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Review

Targeted Therapies for Pancreatic Cancer and Hurdles Ahead

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Abstract. *Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers with a median survival of 6 months after diagnosis. Intrinsic resistance to chemotherapeutics and lack of effective targeted therapies are the major factors contributing to dismal prognosis. Several important genetic alterations (i.e., mutations, deletions) have been identified to be involved in the initiation and progression of pancreatic cancer, including KRAS and inactivation of tumor suppressors, such as TP53, SMAD4 and CDKN2A. Unique tumor microenvironment with excessive stroma due to desmoplastic reaction is one of the major characteristics of PDAC, promoting tumor growth and leading to treatment failures. In addition, tumor stroma represents an important biological barrier for drug delivery and successful treatment of PDAC. Small interfering RNA (siRNA) has recently emerged as a potential and targeted therapeutic approach which is now evaluated in clinical trials. However, siRNA-based therapeutics face important challenges, including rapid serum degradation, poor tumor cell uptake and cellular uptake, leading to off-target effects. Therefore, there is a great need for the development of safe and effective nanoparticles for better tumor-specific delivery of anti-cancer therapeutics. In this article, the main challenges in the treatment of pancreatic cancer and recent advancements on nano delivery systems of chemotherapeutics and gene-targeted agents, used both in preclinical and clinical trials are reviewed.*

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies and the fourth leading cause of cancer-related deaths. Currently, median patient survival is about 6-8 months after diagnosis (5-year survival 1-5%) and accounts for approximately 42,000 deaths per year in the US (1). PDAC incidence is on the rise and predicted to be the second cause of cancer-related deaths by 2030 (2). Despite significantly increased knowledge in biology and genetics and efforts in the development of new methods for early detection and therapeutic approaches, poor survival rates have not significantly changed in the last 30 years and PDAC is still associated with an extremely poor prognosis (1). The poor survival rates are attributed to its highly aggressive nature, intrinsic resistance to chemotherapeutics and lack of effective therapeutics, as well as late diagnosis due to non-specific symptoms and unavailability of early diagnostic tools.

Although both the endocrine and exocrine cells of the pancreas can transform to cancerous cells, pancreatic cancer (PaCa) formed from exocrine cells is much more common and nearly all of PDAC tumors are adenocarcinomas. The overwhelming majority (~85%) of patients present with locally advanced or metastatic disease and progression of disease is virtually unstoppable with current therapies having a minimal impact on survival. Beside single-agent treatments including gemcitabine, 5-fluorouracil (5-FU), irinotecan, oxaliplatin, albumin-bound paclitaxel (*i.e.*, Abraxane), cisplatin, paclitaxel (Taxol), docetaxel (Taxotere), different combinations of chemotherapeutics (2 or more drugs) are used. In the metastatic

PaCa, FOLFIRINOX (folinic acid, 5 fluorouracil, irinotecan, and oxaliplatin), and nab paclitaxel plus gemcitabine have yielded only modest improvements in survival (3). FOLFIRINOX (FOL-folinic acid (leucovorin), a vitamin B9 derivative that reduces the side-effects of fluorouracil; F–fluorouracil (5-FU), a pyrimidine analog and antimetabolite which incorporates into the DNA molecule and stops DNA synthesis; IRIN–irinotecan, a topoisomerase inhibitor, which prevents DNA from uncoiling and duplicating; and OX–oxaliplatin, a platinum-based antineoplastic agent, which inhibits DNA repair and/or DNA synthesis) has been tested in clinical trials of PaCa therapy and showed advantage over gemcitabine since it slightly but significantly increased in median survival (4). The overall survival was increased when gemcitabine was used in combination with nab-paclitaxel (8.5 months) compared to treatment with gemcitabine alone (6.7 months) (5). However, during the last three decades, the outcomes of patients with PDAC have not changed significantly. Chemotherapy can be given along with radiation, which is known as chemoradiation or chemoradiotherapy. However, these combinations can also cause more severe side-effects, limiting their common use in clinical practice. Other treatment options for PaCa are being investigated, with the main focus on the development of different small-molecule inhibitors targeting specific receptors on tumor cells, their use in combination with approved chemotherapeutic agents, or inhibition of overexpressed genes by siRNA molecules. For improved drug and siRNA delivery, efficient nanoparticles have been developed. Nanoparticle-mediated targeted delivery is also being investigated to lower drug-related toxicity, to improve drug bioavailability and increase efficacy of drugs in PDAC.

In this article, the genetics and biology of PDAC and the recent advancements in targeted therapies are reviewed with a special focus on nanotherapeutics. Particular attention is paid to highly specific potential molecular targets and nanoparticles used for either targeted delivery or gene therapy.

Etiology, Genetics and Biology of Pancreatic Ductal Adenocarcinoma

The pancreas is an organ located in the abdominal cavity and consists of four main anatomical components, head, neck, body and tail, where the head is oriented towards lower parts of the duodenumand the tail is ending near spleen (Figure 1). Pancreas constists of two functional units, exocrine and endocrine pancreas. Endocrine pancreas constitutes only 2% of the total pancreatic mass. Exocrine cells including acinar and duct cells are responsible for secretion of digestive enzymes, while endocrine cells also called islets of Langerhans mainly function in the regulation of metabolism and glucose homeostasis through hormone (*i.e.*, insulin) secretion (6). Most pancreatic cancers arise from cells in the ductal epithelium located at the head of the pancreatic gland. The most common type of pancreatic cancer is ductal adenocarcinoma and accounts for about 90% of tumors in the exocrine pancreas (7). Other types of pancreatic cancer are less common and include neuroendocrine tumors such as insulinomas, glucagonomas and gastrinomas.

Even though factors causing PDAC are not known; smoking, alcohol, chronic pancreatitis, obesity and longstanding diabetes represent the main risk factors for the development of pancreatic tumors (8). Genetic factors also represent a risk for PDAC and about 5-10% can be inherited (9). The occurrence of pancreatic cancer increases with age and people over 65 years old have a poorer prognosis (10, 11).

PDAC in most cases evolves from pre-invasive lesions named as microscopic pancreatic intraepithelial neoplasia (PanIN) (12). Loss of cellular polarity and enlargement are observed within three types of lesions also classified as grades (13). Low-grade lesions are observed in the PanIN-1A and PanIN-1B, moderate in PanIN2 and severe cytological and architectural atypias are dominant in PanIN3 (13, 14) (Figure 1). PanIN3 grade lesions are also called "carcinoma *in situ*" (13, 14) and are closely associated with aggressive and metastatic PDAC. Unfortunately, most patients are not diagnosed prior to this stage and most metastatic cells already have escaped to surrounding tissues. Even though the exact genetic features that drive the progression of these precursor lesions to advanced disease are not fully understood, cells continue to grow and expand and acquire additional mutations during the progression of PanIN (15).

There are several well-defined driver genes that are important in the tumorigenesis and progression of PDAC. The most commonly mutated gene in pancreatic cancer is *KRAS* (Kirsten Rat Sarcoma) seen in approximately 90-95% of all pancreatic cancer cases. *KRAS* gene encodes a protein of the RAS family that is involved in many cellular functions such as proliferation and cell survival (16). Oncogenic *KRAS* mutation is present from the early beginning of the low-grade lesions and its frequency increases as the grade of PanIN lesions is increased and detected in about 87% in PanIN2 and PanIN3 (17). The most frequently observed genetic abnormalities in PanINs and invasive PDAC include inactivation of tumor suppressor genes (*i.e.*, *TP53, SMAD* and *CDKN2A*) as well as epigenetic alternations, chromosomal changes (rearrangements, losses and amplifications) and other gene mutations (16).

Tumor suppressor genes that are inactivated in PDAC include *TP53* (mutated in 75-90% PDAC cases), P16/*CDKN2A* (mutated in 50-98%) and *SMAD4* (mutated in 20-50%) (12). *TP53* encodes the p53 protein, the guardian of the genome, which is involved in many cellular processes, in DNA damage and in the regulation of cell-cycle checkpoints, cell-cycle arrest, and apoptosis (18). The mutation inactivates the antiproliferative properties of TP53, leading to promotion of tumor growth and metastasis (18). Inactivation of cyclin-dependent kinase inhibitor 2A (*CDK2A*) gene encoding p16,

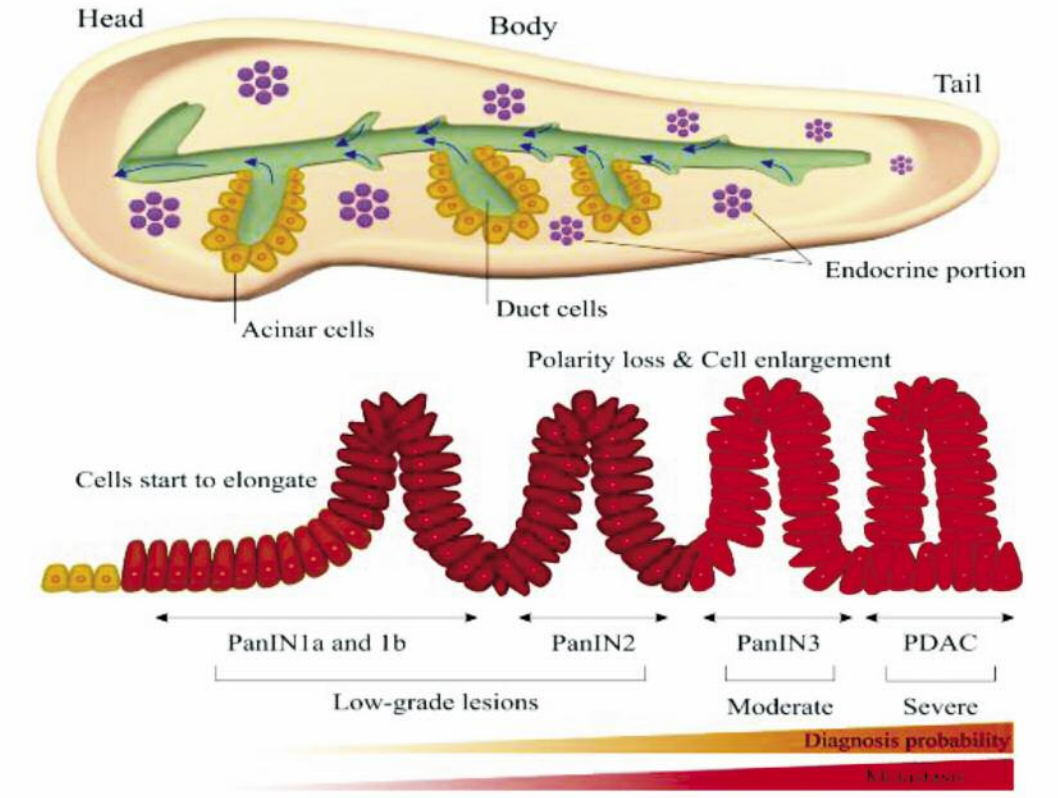

Figure 1. *General overview of the anatomy of pancreas and development and progression of the pancreatic cancer with its subtypes.*

results in the loss of function of this regulatory protein involved in the G_1-S checkpoint of the cell cycle leading to an increase in cell proliferation. Mutations in the *SMAD4* gene result in the aberrant activation of the transforming growth factor (TGFβ) signaling pathway that is important for cellular growth and differentiation (19, 20). Other mutations affect signaling pathways such as Notch, Wnt, DDR, Hedgehog (SHH), or various cellular processes including stem cell growth and maintenance, apoptosis and epigenetic regulation. Mutations in epigenetic regulators or deletions in genes, such as *ARID1A* can result in defective pathways regulating DNA damage (21) and can promote cell survival and oncogenic signals (22). Mutations in other genes including *BRCA1, BRCA2, ATM, ERBB2, EGFR, MET,* and *FGFR1* have been reported to be important in PDAC development (12, 22, 23). Amplification of *MYC* has been reported to be crucial for both initiation and progression of PDAC (22). Epidermal growth factor receptor (EGFR) has certain significance as it is expressed in all grades of PanIN lesions, and is essential for the generation of *KRAS* oncogene lesions (24). EGFR expression in pancreatic lesions can also activate STAT and AKT signaling pathways, which are also thought to be involved in PDAC development and progression (24).

