel anticancer target for pancreatic cancer. In this regard, studies have been carried out in which porous silicon NPs (pSiNPs) functionalized with polyethyleneimine (PEI) encapsulating anti-circFRASA siRNA have been synthesized. In vitro studies performed on SW1990, PANC-1, COLO-357, BxPC-3, and HDPE6C7 lines revealed a significant decrease in malignancy processes such as proliferation and migration, and the biosafety of the NPs was tested in in vivo assays (Yuan et al. 2022). Nerve growth factor (NGF) was the target of Lei et al. who presented a complex of gold nanoclusters (GNC) and siRNA against NGF. Experiments were carried out on the PANC-1 cell line; CNG-siRNA showed no cell toxicity in the range of 0 to 50 nM. In addition, it is noteworthy that tumor volume was reduced by 52% in mice treated with CNG-siRNA,

TABLE 3 | Summary of the most relevant characteristics of the polymeric nanoparticles.

Reference	Cell lines	Type of nanoparticle	Drug loaded	Target gene	In vitro results	In vivo results
Jung et al. 2021	Blue#96, Blue#96 expressing ovalbumin (Blue-OVA)	PLGA NP	—	PD-L1	PD-L1 silencing in Blue-OVA cells increased CTL proliferation	Increased apoptosis, inhibition of tumor growth and activation of infiltrating lymphocytes in Ptf1aCre xenograft model
Y. Wang et al. 2020	Panc02	Polymeric NPs (LYiCluster) delivering TGF-β receptor inhibitors (LY2157299)	—	PD-L1	PD-L1 silencing increases cytotoxic T-lymphocyte-mediated cell lysis	The combination of LY2157299 and siPD-L1 increases CD8+ T-cell infiltration and cytotoxicity, leading to tumor growth suppression in Panc02 xenograft model
Agbaria et al. 2023	Dan-G, PANC-1	PLGA NPs loaded with LL37 peptide	—	VAV1	Inhibition of cell proliferation and migration	All the NPs reduced the tumor volume. siVAV1-LL37 reduced VAV1 mRNA and protein expression, increased mice survival, and decreased metastasis in Dan-G xenograft model
Y. Li et al. 2017	PANC-1	PTP peptide-modified PSPG NPs	PCX	TR3	Dose-dependent cytotoxicity and decreased the expression of Nur77 and Bcl-2	High tumor penetration of PSPGP/Rho-PCX/FAM-siRNA and tumor growth inhibition in PSPGP/PCX/siTR3, decreased expression of Nur-77, and apoptosis induction in PANC-1 xenograft model
Pan et al. 2015	BxPC-3	PLGA/poloxamer NPs	—	EPAS1	Decreased cell proliferation, increased apoptosis and decreased EPAS1 protein expression	Decreased tumor volume and reduction of VEGF expression in BxPC-3 xenograft models
Zhao et al. 2015	PANC-1	Lipid-polymer hybrid NPs	GEM	HIF α	Decreased cell proliferation and HIF1α protein expression	Decreased tumor growth in the LENP-GEM-si-HIF1α group compared to LENP-GEM and LENP-si-HIF1α. Decreased metastasis in PANC-1 xenograft model
R. Wang et al. 2023	Panc02	Polyamino acid-based NP	Calcipotriol	CXCL12	Decreased expression of the activated PSC marker α-SMA and PSC inhibition	Reduced tumor growth, increased mice survival, reduced desmoplasia with no toxicity showed in Panc02 xenograft model
Teo et al. 2016	MIA PaCa-2, HPAF-II, HPDE	Star polymers (1,2,3) of PDMAEMA and POEGMA	—	Tubulin βIII	Cell growth inhibition and reduced βIII-tubulin protein expression	Correct internalization and decreased βIII-tubulin expression of star 3-siRNA in MIA PaCa-2 and HPAF-II xenograft model

(Continues)

TABLE 3 | (Continued)

