Chapter 2

Pancreatic Cancer, Leptin, and Chemoresistance: Current Challenges

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http://dx.doi.org/10.5772/intechopen.76400

Abstract

Pancreatic cancer (PC) remains a leading cause of cancer-related deaths. Currently, conventional chemotherapies have showed only limited benefits for PC patients. Main factors affecting PC treatment failures are due to late detection, lack of early symptoms and biomarkers, and the development of desmoplasia and chemoresistance. Various mechanisms have been implicated in PC chemoresistance that includes stem cells, epigenetic changes, and alteration of signaling pathways, among others. Obesity is a modifiable factor for PC risk, which is characterized by high levels of the adipokine leptin that is a proinflammatory, proangiogenic, survival factor that affects chemotherapy effectiveness. Here, we will discuss on the mechanisms of PC chemoresistance and the influence of obesity and leptin signaling. Furthermore, the potential use of nontoxic leptin antagonists as a novel sensitization strategy for PC chemotherapeutics will also be discussed.

Keywords: leptin, notch, chemoresistance, pancreatic cancer, obesity

1. Introduction

Pancreatic cancer (PC) is a highly aggressive cancer, characterized by early spread with local diffusion and early metastasis to distant organs. PC is a silent disease, without reliable biomarkers that are commonly detected at an advanced stage. The deep position of the pancreas is an additional factor influencing the late detection of most symptoms of PC, when the disease is at final stages and the tumor size is large enough to interfere with the liver, gallbladder, stomach, or duodenum functions [1]. Patients have rapid disease progression, and few of them survive more than a year. Even for patients with localized disease at the time of diagnosis and

undergoing curative surgical treatment, the median survival remains low, around 18 months. The overall 5-year survival rate is only 8.2% for all stages of PC [2]. Despite the advances in understanding PC biology, survival rates remain unmodified in the past years [3]. The underlying causes for PC dismal prognosis, among others, are the lack of viable methods for patient screening, late detection of specific symptoms, especially in the early stages, and few targeted therapies that remain relatively ineffective [4].

2. Pancreas and pancreatic cancer

2.1. Pancreas: structure and function

The pancreas functions as an accessory gland of the digestive system and is composed anatomically and functionally of a mixed, exocrine, and endocrine component. Most of the pancreatic tissue (99%) is made up of exocrine tissue that is composed of closely packed serous acini that secrete digestive enzymes (proteases, lipases, and amylases). Some of the enzymes (e.g., trypsinogen, chymotrypsinogen, and proelastase) are secreted as inactivated precursors, to prevent pancreatic cell damage, and are activated upon release in the duodenum. Other key digestive enzymes, such as α-amylase and lipase, are present in the pancreas in their active forms. The duct cells secrete a watery, bicarbonate-rich fluid that carries the enzymes and neutralizes the acidity in the small intestine. The endocrine pancreas is composed of islets of Langerhans, clusters of about 3000 cells supported by reticulin fibers, in close contact with fenestrated capillaries. They contain three types of cells that secrete the three pancreatic hormones: α cells secrete glucagon that rises the glucose blood levels, while β cells secrete insulin that decreases the glucose blood levels and Δ cells secrete somatostatin that regulates the endocrine system and affects the neurotransmission and cell proliferation. The islet cells appear paler on hematoxylin and eosin stain (**Figure 1**) [5].

2.2. Pancreatic cancer

The incidence of PC continuously raised in the past years, and it is estimated to become the second leading cause of cancer-related deaths by 2030 [6]. The highest PC incidence occurred in Northern America (7.4 per 100,000 people) and Western Europe (7.3 per 100,000 people), followed by other regions of Europe and Australia (equally about 6.5 per 100,000 people). The lowest rates (about 1.0 per 100,000 people) were observed in Middle Africa and South-Central Asia. More than half of new cases (55.5%) were registered in the more developed regions [7]. PC has been correlated to exposure to risk factors concerning lifestyle, such as obesity, or the environment [8]. The incidence of PC is higher in men than in women [9]. PC is a disease of the elderly, with most of the cases being diagnosed after the age of 55 [10]. African-Americans have the highest incidence rate of PC, that is 28-59% higher than those of other racial/ethnic groups [11].

Most pancreatic tumors are derived from the exocrine tissue. More than 80% of the exocrine PCs are classified as pancreatic adenocarcinomas (PAs). Microscopically, these cancers are characterized by infiltrating small glands that are lined with low-columnar, mucin-containing

