Nanocarriers-Based Targeted Therapies for Pancreatic Cancer and Challenges Ahead

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Abstract

Pancreatic cancer is one of the deadliest types of cancer and is considered the seventh leading cause of cancer. Pancreatic cancer incidence and mortality have been steadily increasing. Over the last decade, advancements in diagnosis, pancreatectomy surgery, radiotherapy technique, and systemic therapies have made advances, but relatively small improvements in patient outcomes. Furthermore, in pancreatic cancer, most of the chemotherapy drugs respond poorly or intrinsic resistance to chemotherapeutics, and lack effective target therapies that are the key factors contributing to a dismal prognosis. Recently, significant attempts have been made to provide targeted-based nanocarriers to treat pancreatic cancer. This

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chapter tries to give information about the new possibilities of targeting pancreatic cancer via nanocarriers and challenges ahead.

Keywords

Pancreatic cancer · Targeted therapy · Signalling pathway · Receptors · Resistance · Challenges

7.1 Introduction

The pancreas is a vital organ in the digestion and absorption as well as the use and storage of energy. The pancreas consists of two structurally different but functionally linked glandular systems, the exocrine and endocrine pancreas, which developed from the primitive gut. The exocrine pancreas secretion is regulated by neural and hormonal signals, mostly in the form of gastrointestinal peptide hormones. Pancreatic cancer (PC) is the seventh leading cause of cancer with 496,000 cases and 466,000 deaths due to its poor prognosis in both genders (Sung et al. 2021). There are many factors contributing to the poor prognosis of pancreatic cancer, such as late diagnosis, high inherent resistance to conventional chemotherapy, a lack of biomarkers, and lack of effective therapeutic alternatives. From an epidemiological viewpoint, high-risk factors for pancreatic cancer include age, genetic predisposition, diabetes mellitus, family or personal history of pancreatitis, and especially the lifestyle of a person such as chain smoking, drinking alcohol, tobacco, and obesity, which increase the chance of development of PC by up to 50–60 percent (Dariya et al. 2019; Brand et al. 2007), whereas 5–10% is due to genetic mutations such as Kristen rat sarcoma (Kras). In addition to genetic mutation, PC is associated with epigenetic aberration abnormality of oncogenes. Silencing of tumour suppressor genes such as p16, TP53, and cyclin-dependent kinase inhibitor 2A (CDKN2A) is the risk hallmark of PC (Dariya et al. 2019). PC will soon be the second most malignant cancer in the world, with an overall survival rate of 26% for 5 years in advanced stages of the disease, and 22% for early-stage detection with surgical resection of the tumour.

Currently, treatment options are limited and chemotherapy is one of the choices for the treatment of cancer. Chemotherapy often fails to completely treat and cure cancer due to the high dose of drug required, poor accessibility of antineoplastic agents to tumour, and significant toxic effects due to their nonselective nature. As a result, nanocarrier-based targeted therapies have the potential to significantly enhance cancer treatment by delivering a therapeutically effective concentration of drug at the tumour site. Nanocarriers-based targeted therapies have advantages over conventional therapy such as (1) reducing cytotoxic compound effects on healthy cells, (2) fighting drug-resistant cancerous cells, and (3) reduction in dose-limiting adverse effects (Attia et al. 2019).

7.1.1 Pathophysiology of Pancreatic Cancer and their Types

Pancreatic cancer develops from epithelial cells that are responsible for the formation of digestive pancreatic enzymes and line the pancreatic duct, known as pancreatic exocrine or pancreatic ductal adenocarcinoma (PDAC). There are many characteristic precursor lesions in PDAC, including intraductal papillary mucinous neoplasm, mucinous cystic neoplasm, and pancreatic intra-epithelial neoplasia (most common). Others include the type of exocrine that includes acinar cell carcinoma, intraductal papillary-mucinous neoplasm, and mucinous cystadenocarcinoma, which are less prevalent but common (Chiaravalli et al. 2017). Pancreatic cancer may also grow from the pancreas's endocrine cells called islets of Langerhans, which produce hormones such as glucagon and insulin that are released into the blood circulation and regulate the blood glucose level in the body. This particular cancer is referred to as pancreatic endocrine cancer. It is necessary to identify the type of tumour that has developed in order to treat it effectively because they act and respond differently to treatment. Exocrine tumours are the most common type of pancreatic cancer, accounting for about 93% of cases and nearly 7% of neuroendocrine tumours (pancreatic NETs or PNETs), also known as islet cell tumour. Pancreatic NET grows slower than the endocrine tumour. PNET is generally non-functional (i.e. it does not produce hormones), but in some cases it may be functional (produce hormones), which make it important due to a major glucose homeostasis imbalance. Pancreatic cancer was described in revolutionary terms by Alvin et al. (Makohon-Moore and Iacobuzio-Donahue 2016) They classify it into three stages: the initiation of normal cells driven by gene mutation caused by environmental exposure and clone expansion, in which mutant cells continue to divide; the formation of a clonal population; and lastly, the introduction into a foreign microenvironment, in which tumour cells break through the basement membrane and invade the surrounding stroma. Multiple pancreatic cancer begins with pancreatic intraepithelial neoplasia (PanIN-IA, IB, II, and III), which is caused by a gene mutation, and progresses to invasive neoplastic lesions in the pancreas (Dariya et al. 2019). PanIN are the histological precursors of PC, characterized by enlarged nuclei, polarity loss, crowding of the nucleus, and pseudo-stratification hyper-chromatin. The process begins with dysplastic epithelial cells such as PanIN-IA and B, progresses into dysplasia cells including PanIN-II and III, and ultimately transforms into invasive carcinoma characterized by mutations in oncogenes like Kras and tumour suppressor genes like TP53, CDKN2A, and SMAD4 (Aslan et al. 2018). In one manner, pancreatic cancer is caused by epigenetic modulation and digestive enzyme inhibition that begin in childhood and eventually become chronic in adults (Table 7.1).