Reference	Cell lines	Type of nanoparticle	Drug loaded	Target gene	In vitro results	In vivo results
S. Tang et al. 2023	KPC806, PANC-1	Polycation (PAMD) conjugated to TOC	—	STAT3	Increased cell apoptosis, decreased colony formation and migration, cell cycle arrest, and apoptosis induction	Inhibited tumor growth, decreased Bcl-2 expression and increased mice survival in PANC-1 xenograft model
Burks et al. 2018	AsPC-1, BxPC-3, PANC-1	CCK-B receptor-targeted polyplex NPs	—	Gastrin	Cell proliferation inhibition	Decreased tumor weight and metastasis, increased metalloprotease activity and reduced fibrosis in PANC-1 xenograft model
H. S. Min et al. 2018	BxPC-3	Micellar NPs with antibody fragments (Fab′)	—	PLK1	3 (Fab′) showed the highest cell-specific recognition, reduction in PLK1 expression	Good internalization and reduction of PLK1 expression in BxPC-3 xenograft model
S. Tang et al. 2021	KPC8060, S2-013	PAMD-CHOL NPs	GEM	PLK1	Combined treatment resulted in decreased cell viability, apoptosis induction and inhibition of colony formation	PAMD-CHOL achieved higher accumulation in tumors compared to PAMD and produced a strong tumor growth inhibition in KPC8060 and S2-013 xenograft model
C. Yang et al. 2019	PANC-1, MIA PaCa-2	NPs consisting of BCPV and GQD	DOX	KRAS	Decreased KRAS protein expression, apoptosis induction, and decreased colony formation	—
Y. Wang, Yang, et al. 2015	PANC-1, MIA PaCa-2	PAH hydrochloride or PEI hydrochloride NPs	—	KRAS	High biocompatibility and improved cell-targeting of FA-functionalized NPs	—
Lo et al. 2018	MIA PaCa-2, PANC-1, D8-175	TPN NPs using the PEG-conjugated iRGD cyclic peptide	—	KRAS	Decreased KRAS expression	Decreased genetic expression of KRAS and tumor growth in MIA PaCa-2 xenograft model
R. Hu et al. 2015	MIA PaCa-2	Polymeric NPs, Pluronics F127, and pegylated phospholipids loaded with AIE dye	—	KRAS	Increased cell apoptosis, decreased cell proliferation, migration, and invasion	No toxicity showed in the mice by the nanoparticles in MIA PaCa-2 xenograft model

(Continues)

TABLE 3 | (Continued)

Reference	Cell lines	Type of nanoparticle	Drug loaded	Target gene	In vitro results	In vivo results
Lin et al. 2013	PANC-1	Cationic CPLA NPs	—	KRAS	Nanoparticles did not show cell toxicity	—
C. Yang et al. 2015	MIA Paca-2	BCPV NP	—	KRAS	Decreased cell proliferation, metastasis and migration capacity, and increased apoptosis	—
Strand et al. 2019	KPC-1, BxPC3, KCKO, Capan-1	Peptide-based endosomolytic NP	—	KRAS	KRAS-siRNA caused cell death in KPC-1 cells	KRAS-siRNA efficiently targeted the mouse pancreas, avoiding immune response. Decreased KRAS protein expression and reduced tumor growth in KPC-1 xenograft model

Abbreviations: AIE: aggregation-induced emission; BCPV: biodegradable polyester vectors; CPLA: cationic poly(lactic acid); CTL: cytotoxic T lymphocytes; DOX: doxorubin; FA: folic acid; GEM: Gemcitabine; GQD: graphene quantum dots; LENP: ε-polylysine co-polymer; NP: nanoparticle; PAH: poly(allylamine hydrochloride); PAMD-CHOL: CXCR4 antagonist polymer; PCX: paclitaxel; PD-L1: programmed death ligand 1; PDMAEMA: poly(dimethylaminoethyl methacrylate); PEG: polyethylene glycol; PEI: polyethylenimine; PLGA: poly(lactic-co-glycolic acid); PLK1: Polo-like kinase 1; POEGMA: poly[oligo(ethylene glycol) methyl ether methacrylate]; PSC: pancreatic stellate cells; PTP: plectin-1 targeted peptide; TOC: α-tocopherol; TPN: tumor penetration.