http://dx.doi.org/10.5772/intechopen.76400

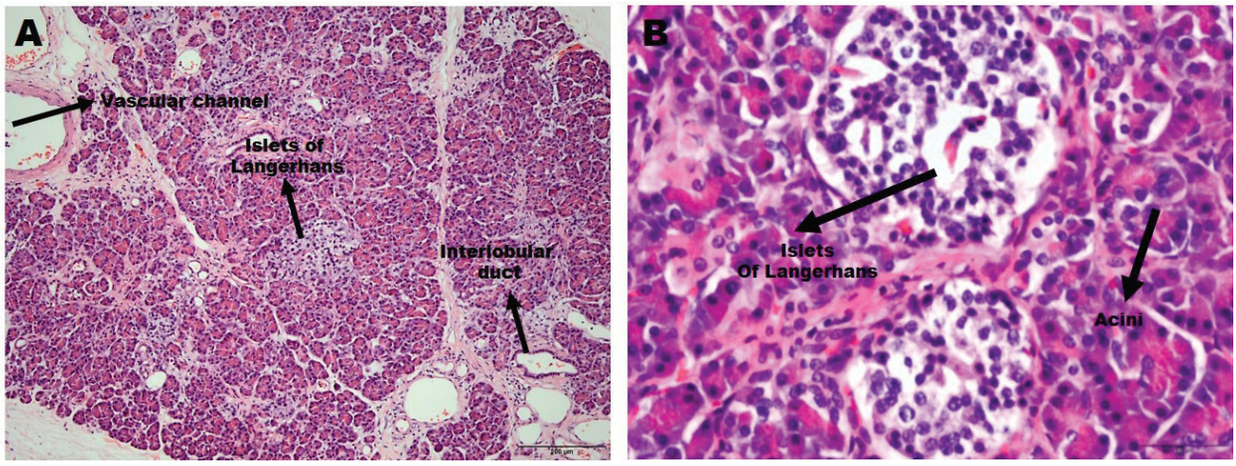

Figure 1. Representative pictures from hematoxylin and eosin staining of pancreatic tissue. (A) Pancreatic parenchyma composed in the vast majority by the exocrine pancreas composed of tightly packed acini that secrete enzymes via a duct system in the duodenum. The endocrine pancreas is composed of islets of Langerhans, which appears as clusters of pale colored cells (10×). (B) High magnification of pancreatic tissue shows exocrine tightly packed acini and endocrine islets of Langerhans. The islets appear pale due to less intracytoplasmic ribosomal content (40×).

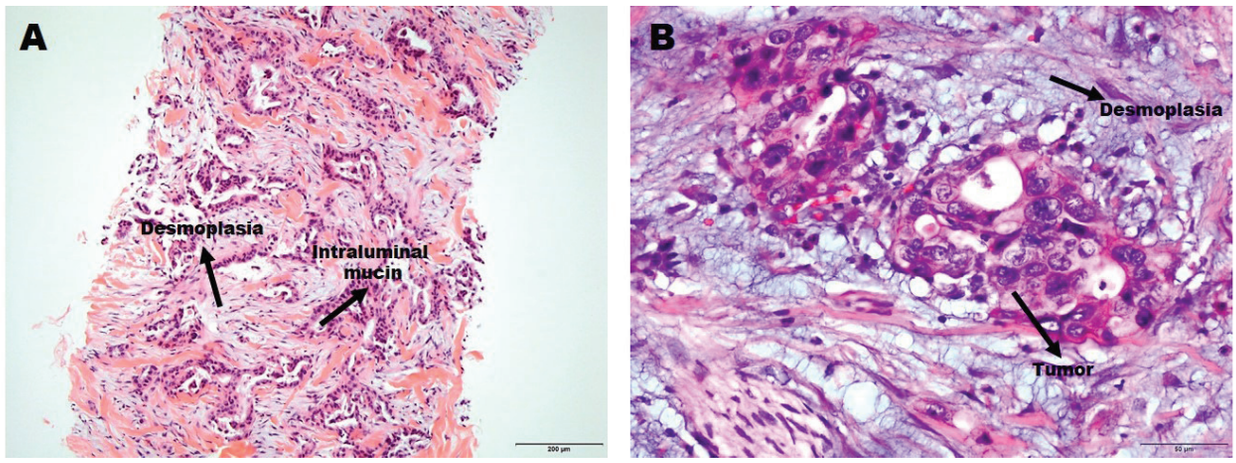

Figure 2. Representative pictures from hematoxylin and eosin staining of PC tissue. (A) Biopsy of pancreatic adenocarcinoma. The malignant glands invade tissue eliciting a strong desmoplastic reaction. Focally intraluminal mucin may be seen (10×). (B) Higher magnification of pancreatic adenocarcinoma shows malignant irregular glands composed of cell with loss of polarity, large nuclei with high nuclear-to-cytoplasmic ratio. The nuclei show irregular shape and are hyperchromatic or vesiculated with prominent nucleoli (40×).

cells. Cell nuclei often show polymorphism, hyperchromasia, loss of polarity, and proeminent nucleoli [12]. PA shows strong desmoplastic reaction that occurs around cancer cells, which is considered a hallmark for this cancer type and may account to up to 90% of the tumor volume (**Figure 2**). The stroma surrounding the cancer cells is actively involved in tumor growth and dissemination. Desmoplastic stroma is composed of extracellular matrix (ECM), cancer-associated fibroblasts, stellate and inflammatory cells, and small blood vessels. Desmoplastic stroma shows high levels of cytokines and growth factors. The desmoplastic stroma creates a barrier for chemotherapeutic drug delivery. Targeted therapies against PC stromal components have so far failed to translate into significant clinical benefits [13].

Pancreatic neuroendocrine tumors (PNETs), representing 1–2% of PC, are commonly called islet cell carcinomas. Functional PNET secretes biologically active hormones (insulin, glucagon, somatostatin, or vasoactive intestinal peptide), causing a clinical syndrome. Nonfunctioning PNET does not cause clinical symptoms [14]. Other types of exocrine PC include acinar cell carcinomas, adenosquamous carcinomas, colloid carcinomas, hepatoid carcinomas, intraductal papillary mucinous neoplasms and pancreatoblastomas [15].