Cancer cells have a high degree of plasticity (i.e. the capacity to change to adapt to the intense tumour environment), which mostly involves the transformation from epithelial to mesenchymal form and metabolic alterations. These alterations provide cancer cells unique phenotype characteristics, including strong invasiveness and resistance to apoptosis. Changes in environmental factors have been suggested as potential causes of cell plasticity. However, genetic alteration (p53 and NFATc1

Table 7.1 A comparison of key features of different types of pancreatic cancer

Type of pancreatic cancer	Features	
Exocrine		References
Pancreatic adenocarcinoma	Highly aggressive Presence of typical precursor lesions viz. pancreatic intraepithelial Prevalence: > 95% of reported cases Neoplasia (most common), mucinous cystic Neoplasm, and intraductal papillary mucinous neoplasm	Desai et al. (2019)
Acinar cell carcinoma	Vary rare type Possibility of excessive pancreatic lipase secretion	Desai et al. (2019)
Intraductal papillary-mucinous neoplasm	Potential precursor of PDAC Main duct carcinoma is more severe Pancreatic duct carcinoma (grows from main pancreatic duct or branches of the duct)	Desai et al. (2019)
Mucinous cystadenocarcinoma	Rare type More commonly observed in tail of pancreas Type of cystic tumour Predominant in women	Desai et al. (2019)
Pancreatic neuroendocrine tumours (pancreatic NETs or PNETs) islet cell tumours		
Prevalence: < 5% of reported cases Less aggressive than PDAC Can be functional (produce hormones) or non-functional (produce no hormones) Majority are non-functional tumours		Desai et al. (2019)

signalling activity) is the most common cause. The classic desmoplastic/stromal reaction is an important histopathological feature of pancreatic cancer, and it is caused by the interaction of various factors including pancreatic stellate cells (PSCs), fibroblasts, inflammatory cells, and derived factors including fibronectin, fibronectin, extracellular matrix (ECM) proteins, and different growth factors (Liu et al. 2016; Apte et al. 2013). Pancreatic cancer cells upregulate various growth factors that cause tumourigenesis and contribute to stromal reaction. These factors include hepatocyte growth factors, transforming growth factors-β, insulin-like growth factor 1, fibroblast growth factor, epidermal growth factor, vascular endothelial growth factor (VEGF), platelet-derived growth factor, and connective tissue growth factors.

7.2 Targeted Therapy

As previously stated, PDAC is genetically heterogeneous, and traditional treatments that target distinct biological processes fail to differentiate between malignant and normal cells, resulting in severe side effects. Hence, small-molecule inhibitors and monoclonal antibodies targeting cancer cell surface receptors, growth factors, or

other proteins that contribute to disease development are needed for targeted treatments utilizing small molecules.

7.2.1 Targeting Surface Receptors

Multiple surface receptors have been linked to pancreatic cancer progression. Only a few inhibitors have been developed, including the EGFR inhibitor erlotinib, but they do not substantially enhance patient survival. However, despite the development of new inhibitors for various receptors, more precise therapeutic targets remain a need.

7.2.1.1 Epidermal Growth Factor Receptor (EGFR), VEGF and IGF Receptor Targeted Delivery

Epidermal growth factor receptor (EGFR or HER1) (Philip et al. 2010), VEGF (Martin et al. 2012), and IGF receptors have been targeted by monoclonal antibodies (Mab) and have been evaluated in both preclinical and clinical trials. Using EGFR inhibitors in a phase III clinical study did not improve outcomes in patients with advanced pancreatic cancer compared to use of gemcitabine when used alone in a patient.

7.2.1.2 Targeting Transferrin Receptors (TFRC)

The membrane-bounded protein TFRC is overexpressed in 90 percent of pancreatic cancer cells, and it is thought to be a marker of malignant cells (Ryschich et al. 2004). TFRC plays a critical role in the development and progression of pancreatic cancer. Along with gemcitabine, a tumour-specific liposome-based nanocomplex conjugated with a single-chain antibody fragment (TfRsFv) targeted the transferrin receptor in vivo mice model with PDAC (Camp et al. 2013). This complex system was demonstrated to be effectively located inside the tumour tissue through the transferrin receptor, to significantly reduce tumour growth and to extend the median survival of a metastatic pancreatic cancer mouse model.