while those treated with free siRNA alone had a 30% reduction in tumor volume (Lei et al. 2017). The synthesis of an NP for simultaneous transport of GEM and siRNA targeting the Bmi-1 oncogene was carried out by Li et al. This polyethylenimine-based NP showed a cooperative antitumor effect both in vitro in the PANC-1 cell line, where a decrease in cell proliferation, migration, and invasion was observed, and in vivo (J. Li et al. 2016). On the other hand, Han et al. formulated gold NPs that can release holo-transretinoic acid (ATRA), an inducer of pancreatic stellate cell quiescence and siRNA against heat shock protein 47 (HSP47). After carrying out studies in PANC-1 and MIA PaCa-2, a decrease in HSP47 protein expression of approximately 79% was observed, thus reducing the synthesis and secretion of collagen and fibronectin, the main components of the extracellular matrix in pancreatic cancer. In vivo studies also showed a significant reduction in tumor metastasis (Han et al. 2018). MUC4 is a gene that is overexpressed in pancreatic cancer, in this sense, Pu et al. developed polyethylenimine (PEI)-modified superparamagnetic iron oxide (SPION) NPs for the delivery of anti-MUC4 siRNA into the BxPC-3 cell line. The authors concluded that PEI-SPION-siRNA could be considered as a new effective tool for the treatment of pancreatic cancer after demonstrating the correct internalization of the NP in cells and the reduction of MUC4 gene expression involved in cell proliferation, infiltration, and apoptosis, with MUC4 down-regulation implying the negative regulation of human epidermal growth factor (HER2) and other ErbB receptors (Pu et al. 2023). Yu et al. developed an anti-PLK1 siRNA delivery system based on a perfluoroalkyl NP coated with hyaluronic acid. Experiments were carried out in the KPC8060 line, after treatment with HA@F-PaP/siRNA, cell cycle arrest was observed in the G_2/M phase. In addition, the expression of SSAT and SMOX enzymes was increased, and cell apoptosis was higher than 70% (Yu et al. 2022). Similarly, Mahajan et al. proposed superparamagnetic iron oxide NPs (SPIONS) conjugated with anti-PLK1 siRNA for the treatment of pancreatic cancer. After conducting assays on the Stav-SPION-siPLK1-treated 6606PDA line, cell cycle arrest in G_2/M phase, reduced toxicity, and decreased reactive oxygen species (ROS) generation were observed compared to Stav-SPION-treated cells (Mahajan et al. 2016). Finally, Wang et al. studied the EMT transcription factor SNAI1 that represses transcription of the tumor suppressor microRNA let-7, which contributes to the acquisition of stem cell characteristics. For this purpose, they developed a type of hyaluronic acid-targeted mesoporous silica NPs (HA-MSN) loaded with anti-SNAI1 siRNA. The results evidenced that treatment of PANC-1 cell line with HA-MSN-siSNAI1 increased the expression of let-7 inhibiting stemness. In vivo studies demonstrated the biosafety of these NPs (H. Wang et al. 2021) (Table 4).

4 | Discussion

Pancreatic cancer continues to be the subject of research in the search for new therapies and treatment approaches. Its low survival rate (Siegel et al. 2021) and the presence of several resistance mechanisms (Beatty et al. 2021) justify the need for researching about more effective therapeutic options. In recent years, nanomedicine has experienced remarkable growth due to its promising implications in the treatment of various diseases. Nanoparticle-based drug delivery systems are designed to take

TABLE 4 | Summary of the most relevant characteristics of the inorganic nanoparticles.

Reference	Cell lines	Type of nanoparticle	Drug loaded	Target gene	In vitro results	In vivo results
Luo et al. 2021	PANC-1, BXPC-3, CFPAC-1, HPDE6-C7	Polyethylenimine-modified hydroxyapatite NPs	—	KRAS	Reduced cell viability in PANC-1, BXPC-3, and CFPAC-1, with slight toxicity in HPDE6-C7 cells	—
Pei et al. 2019	PANC-1, Bxpc-3	LCP biomimetic NPs	—	KRAS	KRAS protein expression inhibition and increased cell apoptosis	Reduction of tumor volume, increase in survival without glomerular inflammation or systemic toxicity in PANC-1 xenograft model
Xu et al. 2022	PANC-1	LDH NPs	—	KRAS and GLS1	Decreased cell proliferation, induction of cell cycle arrest and decreased ATP levels	Inhibition of tumor growth, decreased protein expression of GLS1, Glut1, PKM2, and HK2. Decreased ATP, glycine, glutamine, and glucose levels without any toxicity detected in PANC-1 xenograft model
Yuan et al. 2022	SW1990, PANC-1, COLO-357, BXPC-3, HDPE6C7	Porous silicon NPs	—	circFARSA	Apoptosis induction, decreased proliferation and cellular migration	Reduced tumor growth without any systematic toxicity detected in pancreatic cancer tissues xenograft models
Lei et al. 2017	PANC-1, PANC-1-luc	Gold NPs	—	NGF	CNG-siRNA reduced cell proliferation and migration	Reduced tumor growth and inhibition of NGF protein expression. The NPs showed low toxicity and high biosafety in PANC-1 xenograft model
J. Li et al. 2016	PANC-1	Iron oxide NP targeting scFv	GEM	Bmi-1	Decreased cell proliferation, cell cycle arrest and reduced cell clonogenicity, and migration. GEM combination improved apoptosis induction	scFv-Gem-siBmi-1-NPs achieved the greatest reduction in tumor volume, high levels of cell necrosis and reduced Bcl-2 protein expression in PANC-1 xenograft model
Han et al. 2018	PANC-1, MIA PaCa-2, PANC-1-luc	PEGylated PEI-coated gold NPs loaded with ATRA	GEM	HSP47	Inhibition of HSP47 protein expression, decreased cell proliferation, reduction of α-SMA levels and collagen synthesis inhibition	Decreased ECM in tumor tissues, NP-siR, and GEM combination produced the highest tumor growth and metastasis inhibition in PANC-1 xenograft model
Pu et al. 2023	BxPC-3	PEI-SPION NPs		MUC4	Reduced MUC4 protein expression	—
Yu et al. 2022	KPC8060	Perfluoroalkyl (F-PaP) NPs	—	PLK-1	Cell cycle arrest induction, increased SSAT and SMOX protein expression and apoptosis induction	High tumor uptake and penetration, reduction of metastasis with no toxicity in KPC8060 xenograft model