PaCa microenvironment is composed of a highly heterogeneous collection of cancer cells, extracellular matrix and various stromal, endothelial cells, inflammatory cells, immune cells (T cells, tumor-associated macrophages) and cancer stem cells that have tumor-promoting functions (25, 26). Extreme desmoplastic reaction and excess stroma formation is an important charasteristic of PDAC, promoting tumor growth, progression and drug resistance (27). There is a dynamic bi-directional crosstalk between stromal and cancer cells, leading to activation of oncogenic signaling pathways and immune suppression.

Pancreatic stellate cells (PSCs) are tumor-associated fibroblast-type cells that play a key role in the formation of tumor stroma (26). PSCs carry characteristics of myofibroblasts, and can migrate, proliferate and secrete collagen proteins, growth factors and other components of the extracellular matrix that support the progression of cancer. PSCs are activated by molecules such as growth factors, including PDGF, TGF-beta, TNF-alpha and cytokines such as IL-6 and IL-10 (26). Activated PCSs also secrete various growth factors such as TGF-β, PDGF and sonic hedgehog (sHH) and activate signaling pathways promoting tumor growth (7, 28).

Treatment of Pancreatic Ductal Adenocarcinoma

Only a small percentage of patients diagnosed with early-stage pancreatic cancer is eligible for surgical resection and their 5-year survival rate has increased to about 20% (2, 29). Most PDAC patients receive treatments such as chemotherapy and/or radiotherapy along with or after surgery, however, these treatments have a minimal impact on survival rate because of their intrinsic resistance to apoptosis. Thus, identification of new biomarkers for early diagnosis and identification of novel molecular targets involved in the progression of pancreatic cancer are urgenty needed. There are various therapy options available for the treatment of non-metastatic and advanced-stage PaCa. Besides primary chemotherapy, radiation is also a promising approach that can slightly improve survival and the quality of life of patients. In contrast to other types of cancer, targeted therapies do not have a relative impact on metastatic pancreatic cancer due to the complex molecular mechanisms involved. The most promising strategy for the treatment of PDAC seems to be the inhibition of mutated genes, such as *KRAS* and molecules that are part of signaling pathways that highly contribute to the progression of the disease. Targeted agents used in combination with chemotherapy have been extensively studied, but most of the gene inhibition approaches are still only available in preclinical settings. More importantly, recent studies indicate that the major challenge for the successful treatment of PDAC is the effective delivery of therapeutic agents into tumor and tumor microenvironment, highlighting the significance of nanoparticles and nanodelivery.

Conventional Therapies

There are several drugs and drug combinations that have been approved by FDA for pancreatic cancer among which 5-flourouracil, albumin bound paclitaxel, gemcitabine, and FOLFIRINOX are the most commonly used. Since gemcitabine has a short half-life and experiences rapid body clearance, it is usually administered in higher and repeated doses that lead to many negative side effects (30, 31). It has also been shown to rapidly induce resistance in cancer cells through many different but unclear mechanisms (32). Gemcitabine and erlotinib (EGFR inhibitor) remain the only two agents approved for use in advanced disease despite their modest benefits. Despite the survival benefits observed in clinical studies, only 5-10% of PDAC cases exhibit response to gemcitabine therapy. Combination of gemcitabine with erlotinib prolongs one-year survival by only 23% (33). FOLFIRINOX significantly improves overall survival in patients with metastatic PDAC by about 4 months (11.1 months), compared to gemcitabine showing the overall survival of 6.8 months (4).

There are several reasons why pancreatic cancer is still difficult to treat with conventional therapies. High frequency of genomic changes seen in PDAC leads to significant genomic instability and may limit the effectiveness of therapy, especially of targeted agents, by contributing to secondary or acquired chemoresistance. Mutations in some genes including p53 can result in loss of their suppressor functions that can later lead to more genetic instability, decrease of apoptosis and reduced response to therapeutics.The number of gene mutations is shown to be strongly correlated with overall survival of patients (34). These combined mutations make PDAC patients respond weakly to the conventional treatments. There is an extremely complex network of signaling and genetic alternations and cross talk between cells and microenvironment that make it more difficult to treat. Approximately 63 genetic alternations are defined in 12 different signaling pathways that are usually abnormally activated in the majority of PDAC cases (35). Hypovascularity and high desmoplastic reaction are thought to act as an important barrier for delivery of chemotherapeutic agents (36) leading to reduced exposure of PDAC cells to inadequate doses.

Targeted Therapies

As mentioned above, PDAC has a genetically heterogenous nature, and conventional therapies that target different cellular processes fail to distinguish between cancerous and normal cells leading to unacceptable side-effects. Therefore, there is a need for targeted therapies using small-molecule inhibitors and monoclonal antibodies targeting either cancer cell surface receptors, growth factors or other proteins that contribute to the overall progression of the disease.

Targeting Signaling Pathways in PDAC

Abnormal activation or deregulation of different signaling pathways highly contribute to the heterogeneity of pancreatic cancer. Many efforts have been made towards development of effective inhibitors such as biological or small molecule agents, however most of these signaling inibitors are still under investigation, and have not been yet aproved for clinical usage. Comparing to the conventional agents used in the clinic, targeted therapies work more precisely by inhibiting a significant signaling pathway that functions as a critical driver of cell proliferation, survival, metastasis and progression.

KRAS signaling; *KRAS* mutation is detected in about 90% of PDAC cases and represents one of the most important therapeutic targets since activating mutations of this oncogene are the major driver of the disease and its onset (37). *KRAS* proto-oncogene encodes a protein that has GTP-ase activity. The *KRAS* Mutation G12D leads to constitutive phosphorylation and activation of this pathway (38). *KRAS* mutations activate different signaling pathways such as RAF, MEK, ERK as well as P13K/AKT pathway (39) which play important roles in cell division, survival, and drug resistance inhibiting apoptosis, promoting tumor growth and progression. KRAS activity can influence the microenvironment of PDAC by producing factors

such as Sonic Hedgehog (40), interleukin-6 (41), prostaglandin E (42), and in this way regulate stroma maintenance (43). KRAS signaling was also shown to act in the promotion of immunosuppression (43).

Although over 90% of PDAC harbor activating mutations in *KRAS*, one of the most potent of all human oncogenes, still there are no *KRAS* targeted therapies in the clinic. Direct inhibition of KRAS has proven challenging due to relatively smooth surface of 3D structure, classifying KRAS as an undrugable target (44, 45). Instead, multiple groups have investigated the efficacy of targeting KRAS indirectly and targeted its downstream mediators such as MEK and PI3K pathways. However, inhibition of PI3K is complicated by the existence of multiple isoforms of the PI3K protein and the fact that not all isoforms of PI3K interact with KRAS (46). New strategies for the treatment of advanced stage PDAC need to be developed to overcome the high drug resistance and increase survival of patients. Targeting KRAS opens many possibilities to develop novel strategies for suppressing KRAS activity. Allosteric compounds that access an inducible pocket formed in the structureof KRAS, have been reported, but these compounds require further optimization to enable their advancement into drugs that can be used in the clinic. Recently, covalent inhibitors of KRAS G12C have been reported (47, 48) a few of which could be tested in clinical trials by 2020.

TGF-β signaling; TGF-β plays an important role in homeostasis and various cellular processes, including cell growth, differentiation and apoptosis (49). At the early stages of tumor progression TGF-β has a tumor suppressive function and inhibits cell growth but with the progression of tumorigenesis, it gains an oncogenic function in PDAC (50). SMAD proteins that can be classified as receptor-regulated, mediators or inhibitors, usually activate TGF-β signaling. Binding of TGF-β ligand to type II receptor leads to SMAD2 and SMAD3 phosphorylation, which then make a complex with SMAD4 tumor suppressor protein (7). Inhibitory SMADs (SMAD6 and SMAD7) can disrupt this signaling *via* binding to the receptor. This pathway is usually up-regulated in patients with PDAC. Mutation in *SMAD4* is observed in about 40% of pancreatic tumor cases, leading to its reduced expression and inactivation (51). *SMAD4* mutation is recognized and seen in high-grade PanIN lesions (51). Activation of pathways due to *SMAD4* mutation promotes tumor cell survival, stimulates angiogenesis and contributes to the resistance to chemotherapy. Mutations in TGF-β1 and TGF-βR-1-2 are observed in most of the pancreatic carcinoma cell lines and patients with advanced pancreatic cancer, and contribute to the poor survival of patients (52). It has been shown that immune activation of *TGF-β* gene knockdown mice can result in tumor cell apoptosis and prolonged survival. Targeting TGF-β with siRNA induced apoptosis *via* activation of RIG-I signaling, significantly reduced TGF-β serum levels, and demonstrated antitumor activity upon systemic treatment of orthotopic PDAC mice models (53). One of the molecules that negatively regulate TGF-β pathway is SnoN (Ski-related novel protein N) protein and its silencing leads to a decrease in proliferation and increase in apoptosis of cancer cells *in vitro* (54), suggesting that TGF-β signaling represents and important molecular target in PDAC tumors.

Hedgehog signaling; Overexpression of Sonic Hedgehog ligands (SHH) in PDAC tumors results in cancer initiation and metastasis and in a significant desmoplastic reaction (36). It binds to the PTCH1 receptor that acts in the regulation of Smoothened protein (SMO) and downstream of the pathway. Overexpression of Hedgehog SMO results in aberrant activation of the sHH pathway in pancreatic cancer (55). Currently, many ongoing studies investigate the role of inhibition of SMO receptors in PDAC. Inhibition of SMO receptor by saridegib in combination with gemcitabine significantly decreased desmoplasia and collagen deposition and increased intratumoral gemcitabine concentration improving overall survival of mice (36). However, promising results were not obtained in clinical trials by using combination of hedgehod signaling inhibitor with gemcitabine. 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 (56) (NCT01064622). Targeting any molecule in the Hedgehog pathway seems a good strategy as it can affect both the tumor and its surrounding stroma, as well as their interaction.