7.2.1.3 Folate Receptor (FR)

FR, a glycosylphosphatidylinositol-anchored receptor, is another potential target for pancreatic cancer treatment, since it is expressed at moderate to high level in more than 80% of patients with PDAC tumours (Cai et al. 2017). FR is mostly expressed in tumour cells with limited expression in normal cells (Zwicke et al. 2012) and may serve as a good receptor for nanoparticle-based targeted drug delivery.

7.2.2 Targeting Signalling Pathway in PDAC

The abnormal activation or dysregulation of several signalling pathways significantly contributes to pancreatic cancer heterogeneity. Numerous attempts have been made to create effective inhibitors, including biological and small molecules. Most of these signalling inhibitors are still being investigated and have not yet been

approved for clinical use. Targeted treatments are more precise than conventional drugs in the clinic because they block a key signalling pathway that is crucial for cell proliferation, survival, metastasis, and progression.

7.2.2.1 KRAS Signalling

About 90% of PDAC patients have a KRAS mutation, making it an excellent therapeutic target since KRAS triggering mutations of this oncogene are the primary cause of the disease and its development (Collins et al. 2012). The KRAS proto-oncogene is responsible for the production of the GTPase protein. The KRAS mutation G12D leads to constitutive phosphorylation and activation of this pathway (Eser et al. 2014). Many distinct signalling pathways are activated when KRAS mutations occur. These signalling pathways include RAF, MEK, ERK, and the P13K/AKT pathways (Knickelbein and Zhang 2015). These pathways play key roles in cell division, survival, and drug resistance. KRAS activity affects the microenvironment of PDAC by generating sonic Hedgehog (Ji et al. 2007), interleukin-6 (Lesina et al. 2011), and prostaglandin E (Charo et al. 2013) and therefore regulates stroma maintenance (Pylayeva-Gupta et al. 2012). KRAS signalling has also contributed to the promotion of immunosuppression (Pylayeva-Gupta et al. 2012).

Although KRAS, one of the most potent of all human oncogenes, is activated in over 90% of PDAC, there are currently no KRAS-targeted treatments in the clinic. Due to the relatively smooth surface of the 3D structure, it has been difficult to inhibit KRAS directly, making it an untreatable target (Van Cutsem et al. 2004; Zeitouni et al. 2016). Several groups have been studied and the efficacy of targeting KRAS indirectly, as well as its downstream mediators PI3K and MEK pathway. However, it is difficult to suppress PI3K due to the presence of multiple isoforms of PI3K protein and not all isoforms of PI3K interact with KRAS (Vanhaesebroeck et al. 2010). To overcome the high levels of drug resistance and improve patient survival, new therapeutic strategies for PDAC at an advanced stage are required. Inhibiting KRAS activity by targeting it offers many opportunities for new drug development. Although compounds that access an inducible pocket generated in the KRAS structure have been discovered, more optimization is needed before these compounds can be developed into a clinically useful drug.

7.2.2.2 TGF-β Signalling

TGF is involved in a variety of biological processes, including homeostasis and cellular processes such as cell proliferation, differentiation, and apoptosis (Elliott and Blobe 2005). TGF- suppresses cell proliferation and has tumour suppressive activity in the early stages of tumour development, but as tumourigenesis progresses, it takes on an oncogenic role in PDAC (Shen et al. 2017). TGF-signalling is typically activated by SMAD proteins that are classified as receptor-regulated, mediators, or inhibitors. TGF-ligand binding to the type II receptor phosphorylates SMAD2 and SMAD3, which then form a complex with the tumour suppressor protein SMAD4 (Singh et al. 2015). This signalling may be disrupted by inhibiting SMADs (SMAD6 and SMAD7) from interacting with the receptor. This pathway is usually

upregulated in patients with PDAC. In about 40% of pancreatic tumour cases, SMAD4 mutations are observed, resulting in decreased SMAD4 expression and inactivation (Malkoski and Wang 2012). The SMAD4 mutation is recognized and seen in high-grade PanIN lesions (Malkoski and Wang 2012). Most pancreatic carcinoma cell lines and patients with advanced pancreatic cancer have mutations in TGF-β1 and TGF-βR-1-2, which contribute to their poor prognosis. It has been shown that immune activation of TGF-β gene knockdown mice can result in tumour cell apoptosis and prolonged survival (Javle et al. 2014). TGF-siRNA induced apoptosis by activating RGI-I signalling, and TGF-siRNA decreased serum levels and anticancer efficacy in an orthotopic PDAC mouse model (Ellermeier et al. 2013). The SnoN (Ski-related novel protein N) protein is a molecule that negatively regulates the TGF- pathway, and its silencing leads to a decrease in cancer cell proliferation and an increase in apoptosis in vitro, indicating that TGF- signalling is an important molecular target in PDAC tumours.