(Continues)

TABLE 4 | (Continued)

Reference	Cell lines	Type of nanoparticle	Drug loaded	Target gene	In vitro results	In vivo results
Mahajan et al. 2016	6606PDA	StAv-conjugated SPION NPs	—	PLK1	Cell cycle arrest induction, decreased cell proliferation and migration	High tumor uptake and reduced tumor volume. PLK1 expression inhibition and apoptosis induction. Increased mice median survival in 6606PDA xenograft model
H. Wang et al. 2021	PANC-1	HA-conjugated mesoporous silica (MSN) NPs	—	SNAI1	Decreased SNAI1 protein expression and decreased size of the spheroids formed	Decreased SNAI1 mRNA expression and reduction of tumor volume in PANC-1 xenograft model

Abbreviations: GEM: gemcitabine; HA: hyaluronic acid; LCP: lipi-coated calcium phosphate; LDH: layered double hydroxide; NGF: nerve growth factor; PEI: polyethylenimine; PLK1: polo-like kinase 1; ROS: reactive oxygen species; SPION: superparamagnetic iron oxide nanoparticles; StAv: streptavidin.

advantage of the unique characteristics of these structures at the nanometer scale. One of the main advantages lies in their ability to specifically target cells or tissues, thus minimizing unwanted side effects on healthy circulating tissues (T. Sun et al. 2014). In addition, nanoparticles can be designed to release drugs in a controlled and sustained manner at the site of action, allowing optimal therapeutic concentrations to be maintained for prolonged periods, improving treatment efficacy and reducing the frequency of drug administration (Dadwal, Baldi, and Kumar Narang 2018). They are widely used in numerous types of cancer such as breast cancer (Marshall et al. 2022), lung cancer (Reda et al. 2022), colorectal cancer (D. Sun et al. 2022). In addition, siRNAs also have an important role as a new therapy for cancer treatment. siRNA has advantages over other drugs because it performs its function by base pairing with the mRNA of the target gene, while other treatments such as small molecule drugs and monoclonal antibodies need to recognize the conformation of certain proteins (B. Hu et al. 2020). The proposal to carry out this systematic review arose as a result of the advantageous characteristics of both treatment alternatives. In this sense, the idea of analyzing the literature described in the last 10 years in relation to the strategy of siRNA encapsulation in nanoparticles has been carried out (Figure 4). The choice of this period allows us to address the most recent and relevant trends in the application of this therapeutic strategy, which will contribute to consolidate the existing knowledge and to identify possible areas for improvement or future development.

According to the articles analyzed in this systematic review, although the three main types of nanoparticles included appear to have great therapeutic potential as siRNA and drug carriers, polymeric nanoparticles appear to be the vehicle most widely used by researchers for their studies. This type of nanoparticle can be synthesized using both natural and synthetic materials, or from already formed monomers or polymers, offering a wide range of possible structures and properties. They can be designed to precisely control several of their characteristics and are often efficient vehicles for the delivery of therapeutics due to their biocompatibility and simple formulation (Mitchell et al. 2021). Therapeutic agents can be encapsulated within the nanoparticle core, trapped in the polymer matrix, chemically conjugated to the polymer, or attached to the nanoparticle surface. This allows the delivery of different types of compounds, both hydrophobic and hydrophilic, as well as substances of various molecular weights, including small molecules, biological macromolecules, or proteins (Caldorera-Moore, Vela Ramirez, and Peppas 2019; L. Zhang et al. 2020), which makes them ideal for co-management (Afsharzadeh et al. 2018). By adjusting characteristics such as composition, stability, responsiveness, and surface charge, it is possible to precisely control the charge efficacy and release kinetics of therapeutic agents (Patra et al. 2018; Volpatti et al. 2020).

