

Pancreatic Cancer and Obesity: Some Molecular Perspectives

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Introduction

Obesity, pandemic in USA, is associated with increased risk of different types of cancer [1], including pancreatic cancer (PC). In the NIH-AARP Diet and Health Study, it was observed that an excess of body weight across a lifetime remains significantly associated with increased risk of PC, in particular when comparing the category with BMI>25 to BMI=8.5-22.5 [2]. Results from several preclinical models suggest that obesity can initiate pancreatic carcinogenesis and promote metastasis [3]. A high fat diet activates oncogenic K-Ras and Cox2, causing inflammation and fibrosis in the pancreas, with subsequent pancreatic intraepithelial neoplasia (PanINs) and PC onset [4]. A recent study showed that the number of PanINs is correlated with intravisceral fat. Moreover, the presence of PanINs was associated with intralobular fat accumulation [5]. It appears that a fat diet leading to pancreatic fatty infiltration could play an important role in PC [6]. Excess body weight worsens the prognosis already dismal for PC patients [7-9]. It is estimated that in 2016, 53,070 people will be diagnosed with PC, while 41,780 people will die of this disease, bringing PC as the third cause of cancer related death. Lack of early clinical symptoms and specific tumor markers are the reasons for late diagnosis in PC [10].

Pancreatic Cancer Therapy

Surgical excision remains the primary therapy and the efficacy of conventional chemo radiotherapy for PC is limited. Correct staging is important for PC, since prognosis and treatment depend on this. In recent years, PC patients without distant metastasis, but showing blood vessel involvement and "borderline resectable" tumors have been treated with neoadjuvant chemotherapy followed by surgical resection of the tumor. For unresectable PC, chemotherapy has been used (e.g., Gemcitabine/Erlotinib, FOLFIRINOX that is a combination regimen of oxaliplatin, 5-fluorouracil or 5-FU, Leucovorin and Irinotecan; Gemcitabine/NAB-paclitaxel, Gemcitabine/Capecitabine and Capecitabine/Oxaliplatin or XELOX) [11]. However, PC is characterized by its early metastasis and resistance to standard chemotherapy or radiation therapy. Desmoplasia, a result of the proliferation of cancer associated fibroblasts and increased deposition of extracellular matrix, leads to reduced elasticity of tumor tissue with a concomitant increase in tumor interstitial fluid pressure, which results in a decreased rate of perfusion of therapeutic agents and consequently decreased efficacy [12]. Studies have shown that in obesity, the crosstalk between adipocytes, tumor associated neutrophils and pancreatic stellate cells promotes desmoplasia in mouse models of PC, and leads to accelerated tumor growth [13].

Pancreatic Cancer Chemoresistance

Unfortunately, none of the established targeted therapy agents that have been effective on other tumor types show similar effects on PC, suggesting that there are unique elements in the microenvironment of PC that facilitate its dramatic chemoresistance [14]. Studies have indicated that various mechanisms of drug resistance are involved in PC, such as changes in individual genes or signaling pathways, the influence of the tumor microenvironment, as well as the actions of the PC stem cells (PCSC) [15]. These cells are capable of dividing, renewing themselves and differentiating into less tumorigenic cells. Therefore, PCSC are thought to be involved in tumor recurrence post-treatment. Although stem cells exist as a small population in the cancer tissues, recent evidence shows that PCSC contribute to tumor initiation, growth, metastasis, and resistance to therapy [16,17]. Furthermore, a recent study shows that apoptosis-resistant PC cells have PCSC-like properties. These cells are characterized by their ability to initiate sphere formation when cultured in low attachment plates. Additionally, PCSC can express stem cell genes and respond to epithelial to mesenchymal transition (EMT) stimulation [18]. Gemcitabine increases cell populations expressing PCSC markers (CD24+ and CD133+) and stemness-associated genes such as *Bmi1*, *Nanog*, and *Sox2*. The enhancement of stemness after Gemcitabine treatment was accompanied by increased cell migration, chemoresistance, and tumorigenesis [19]. Isolation of side population (SP) cells from a human PC cell line led to their characterization as potential PCSC. SP cells showed highly tumorigenic and metastatic characteristics after being orthotopically injected into mice. In culture, SP cells showed an increased resistance to Gemcitabine, but not to 5-FU [20]. PCSC possess escape mechanisms to avoid drug effects that are shared with normal stem cells, such as overexpression of ATP binding cassette multidrug transporters (ABC-family of proteins). ATP-binding cassette sub-family B member 1 (ABCB1 or P-glycoprotein 1, also known as multidrug resistance protein 1, MDR1 or CD243) was significantly increased in CD44+ PC cells during acquisition of resistance to Gemcitabine. CD44 expression in PC was correlated with higher tumor histological grade and worse prognosis [21].