Notch and Wnt signaling; Overexpression of Notch genes, Notch receptors and ligands were recognized even in early PanIN lesions (57, 58). Up-regulation of Notch signaling pathway leads to the invasive phenotype of pancreatic cancer by interacting with oncogenic pathways and deregulating EGFR and NF-kB signaling (59). Notch 1 has been shown to be highly expressed in BxPC-3, HPAC, and PANC-1 pancreatic cancer cell lines and its inhibition by siRNA significantly decreased cell growth and induced apoptosis (60).

Wnt signaling pathway was also shown to be abnormally activated in PDAC (59, 61). Wnt receptor is activated by ligand binding, resulting in the activation of β-catenin. In normal cells, β-catenin is inactive, however, in pancreatic carcinoma, active β-catenin levels are increased. Extracellular proteins can act as positive regulators, such as Hsulf-1, 2, of Wnt signaling pathway and are usually shown to be overexpressed in tumor cells but not in normal cells (62), suggesting that Wnt signaling pathway is constitutively activated and represents a therapeutic target. Biological therapeutic agent OMP-54F28, a type of decoy receptor protein that binds to the Wnt ligands was recently developed, and is being used as part of a phase I clinical trial in combination with paclitaxel and gemcitabine in patients with stage IV pancreatic cancer. Results of the mentioned study are yet to be announced (NCT02050178).

Targeting Surface Receptors

Expression of several surface receptors has been associated with progression of pancreatic cancer. Only a few inhibitors have been developed including the EGFR inhibitor erlotinib, but did not improve survival of patients significantly. New inhibitors are being developed against various receptors, but more specific therapeutic targets are still needed.

Targeting Transferrin Receptors (TFRC); TFRC is a membrane bound protein overexpressed in 93% of pancreatic cancer cells and is considered a specific marker of malignant cells (63). It has an essential role in the progression and growth of pancreatic cancer (63, 64). Tumor-specific liposome-based nanocomplex conjugated with a single- chain antibody fragment (TfRscFv) for targeting transferrin receptor has been used *in vivo* in PDAC models in mice (65) in combination with gemcitabine. This complex system was shown to successfully localize within tumor tissue *via* transferrin receptor, to successfully decrease tumor growth and to prolong median survival of metastatic pancreatic cancer mouse model.

Folate receptor (FR); FR a glycosylphosphatidylinositol-anchored receptor is another promising target for pancreatic cancer therapy, as it is expressed in more than 80% of analyzed patients with PDAC tumors, with intermediate or high expression levels (66). FR is expressed mostly on tumor cells, with limited expression in normal cells (67) and may serve as an excellent receptor for targeted drug delivery by nanoparticles.

Epidermal growth factor receptor (EGFR), VEGF and IGF receptor targeted delivery; Epidermal growth factor receptor (EGFR or HER1) (68), VEGF (69), and IGF receptors have been targeted by monoclonal antibodies (Mab), and have been evaluated in both preclinical and clinical trials. Targeting EGFR in phase III clinical trial did not improve the outcome of patients with advanced pancreatic cancer compared to the use on gemcitabine alone.

Tumor-specific Nanotherapeutics for Targeting PDAC

The use of nanoparticles (NPs) for the treatment of pancreatic cancer has emerged in recent years because of their unique properties. Cellular internalization of nanoparticles is depended on their size, shape and charge. In addition to avoiding unwanted and dose-limiting toxicity to normal cells, specific and selective targeting by tumor targeting nanodelivery systems is critical to improve efficacy of the anti-cancer therapeutics in PDAC. Conjugation or encapsulation of drugs to the nanoparticles improve their stability and half-life and allow them to be released in a more controlled manner. Modified nanoparticles can significantly increase pharmacokinetics and biodistribution profile of drugs. Various features of nanoparticles such as size play important role in determining the biodistribution of cargo drugs and allow them to penetrate tumors through enhanced permeability and retention effect (EPR). They can easily penetrate the cell membrane, interact with different biological molecules and accumulate within tumors. Charged particles develop electrostatic attraction or repulsion with charged components, which further hinders their diffusion. Their stability can be increased by functionalizing their surfaces with molecules that prolong their circulation throughout the body. Chemotherapeutic agents or therapeutic RNA molecules (siRNA) for gene therapies can be conjugated or encapsulated within nanoparticles that acts as nanocarriers to increase the intracellular delivery of a drug to cancer cells, but also for the knockdown of an abnormal gene or protein expression in cancer. All of these properties of NPs represent an advantage for utilizing nanoparticle-mediated targeted delivery that can significantly lower the drug dosage and toxicity, and improve drug bioavailability and gene therapy for better prognosis of hardly treated pancreatic cancer.

Mechanisms of NPs Accumulation in Tumors

Tumor targeting can be classified as passive or active. In the passive targeting, NPs accumulate within neoplastic tissue as a result of EPR effect. Besides NP size, EPR effect is based on two main characteristics, leaky and defective vasculature and impaired lymphatic drainage that are distinctive properties of cancerous tissues (70). The permeability of vasculature and retention lead to the accumulation of nanoparticles, thus significantly increasing their tumor concentration (71). Passive targeting happens mostly through diffusion-mediated transport. Therefore, size, shape, geometry and surface properties of nanoparticles are critically important factors for their internalization into cells. Active targeting is achieved by receptor-mediated targeting into the tumor or tumor microenvironment (71). Some receptors that are specifically overexpressed or only unique to cancer cells enable and facilitate the uptake of NPs and therapeutic cargo accumulation of chemotherapeutic agents in the tumor. Targeting moieties, such as high affinity ligands (*i.e.*, antibodies, Ab-fragments, peptides, aptamers) that bind to the specific receptors on tumors are usually used to functionalize the surface of the delivery system to increase NP/drug uptake.

NP-mediated Delivery of Chemotherapeutic Drugs

Gemcitabine is incorporated into nanoparticles to increase its distribution into cancer cells at lower doses. Rejiba *et al.*, showed increased efficacy of Gem (4-(*N*)-*tris*-nor-squalenoyl-gemcitabine (SQ-Gem) nanoparticle formulation (72) in PDAC tumor models (73). This nano-formulation had an anti-tumor effect through inhibiting cell proliferation, and inducing

apoptosis when administered at a concentration of 5 μM in resistant Panc1 cells that resulted in 40% of apoptotic cells, while the treatment with free gemcitabine resulted in only 10% of apoptotic cells and significantly inhibited tumor growth and prolonged survival of mice. Similar results were obtained with gemcitabine–squalene nanoassemblies both *in vitro* and *in vivo* mouse model, where inhibition of tumor volume by approximately 70% was observed (74).

Inorganic NPs. These NPs such as iron oxide, carbon nanotubes (CNT), quantum dots (QDs), gold nanoparticles (AuNP) functionality have been investigated as drug or gene carriers to improve drug therapeutic efficacy and prolong survival of pancreatic cancer models in preclinical and patients in clinical studies (75). Administration of (intravenous) IONPs conjugated with IGF-1 and loaded with Dox (size: 20.4 nm) into orthotopic pancreatic PDX models (76) have demonstrated increased specificity and accumulation of nanoparticles within a tumor area (Figure 2) and lead to significant inhibition of cell proliferation and tumor growth (untreated *vs*. IGF1-IONP-Dox). Selective targeting against IGF-1R resulted in higher levels of Dox within a tumor (5 mg/kg dose) that also contributed to the reduction of tumor mass.

Gelatin-based drug delivery: Gelatin has been suggested as an effective gemcitabine carrier due to its safety, biocompatibility and biodegradability (77). To facilitate uptake of this NP by pancreatic cancer cells, EGFR peptide was conjugated on the surface of gelatin through a PEG linker (MW 2,000 Da, size: 150 to 250 nm) for targeted delivery. PEGyation is known to provide better and longer systemic circulation. Gemcitabine release into Panc-1 cells from Gem-Gel-PEG-EGFR nanoparticle occurs following disulfide bonds cleavage. Intravenous injection (once per week for 4 weeks) of EGFR-targeted Gem-Gel-PEG nanoparticle significantly diminished tumor volume (about 70%). Even though nanoparticles were also trapped in liver or spleen, no side effects were determined.

Dual or multidrug delivery can be achieved by incorporating two chemotherapeutic agents into the nanoparticle. Self-assembled so-called nanoscale coordination polymers (NCPs)-based nanoparticles containing two agents (oxaliplatin and gemcitabine) demonstrated good antitumor effect by induction of apoptosis both *in vitro* by 75% in AsPc-1 and 80% in BxPc-3 cells, and *in vivo* by 80% (78). This formulation inhibited tumor growth in subcutaneous AsPc-1 xenograft models resulting in 11-fold smaller tumor size compared to that of the control group.

NP-based Delivery of siRNAs

Gene silencing can be achieved by three main ways that are part of the RNA interference (RNAi) pathway: small interfering (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA). siRNA also called small interfering RNA or silencing RNA are non-coding RNAs that are produced from double-stranded RNA molecules and are 20-25 base pairs in length. These molecules act as a part of the RNAi mechanism. Once siRNA is introduced into cells, it is cleaved by the enzyme Dicer into short fragments, that later guides loading of siRNA molecules to a protein complex called RNA-inducing silencing complex (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 (79).

In many preclinical and clinical studies, RNAi has been used for the inhibition of oncogenes, growth or angiogenic factors or receptors that are overexpressed in tumor cells and involved in tumor progression. siRNA-based therapeutics have many advantages compared to other oligonucleotides. They can be easily chemically synthesized and efficiently suppress gene expression. It carries a high degree of safety and since it does not bind directly to DNA, there are no risks for generation of new mutations during gene therapy (80). Although siRNA have many advantages, there are also some limitations for its use in cancer therapy. 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 (76).

Polymeric based NPs for siRNA delivery into PDAC: Polymer-based NPs have been used as carriers for delivery of siRNA targeting KRAS or other target genes both *in vitro* and *in vivo* (80). LODER (Local Drug EluteR) local intratumoral delivery system based on a polymer-scaffold has been shown to be stabile, safe and effective in a PDAC mouse model (81). LODER sufficiently delivered KRAS siRNA into PDAC tumors and inhibited tumor growth and prolonged overall survival of a mouse model.

Cationic poly (lactic acid) (CPLA) biodegradable nanocapsules (CPLA-NC) with zeta potentials of +45 MV and 32 nm in diameter) have been tested for silencing the KRAS oncogene in PDAC models (82). Negatively charged siRNA was attached to its surface through electrostatic interactions. CPLA-NC carrying siRNA against KRAS was able to suppress the expression of *KRAS* gene by almost 50% in PDAC models *in vivo*. This complex did not exert any nanoparticle-based cytotoxicity, suggesting its safe use for *in vivo* applications.