The majority of PC develops silently from pancreatic intraepithelial neoplasia (PanIN) over a long period of time that highlights the importance and the challenge for early diagnosis [16]. Survival of patients with PC depends on the tumor stage at the time of diagnosis. The American Joint Committee on Cancer staging system has defined the relationship of pancreatic tumor with surrounding tissues, lymph nodes, vessels, and distant organs [17]. The first clinical stage of PC refers to tumors that are confined within the pancreas. The second stage involves PC that is spread to the adjacent tissues, especially to the lymph nodes. In Stage 3, the disease has already spread to the blood vessels, while in Stage 4, the metastasis has occurred in distant organs. Unfortunately, at the time of diagnosis, most of the patients have already invasion of vascular, lymphatic, and perineural tissue. The most common sites for distant metastasis are the liver, lung, pleura, peritoneum, and adrenal glands. Surgery may be offered to <20% of patients with PC. An additional challenge is that surgery success rate is gravely limited by the extent of early or occult micro metastases [18].

3. Risk factors for pancreatic cancer

There are several factors that pose high risk for PC, such as obesity, chronic pancreatitis, diabetes, tobacco, and alcohol usage, exposure to chemicals, such as dyes and pesticides, age, and epigenetic changes. High-fat diets activate oncogenic Kras and Cox-2, causing inflammation and fibrosis in the pancreas, leading to PanINs and PC onset. Fat diet that induces pancreatic fatty infiltration could play an important role in PC. Moreover, the presence of PanINs was associated with intralobular fat accumulations [19]. The risk of PC increases with age, more than half of new cases occur in patients over 70 years old. ABO blood types and genetic variants may also influence PC risk [20]. Cigarette smoking increases the risk for PC by 75% when compared with nonsmoking individuals, and the risk persists for 10 years after smoking cessation [7]. Although several risk factors have been identified, the causes of PC are not well known. Understanding the mechanisms through which the risk factors might affect PC progression and survival is the key to develop a prevention strategy for this disease.

3.1. Obesity

Obesity is pandemic in the USA and has been associated with poor prognosis of several malignancies, including prostate, colon, breast, endometrial cancer, and PC. Both general and abdominal obesity are associated with increased PC risk. Moreover, physical inactivity has been linked with increased PC risk [7]. Obesity was linked with increased mortality from PC [21] and the promotion of stromal desmoplasia [22].

The most common method for obesity detection is the determination of the body mass index (BMI) that is calculated based on the relationship between body height and weight (BMI 18.5–24.9, normal; 25.0–29.9, overweight; ≥30, obese). Obesity strongly correlates with body fat levels. Adipose tissue has a very strong endocrine function, secreting various adipokines that are involved in cancer development and progression, and insulin resistance. Leptin, IL-6, and tumor necrosis factor-alpha (TNF-α) are inflammatory factors increased in cancers, but adiponectin is protective against tumorigenesis, and its serum levels are usually decreased. Cancer patients show higher baseline levels of C-reactive protein and soluble TNFα receptor 2. Lipocalin 2 was associated with tumor invasiveness. Resistin, another proinflammatory adipokine, was increased in colon, breast, and prostate cancer. To date, many adipokines have been associated with cancer, contributing to enhanced inflammation, angiogenesis, cellular proliferation, and tumorigenesis [23].

3.1.1. Leptin

One of the main adipokines is leptin, a small protein (16 kDa), which is secreted by white, brown adipose tissue and cancer cells [24]. Leptin binding to its receptor, Ob-R, in the hypothalamus controls food intake and energy expenditure. Leptin also influences the reproductive function and is a long-term regulator of body weight. Leptin is also expressed in placenta, ovaries, skeletal muscle, stomach, and mammary epithelial cells. Leptin can inhibit bone formation. It regulates the ovulatory cycle and plays an important role in embryo implantation [25]. Obese and overweight individuals have high levels of leptin in blood but exhibit leptin resistance, failing to control food intake. Leptin blood levels in obese patients are 10 times higher (40 ng/ml) than in normal individuals (4 ng/ml). The underlying mechanism of leptin resistance in obese individuals is multifactorial that includes impairment of Ob-Rb signaling, hypothalamic neuronal wiring, leptin transport into the brain and Ob-R trafficking, endoplasmic reticulum (ER) stress, and inflammation [26]. High-leptin levels can induce cancer cell proliferation and thus can provide a link between obesity and cancer progression.

Several cancer cell types express leptin [25, 27, 28]. Both in vitro preclinical studies and patient, data suggest that leptin signaling is linked to the development of PC, breast, endometrial, colon, esophagus, stomach, thyroid gland, prostatic, hepatic, skin, brain, ovarian, lung and colon cancers, and leukemia [28–32]. Leptin can induce the development of nonalcoholic fatty liver disease, one of the major causes of hepatocellular carcinoma [33]. Leptin increases the proliferation of human myeloid leukemia cell lines and prostate cancer [34, 35]. In breast cancer, leptin increases the cancer cell proliferation and the expression of antiapoptosis-related proteins like Bcl-2 [36, 37]. Moreover, leptin induces the tumor angiogenesis, by promoting the expression of angiogenic factors, such as vascular endothelial-growth factor (VEGF) and fibroblast-growth factor 2 (FGF-2) [38]. Leptin has a direct effect on the proliferation of endothelial cells that were similar to VEGF [39]. Overall, leptin induces the production of inflammatory cytokines (IL-1, IL-6, and TNF-α), which can promote tumor invasion and metastasis [40].