To ensure the effective development of research in the field of nanomedicine, it is essential to address the correct internalization of nanoparticles inside cells. This step requires not only accurate assessment methods, but also an understanding of the mechanisms involved in the interaction between nanoparticles and cells. While there are several techniques available to assess the cellular internalization of nanoparticles, most of the articles reviewed choose to use fluorescence as the main tool to measure

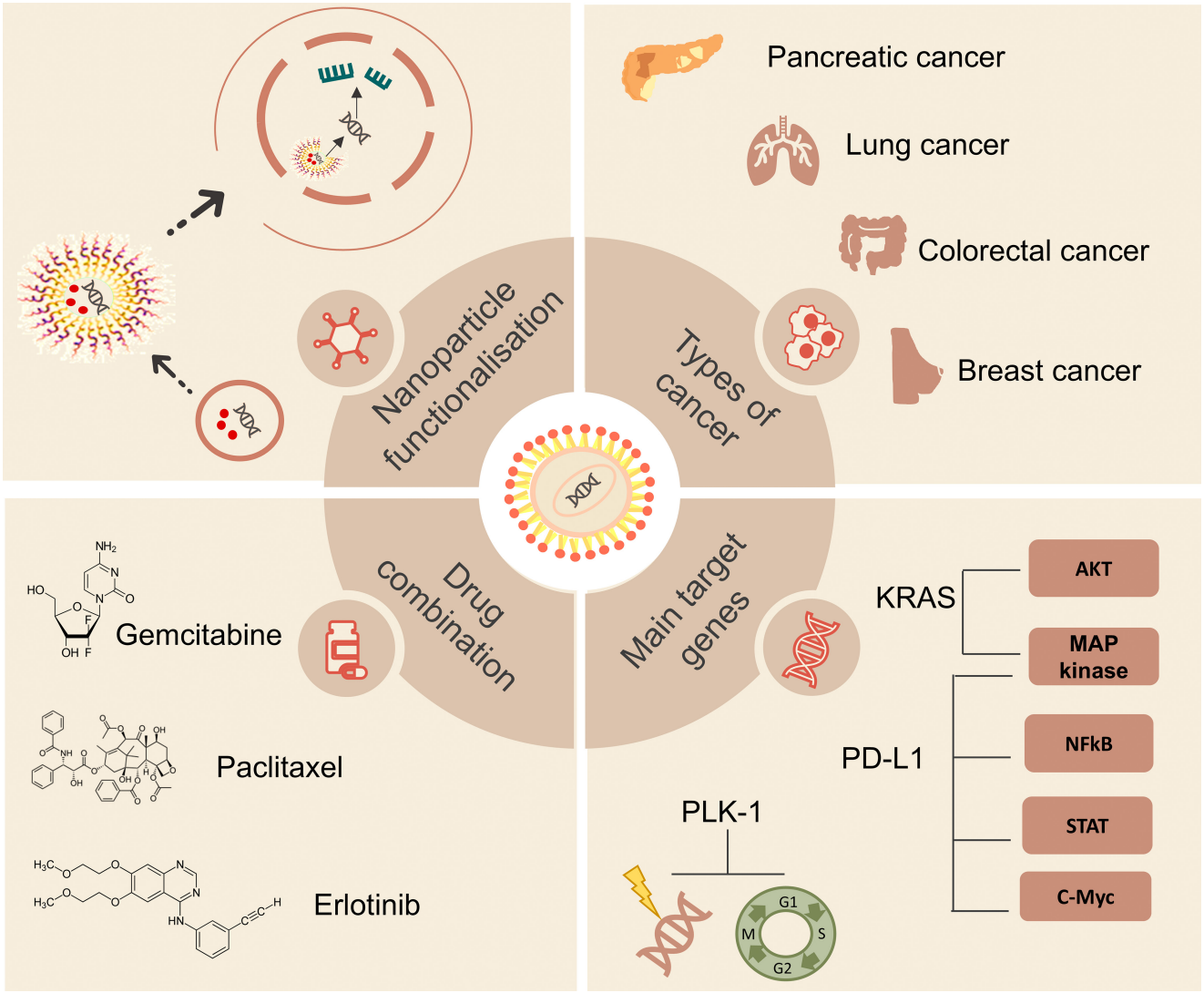

FIGURE 4 | Graphic representation of the use of siRNA encapsulated in nanoparticles, including the types of cancer in which it is used, the main genes targeted by siRNAs, drugs used as cancer co-treatment, as well as the mechanism of functionalization of the nanoparticles for better siRNA delivery and avoidance of resistance mechanisms.

this capability. Fluorescence offers several advantages, such as its sensitivity and specificity, which make it particularly suitable for this purpose. However, it is important to emphasize that the choice of the evaluation technique must be made carefully, considering the specific characteristics of the nanoparticles and the cell line under study, as well as the objective of the research. Examples of researchers using this technique to evaluate the internalization of their nanoparticles include Xie et al. who developed polymeric nanoparticles antagonistic to CXCR4, H. Tang et al. who used membrane-coated or membrane-impregnated NPs or Jung et al. in PLGA nanoparticles targeting PD-L1.

To improve treatment with NPs using gene therapy, some authors opted for the combination of this therapy with drugs indicated for the treatment of pancreatic cancer, gemcitabine being one of the drugs of choice. In the studies carried out, it has been confirmed that the combined treatment of siRNA-loaded NPs plus the drug enhances the effect of the gene therapy, obtaining a greater antitumor effect in both in vitro and in vivo studies. An example of this is the trial conducted by Anthiya et al. who observed a higher rate of cell apoptosis in pancreatic cancer cells treated with the combination therapy than with gemcitabine alone, and