7.2.2.3 Hedgehog Signalling

In PDAC tumours, overexpression of Sonic Hedgehog ligands (SHH) leads to cancer initiation and metastasis, as well as a substantial desmoplastic response (Olive et al. 2009). It binds to the PTCH1 receptor, which is involved in the regulation of Smoothened protein (SMO) and downstream pathways. The sHH pathway is abnormally activated in pancreatic cancer when Hedgehog SMO is overexpressed (Honselmann et al. 2015). Currently, many ongoing studies investigate the role of inhibition of SMO receptors in PDAC. The combination of saridegib and gemcitabine, which inhibits the SMO receptor, reduced desmoplasia and collagen deposition, while increasing the intratumoural gemcitabine concentration and improved overall mice survival (Olive et al. 2009). However, in clinical studies combining hedgehog signalling inhibitors with gemcitabine failed to provide positive outcomes. As a part of one pilot study and phase II clinical trial, small-molecule SMO antagonist vismodegib showed disappointing results in patients with metastatic pancreatic cancer (Kim et al. 2014) (NCT01064622). Targeting any Hedgehog pathway molecule seems to be a viable approach since it can impact both the tumour and its surrounding stroma, as well as their interaction.

7.2.2.4 Notch and Wnt Signalling

Overexpression of Notch genes, Notch receptors, and ligands were recognized even in early PanIN lesions (Guo et al. 2016; Mazur et al. 2010). Notch signalling pathway activation upregulates the invasive phenotype of pancreatic cancer by interfering with oncogene pathways and decreasing EGFR and NF-kB signalling (Mccleary-Wheeler et al. 2012). Pancreatic cancer cell lines BxPC-3, HPAC, and PANC-1 exhibited a high level of Notch 1 expression, and siRNA-mediated suppression of Notch 1 substantially reduced cell proliferation and induced apoptosis (Wang et al. 2006). However, the abnormal activation of the Wnt signalling pathway was also discovered in PDAC (Mccleary-Wheeler et al. 2012; Zeng et al. 2006). Wnt receptor activation is caused by ligand binding, which in turn activates β-catenin. Normally, β-catenin is inactive; however, active-catenin levels are elevated in

pancreatic cancer. Extracellular proteins, such as Hsulf-1,2, may serve as positive regulators of the Wnt signalling pathway and are often overexpressed in tumour cells but not in normal cells (Nawroth et al. 2007), indicating that the Wnt signalling pathway is constitutively active and therefore a therapeutic target. Patients with stage IV pancreatic cancer are being treated with biological therapeutic agent OMP-54F28, a type of decoy receptor protein that binds to Wnt ligands. This treatment is being utilized in conjunction with paclitaxel and gemcitabine as part of a phase I clinical study. The study's findings have not yet been made public (NCT02050178).

7.2.3 Tumour-Specific Nanotherapeutics for Targeting PDAC

In pancreatic cancer, use of nanoparticles has recently emerged as a therapeutic option for pancreatic cancer. The shape, size, and charge of nanoparticles affect their ability to enter into the cell. Tumour-specific nano-delivery systems are important for improving the effectiveness of anticancer therapies in PDAC because they minimize undesired and dose-limiting damage to normal cells while targeting tumours specifically and selectively. Drugs conjugated or encapsulated into nanoparticles have improved stability and half-life, allowing for more controlled release. Using modified nanoparticles, it is possible to improve the pharmacokinetics and biodistribution profiles of drugs substantially. Size influences cargo drug biodistribution and allows them to enter tumours via the increased permeability and retention effect of nanoparticles (EPR). They can easily penetrate the cell membrane, interact with various biological molecules, and accumulate inside tumours. Charged particles generate electrostatic attraction or repulsion with other charged particles, further impeding their diffusion. Their stability can be increased by functionalizing their surfaces with molecules that prolong their circulation throughout the body. These nanoparticle nanocarriers may be used to enhance the intracellular delivery of drugs to cancer cells while also knocking down abnormal gene or protein expression in cancer cells when combined with chemotherapy drugs or therapeutic RNA molecules (siRNA) for gene therapy. Nanoparticle-mediated targeted delivery may substantially reduce drug dosage and toxicity while improving drug bioavailability and gene therapy for improved prognosis in the case of hardly treated pancreatic cancer.

7.2.3.1 Chemoprotective Drug Delivery Via NPs

Low dosages of gemcitabine are administered using nanoparticles, which enhance distribution in cancer cells and enhance efficacy. In PDAC tumour models, Rejiba et al. demonstrated increased efficacy of Gem (4-(N)-tris-nor-squalenoyl-gemcitabine (SQ-Gem) nanoparticle formulation (Couvreur et al. 2008). It inhibited cell proliferation and induced apoptosis in resistant Panc1 cells when administered at a concentration of 5 microM, resulting in 40% of apoptotic cells. In contrast, treatment with free gemcitabine killed only 10% of the cells and had no effect on tumour growth or survival in mice. The gemcitabine–squalene nanoassemblies

produced similar results in vitro and in vivo, with a 70% reduction in tumour volume (Maksimenko et al. 2015).