There is a correlation between increased leptin levels and PC. Overexpression of leptin promotes the growth of human PC xenografts and lymph node metastasis in mice [41]. Ob-R is expressed by pancreatic cells, but its expression is increased in PC cells. Leptin binding to Ob-R induces proliferation, migration, angiogenesis and reduces PC cell apoptosis. The receptor

long isoform, Ob-Rb, is found more often in cancer cells and has full signaling capabilities, in contrast to the short isoform. Leptin and Ob-R have absolute affinity for binding. Leptin binding to Ob-R activates canonical (JAK2/STAT3, MAPK, PI-3 K/AKT1) and noncanonical signaling pathways (p38MAK, JNK, AMPK). The first leptin signaling event is the activation of JAK2, which phosphorylates Ob-R intracytoplasmic tail, leading to the phosphorylation of a tyrosine residue of STAT3 (pSTAT3). pSTAT3 forms a dimer that is translocated to the nucleus, inducing the transcription of specific genes, such as SOCS3, which acts as a potent negative feedback regulator of the JAK/STAT pathway [26]. Recently, it was reported that the central or peripheral administration of an Ob-R antagonist induced comparable changes in food intake, body weight, and hypothalamic SOCS3 expression in lean and diet-induced obesity (DIO) mice. These results suggest that endogenous Ob-R signaling may not be reduced in the context of DIO, thus challenging the established concept of leptin resistance under dietary-induced conditions [42].

4. Mechanisms of chemoresistance in PC

Cancer chemoresistance is a current PC challenge. Intrinsic chemoresistance occurs when chemotherapy is ineffective from the start of treatment, whereas acquired chemoresistance develops only after exposure to anticancer drugs. Although PC cells are more susceptible to Gemcitabine when compared with other anticancer agents, most patients develop resistance within weeks of treatment initiation, leading to poor survival [2]. Mechanisms of cancer chemoresistance include drug modification, reduction or inhibition of drug-induced apoptosis, overexpression of drug efflux proteins, increased expression of survival factors and deregulation of pathways, such as Notch, and expansion of cancer stem cells (CSCs), among others [43].

4.1. Pancreatic cancer stem cells (PCSCs)

The hierarchical model of cancer states that tumors arise from CSC or cancer-initiating cells that can reproduce all tumor cell types. CSCs have common characteristics associated to normal stem cells. CSCs are tumorigenic, show self-renewal capabilities, and can be differentiated into multiple cancer cell types. CSCs hide in the tumor niche causing relapse and metastasis. The tumor niche is composed of stromal and inflammatory cells, cytokines, ECM, and vasculature. It provides signals helping CSCs to maintain their undifferentiated state. The accumulation of ECM destroys the normal PC architecture and enhances the expression of PCSC markers [44].

PCSCs express various markers, including CD24+CD44+, CD133+, CD24+CD44+ESA+, ALDH+, or c-Met+. Metastatic PCSCs express CXCR4+CD133+. PCSC markers CD133 and CD44 correlated to CXCR1 expression. PCSC could be identified using Hoechst 33342 dye by flow cytometry. Hoechst-negative cells were called “side population” and were linked to chemoresistance [45]. ALDHs are a class of enzymes that oxidize aldehydes. ALDH + PCSC show clonogenic and metastatic potential that affects survival in PC. Positive PC cells for PCSC markers form tumors in mice, in contrast to negative PC cells. ALDH1 mediates resistance to Cyclophosphamide and

Gemcitabine in PC. TGF-β negatively regulates ALDH1 in PC in a SMAD-dependent manner. That can be disrupted by SMAD4 mutations and deletions. Therefore, targeting PCSC could induce sensitization of PC to chemotherapeutic treatment [46].

Chemotherapeutic agents target the bulk of the tumor but unfortunately allow the proliferation of CSC that exhibits chemoresistance. Gemcitabine kills tumor cells but increases PCSC (CD24+ and CD133+) that expresses stemness-associated genes, such as Bmi1, Sox2, and Nanog. PCSC expansion increased cell migration, chemoresistance, and tumorigenesis [47]. Drug resistant cells showed activated c-Met and increased expression of CD24, CD44, and ESA. The use of a c-Met+ cell inhibitor (Cabozantinib) abrogated Gemcitabine resistance in PC patients [48]. Administration of anti-CD44 monoclonal antibody to a human PC xenograft mouse model increased Gemcitabine sensitivity [49]. Similarly, Metformin enhanced the antiproliferation effects of Gemcitabine by inhibiting the proliferation of CD133+ cells in PC [50].

Another PCSC marker, Dclk1, was found in PanIN lesions, and PC at invasive stages [51], suggesting that PCSC may be used as diagnosis biomarkers. PCSCs show transcription factors found on embryonic stem cells (Oct-4, Sox-2, and Nanog). Increased levels of Oct-4 and Nanog correlate with early stages of carcinogenesis and worse prognosis. Oct-4 contributes to metastasis and cancer multidrug resistance. Sox-2 expression alone in PC could induce self-renewal and differentiation [24].

PCSC marker expression correlates with lymph node metastasis and poor survival. There are several factors that could affect PCSC maintenance and proliferation. For example, PCSC maintenance and survival are affected by miRNA34. In addition, stem cell factor (SCF) binding to its receptor, c-Kit, induces an increase in HIF-1α synthesis, which is involved in PC progression and chemoresistance [26].

Our data suggest that 5-FU (a common chemotherapeutic used in PC treatment) decreased PC tumorsphere formation. PC cells that expressed CD24 + CD44+, CD24 + CD44 + ESA+, and pluripotency (Oct-4, Sox-2, Nanog) markers were spared by the 5-FU treatment [30]. Therefore, the development of specific treatments against PCSC remains a challenge.