PLGA/poloxamer (polyethyleneimine-poly (lactide-coglycolide) nanoparticle for siRNA delivery in to PDAC. Hypoxia-inducible factor 2α (HIF-2α) also called endothelial PAS domain protein-1 (EPAS1) was shown to be up-regulated in most of the cancers (83) and in about 67% of PDAC patients (84). Overexpression is correlated with poor prognosis, advanced stage and lymph node metastasis, thus making it a potential therapeutic target in PDAC. *In vivo* targeting of EPAS1 by siRNA encapsulated in PLGA/poloxamer

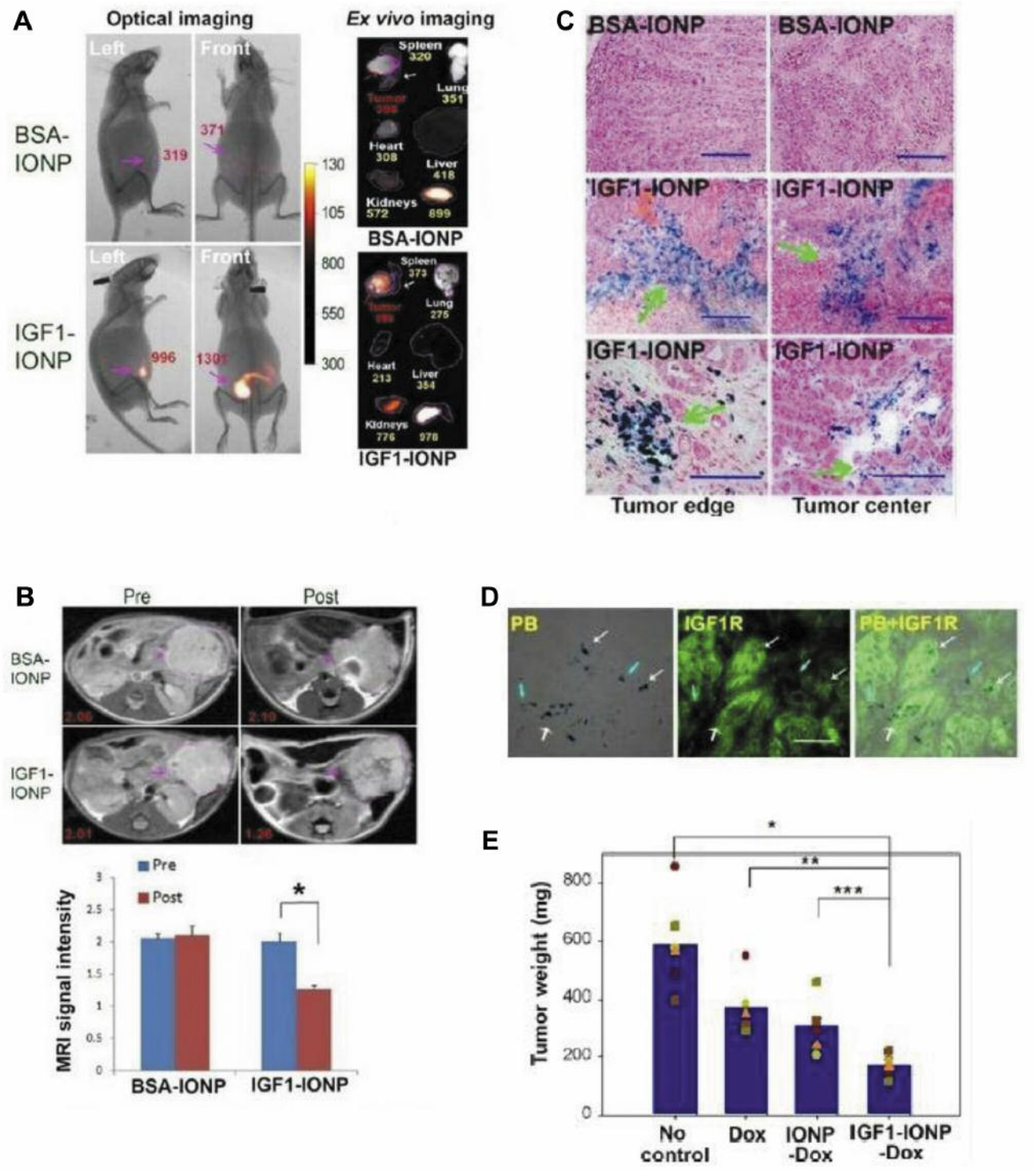

Figure 2. *Targeted delivery of IGF1-IONPs into orthotopic pancreatic PDX tumors and in vivo antitumor effect. (A) NIR optical imaging of the whole body 24 h after IONP administration; Ex vivo optical imaging of representative tumors and organs following sacrificing the mice (B) Pre and post 24 h T2-weighted MR images. (C) Prussian blue staining of frozen tumor sections indicating the presence of IONPs in both tumor edge and tumor center (green arrows) after IGF1-IONP administration via the tail vein. Blue: IONP-positive cells. Red: Nuclear fast red. (D) IGF1R antibody labeled tumor tissue sections obtained from IGF1-IONP-treated mice dual stained with Prussian blue staining. IONPs (blue) were detected in IGF1R highly expressing tumor cells (white arrows) and intermediate IGF1R expressing tumor stromal cells (light blue arrows). (E) Tumor growth inhibition. The mean tumor weight (navy bar) and individual tumor weight distributions as color symbols after the treatment are shown. *p<0.0001; **p<0.0006; ***p<0.005. Reprinted with permission from (76) © 2015 American Chemical Society.*

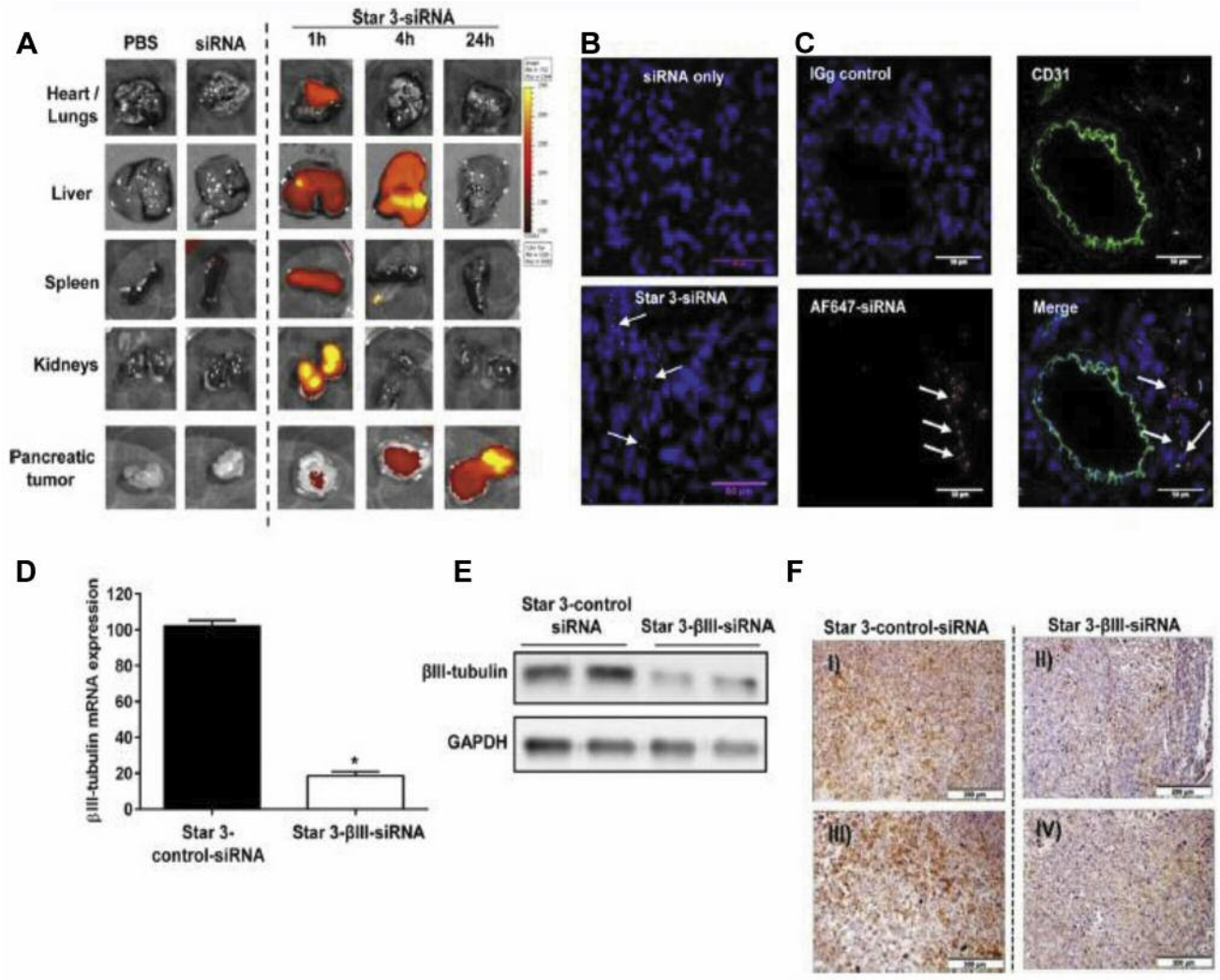

Figure 3. *Biodistribution and gene-silencing activity of star 3-βIII-tubulin siRNA in orthotopic pancreatic tumors in mice. (A) Ex vivo fluorescent images of heart, lungs, liver, spleen, kidney and orthotopic pancreatic tumors from mice injected (via the tail vein) with PBS, fluorescent siRNA (AlexaFluor-647), or star 3-fluorescent siRNA (AlexaFluor-647). (B, C) Confocal images of frozen sections of orthotopic pancreatic tumors showing the presence of fluorescent siRNA (red) in tumor tissue in mice administered with star 3-fluorescent siRNA, 24 h postinjection. Fluorescent siRNA was extravasated from the tumor vasculature into the surrounding tumor cells. Mice injected with fluorescent siRNA (AlexaFluor-647) alone served as controls. Fluorescent siRNA (red) and nuclear DNA (blue), tumor vessels (green) (white arrows indicate the location of siRNA). (D) Graph showing knockdown of βIII-tubulin mRNA expression in orthotopic pancreatic tumors in mice 24 h after the final injection with star 3-βIII-tubulin siRNA (4 mg/kg). (E, F) Western blot and immunohistochemistry staining from 2 individual mice showing reduced βIII-tubulin protein expression in orthotopic pancreatic tumors in mice injected with star 3-βIII-tubulin siRNA (4 mg/kg) 24 h after the final injection. Reprinted with permission from (86) © 2016 American Chemical Society.*

(polyethyleneimine-poly (lactide-coglycolide) nanoparticle led to better intracellular uptake (85). PLGA has been used for many years for siRNA delivery, however, due to the low electrostatic interaction between PLGA and siRNA, cationic polyetheyleneimine (PEI) polymer is coated on the surface of PLGA to overcome this limitation. *In vivo* testing in PDAC models suggested the significant diminishing in tumor volume upon treatment of a nude mouse model with EPAS1 siRNA nanoparticles.