(a) *Gelatin-based drug delivery*—Gelatin has been proven as an effective gemcitabine carrier owing to its safety, biocompatibility, and biodegradability (Maksimenko et al. 2015). To enhance absorption of NP by pancreatic cancer cells, the EGFR peptide was attached to gelatin through a PEG linker (MW 2000 Da, size: 150–250 nm) for targeted drug delivery. PEGylation is well recognized to improve and prolong the systemic circulation. Gemcitabine release into Panc-1 cells from Gem-Gel-PEG- EGFR nanoparticles occurs following a disulfide bond cleavage. Intravenous injection of an EGFR-targeted Gem-Gel-PEG nanoparticle (one per week for four weeks) substantially decreased tumour volume (approximately 70%). However, nanoparticles were also found in the liver and spleen, and no adverse effects were found. Two chemotherapeutic drugs may be incorporated into nanoparticles to enable dual or multidrug delivery. Self-assembled nanoscale coordination polymers (NCPs)-based nanoparticles containing two agents (oxaliplatin and gemcitabine) showed a significant anticancer impact by inducing apoptosis by 75% in AsPc-1 and 80% in BxPc-3 cells in vitro and 80% in vivo (Poon et al. 2015). AsPc-1 xenograft models treated with this formulation showed an 11-fold reduction in tumour size compared to the controls, indicating that it prevented tumour development.

(b) *Inorganic nanoparticles*—Some of these NPs, including iron oxide, carbon nanotubes (CNT), quantum dots (QDs), and gold nanoparticles (AuNP), have been studied as drugs or gene carriers to enhance drug treatment effectiveness and extend the lifespan of pancreatic cancer models in preclinical and clinical trials (Hwang et al. 2012). The administration of (intravenous) IONPs coupled with IGF-1 and loaded with Dox (size: 20.4 nm) into an orthotopic pancreatic PDX model (Zhou et al. 2015) showed enhanced nanoparticle selectivity and accumulation within the tumour region, resulting in substantial suppression of cell proliferation and tumour development (untreated vs. IGF1-IONP- Dox). Using a drug that selectively targets IGF-1R led to increased amounts of Dox (5 mg/kg dose) in the tumour, which helped reduce tumour mass.

7.2.3.2 Nanoparticle-Based Delivery of siRNAs

In the RNA interference (RNAi) pathway, there are three primary methods to silence genes: small interfering RNAs, microRNA, and short hairpin strands of RNA (shRNA). Non-coding RNAs, such as siRNA, also known as small interfering RNA or silencing RNA, are made from double-stranded RNA molecules and have a length of 20–25 base pairs. These molecules act as part of the RNAi mechanism. Once siRNA has been delivered into cells, the enzyme Dicer cleaves it into small fragments that direct the loading of siRNA molecules into a protein complex known as the RNA-inducing silencing complex later on (RISC). RISC proteins act in the unwinding of siRNA and cleavage of siRNA sense strand, leaving anti-sense strand free to complementary bind to mRNA and induce post-transcriptional gene silencing

(Guo et al. 2013). RNAi has been used in many preclinical and clinical investigations to suppress tumour-associated oncogenes, growth factors, and angiogenesis-promoting receptors that are overexpressed and contribute to tumour development; compared to other oligonucleotides, siRNA therapies offer many benefits. They can be easily chemically synthesized and efficiently suppress gene expression. Since it doesn't directly attach to DNA, there are no concerns of new mutations being generated during gene therapy. While siRNA has many benefits, it also has certain drawbacks when it comes to cancer treatment. It is highly unstable in body fluids and serum due to nuclease-induced degradation. For successful delivery of siRNA to cancer cells, many delivery systems including liposomes, polymers, and inorganic nanoparticles have been developed and conjugated with cancer-specific targeting molecules.

Polymeric-Based Nanoparticles for siRNA Delivery into PDAC

NPs based on polymers have been utilized in vitro and in vivo as carriers for siRNA delivery that specifically targets the KRAS gene or other target genes (Xu and Wang 2015). Stability, safety, and effectiveness have been shown in a PDAC mouse model for the local intratumoural delivery system LODER (Local Drug EluteR) (Khvalevsky et al. 2013). When used in a mouse model of PDAC tumours, LODER effectively delivered KRAS siRNA, reduced tumour development, and extended overall survival.

Cationic poly (lactic acid) (CPLA) biodegradable nanocapsules (CPLA-NC with zeta potentials of +45 MV and diameters of 32 nm) were evaluated for their ability to silence the KRAS oncogene in PDAC models (Lin et al. 2013). Through electrostatic interactions, negatively charged siRNA was attached to the surface. In PDAC models, CPLA-NC containing anti-KRAS siRNA could reduce the expression of the KRAS gene by nearly 50%. This complex did not have any nanoparticle-based cytotoxicity, indicating that it is safe for use in in vivo experiments.