4.2. ATP-binding cassette proteins

Overexpression of drug efflux proteins (ATP-binding cassette proteins and ABC family of proteins) increases the elimination of anticancer drugs and decreases their accumulation inside the cancer cells. ABC proteins (ABCB1, ABCC1, and ABCG2) are found in PCSC and contribute to their resistance to Gemcitabine [52]. Indeed, ABCB1 was significantly increased in CD44+ PC cells during the acquisition of resistance to Gemcitabine [53]. PC chemoresistance correlated with increased expression of CXCR4, CD133, and ABCB1 by PCSC [54]. Interestingly, ABCG2 localization and activity were not confined only to the plasma membrane, as intracellular vesicles containing ABCG2 were detected within CSC in PC, colorectal, and hepatocellular cancers. Moreover, a direct relationship between the presence of these vesicles in CSCs and the maintenance of their stem-like properties, including chemoresistance, was found. Furthermore, the vesicles accumulated ABCG2-dependent substrates, such as the fluorescent vitamin riboflavin (vitamin B2). In addition, the vesicles could accumulate

ABCG2-depedent therapeutics, such as Mitoxantrone, to avoid apoptotic cell death [55]. Our data showed that PC tumorspheres treated with 5-FU were enriched in cells that overexpressed ABCC5 and ABCC11 efflux proteins [30].

4.3. Epithelial to mesenchymal transition (EMT) and PC metastasis

To gain invasive and migratory capacity, and resistance to apoptosis, cancer epithelial cells undergo EMT. The expression of transcription factors, including Snail, Slug, zinc finger E-box-binding homeobox 1 (ZEB1), and Twist, among others, induces EMT. ZEB1 deletion had a negative effect on tumor progression, invasiveness, and metastasis, reaffirming EMT's role in PC metastasis [55]. Gemcitabine-resistant PC cells had increased Vimentin and decreased E-cadherin expression. These alterations are hallmarks of EMT.

Our data showed that the use of 5-FU rendered different outcomes on EMT markers in tumorspheres derived from different PC cell lines. In BxPC-3 tumorspheres, 5-FU did not change the levels of expression of EMT markers (Vimentin and N-cadherin), while in MiaPaCa-2 tumorspheres, it slightly increased the expression of N-cadherin. Moreover, 5-FU spared PC cells that were N-cadherin+ [30]. Recently, the EMT concept was challenged by studies demonstrating the existence of a hybrid epithelial/mesenchymal phenotype in cells transitioning from EMT to mesenchymal to epithelial transition (MET). Because MET has been considered crucial for metastasis seeding in distant organs, this hybrid phenotype seems to be linked to drug resistance and tumor-initiating potential. Moreover, MET could allow tumor cells to collectively migrate in clusters to form metastases in a more effective way than pure EMT single cells [55].

4.4. Tumor microenvironment

PC desmoplasia results from proliferation of cancer-associated fibroblasts and increased deposit of ECM. This process reduces elasticity of tumor tissue and increases interstitial pressure, leading to decreased perfusion of chemotherapeutic agents [56]. The proliferative pancreatic stellate cells are the primary source of many of the ECM components in PC. These cells show increased proliferation and sensitivity to mitogenic factors. Fibrous proteins (e.g., collagen) and polysaccharide chain glycosaminoglycans (e.g., hyaluronan) are ECM factors that constitute the noncellular components of PC desmoplastic tissue. A significant overproduction of ECM components can be described as the failed resolution of a healing wound, which leads to fibrosis in PC. Immune cells (macrophages, neutrophils, and regulatory T cells [Treg]) contribute to PC desmoplasia. Therapeutics reducing the contribution of the desmoplastic reaction to chemoresistance are being actively pursued as a potential therapeutic approach [57].

4.5. Changes in signaling pathways

From the early lesions, PC cells harbor alterations in signaling pathways that remain throughout carcinogenesis. These changes not only impact tumor cells but also the surrounding stromal cells. Components of the Hedgehog (Hh) signaling pathway have essential roles in PC pathogenesis. In a global genomic analysis of PC, all tumors tested had alterations in at least one of the Hedgehog signaling genes. Hh signaling induced desmoplasia, playing a key role in chemoresistance [56]. Wnt signaling pathway is mainly involved in PC cell growth. The Wnt pathway is activated when ligands bind to the cell membrane Wnt receptor, resulting in the release of

β-catenin into the cytoplasm. Increased β-catenin levels and activity have been found in PC but not in the normal pancreas [58]. Wnt pathway induces PC formation by actions not only on the tumor cells but also on the stromal compartment through increases in ECM formation [59].

There are other dysregulated pathways in PC. The nuclear factor-κB (NF-κB) proteins constitute a family of transcription factors associated with mediating inflammatory responses. However, these transcription factors also control diverse genes involved in development, apoptosis, and cell proliferation. NF-κB has an important role in PC. Additionally, Notch and IL-1 induce NF-κB in PC [60]. NF-κB signaling crosstalks with other signaling pathways, oncogenic or cancer-related proteins, such as STAT3, p53, ALDH1, PI-3 K, and MAPK. A recent study that evaluated a large number of human PC samples along with a few PanIN lesions found amplification of c-Myc in 30% of the tumors [61]. c-Myc deregulation, in cooperation with other oncogenic pathways, such as Kras, is sufficient to promote tumorigenesis [62]. The complexity of the PC altered signaling pathways affects pathogenesis and could explain why there is no successful PC treatment. Relationships among tumor cells, stroma, and signaling pathway crosstalks demonstrate the importance of developing combined therapies targeting both compartments and altered signaling in PC.