Reversible addition–fragmentation chain transfer polymerization based NPs was used for the synthesis of star polymers to be used as carriers for delivery of siRNA to pancreatic cancer cells (86). Combination of poly (dimethylaminoethyl methacrylate) (PDMAEMA) and poly oligo (ethylene glycol) methyl ether methacrylate) (POEGMA) in different amounts, that were conjugated with anti-siRNA targeting βIII-tubulin, type of proteins of the cytoskeleton could lead to effective delivery of siRNA.

PEGylation of nanoparticles with POEGMA (38 nm) was used to overcome the problem of opsonization and increase the efficcay of gene silencing. High transfection efficiency and uptake of fluorescently-labeled siRNA star-POEGMA nanoparticles into MiaPaca-2 cells were observed (Figure 3). There was a significant, more than 80%, reduction in βIII-tubulin gene expression following systemic delivery (4 mg/kg) of star nanoparticle. 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 (86).

Inorganic nanoparticles (INPs). INPs (ranges from 1-100 nm) such as iron, silver, gold, and copper sulfide have been investigated for the delivery of siRNA for *in vitro* and *in vivo* gene knockdown (87, 88). Multifunctional superparamagnetic iron oxide nanoparticles (SPIONs) have been developed and used for silencing PLK1 (Polo-like kinase) through RNAi mechanism and imagining both nanoparticle delivery *in vivo*, tumor progression and response to treatment (88). PLKs are a type of serine-threonine-kinases which, in most of pancreatic cancer patients, are involved in cell-cycle processes and are thought to contribute to the oncogenesis of pancreatic cancer (89). SPIONs were coated with dextran and functionalized with streptavidin for the conjugation of biotinylated EPPT1, which is a peptide targeting overexpressed underglycosylated MUC1 antigen on the surface of the pancreatic cancer cells. Biotinylated myristoylated polyarginine peptides (MMAP) (increased tumor-specific targeted delivery of nanoparticles (123 nm) with PLK siRNA and improved cellular uptake of nanoparticles followed by sufficient release of siRNA for silencing of PLK1. Delivery of nanoparticles specifically to a tumor tissue in a mouse model after intravenous injection (3qD/ 4 weeks, 5 mg/kg of iron) was successful and led to a reduction in tumor volume. *In vivo* silencing of PLK1 was shown to induce apoptosis, and inhibit proliferation of cancer cells. The main problem of siPLK1-StAv-SPIONs was the relatively short half-life of siPLK1-StAv-SPIONs that can result in rapid body clearance, therefore limiting its therapeutic efficacy (88).

Overall, studies suggest that application of siRNA as a therapeutic agent for pancreatic cancer with the possibility for *in vitro* and *in vivo* imaging of tumor response, represents a viable strategy, that has many advantages over conventional therapies used in the treatment of this devastating disease.

Photothermal Therapy by Inorganic Nanomaterials

An important characteristic of some nanomaterials is that they are able to convert light energy into heat energy, making them effective therapeutics for targeting cancer cells. There are several advantages of using this strategy to treat cancer, including minimal invasion, fewer side-effects, controllability and specificity to certain tumor regions. Inorganic nanoparticles such as gold, carbon nanotubes or copper sulfide nanoparticles were shown to successfully convert photoenergy into thermal energy (90).

Carbon nanotubes; Photothermal effects of multi-walled carbon nanotubes functionalized with PEG were shown on PANC-1 cells at different concentrations of nanoparticles (5, 10, 50 μg/ml) (91). In the presence of laser irradiation, a significant increase in apoptotic cells was detected at doses over 10 μg. Exposure to 50 μg/ml resulted in a significant induction of reactive oxygen species (ROS)and 57% of pancreatic cancer cells were ROS-positive.

Gold nanorods, recently, received much attention due to their plasmonic photothermal therapy characteristics. Gold nanorods are able to produce vapor nanobubbles known as plasmonic nanobubbles after irradiation with short laser pulses. However, tumor specific delivery and targeting is a key factor in order to avoid the damaging effect on bystander cells. In this regard, Patino *et al*., (92) functionalized the surface of gold nanorods with thiol-PEG-biotin in order to get rid of cetyltrimethylammonium bromide (CTAB) layer on the surface of gold nanorod, which is known to be highly toxic for cells. In addition, in order to achieve a targeted delivery by MUC-1 marker and increase the cellular uptake of the gold nanorods, they conjugated the nanorods with EPPT (MUC-1 specific peptide) and MPAP (myristoylated polyarginine peptide) peptides and showed a highly selective destruction of cells upon laser irradiation with no obvious damage in surrounding cells. In photothermal therapy approach a high loading of nanomaterials is generally required to achieve sufficient heating and, in this case, the uptake rate of gold nanorods was increased by dual (EPPT and MPAP) conjugation. In another study, Yin and colleagues (93) studied the triple effect of *KRAS* gene silencing, doxorubicin and photothermal therapy as a pancreatic cancer therapy. They used a layered structure and coated the surface of gold nanorods with negatively charged PSS polymer to capture doxorubicin and also coated the surface with positively charged PAH polymer to capture siRNA. Exposure to 665 nm light provided the controlled release of doxorubicin and KRAS siRNA to the tumor cells and suppressed t tumor growth for at least 25 days (Figure 4).

Combination Therapies with Gene Targeting

Co-delivery of chemotherapeutic agents and siRNAs incorporated within one nanocarrier gain much attention and has become an important part of both preclinical and clinical applications for cancer treatments. Recent studies have also focused on targeting more than a single gene using siRNA for development of more effective combination therapies.

Zhao *et al*., employed the delivery of siRNA targeting HIF1α and gemcitabine both *in vitro* and *in vivo* to pancreatic cancer *via* lipid-polymer hybrid nanoparticles (94). High

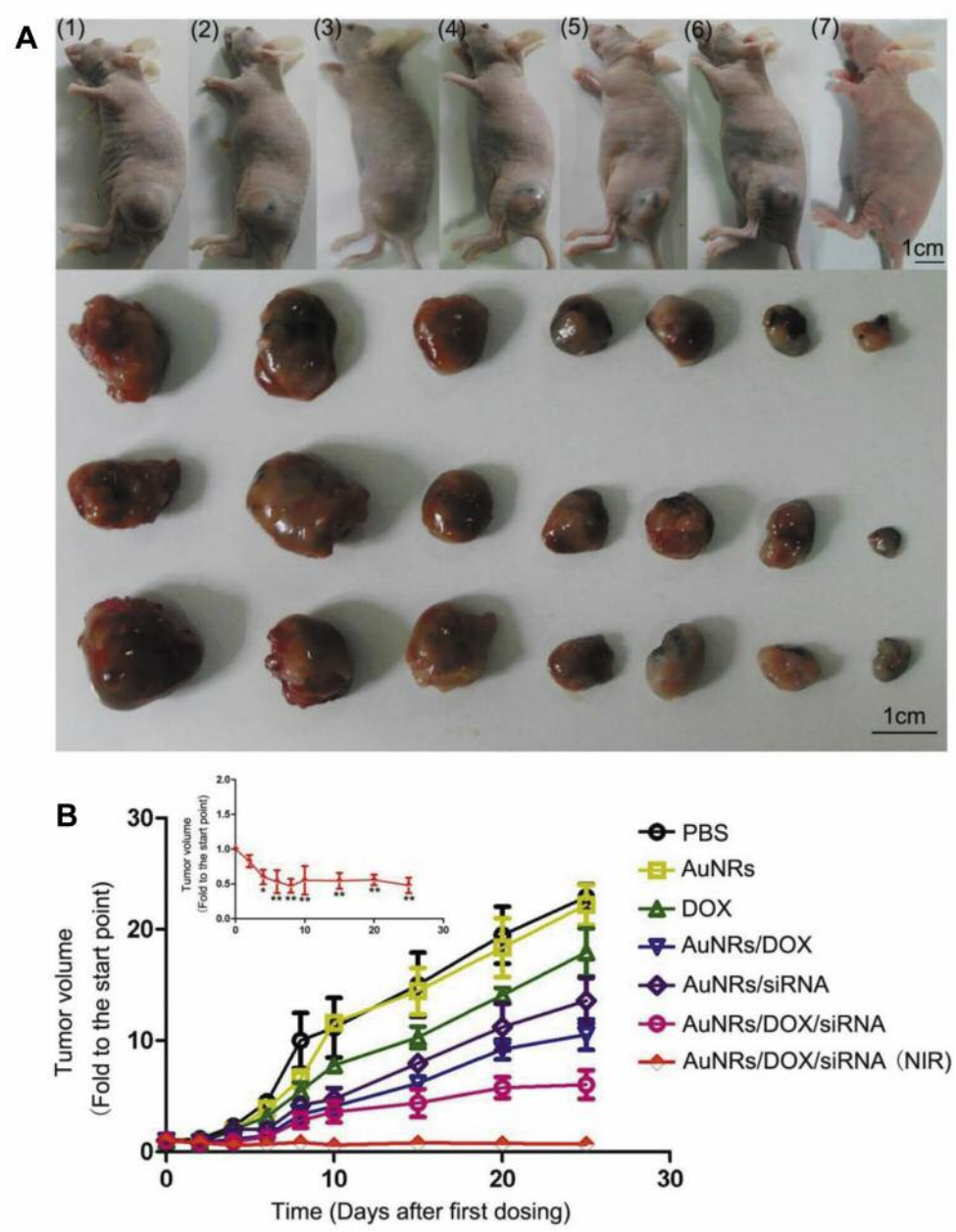

Figure 4. *Antitumor activity of AuNRs/DOX/KRas siRNA in a Panc-1 xenograft animal model. (A) Representative images of mouse and tumor tissues treated with (1) PBS, (2) free AuNRs, (3) free DOX, (4) AuNRs/DOX, (5) AuNRs/KRAS siRNA, (6) AuNRs/DOX/KRASsiRNA or (7) AuNRs/DOX/KRASsiRNA with 665 nm light irradiation. For mice treated with both AuNRs/DOX/siRNA and 665 nm light, the strongest inhibition rate was observed. (B) Relative changes in tumor volume versus time for mice treated by PBS, free AuNRs, free DOX, AuNRs/DOX, AuNRs/siRNA, AuNRs/DOX/siRNA or AuNRs/DOX/siRNA with 665 nm light, respectively. Relative tumor volume was defined as (V-V0)/ V0, where V and V0 indicate the tumor volume on a particular day and day 0, respectively. Error bars represent SEMs for triplicate data. Mean tumor volumes were analyzed using one-way ANOVA. *p<0.05, **p<0.01 (n=5-7 tumors). Reprinted with permission from (93) © 2015 Ivyspring International Publisher.*

hypoxia in pancreatic cancer led to overexpression of HIF (hypoxia-inducible factors) transcription factors that play important role in activating other signaling pathways that regulate tumor progression, mainly proliferation and metastasis of cancer cells, but are also involved in drug resistance. Gemcitabine was loaded in the core of the hybrid lipid-polymer nanoparticles, while the surface is coted with ε-polylysine co-polymer (ENPs) and siRNA was effectively bound through electrostatic interactions, forming LENP-Gem-siRNA nanoparticle with an average diameter of about 140 nm.