PLGA/poloxamer (polyethyleneimine-poly (lactide-coglycolide) nanoparticle for siRNA delivery into PDAC. About 67% of PDAC patients had elevated levels of hypoxia-inducible factor 2 (HIF-2), also known as endothelial PAS domain protein 1 (EPAS1). Overexpression is associated with a poor prognosis, an advanced stage, and lymph node metastases, making it a possible therapeutic target in PDAC. In vivo targeting of EPAS1 with siRNA encapsulated in a PLGA/poloxamer (polyethyleneimine-poly (lactide-coglycolide)) nanoparticle resulted in improved intracellular uptake (Pan et al. 2015). PLGA has been used for many years for siRNA delivery. However, due to the low electrostatic interaction between PLGA and siRNA, a cationic polyetheyleneimine (PEI) polymer is coated on the surface of PLGA to overcome this limitation. Treatment of a nude mouse model with EPAS1siRNA nanoparticles resulted in a substantial decrease in tumour volume, according to in vivo tests in PDAC models.

To address the issue of opsonization and improve the efficacy of gene silencing, nanoparticles were PEGylated with POEGMA (38 nm). High transfection efficiency and uptake of fluorescently labelled siRNA star-POEGMA nanoparticles into

MiaPaca-2 cells were observed. There was a significant, more than 80%, reduction in βIII- tubulin gene expression following systemic delivery (4 mg/kg) of star nanoparticles. The stability of nanoparticles with POEGMA was achieved and star polymeric nanoparticles carrying siRNA against βIII-tubulin showed a therapeutic effect in an in vivo orthotopic pancreatic mouse model (Teo et al. 2016).

The overall study suggested that using siRNA as a therapeutic agent for pancreatic cancer with the ability to image tumour response in vitro and in vivo offers a feasible approach, with numerous benefits over conventional treatments.

7.2.3.3 Photothermal Therapy by Inorganic Nanomaterials

Some nanomaterials can transform light energy into heat energy, which makes them potent therapies for targeting cancerous tissues. Using this approach to treat cancer has many benefits, including less invasion, fewer side effects, controllability, and specificity to particular tumour areas. Inorganic nanoparticles such as gold, carbon nanotubes, and copper sulfide nanoparticles were shown to successfully convert photo energy into thermal energy (Bao et al. 2016).

- Gold nanorods
  Recently, gold nanorods have gained considerable interest owing to their plasmonic photothermal treatment properties. After being irradiated with a short laser pulse, gold nanorods produce vapour nanobubbles called plasmonic nanobubbles. Patino et al. (Patino et al. 2015) functionalized the surface of gold nanorods using thiol-PEG-biotin to remove the cetyltrimethylammonium bromide (CTAB) layer on the nanorod's surface, which is known to be hazardous to cells. Additionally, they coupled gold nanorods with EPPT (MUC-1-specific peptide) and MPAP (myristoylated polyarginine peptide) peptides to enable targeted delivery by MUC-1 markers and enhance cellular uptake of gold nanorods. This resulted in extremely selective apoptosis after laser irradiation with no harm in surrounding cells. High loading of nanomaterials is usually needed in photothermal treatment approaches to produce adequate heating, and in this instance, the uptake rate of gold nanorods was enhanced by dual conjugation (EPPT and MPAP). Yin and colleagues (Yin et al. 2015) investigated the triple impact of KRAS gene silencing, doxorubicin, and photothermal treatment in pancreatic cancer therapy. They utilized a multilayer arrangement to cover the surface of gold nanorods with a negatively charged PSS polymer for doxorubicin capture and a positively charged PAH polymer for siRNA capture. Doxorubicin and KRAS siRNA were released into tumour cells under the control light (665 nm), which inhibited tumour development for at least 25 days.
- Carbon nanotubes
  PEG-functionalized multi-walled carbon nanotubes showed photothermal effects on PANC-1 cells at varying nanoparticle concentrations (5, 10, 50 g/ml) (Mocan et al. 2014). At dosages of more than 10 μg, laser irradiation significantly increased the number of apoptotic cells. Exposure to 50 μg/ml resulted in a substantial increase in reactive oxygen species (ROS), with 57% of pancreatic cancer cells expressing ROS.

7.3 Clinical Trials

In clinical trials, the entry of nanocarriers-based formulations opens a new pathway for the treatment of pancreatic cancer. A significant number of nanocarrier-based formulations are now in various phases of clinical trials. This formulation includes polymeric nanoparticles, liposomes, amphiphilic polymers nanoparticles, small interfering ribonucleic acid (siRNA) nanoparticles, dendrimers, carbon nanotubes, gold nanoparticles, quantum dots, inorganic nanoparticles, and magnetic nanoparticles (Table 7.2).