4.6. Inhibition of apoptosis

Apoptosis or programmed cell death regulates the tissue homeostasis. Chemoresistance is in part due to impairment of apoptosis in cancer cells. Antiapoptotic protein Bcl-2 is not frequently overexpressed in PC, which differs from other cancer types. In contrast, an imbalance between antiapoptotic Bcl-XL and proapoptotic Bax was found in the TGF-α murine model of PC [63]. Moreover, inhibitors of apoptosis, such as survivin, are overexpressed in PC when compared with normal pancreatic tissue. Resistant PC cells can be sensitized to death receptor–mediated apoptosis by inhibiting the NF-κB prosurvival pathway or by decreasing the expression of antiapoptotic proteins. The p53 pathway plays an important role in cancer cells avoiding the apoptosis, with mutations in p53 gene leading to increased drug resistance in PC cell lines and poor survival in PC patients [63]. Our data showed that 5-FU treatment of PC tumorspheres reduced RIP and Bcl-XL levels and increased Bax. Moreover, 5-FU increased caspase-3 activation and decreased uncleaved PARP in PC [30]. These data indicate that 5-FU actions on PC induce apoptosis through several components of the pathway. Numerous chemotherapeutic drugs target DNA synthesis in cancer cells, leading to increased apoptosis.

4.7. Leptin and chemoresistance mechanisms in pancreatic cancer

Leptin induces a wide range of prooncogenic effects. We have shown, for the first time, that leptin could be secreted by PC cells and derived tumorspheres. Moreover, leptin induced PCSC in tumorspheres [28]. In line with these data, a study of a pool analysis from PC patients showed that leptin levels and elevated Ob-R expression correlated to Oct-4 [64]. Our data demonstrated that leptin increased PC cell proliferation, tumorsphere formation, and xenograft growth in an immunocompromised mouse model. Moreover, leptin induced cell cycle progression, PCSC markers (CD24 + CD44 + ESA+, ALDH+), and ATP-binding cassette protein expression (ABCB1) in PC cells [28]. Leptin has been shown to increase the expression of miR21, while the tumor suppressors (miR200a, miR200b, and miR200c) decrease the expression of Ob-R. Furthermore, these tumor suppressors could also interact with some of the

PCSC markers (c-Met, ABCB1, and CD44), which decrease their expression. Oncogenic miR21 increases the expression of ABCB1, ALDH, and CD44.

Leptin can directly regulate the expression of HDAC4 and HDAC5 and indirectly affect the expression of other HDAC via microRNA or PCSC markers. We have suggested that leptin can increase the expression of miR21, which in turn can increase the expression of HDAC3. Analysis of data from PC biopsies (TCGA databank) suggested that HDAC, miRNA21/200, and leptin could have complex signaling crosstalk that could be a novel therapeutic target for obese PC patients. We further determined the effects of leptin on HDAC expression in PC tumorspheres. HDAC3 and HDAC8 expression was increased by leptin. Furthermore, the Gemcitabine-induced decreased expression of HDAC2, HDAC3, and HDAC8 was reversed by leptin. Thus, we have shown that leptin through its effects on PCSC, ABCB1, and HDAC could be involved in PC chemoresistance [65]. Moreover, using another chemotherapeutic agent commonly used in PC treatment, 5-FU, we demonstrated that leptin impaired 5-FU cytotoxicity by increasing the expression and number of PCSC+, pluripotency+, and EMT+ PC cells. ABCC5 and ABCC11 expression as well as the number of positive cells for these ATP-binding cassette proteins were increased by leptin in PC tumorspheres. These leptin's effects protected the survival of PC tumorspheres treated with 5-FU and reduced its cytotoxicity. The survival of PC tumorspheres treated with 5-FU and leptin was linked to reduced apoptosis. Leptin increased the levels of PARP, Bcl-XL, and RIP and decreased Bax. 5-FU increased caspase-3 activation, which was reduced by leptin. These data could help to unravel the multiple mechanisms through which leptin signaling contributes to drug resistance in PC [30].

4.7.1. Leptin-Notch crosstalk in pancreatic cancer

Notch signaling controls the cell proliferation, PCSC maintenance and differentiation, apoptosis, invasion, and metastasis in cancer. Overexpression of Notch receptors (Notch1 and Notch2) was found in PCSC when compared with nonmalignant pancreatic stem cells [66]. DLL4 increase in PC cells stimulated the expression of Oct-4, Nanog, and stem cells [67]. PCSCs that express Oct-4, Sox-2, and Nanog show an increased aggressivity and chemoresistance. Notch4 overexpression was linked to PC chemoresistance to Docetaxel [68]. Expression of Notch3 and Hey1 was associated with reduced survival in PC [69]. Resistance to Gemcitabine correlated with Notch2, Notch4, and JAG1 overexpression [70]. The inhibition of Notch1 by siRNA suppressed proliferation, induced apoptosis, and reduced migration and invasion of PC cells [71].

Notch signaling induced EMT phenotype in Gemcitabine-resistant PC cells overexpressing Notch2, Notch4, and JAG1. Furthermore, the inhibition of Notch signaling decreased EMT markers, including Vimentin, Snail, Slug, and ZEB1, in human PC cell lines [72]. MiR200 members increased Notch activation by ZEB1 that regulates the expression of JAG1 and the mastermind-like coactivators (Maml2 and Maml3). In PC cells, miR200 expression showed an inverse correlation with JAG1 and ZEB1 levels [73]. Therefore, miR200 inhibits EMT by interacting with ZEB1/2 and the Notch pathway and represses self-renewal and differentiation in CSC. MiR200 is also involved in apoptosis [72].