LENP-Gem-siRNA successfully penetrated across the cell membrane delivering gemcitabine and siRNA to PANC-1 cells *in vitro*, that resulted in the reduction of their viability and a 90% decrease in HIF1α mRNA expression. The stability of nanoparticles was confirmed upon administration intravenously and contributed to longer systemic circulation and ability to specifically and selectively target and accumulate within tumor tissue, silencing HIF1α *in vivo* and inhibiting tumor growth. This hybrid nanocomplex could suppress primary tumor growth but also inhibit metastasis of the tumor and is suggested for use in combination therapies carrying different siRNAs for gene knockdown.

In addition to the already mentioned aberrantly activated signaling pathways in PDAC, STAT3 (signal transducer and activator of transcription 3) signaling was shown to be involved in the poor outcome of patients through regulating the expression of genes that play a role in proliferation and apoptosis of cancer cells. STAT3 is also thought to be activated in cancer stem cells, thus promoting resistance to chemo and radiotherapy. Wu *et al*., prepared liposome vehicle carrying STAT3 inhibitor FLLL32 and investigated its antitumor efficacy both *in vitro* and in PANC-1 xenograft mouse model (95). Lip-FLLL32 could inhibit the cell proliferation in several pancreatic cancer cell lines, with an IC_{50} value two times higher than that of FLLL32 alone. Administration of 15 mg/kg Lip-FLLL32 *in vivo* significantly decreased STAT3 levels, as well as its target genes including Bcl-xL, Survivin and CCND1. Down-regulating the expression of STAT3 with Lip-FLLL32, demonstrated its ability to induce apoptosis and reduce tumor growth. This nanoformulation sensitized PANC-1 cells to gemcitabine, and in combination with X-ray radiation significantly reduced tumor size, compared to the treatment with radiation or Lip-FLLL32 alone.

Zeng *et al*., packaged siRNA against *KRAS* oncogene into a nanocarrier formed from poly (ethylene glycol)-*block*-poly (l-lysine) (PEG-PLL), and prepared another vehicle from poly (ethylene glycol)-*block*-poly (dl-lactide) (PEG-PDLLA) to encapsulate arsenic (As) for co-delivery and efficient treatment in pancreatic cancer (96). With high transfection efficiency (100 nM siRNA) in PANC-1 cells, KRAS mRNA levels were down-regulated to ~40%, and cell-cycle arrested. Combined synergetic effect of siRNA and As-NPs (si + As-NPs) resulted in a significantly higher number of apoptotic cells (~40%) compared to that of the control and single NP treatment groups. This NP approach led to a more than 60% inhibition of the tumour compared with the PBS-treated control group.

To overcome the problem with gemcitabine resistance, de la Fuente *et al*., investigated the antitumor effect after co-administration of gemcitabine with polypropylenimine dendrimer-coupled with siRNA or shRNA targeting ubiquitin ligase ITCH that is a part of the p73 pathway involved in apoptotic events in cells (97). These dendrimer complexes exhibit fast cell uptake and intracellular trafficking and provide silencing targeted gene expression by 40%. In the delivery with gemcitabine (100 mg/kg), tumor growth was postponed, meaning that down-regulation of ITCH could inhibit the cascade of events, therefore, inducing apoptosis and promoting sensitivity of pancreatic cancer cells to gemcitabine.

Conjugating siRNA targeting mutated *KRAS* gene on the surface of gold nanorods (AuNR) and loading doxorubicin as a chemotherapeutic agent led to the novel nanoformulation named as AuNRs/DOX/K-RAS siRNA nanoplex for delivery both *in vitro* and *in vivo* (93). Exposing to NIR light leads to a significantly higher amount of siRNA and chemotherapeutic agent release into cells for more improved antitumor activity. The combination of chemotherapy and photothermal ablation using AuNRs represents a good approach for effective treatment of pancreatic cancer *in vivo*. Designing such nanoformulations can be a good strategy to implement personalized medicine.

In Table I, the nanotherapeutics that are developed or are under research for pancreatic cancer therapy as a part of *in vivo* preclinical studies in mice are summarized.

Conclusion Remarks

Over the recent years, there have been attempts to develop different nanoparticles for delivery of chemotherapeutic agents such as gemcitabine and gene inhibitors such as siRNA. In addition to attempts to improve delivery of gemcitabine, a front line therapeutic in PDAC, various nanoparticles, including polymers, inorganic and lipid-based NPs have been developed to inhibit PDAC growth and metastasis. To overcome drug resistance, decrease off-target toxicity and improve antitumor efficacy of chemotherapeutic agents, siRNA therapeutics have also been used with the hope of suppressing the progression of pancreatic cancer.

Targeting *KRAS*, *EGFR* and other genes have been shown to be an effective strategy for treatment of PDAC. Designing of nanoparticles to encapsulate both siRNA molecules and chemotherapeutic agents and targeting them to cancer specific receptors to improve active targeting of cancer cells, emerged as a potential therapy of PDAC. Most nanoparticles exert desired properties *in vitro* in terms of toxicity as well as stability, but *in vivo* safety and toxicity profiles could be different. Thus, extensive safety/toxicity studies should be performed after *in vivo* administrations. Novel therapies are also expected to target both cancer cells and tumor microenvironment and disrupt stroma cancer cell communication networks. Targeting signaling pathways that are both active in the stroma and tumor compartments may represent a valuable approach. In conclusion, NPs are expected to be utilized more in the era of personalized medicine for the development of single- or multi-gene

Table I. *Review of approaches targeting important genes and receptors along with the effective therapeutic agents for pancreatic cancer therapy.*

Type of therapy	Targeted gene/ Receptor	Therapeutic approach	Type of nanoparticle	Ref.
NP-mediated delivery of chemotherapeutic drugs	-	Gemcitabine delivery	Squalene nanoassamblies	(73, 74)
	IGF-1	Doxorubicin delivery	IONP	(76)
	EGFR	Gemcitabine	Gel-PEG	(77)
	-	Oxaliplatin and gemcitabine	Nanoscale coordination polymers (NSP)	(78)
NP-based delivery of siRNAs	KRAS	siRNA	LODER (polymeric matrix) NPs	(81)
	HIF-2α	siRNA	PLGA/poloxamer NP	(85)
	βIII-tubulin	siRNA	Star-POEGMA NPs	(86)
	PLK1	siRNA	SPIONs coated with dextran	(88)
	p53	Liposomes with TfRscFv for gemcitabine delivery	Liposome-based Nanocomplex	(65)
Receptor-mediated targeting	HIF1α	siRNA and gemcitabine	Hybrid lipid-polymer NP	(94)
Combination therapy	STAT3	STAT3 inhibitor FLLL32	Liposome	(95)
and Photothermal ablation	KRAS	siRNA and As-NPs	PEG-PLL & PEG-PDLLA	(96)
	ITCH	Co-administration of gemcitabine and siRNA or shRNA	Polypropylenimine dendrimer complex	(97)
	KRAS	Chemotherapy and photothermal ablation	Gold nanorods coated with PSS and PAH	(93)

targeting therapeutic approaches as well as in combination with chemotheapeutics or small molecule inhibitor agents.

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References

1 Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. CA Cancer J Clin *66*: 7-30, 2016.
2 Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM and Matrisian LM: Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res *74*: 2913-2921, 2014.
3 Garrido-Laguna I and Hidalgo M: Pancreatic cancer: from state-of-the-art treatments to promising novel therapies. Nat Rev Clin Oncol *12*: 319-334, 2015.
4 Conroy T, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet J-B, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C and Ducreux M: FOLFIRINOX *versus* Gemcitabine for metastatic pancreatic cancer. N Engl J Med *364*: 1817-1825, 2011.
5 Von Hoff DD, Ervin T, Arena FP, Chiorean EG, Infante J, Moore M, Seay T, Tjulandin SA, Ma WW, Saleh MN, Harris M, Reni M, Dowden S, Laheru D, Bahary N, Ramanathan RK, Tabernero J, Hidalgo M, Goldstein D, Van Cutsem E, Wei X, Iglesias J and Renschler MF: Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. N Engl J Med *369*: 1691-1703, 2013.
6 Kierszenbaum AL and Tres LL: Endocrine system. *In*: Histology and Cell Biology – An Introduction to Pathology. Philadelphia, Elsevier Inc., pp. 581-608, 2012.
7 Singh D, Upadhyay G, Srivastava RK and Shankar S: Recent advances in pancreatic cancer: biology, treatment, and prevention. Biochim Biophys Acta *1856*: 13-27, 2015.
8 Yadav D and Lowenfels AB: The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology *144*: 1252-1261, 2013.
9 Klein AP: Genetic susceptibility to pancreatic cancer. Mol Carcinog *51*: 14-24, 2012.
10 Tas F, Sen F, Keskin S, Kilic L and Yildiz I: Prognostic factors in metastatic pancreatic cancer: Older patients are associated with reduced overall survival. Mol Clin Oncol *1*: 788-792, 2013.
11 Hentic O, Dreyer C, Rebours V, Zappa M, Levy P, Raymond E, Ruszniewski P and Hammel P: Gemcitabine in elderly patients with advanced pancreatic cancer. World J Gastroenterol *17*: 3497-3502, 2011.
12 Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A and DePinho RA: Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev *30*: 355-385, 2016.
13 Distler M, Aust D, Weitz J, Pilarsky C and Grutzmann R: Precursor lesions for sporadic pancreatic cancer: PanIN, IPMN, and MCN. Biomed Res Int *2014*: 1-11, 2014.
14 Zamboni G, Hirabayashi K, Castelli P and Lennon AM: Precancerous lesions of the pancreas. Best Pract Res Clin Gastroenterol *27*: 299-322, 2013.
15 Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B, Kamiyama M, Hruban RH, Eshleman JR, Nowak MA, Velculescu VE, Kinzler KW, Vogelstein B and Iacobuzio-Donahue CA: Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature *467*: 1114-1117, 2010.
16 Pylayeva-Gupta Y, Grabocka E and Bar-Sagi D: RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer *11*: 761-774, 2011.
17 Lohr M, Kloppel G, Maisonneuve P, Lowenfels AB and Luttges J: Frequency of K-ras mutations in pancreatic intraductal neoplasias associated with pancreatic ductal adenocarcinoma and chronic pancreatitis: a meta-analysis. Neoplasia *7*: 17-23, 2005.

18 Morton JP, Timpson P, Karim SA, Ridgway RA, Athineos D, Doyle B, Jamieson NB, Oien KA, Lowy AM, Brunton VG, Frame MC, Evans TR and Sansom OJ: Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc Natl Acad Sci USA *107*: 246-251, 2010.

19 Makohon-Moore A and Iacobuzio-Donahue CA: Pancreatic cancer biology and genetics from an evolutionary perspective. Nat Rev Cancer *16*: 553-565, 2016.