the survival rate of mice in in vivo experiments increased to 43% in mice treated with NP-siRNA relative to those treated with gemcitabine (29%). These results are supported by Han et al. who, when carrying out the combined treatment of NP-siRNA plus gemcitabine in mice, observed a reduction in tumor size of 69.3%. These data confirm that the combination of gene therapy with drugs is an effective strategy for the treatment of pancreatic cancer.

After carrying out the analysis, it has been observed that the target gene of most of the articles is KRAS, representing the 85% of the mutations that lead to the development and progression of cancer (Cox et al. 2014). The KRAS gene encodes a member of the Ras family of small GTPases; mutation of Ras is normally a biomarker associated with poor prognosis and poor response to specific agents (Stephen et al. 2014). Ras mutations are of great importance in cancer as Ras triggers signals to several effector pathways whose activation promotes oncogenic transformation.

These pathways include the MAP-kinase (MAPK) pathway, the PI—kinase (PI3K)/AKT/mTOR pathway, the small GTPases Rho, Rac, and Ral, as well as phospholipase C. Together, these pathways regulate key cellular processes such as proliferation, growth, survival, metabolism, cell motility, and gene expression (Pylayeva-Gupta, Grabocka, and Bar-Sagi 2011). In this context, studies have also been carried out using the strategy of inhibiting KRAS to reduce tumor malignancy, in this case, Strickler et al. (2023) evaluated the efficacy of the drug sotorasib to inhibit $KRAS^{G12}$ in a phase 1–2 clinical trial, it was found to be clinically effective and with an acceptable safety profile in patients with metastatic pancreatic cancer with a $KRAS^{G12}$ mutation, 21% of patients showed a confirmed response, with a median response time of 1.5 months. Furthermore, disease control was observed in 84% of patients, with an overall survival of 6.9 months. Also, Bryant et al. used an anti-KRAS siRNA and showed that inhibition of the KRAS-activated ERK signaling pathway reduced both glycolytic and mitochondrial function in cancer cells, making them more dependent on autophagy for energy (Bryant et al. 2019).