Our data showed that leptin induced the expression of Notch family components in PC (Notch1–4, DLL4, JAG1, survivin, and Hey2), PCSC markers (CD24CD44ESA, ALDH, CD133,

and Oct-4), ABCB1 (MDR1), tumorsphere formation, cell cycle progression, proliferation, and tumorigenesis. These effects were reduced by GSI [28]. Moreover, mouse and human PC and cell lines treated with adiponectin, or an adiponectin receptor agonist, AdipoRon, suppressed leptin-induced STAT3 signaling in vitro and reduced PC growth in vivo [74]. The addition of leptin to 5-FU treated tumorspheres decreased 5-FU-induced cytotoxicity and increased colony forming ability, number of cells expressing pluripotency and EMT markers, drug efflux proteins (ABCC5 and ABCC11), and Notch. Leptin also reduced the 5-FU effects on apoptosis by decreasing proapoptotic (Bax, caspase-3 activation, and PARP degradation) and increasing antiapoptotic factors (RIP and Bcl-XL). Leptin's effects on PC tumorspheres were mainly Notch signaling dependent [30]. Therefore, the leptin-Notch axis could be a target to develop novel strategies for PC treatment.

5. Pancreatic cancer treatment

5.1. Chemotherapy

To decrease the risk of local and distant metastasis, adjuvant therapy is usually started 1–2 months after PC surgery. Although no regimen has been proven significantly more effective than others, a regimen based on 5-FU or Gemcitabine for 6 months is usually the option used to reduce PC patients' mortality [75]. The activity of 5-FU/Leucovorin has been compared to Gemcitabine as an adjuvant therapy in the European Study Group for PC (ESPAC)-3 trial [76]. However, the study showed that median overall survival for patients treated with 5-FU/Leucovorin was 23 months when compared with 23.6 months for patients treated with Gemcitabine. The ESPAC-4 study measured the efficacy of a combination treatment with Gemcitabine plus Capecitabine when compared with monotherapy with Gemcitabine alone. The results showed a survival of 28 months in the combined therapy when compared with 25.5 months in the monotherapy group. Because the dual therapy was well tolerated, the combination of Gemcitabine and Capecitabine has been used as a standard in the clinical setting [77]. Currently, regimens with Gemcitabine plus nanoparticle albumin-bound Paclitaxel (nab-Paclitaxel) and a combination of 5-FU, Irinotecan, and Oxaliplatin (FOLFIRINOX) are evaluated in the clinical setting [78]. Gemcitabine has usually some efficacy as an adjuvant therapy, but often patients develop chemoresistance. Nab-Paclitaxel, a water-soluble compound, has enhanced distribution properties within the tumor microenvironment when compared with Paclitaxel. However, studies have shown that nab-Paclitaxel treatment neither decreased tumor stroma nor increased tumor vascular perfusion in a mouse patient-derived xenograft (PDX) tumor model [79]. The infiltration of neoplastic lesions by CD8+ T lymphocytes is associated with improved prognosis. However, a CD40 monoclonal antibody that activated CD8+ T cells in Phase I clinical trial had only a partial response [80]. FOLFIRINOX and nab-Paclitaxel plus Gemcitabine have the potential to downstage local advanced disease and to improve tumor resection rates. The use of chemoradiation therapy as an adjuvant is controversial and with minimal effects on survival in clinical trials so far [81]. New studies that incorporate modern radiation techniques and current chemotherapy regimens are still needed to determine if radiation is beneficial in PC treatment.

5.2. Targeted therapy

A comprehensive genetic analysis of PC showed that these tumors contain an average of 63 genetic alterations in 12 cellular signaling pathways, including Notch pathway [82]. A Phase Ib trial for PC using a combination of Demcizumab (OMP-21 M18), a monoclonal antibody against Notch ligand, DLL4, with Gemcitabine and Abraxane, showed some clinical benefits [60]. An antibody against Notch2 and Notch3, Tarextumab, was tested in Phase 2 clinical trials in combination with Gemcitabine and nab-Paclitaxel in patients with metastatic PC. For these patients, the median progression-free and overall survival were 5.6 and 11.6 months, respectively. Gamma secretase inhibitors (GSIs) have been used in clinical trials in PC. For example, a GSI called RO4929097 was safely tolerated in combination with Gemcitabine and achieved clinical antitumor activity and more than 4 months of stable disease. However, the use of GSI has limitations and still represents a challenge because of the increased drug toxicity and lack of high specificity to Notch besides other substrates of γ-secretase [83].

Desmoplasia is a target in PC treatment. Hyaluronan, a component of the ECM of PC, is a naturally occurring nonsulfated glycosaminoglycan that was targeted using pegylated hyaluronidase (PEGPH20). In a Phase II study combining Gemcitabine, nab-Paclitaxel, and PEGPH20, there was no difference seen in the survival of PC patients that had this addition to their treatment. Also, due to the ubiquitous nature of hyaluronan, there were unexpected side effects, such as thrombosis. For the Gemcitabine, nab-Paclitaxel, and PEGPH20 study, a subset analysis was performed on the high-hyaluronan patients. In the arm receiving PEGPH20, the response rate was 45% when compared with 31% in controls, which was encouraging, and led to a Phase III clinical trial (HALO301) for patients that had high hyaluronan. In these studies, Lovenox was included for anticoagulation [84].