20 Xia X, Wu W, Huang C, Cen G, Jiang T, Cao J, Huang K and Qiu Z: SMAD4 and its role in pancreatic cancer. Tumour Biol *36*: 111-119, 2015.

21 Shen J, Peng Y, Wei L, Zhang W, Yang L, Lan L, Kapoor P, Ju Z, Mo Q, Shih Ie M, Uray IP, Wu X, Brown PH, Shen X, Mills GB and Peng G: ARID1A deficiency impairs the DNA damage checkpoint and sensitizes cells to PARP inhibitors. Cancer Discov *5*: 752-767, 2015.

22 Witkiewicz AK, McMillan EA, Balaji U, Baek G, Lin WC, Mansour J, Mollaee M, Wagner KU, Koduru P, Yopp A, Choti MA, Yeo CJ, McCue P, White MA and Knudsen ES: Whole-exome sequencing of pancreatic cancer defines genetic diversity and therapeutic targets. Nat Commun *6*: 1-11, 2015.

23 Holter S, Borgida A, Dodd A, Grant R, Semotiuk K, Hedley D, Dhani N, Narod S, Akbari M, Moore M and Gallinger S: Germline BRCA mutations in a large clinic-based cohort of patients with pancreatic adenocarcinoma. J Clin Oncol *33*: 3124-3129, 2015.

24 Navas C, Hernandez-Porras I, Schuhmacher AJ, Schuhmacher AJ, Sibilia M, Guerra C and Barbacid M: EGF receptor signaling is essential for k-ras oncogene-driven pancreatic ductal adenocarcinoma. Cancer Cell *22*: 318-330, 2012.

25 Ercan G, Karlitepe A and Ozpolat B: Pancreatic cancer stem cells and therapeutic approaches. Anticancer Res *37*: 2761-2775, 2017.

26 Nielsen MF, Mortensen MB and Detlefsen S: Key players in pancreatic cancer-stroma interaction: Cancer-associated fibroblasts, endothelial and inflammatory cells. World J Gastroenterol *22*: 2678-2700, 2016.

27 Liu Q, Liao Q and Zhao Y: Chemotherapy and tumor microenvironment of pancreatic cancer. Cancer Cell Int *17*: 1-17, 2017.

28 Rasheed ZA, Matsui W and Maitra A: Pathology of pancreatic stroma in PDAC. In: Pancreatic cancer and tumor microenvironment. Grippo PJ and Munshi HG (eds.). Trivandrum, Transworld Research Network, 2012.

29 Oettle H, Post S, Neuhaus P, Gellert K, Langrehr J, Ridwelski K, Schramm H, Fahlke J, Zuelke C, Burkart C, Gutberlet K, Kettner E, Schmalenberg H, Weigang-Koehler K, Bechstein WO, Niedergethmann M, Schmidt-Wolf I, Roll L, Doerken B and Riess H: Adjuvant chemotherapy with gemcitabine *vs*. observation in patients undergoing curative-intent resection of pancreatic cancer: a randomized controlled trial. JAMA *297*: 267-277, 2007.

30 Robinson K, Lambiase L, Li J, Monteiro C and Schiff M: Fatal cholestatic liver failure associated with gemcitabine therapy. Dig Dis Sci *48*: 1804-1808, 2003.

31 Patra CR, Bhattacharya R, Mukhopadhyay D and Mukherjee P: Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer. Adv Drug Deliv Rev *62*: 346-361, 2010.

32 Chen YW, Liu JY, Lin ST, Li JM, Huang SH, Chen J-Y, J.Y. C, Wu JY, Kuo CC, Wu CL, Lu YC, Chen YH, Fan CY, Huang PC, Law CH, Lyu PC, Chou HC and Chan HL: Proteomic analysis of gemcitabine-induced drug resistance in pancreatic cancer cells. Mol Biosyst *7*: 3065-3074, 2011.

33 Moore MJ, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M and Parulekar W: Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol *25*: 1960-1966, 2007.

34 Yachida S, White CM, Naito Y, Zhong Y, Brosnan JA, Macgregor-Das AM, Morgan RA, Saunders T, Laheru DA, Herman JM, Hruban RH, Klein AP, Jones S, Velculescu V, Wolfgang CL and Iacobuzio-Donahue CA: Clinical significance of the genetic landscape of pancreatic cancer and implications for identification of potential long-term survivors. Clin Cancer Res *18*: 6339-6347, 2012.

35 Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE and Kinzler KW: Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science *321*: 1801-1806, 2008.

36 Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, Madhu B, Goldgraben MA, Caldwell ME, Allard D, Frese KK, Denicola G, Feig C, Combs C, Winter SP, Ireland-Zecchini H, Reichelt S, Howat WJ, Chang A, Dhara M, Wang L, Ruckert F, Grutzmann R, Pilarsky C, Izeradjene K, Hingorani SR, Huang P, Davies SE, Plunkett W, Egorin M, Hruban RH, Whitebread N, McGovern K, Adams J, Iacobuzio-Donahue C, Griffiths J and Tuveson DA: Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science *324*: 1457-1461, 2009.

37 Collins MA, Bednar F, Zhang Y, Brisset JC, Galban S, Galban CJ, Rakshit S, Flannagan KS, Adsay NV and Pasca di Magliano M: Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. J Clin Invest *122*: 639-653, 2012.

38 Eser S, Schnieke A, Schneider G and Saur D: Oncogenic KRAS signalling in pancreatic cancer. Br J Cancer *111*: 817-822, 2014.

39 Knickelbein K and Zhang L: Mutant KRAS as a critical determinant of the therapeutic response of colorectal cancer. Genes Dis *2*: 4-12, 2015.

40 Ji Z, Mei FC, Xie J and Cheng X: Oncogenic KRAS activates hedgehog signaling pathway in pancreatic cancer cells. J Biol Chem *282*: 14048-14055, 2007.

41 Lesina M, Kurkowski MU, Ludes K, Rose-John S, Treiber M, Klöppel G, Yoshimura A, Reindl W, Sipos B, Akira S, Schmid RM and Algül H: Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. Cancer Cell *19*: 456-469, 2011.

42 Charo C, Holla V, Arumugam T, Hwang R, Yang P, Dubois RN, Menter DG, Logsdon CD and Ramachandran V: Prostaglandin E2 regulates pancreatic stellate cell activity *via* the EP4 receptor. Pancreas *42*: 467-474, 2013.

43 Pylayeva-Gupta Y, Lee KE, Hajdu CH, Miller G and Bar-Sagi D: Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. Cancer Cell *21*: 836-847, 2012.

44 Van Cutsem E, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y and Von Hoff D: Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. J Clin Oncol *22*: 1430-1438, 2004.

45 Zeitouni D, Pylayeva-Gupta Y, Der CJ and Bryant KL: KRAS mutant pancreatic cancer: no lone path to an effective treatment. Cancers (Basel) *8*: 1-22, 2016.

46 Vanhaesebroeck B, Guillermet-Guibert J, Graupera M and Bilanges B: The emerging mechanisms of isoform-specific PI3K signalling. Nat Rev Mol Cell Biol *11*: 329-341, 2010.

47 Zeng M, Lu J, Li L, Feru F, Quan C, Gero TW, Ficarro SB, Xiong Y, Ambrogio C, Paranal RM, Catalano M, Shao J, Wong KK, Marto JA, Fischer ES, Janne PA, Scott DA, Westover KD and Gray NS: Potent and selective covalent quinazoline inhibitors of KRAS G12C. Cell Chem Biol *24*: 1005-1016, 2017.

48 Ostrem JM, Peters U, Sos ML, Wells JA and Shokat KM: K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature *503*: 548-551, 2013.

49 Nagaraj NS and Datta PK: Targeting the transforming growth factor-beta signaling pathway in human cancer. Expert Opin Investig Drugs *19*: 77-91, 2010.

50 Shen W, Tao GQ, Zhang Y, Cai B, Sun J and Tian ZQ: TGF-beta in pancreatic cancer initiation and progression: two sides of the same coin. Cell Biosci *7*: 1-7, 2017.

51 Malkoski SP and Wang XJ: Two sides of the story? Smad4 loss in pancreatic cancer *versus* head-and-neck cancer. FEBS Lett *586*: 1984-1992, 2012.

52 Javle M, Li Y, Tan D, Dong X, Chang P, Kar S and Li D: Biomarkers of TGF-beta signaling pathway and prognosis of pancreatic cancer. PLoS One *9*: 1-8, 2014.

53 Ellermeier J, Wei J, Duewell P, Hoves S, Stieg MR, Adunka T, Noerenberg D, Anders HJ, Mayr D, Poeck H, Hartmann G, Endres S and Schnurr M: Therapeutic efficacy of bifunctional siRNA combining TGF-beta1 silencing with RIG-I activation in pancreatic cancer. Cancer Res *73*: 1709-1720, 2013.

54 Liu C, Zhang H, Zang X, Wang C, Kong Y and Zhang H: The influence of SnoN gene silencing by siRNA on the cell proliferation and apoptosis of human pancreatic cancer cells. Diagn Pathol *10*: 1-7, 2015.

55 Honselmann KC, Pross M, Jung CM, Wellner UF, Deichmann S, Keck T and Bausch D: Regulation mechanisms of the hedgehog pathway in pancreatic cancer: a review. Jop *16*: 25-32, 2015.

56 Kim EJ, Sahai V, Abel EV, Griffith KA, Greenson JK, Takebe N, Khan GN, Blau JL, Craig R, Balis UG, Zalupski MM and Simeone DM: Pilot clinical trial of hedgehog pathway inhibitor GDC-0449 (vismodegib) in combination with gemcitabine in patients with metastatic pancreatic adenocarcinoma. Clin Cancer Res *20*: 5937-5945, 2014.

57 Mazur PK, Einwachter H, Lee M, Sipos B, Nakhai H, Rad R, Zimber-Strobl U, Strobl LJ, Radtke F, Kloppel G, Schmid RM and Siveke JT: Notch2 is required for progression of pancreatic intraepithelial neoplasia and development of pancreatic ductal adenocarcinoma. Proc Natl Acad Sci USA *107*: 13438-13443, 2010.

58 Guo J, Xie K and Zheng S: Molecular biomarkers of pancreatic intraepithelial neoplasia and their implications in early diagnosis and therapeutic intervention of pancreatic cancer. Int J Biol Sci *12*: 292-301, 2016.

59 McCleary-Wheeler AL, McWilliams R and Fernandez-Zapico ME: Aberrant signaling pathways in pancreatic cancer: a two compartment view. Mol Carcinog *51*: 25-39, 2012.

60 Wang Z, Zhang Y, Li Y, Banerjee S, Liao J and Sarkar FH: Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells. Mol Cancer Ther *5*: 483-493, 2006.

61 Zeng G, Germinaro M, Micsenyi A, Monga NK, Bell A, Sood A, Malhotra V, Sood N, Midda V, Monga DK, Kokkinakis DM and Monga SP: Aberrant Wnt/beta-catenin signaling in pancreatic adenocarcinoma. Neoplasia *8*: 279-289, 2006.