10% of the analyzed articles focus on the study of PLK1, the polo-like kinase 1 is the main member of the serine/threonine kinase family (Chiappa et al. 2022). PLK1 has several functions, one of the most important being its role in cell mitosis, as well as the regulation of processes such as centrosome formation and function, the correct organization of the mitotic spindle, and the completion of cytokinesis (Goroshchuk et al. 2019). In addition to these functions during the cell cycle, other PLK1 activities related to the phosphorylation of new emerging substrates have been identified. PLK1 has been observed to be deregulated in several cancer types, contributing to tumor development and progression, PLK1 is known to be overexpressed at both the mRNA and protein level in many tumor types and this overexpression has been linked to worse patient outcomes (Liu, Sun, and Wang 2017). In this context, Z. Yang et al. analyzed the involvement of PLK-1 in pancreatic cancer, their studies revealed that combined inhibition of fibroblast growth factor receptor 1 (FGFR1) and PLK1 induces synergistic toxicity in pancreatic cancer cells with KRAS mutations, furthermore, combined treatment with FGFR1 and PLK1 inhibitors increases reactive oxygen species (ROS) levels, leading to oxidative stress that activates the JNK/p38/E2F1 pathway (Z. Yang et al. 2021). In addition, PD-L1 or programmed death ligand 1, a critical immune checkpoint protein has also been extensively studied. Binding of PD-L1 to PD-1 inhibits T cells from destroying cancer cells by reducing T cell proliferation and activity (Yokosuka et al. 2012). PD-L1 expression in cancer cells is regulated by multiple signaling pathways, including NFkB, MAPK, mTOR, STAT, and c-Myc (Casey et al. 2016; Ritprajak and Azuma 2015). X. Zhang et al. demonstrated that NEK2, a prognostic factor for pancreatic cancer, interacts directly with PD-L1. The results of their studies showed that the combination of a NEK2 inhibitor and an anti PD-L1 antibody had a synergistic effect in suppressing tumor growth and antitumor immune response (X. Zhang et al. 2021).

In recent years, there has been a notable advance in therapeutic approaches based on nanoparticles, which are specifically designed with surface modifications to selectively target disease-affected cells. This innovative approach seeks to overcome several challenges that limit the efficacy of conventional treatments, such as systemic toxicity and multidrug resistance (MDR), achieving precise, and efficient delivery of the drug into the desired site of action. To address this challenge, nanoparticles are being designed with specific features that allow them to circumvent biological barriers and take advantage of cellular uptake mechanisms to selectively target. Functionalized nanoparticles offer additional advantages in this regard by enhancing interaction with cancer cells. These nanoparticle surface modifications may include conjugation of specific ligands that bind to receptors overexpressed on the surface of tumor cells, thus facilitating their selective internalization through processes such as endocytosis (Tariq and Bokhari 2020). In this sense, H. Tang et al. designed membrane-coated NPs for co-administration of gemcitabine and erlotinib and delivery of anti-IRAK4 siRNA to tumor cells (H. Tang et al. 2022). This functionalized NP system has been carried out in other cancer types, such as neuroblastoma, where they functionalized gold NPs with two different thiols on the surface for methotrexate delivery to tumor cells (Salamone et al. 2023). Arachchige et al. functionalized iron oxide nanoparticles with red autofluorescent doxorubicin and fluorescein isothiocyanate allowing tracking of nanoparticle intracellular transport and drug release, enabling more than 20-fold faster drug entry and release in pancreatic cancer cells (Arachchige et al. 2017). In this regard, Huang et al. carried out the synthesis of magnetic iron oxide nanoparticles coated with casein for the treatment of pancreatic cancer, demonstrating effective distribution as well as specific accumulation of the nanoparticles in the tumor, with the modified nanoparticle resulting in improved therapeutic efficacy compared to unfunctionalized nanoparticles (Huang et al. 2016).

This systematic review provides a comprehensive and analytical overview of the efficacy of nanoparticles as transporters and the use of siRNA as a promising tool for the treatment of pancreatic cancer, providing an in-depth understanding of the main targets that can be targeted to address the disease and achieve high therapeutic efficacy. Importantly, this systematic review has considerable applicability as many nanoparticle-based formulations and technologies have already been introduced into the clinic (Anselmo and Mitragotri 2014; Y. Min et al. 2015; Svenson 2012; Torchilin 2014; Wagner et al. 2006). In this context, to broaden the therapeutic applications of nanoparticles, their conjugation with siRNA is also beginning to enter the clinic (Kanasty et al. 2013; Zhou et al. 2013). In fact, a clinical trial (NCT02110563) has been conducted in which patients were treated with siRNA formulated in lipid nanoparticles to silence the MYC oncogene (DCR-MYC), which is deregulated in most human malignancies. A total of 19 patients with 5 dose ranges were treated. The tumor types included in the study were neuroendocrine tumor, metastatic breast tumor, colorectal, ovarian, and appendicular cancer among others. A few treatment-related adverse effects were detected, the most common being fatigue, nausea, and other treatment reactions. Metabolic responses were observed after cycle 1, as well as evident tumor shrinkage in multiple patients. The results obtained in this trial highlight DCR-MYC as a promising MYC inhibitor, showing high clinical and metabolic response rates at various dose levels (Tolcher et al. 2015). Several clinical trials are currently underway involving the administration of siRNA-encapsulating nanoparticles (NCT01437007 and NCT02314052) in liver and hepatocellular carcinoma, but no results are yet available. Although the results

appear to be promising, more research is needed on this type of treatment and the possible adverse effects it may have on patients.