7.4 Challenges for Nanocarriers-Based Targeted Therapies

7.4.1 Physiological Barriers

Pancreatic stellate cells (PSCs) are a major barrier to any antineoplastic nanomedicine or conventional drug delivery to pancreatic tumour cells. PSCs cells stimulate pancreatic cancer and undergo functional and morphological alterations. As a consequence, the ECM is overproduced and deposited, leading to fibrosis of pancreatic stroma. Stromal fibrosis promotes tumour development by creating a favourable environment, and it also plays a key role in distant metastasis. Additionally, stromal fibrosis restricts delivery of drugs to the tumour site, resulting in less sensitivity to drugs and, sometimes, resistance.

PDAC develops in the exocrine area of the pancreas and is graded according to the histology of intraepithelial neoplasms (PanIN-1–3). Each PanIN stage has its own histological features, and the accumulation of mutations at each stage correlates with the progression of the disease (Cowan and Maitra 2014).

The desmoplastic nature of the stroma generates solid stress and/or increased interstitial fluid pressure inside the tumour, resulting in vessel compression and insufficient perfusion and hypo-vascularity, leaving about 80% of the tumour's vessels non-functional. The tumour microenvironment in PDAC is typically hypoxic due to poor perfusion and hypo-vascularity (Chauhan et al. 2013).

PDAC has a high stromal-to-neoplastic tissue ratio and a thick desmoplastic stroma composed of cellular (endothelial, nerve, and immune cells, as well as fibroblasts) and acellular (fibrin, collagen, fibronectin, and hyaluronan) components (Cowan and Maitra 2014; Rucki and Zheng 2014). Neoplastic cells usually make up less than 20% of the tumour mass.

Inflammatory cytokines, such as IL-1 and IL-6, and growth factors, such as tumour necrosis factor (TNF) and transforming growth factor1 (TGF-1), may activate pancreatic stellate cells in the tumour stroma, causing them to secrete copious quantities of extracellular matrix components that serve as a barrier to drug extravasation into the tumour interstitium (Phillips et al. 2003).

Stellate cells secrete matrix metalloproteinases (MMP-1 and MMP-9) that destroy basement membrane proteins, causing the initiation of fibrosis and cancer cell invasion (Li et al. 2010).

Table 7.2 Current status of nanocarriers-based targeted therapies in clinical trials

Intervention	Targets	Phase	Result	NCT identifier
ATI-1123: Liposomal docetaxel	Tubulin	I	Tumour size reduced to 29% from baseline in 1 of 6 patient with PDAC	NCT01041235
BIND-014: Polymeric docetaxel nanoparticle	Tubulin	I	_	NCT01300533
Doxil: PEGylated liposomal doxorubicin (in combination with topotecan)	DNA	I	_	NCT00252889
TKM-080301: Lipid nanoparticles containing PLKI siRNA	PLK1	I	_	NCT01437007
siG12D-LODER: Biopolymeric cylindrical implant	$KRAS^{G12D}$	I II	Decreased CA19–9 levels in 70% of patients; median overall survival (OS) was 15.1 months. Phase II trial ongoing	NCT01188785 NCT01676259
Atu027: Liposomal PKN3 siRNA	Silences PKN3 (a PKC-signalling pathway molecules)	I & II	Ongoing	NCT01808638
SGT-53: Liposomal p53 plasmid DNA (with nab-paclitaxel + gemcitabine)	P53	II	Ongoing	NCT02340117
NC-6004 (Nanoplatin): Micellar polymeric nanoparticles encapsulating cisplatin (with gemcitabine)	DNA	II & III	_	NCT00910741 NCT02043288
MM-398 (onivyde): Liposomal irinotecan (with 5-FU/folinic acid)	Topoisomerase 1 inhibitor	III	Improve PFS, ORR, and OS versus 5-FU/ folinic acid alone The FDA has given its clearance for usage in combination as a second-line treatment.	NCT01494506

7.4.2 Challenges in Clinical

7.4.2.1 Controllable and Reproducible Synthesis

It is necessary to determine the optimum physicochemical parameters for the effective development of therapeutic nanoparticles (NPs). There has been significant progress in understanding the individual factors that contribute to successful immune evasion, cell targeting and internalization, extravasation and diffusion, and controlled drug release (Alexis et al. 2008; Perrault et al. 2009). It is still difficult to systematic parallel screening the wide range of NP attributes, due to the challenges of rapid, precise, and reproducible synthesis of NP libraries with unique characteristics. For the high-speed self-assembly of NPs with smaller size distribution, adjustable physical and chemical properties, and better batch-to-batch repeatability, microfluidic methods have lately gained interest compared to conventional bulk approaches that typically produce NPs with significant polydispersity (Valencia et al. 2010; Chen et al. 2012). In the same way, particle replication in non-wetting template (PRINT) technology has allowed the synthesis of monodispersed nanoparticles with exquisite control over chemical composition, drug loading, surface characteristics, and shape and size (Rolland et al. 2005; Xu et al. 2013).