62 Nawroth R, van Zante A, Cervantes S, McManus M, Hebrok M and Rosen SD: Extracellular sulfatases, elements of the Wnt signaling pathway, positively regulate growth and tumorigenicity of human pancreatic cancer cells. PLoS One *2*: 1-11, 2007.

63 Ryschich E, Huszty G, Knaebel HP, Hartel M, Büchler MW and Schmidt J: Transferrin receptor is a marker of malignant phenotype in human pancreatic cancer and in neuroendocrine carcinoma of the pancreas. Eur J Cancer *40*: 1418-1422, 2004.

64 Jeong SM, Hwang S and Seong RH: Transferrin receptor regulates pancreatic cancer growth by modulating mitochondrial respiration and ROS generation. Biochem Biophys Res Commun *471*: 373-379, 2016.

65 Camp ER, Wang C, Little EC, Watson PM, Pirollo KF, Rait A, Cole DJ, Chang EH and Watson DK: Transferrin receptor targeting nanomedicine delivering wild-type p53 gene sensitizes pancreatic cancer to gemcitabine therapy. Cancer Gene Ther *20*: 222-228, 2013.

66 Cai L, Michelakos T, Ferrone CR, Zhang L, Deshpande V, Shen Q, DeLeo A, Yamada T, Zhang G, Ferrone S and Wang X: Expression status of folate receptor alpha is a predictor of survival in pancreatic ductal adenocarcinoma. Oncotarget *8*: 37646-37656, 2017.

67 Zwicke GL, Mansoori GA and Jeffery CJ: Utilizing the folate receptor for active targeting of cancer nanotherapeutics. Nano Rev *3*: 1-11, 2012.

68 Philip PA, Benedetti J, Corless CL, Wong R, O'Reilly EM, Flynn PJ, Rowland KM, Atkins JN, Mirtsching BC, Rivkin SE, Khorana AA, Goldman B, Fenoglio-Preiser CM, Abbruzzese JL and Blanke CD: Phase III study comparing gemcitabine plus cetuximab *versus* gemcitabine in patients with advanced pancreatic adenocarcinoma: Southwest Oncology Group-directed intergroup trial S0205. J Clin Oncol *28*: 3605-3610, 2010.

69 Martin LK, Li X, Kleiber B, Ellison EC, Bloomston M, Zalupski M and Bekaii-Saab TS: VEGF remains an interesting target in advanced pancreas cancer (APCA): results of a multi-institutional phase II study of bevacizumab, gemcitabine, and infusional 5-fluorouracil in patients with APCA. Ann Oncol *23*: 2812-2820, 2012.

70 Bazak R, Houri M, Achy SE, Hussein W and Refaat T: Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. Mol Clin Oncol *2*: 904-908, 2014.

71 Danhier F, Feron O and Préat V: To exploit the tumor microenvironment: passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. J Control Release *148*: 135-146, 2010.

72 Couvreur P, Reddy LH, Mangenot S, Poupaert JH, Desmaele D, Lepetre-Mouelhi S, Pili B, Bourgaux C, Amenitsch H and Ollivon M: Discovery of new hexagonal supramolecular nanostructures formed by squalenoylation of an anticancer nucleoside analogue. Small *4*: 247-253, 2008.

73 Rejiba S, Reddy LH, Bigand C, Parmentier C, Couvreur P and Hajri A: Squalenoyl gemcitabine nanomedicine overcomes the low efficacy of gemcitabine therapy in pancreatic cancer. Nanomedicine *7*: 841-849, 2011.

74 Maksimenko A, Caron J, Mougin J, Desmaele D and Couvreur P: Gemcitabine-based therapy for pancreatic cancer using the squalenoyl nucleoside monophosphate nanoassemblies. Int J Pharm *482*: 38-46, 2015.

75 Hwang RF, Moore TT, Hattersley MM, Scarpitti M, Yang B, Devereaux E, Ramachandran V, Arumugam T, Ji B, Logsdon CD, Brown JL and Godin R: Inhibition of the hedgehog pathway targets the tumor-associated stroma in pancreatic cancer. Mol Cancer Res *10*: 1147-1157, 2012.

76 Zhou H, Qian W, Uckun FM, Wang L, Wang YA, Chen H, Kooby D, Yu Q, Lipowska M, Staley CA, Mao H and Yang L: IGF1 receptor targeted theranostic nanoparticles for targeted and image-guided therapy of pancreatic cancer. ACS Nano *9*: 7976-7991, 2015.

77 Singh A, Xu J, Mattheolabakis G and Amiji M: EGFR-targeted gelatin nanoparticles for systemic administration of gemcitabine in an orthotopic pancreatic cancer model. Nanomedicine *12*: 589-600, 2016.

78 Poon C, He C, Liu D, Lu K and Lin W: Self-assembled nanoscale coordination polymers carrying oxaliplatin and gemcitabine for synergistic combination therapy of pancreatic cancer. J Control Release *201*: 90-99, 2015.

79 Guo W, Chen W, Yu W, Huang W and Deng W: Small interfering RNA-based molecular therapy of cancers. Chin J Cancer *32*: 488-493, 2013.

80 Xu CF and Wang J: Delivery systems for siRNA drug development in cancer therapy. AJPS *10*: 1-12, 2015.

81 Zorde Khvalevsky E, Gabai R, Rachmut IH, Horwitz E, Brunschwig Z, Orbach A, Shemi A, Golan T, Domb AJ, Yavin E, Giladi H, Rivkin L, Simerzin A, Eliakim R, Khalaileh A, Hubert A, Lahav M, Kopelman Y, Goldin E, Dancour A, Hants Y, Arbel-Alon S, Abramovitch R, Shemi A and Galun E: Mutant KRAS is a druggable target for pancreatic cancer. Proc Natl Acad Sci USA *110*: 20723-20728, 2013.

82 Lin G, Hu R, Law WC, Chen CK, Wang Y, Li Chin H, Nguyen QT, Lai CK, Yoon HS, Wang X, Xu G, Ye L, Cheng C and Yong KT: Biodegradable nanocapsules as siRNA carriers for mutant K-Ras gene silencing of human pancreatic carcinoma cells. Small *9*: 2757-2763, 2013.

83 Zhao J, Du F, Shen G, Zheng F and Xu B: The role of hypoxia-inducible factor-2 in digestive system cancers. Cell Death Dis *6*: 1-9, 2015.

84 Yang J, Zhang X, Zhang Y, Zhu D, Zhang L, Li Y, Zhu Y, Li D and Zhou J: HIF-2alpha promotes epithelial-mesenchymal transition through regulating Twist2 binding to the promoter of E-cadherin in pancreatic cancer. J Exp Clin Cancer Res *35*: 1-10, 2016.

85 Pan X, Zhu Q, Sun Y, Li L, Zhu Y, Zhao Z, Zuo J, Fang W and Li K: PLGA/poloxamer nanoparticles loaded with EPAS1 siRNA for the treatment of pancreatic cancer *in vitro* and *in vivo*. Int J Mol Med *35*: 995-1002, 2015.

86 Teo J, McCarroll JA, Boyer C, Youkhana J, Sagnella SM, Duong HTT, Liu J, Sharbeen G, Goldstein D, Davis TP, Kavallaris M and Phillips PA: A rationally optimized nanoparticle system for the delivery of RNA interference therapeutics into pancreatic tumors *in vivo*. Biomacromolecules *17*: 2337-2351, 2016.

87 Panwar N, Yang C, Yin F, Yoon HS, Chuan TS and Yong KT: RNAi-based therapeutic nanostrategy: IL-8 gene silencing in pancreatic cancer cells using gold nanorods delivery vehicles. Nanotechnology *26*: 1-14, 2015.

88 Mahajan UM, Teller S, Sendler M, Palankar R, van den Brandt C, Schwaiger T, Kuhn JP, Ribback S, Glockl G, Evert M, Weitschies W, Hosten N, Dombrowski F, Delcea M, Weiss FU, Lerch MM and Mayerle J: Tumour-specific delivery of siRNA-coupled superparamagnetic iron oxide nanoparticles, targeted against PLK1, stops progression of pancreatic cancer. Gut *65*: 1838-1849, 2016.

89 Gray PJJ, Bearss DJ, Han H, Nagle R, Tsao MS, Dean N and Von Hoff DD: Identification of human polo-like kinase 1 as a potential therapeutic target in pancreatic cancer. Mol Cancer Ther *3*: 641-646, 2004.

90 Bao Z, Liu X, Liu Y, Liu H and Zhao K: Near-infrared light-responsive inorganic nanomaterials for photothermal therapy. AJPS *11*: 349-364, 2016.

91 Mocan T, Matea CT, Cojocaru I, Ilie I, Tabaran FA, Zaharie F, Iancu C, Bartos D and Mocan L: Photothermal treatment of human pancreatic cancer using PEGylated multi-walled carbon nanotubes induces apoptosis by triggering mitochondrial membrane depolarization mechanism. J Cancer *5*: 679-688, 2014.

92 Patino T, Mahajan U, Palankar R, Medvedev N, Walowski J, Munzenberg M, Mayerle J and Delcea M: Multifunctional gold nanorods for selective plasmonic photothermal therapy in pancreatic cancer cells using ultra-short pulse near-infrared laser irradiation. Nanoscale *7*: 5328-5337, 2015.

93 Yin F, Yang C, Wang Q, Zeng S, Hu R, Lin G, Tian J, Hu S, Lan RF, Yoon HS, Lu F, Wang K and Yong KT: A light-driven therapy of pancreatic adenocarcinoma using gold nanorods-based nanocarriers for co-delivery of doxorubicin and siRNA. Theranostics *5*: 818-833, 2015.

94 Zhao X, Li F, Li Y, Wang H, Ren H, Chen J, Nie G and Hao J: Co-delivery of HIF1alpha siRNA and gemcitabine *via* biocompatible lipid-polymer hybrid nanoparticles for effective treatment of pancreatic cancer. Biomaterials *46*: 13-25, 2015.

95 Wu X, Tang W, Marquez RT, Li K, Highfill CA, He F, Lian J, Lin J, Fuchs JR, Ji M, Li L and Xu L: Overcoming chemo/radio-resistance of pancreatic cancer by inhibiting STAT3 signaling. Oncotarget *7*: 11708-11723, 2016.

96 Zeng L, Li J, Wang Y, Qian C, Chen Y, Zhang Q, Wu W, Lin Z, Liang J, Shuai X and Huang K: Combination of siRNA-directed Kras oncogene silencing and arsenic-induced apoptosis using a nanomedicine strategy for the effective treatment of pancreatic cancer. Nanomedicine *10*: 463-472, 2014.

97 de la Fuente M, Jones MC, Santander-Ortega MJ, Mirenska A, Marimuthu P, Uchegbu I and Schatzlein A: A nano-enabled cancer-specific ITCH RNAi chemotherapy booster for pancreatic cancer. Nanomedicine *11*: 369-377, 2015.

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