However, this systematic review has some limitations that should be considered. One of them lies in the possibility that the established search equation does not cover all the studies conducted on the subject under discussion. An example of this is the non-inclusion of articles related to the subject of the study cited in the sources analyzed; in addition, some articles may have been excluded from the analysis if they did not explicitly mention the type of cancer being studied in the title and abstract. Due to the established inclusion and exclusion criteria, articles focused on other types of cancer, even if they performed experiments using pancreatic cancer cell lines, were not considered for inclusion in the systematic review.

Consequently, it is important to be aware of these limitations when interpreting the findings of this systematic review and to consider them when planning future research on the topic. Addressing these limitations could involve the implementation of broader and more comprehensive search strategies which would contribute to a more complete and accurate understanding of the efficacy of the treatments in question.

5 | Conclusion

Pancreatic cancer is a devastating disease that poses considerable challenges in the field of medicine. Its aggressiveness and lack of satisfactory responses to conventional therapies make it one of the most difficult cancers to treat. However, in recent years, a new strategy has emerged in the form of siRNA-loaded nanoparticle therapy, an innovative strategy that is beginning to stand out as a promising therapeutic option against this deadly disease. This systematic review has been dedicated to comprehensively explore the therapeutic potential of these nanoparticles in the treatment of pancreatic cancer. It has focused on the genes that serve as key therapeutic targets in this emerging strategy. Throughout this detailed analysis, the most relevant genes that play a key role in pancreatic cancer progression, such as KRAS, PLK1, and PD-L1, have been identified and examined. More than 50% of the studies analyzed have focused on these genes, underscoring their importance in the understanding and treatment of this disease. These genes play a critical role in the development and spread of pancreatic cancer and their inhibition by siRNA has proven to be an effective strategy to reduce carcinogenic processes such as cell proliferation and migration. In addition, their targeted therapy has been shown to induce cell cycle arrest and ultimately apoptosis of cancer cells, offering significant hope for the development of more effective treatments against this disease.

One of the most significant findings of this review is the synergy observed between nanoparticle-based gene therapy and the administration of conventional chemotherapeutic agents, with gemcitabine as the featured drug in many cases. This combination has been shown to markedly improve treatment efficacy by suppressing tumor growth and reducing the likelihood of developing resistance to therapy. Despite the significant progress achieved, more research is needed to validate and optimize this promising therapy. Further clinical trials are required to evaluate the safety and efficacy of these therapies in patients with pancreatic cancer, as well as to identify biomarkers that can predict response to treatment and guide patient selection. In addition, a greater understanding of the underlying mechanisms of action of siRNA-loaded nanoparticles, as well as their interaction with the tumor microenvironment and the host immune system, is needed. Successful implementation of these therapies in clinical practice presents significant challenges. From the scalability and efficient production of nanoparticles to the implementation of safe and efficient delivery strategies in clinical settings, there are numerous hurdles to overcome. However, with the continued advancement of technology and the growing accumulation of scientific evidence, it is possible that siRNA-loaded nanoparticle-based therapies may become a fundamental tool in the treatment of pancreatic cancer soon. This is an exciting time in cancer research, and these innovative therapies have the potential to make a significant difference in the lives of patients battling this devastating disease.

Author Contributions

Patricia Lara: data curation (equal), investigation (equal), methodology (equal), writing – original draft (equal). **Francisco Quiñonero:** data curation (equal), investigation (equal), methodology (equal), writing – original draft (equal). **Raul Ortiz:** supervision (lead), writing – review and editing (equal). **Jose Prados:** conceptualization (equal), project administration (equal), resources (equal), writing – review and editing (equal). **Consolación Melguizo:** conceptualization (equal), project administration (equal), resources (equal), writing – review and editing (equal).

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Related Wires Articles

Theranostic small interfering RNA nanoparticles in cancer precision nanomedicine

A review on RNAi therapy for NSCLC: Opportunities and challenges

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