7.4.2.2 Evaluation and Screening

As new biomaterials or nanostructured NPs rapidly develop, in vitro assessment is becoming more essential for identifying biocompatible candidates before moving to animal testing. Additionally, in vitro tests may help us better understand the interaction between the nanoparticle and the cell. However, since traditional in vitro models based on cell culture in multi-well plates lack the complexity of real biological tissue and the ability to regulate fluid flow, they may be unable to represent the complicated interaction of nanoparticles with physiological barriers. Recent attempts to create biomimetic 'organ/tumour on a chip' technology may overcome some constraints associated with existing in vitro models (Toh et al. 2009; Huh et al. 2010; Albanese et al. 2013). Tumour-like spheroids incorporated into a microfluidic channel may provide information on the impact of cell binding, interstitial flow, and diffusion (Albanese et al. 2013). Nanoparticle behaviour in these chip systems may be comparable to those of animals, which may provide a glimpse into the future possibilities of these biomimetic microdevices. To evaluate NP performance in vivo (for example, biodistribution, PK, efficacy, and safety), animal models are required. One well-recognized barrier is the difference seen between efficacy achieved in preclinical studies and the results of clinical trials. This is a significant area due to the paucity of tumour models that adequately replicate human malignancies, despite the fact that certain studies have shown PK scaling across various species (including humans) for different nanotherapeutics (Zuckerman et al. 2014; Schultheis et al. 2014). Many animal models are now available, including orthotopic xenografts, cell line-based subcutaneous, genetically engineered mouse models (GEMMS), and patient-derived xenografts (PDX). However, not a single model can completely replicate all aspects of human malignancy. On the other hand, EPR is usually more consistent in animals than in human cancer patients.

Additionally, since tumour metastases are a significant cause of cancer death, a model of human tumour metastasis will be crucial in evaluating EPR and nanoparticle penetration and targeting in metastasis tumour in comparison to primary tumour.

The translation of nanotherapeutics may be significantly aided by the creation of animal models that accurately replicate the heterogeneity and anatomical histology of human tumours, such as humanized mouse (Shi et al. 2017)models (Rongvaux et al. 2014), high-fidelity PDXs (Lin et al. 2014), and GEMMs with aggressive metastasis.

7.4.3 Manufacturing on a Large Scale

Another challenge to clinical development stems from the escalating complexity in chemistry, manufacturing and controls (CMC) and good manufacturing practice (GMP) requirements as NP technology transitions from preclinical to clinical development, subsequent commercialization and beyond, as long as the product is on the market. Both aim to ensure that a product consistently meets a specified quality standard, although their methods and regulations diverge yet overlap. Additional GMP and CMC difficulties may arise when more complex nanomedicines are scaled up. This could involve changes to current unit operations or the development of novel manufacturing processes.

Complex technology and numerous stages in the NP formulation process make large-scale and repeatable synthesis more challenging (Shi et al. 2017). A change in formulation parameters or technique is almost always required when moving a molecule from the laboratory to clinical trials, thus thinking about scaling up early is critical to NP design and engineering.

7.4.4 Funding

Financial issues are yet another hindrance in the development of nanocarrier-based systems, as it is difficult to demonstrate their efficacy and safety to gain regulatory approval using traditional medicine's guidelines (Rebelo and Reis 2018). The majority of currently approved nano pharmaceuticals are based on already approved conventional drugs, and their contribution is still negligible. Only a small number of nanotherapeutics are currently in the development stage and will get regulatory approval.

7.5 Conclusion

Several attempts have been made recently to deliver chemotherapeutic drugs such as gemcitabine and gene inhibitors such as siRNA via nanoparticles. Various nanoparticles, including polymeric, inorganic and lipid-based NPs, have been created to suppress pancreatic cancer development, and metastasis in addition to efforts

to enhance gemcitabine administration, a front-line treatment in PDAC. There is also optimism that siRNA therapies will be utilized to inhibit pancreatic cancer development by overcoming drug resistance, decreasing off-target toxicity, and improving chemotherapeutic agent antitumour effectiveness. PDAC may be treated well by targeting KRAS, EGFR, and genes. Developing nanoparticles that contain both siRNA molecules and chemotherapeutic drugs and then delivering them to cancer-specific receptors to enhance active cancer cell delivery has emerged as a viable treatment for PDAC. Most nanoparticles have desirable characteristics in vitro, such as toxicity and stability, but in vivo safety and toxicity profiles may vary. As a result, after in vivo administrations, comprehensive safety and toxicology investigations should be performed. Both cancer cells and the tumour microenvironment are anticipated to be targeted by novel formulations as well as communication networks in the stroma that support cancer cells. Targeting signalling pathways active in both the stroma and tumour compartments is effective. In conclusion, nanoparticles are probably more widely used in the era of personalized drugs to create single- or multi-gene targeting therapeutic approaches as well as chemotherapeutic or small molecule inhibitors.

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