STAT3 inhibition has been shown to decreased PC growth in mouse models. Napabucasin decreased STAT3 transcription and tumorsphere formation and showed some efficacy in PC. Napabucasin induced a median progression-free survival of >7.1 months and a median overall survival of >10.4 months in PC patients. Based on these encouraging results, it is now being evaluated in a PC Phase III study in combination with Gemcitabine and nab-Paclitaxel (NCT02993731) [85].

The expression of leptin in gastroesophageal adenocarcinomas was associated with chemoresistance. Therefore, the addition of leptin antagonists to current chemotherapeutic treatment could represent a new strategy to overcome drug resistance and to improve survival of PC patients. SHLA, a leptin antagonist, increased the sensitivity of resistant gastric cancer cell line, AGS Cis5, and the esophageal adenocarcinoma, OE33, to cisplatin [86].

LPrA2 was designed and tested in vitro and in vivo in PC xenograft mouse models in our laboratory. LPrA2 is composed by a leptin sequence corresponding to its binding Site III of the leptin molecule. LPrA2 was conjugated to iron-oxide nanoparticles (IONP-LPrA2) to increase its bioavailability and effectiveness to block leptin signaling in cancer cells [28]. IONP-LPrA2 showed no toxicity and did not affect energy balance (body weight or food intake) or general health when it was administered to mice. IONP-LPrA2 reduced the expression of Ob-R, Notch, and PCSC markers. Furthermore, specific inhibition of leptin signaling by IONP-LPrA2 delayed tumor onset and decreased tumor growth in a PC xenograft mouse model. Our data also showed that IONP-LPrA2 could be used as an adjuvant therapy to 5-FU. In PC cells treated

	Human pancreatic cancer cells							
	BxPC-3 low aggressive				MiaPaCa-2 highly aggressive			
	B	CT	CT+L	CT+L+LI	B	CT	CT+L	CT+L+LI
Proliferation (%)	100	70	88	68[a]	100	32	91	58[a]
Tumorsphere formation (%)	100	96	161	97[a]	100	88	201	70[a]
Notch receptors Notch1+	100	263	234	167[a]	100	144	149	125[a]
Notch3+	100	91	113	111	100	240	325	210[a]
Notch4+	100	143	200	149[a]	100	146	219	186[a]
PCSC CD34+CD44+ESA+	100	122	144	91[a]	100	653	1088	403[a]
c-Met+	100	212	226	168[a]	100	81	142	69[a]
Pluripotency markers Oct-4+	100	141	144	89[a]	100	67	95	44[a]
Sox-2+	100	87	95	69[a]	100	84	116	62[a]
Nanog+	100	148	172	134[a]	100	84	78	57[a]
EMT N-cadherin	100	34	39	19[a]	100	159	276	137[a]
Vimentin	100	92	88	86	100	64	83	54[a]
ABC proteins ABCC5	100	93	123	110	100	55	76	34[a]
ABCC11	100	51	60	50	100	50	83	42[a]
Survival	100	60	104	67[a]	100	61	113	89[a]
Apoptosis Caspase-3 activity	100	980	300	441[a]	100	187	159	159
PARP	100	21	39	28	100	71	92	65[a]
RIP	100	26	38	20[a]	100	62	82	40[a]
Bcl-XL	100	87	109	80[a]	100	72	89	70[a]
Bax	100	136	103	130[a]	100	119	72	70

Table 1. Inhibition of leptin signaling using IONP-LPrA2 resensitizes PC cell lines to chemotherapy.

with 5-FU and leptin, IONP-LPrA2 reduced tumorsphere formation and cell proliferation, the number of Notch+, ABCC5/11+, and PCSC+ cells, and increased apoptosis. Thus, IONP-LPrA2 resensitized PC cells to 5-FU actions [28, 30]. In view of leptin multiple effects on PC and the involvement of Notch signaling in leptin's effects, targeting leptin-Notch crosstalk in PC patients might be a new treatment strategy for this deadly disease (**Table 1**). The addition of leptin antagonists to current chemotherapeutic treatment could represent a new strategy to overcome drug resistance and to improve survival of PC patients.

6. Conclusions

PC is a lethal systemic disease that is difficult to detect and treat. This is mainly due to the fact that even patients diagnosed with early stages eventually develop metastasis. The deep abdominal position of the pancreas is an additional factor that delays the onset of specific PC symptoms. Early PC diagnosis and potential cure remain important challenges due to the lack in screening methods and specific biomarkers. PC risk factors, such as high-fat diet, obesity, tobacco, and alcohol consumption, can be modified, leading to prevention of disease occurrence and

increased survival. PC desmoplastic stroma, which decreases chemotherapeutic drug delivery to the tumor, is an another current challenge to improve PC survival. Currently, combined chemotherapy strategies are used in selected patients with PC metastatic disease. The identification of novel PC targets is the key for the development of new individualized strategy for prevention and treatment. An emerging and promising area is the relationship between obesity and leptin-induced prooncogenic effects in PC, which could also affect chemoresistance and metastasis. In this respect, the use of leptin signaling antagonists as a novel sensitization adjuvant for current chemotherapeutic drugs appears as a potential new strategy to improve treatment effectiveness and patients' survival. The use of leptin signaling antagonists could also make possible the reduction of drug dosage and the improvement of patient quality of life.

Acknowledgements

This work was supported by the National Institute of Minority Health and Health Disparities (NIMHD) at the National Institutes of Health under Award Number S21MD000101 and the National Cancer Institute 5G12 MD0076021, G12 RR026250-03, NIH RR03034, and 1C06 RR18386 to Morehouse School of Medicine.

Conflict of interest

The authors declare that there are no conflicts of interest in writing this chapter.

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