Leptin-Notch Crosstalk

A potential link between obesity and PC progression could be the adipokine leptin, a hormone secreted from adipocytes. In normal-weight individuals, leptin regulates food intake, body weight and energy expenditure. Higher expression of leptin receptor, OB-R, is a characteristic of cancer and embryonic stem cells. OB-R expression in stem cells is mediated directly by the core pluripotency-associated transcription factors Oct4 and Sox2 [22]. Leptin increases proliferation and migration of human and murine PC cell lines expressing the OB-R long and short isoforms. shRNA knockdown of OB-R partially

abrogated the enhanced obesity-mediated growth of orthotopic PC tumors in obese mice [23]. Moreover, the effects of obesity through leptin signaling on cancer growth seem to be involved in chemoresistance developed by other cancer types. Indeed, in gastro-esophageal adenocarcinomas leptin expression was associated with chemoresistance. Additionally, the blockade of leptin signaling with use of a leptin receptor antagonist increased the sensitivity of gastro-esophageal adenocarcinomas to cisplatin [24].

Three key embryonic signaling pathways: the Wnt/ β -catenin, Notch and Hedgehog pathways are up regulated in cancer stem cells (CSC) [25]. Aberrant Notch pathway signaling has been found in CSC from various cancer types. The inhibition of Notch pathway depleted CD133+ glioblastoma cells and inhibited tumor growth neurosphere formation [26]. Breast CSC show Notch4 activity [27]. The use of siRNA and γ -secretase inhibitor (GSI) for Notch-1 inhibition suppressed proliferation, induced apoptosis, reduced migration, and decreased invasion of PC cells [28]. Furthermore, a Phase 1b trial for PC using a combination of Domicizumab (OMP-21M18, a monoclonal antibody against Notch ligand, DLL4) with Gemcitabine and Abraxane showed some clinical benefits [25]. NF-KB is activated in approximately 70% of PC cases. Notch and IL-1 induce NF-kB in PC [28]. Data from mouse models of PC showed that NF-kB is required for oncogenic K-Ras-induced tumor development. Treatments with Gemcitabine alone or in combination with IL-1 inhibitors decreased IL-1 α -induced NF-kB activity, and reduced PC growth [29].

NILCO, RBP-Jk and Pancreatic Cancer

Leptin levels are elevated in obesity, which is a risk factor for breast, PC and endometrial cancers. Leptin-Notch crosstalk likely plays a role

in these cancers [30-32], where it also enhances angiogenic transformation of endothelial cells [33]. Notch, IL-1 and leptin are known factors involved in PC growth, progression, and chemoresistance. We have previously shown that a crosstalk between these factors (Notch, IL-1, leptin crosstalk outcome, NILCO) occurs in breast cancer. NILCO is essential for leptin-induced proliferation/migration and contributes to increased tumor angiogenesis and metastatic potential in breast cancer. Combinatory treatments of leptin signaling inhibitor (Peg-LPrA2) and drugs designed to prevent Notch and IL-1 oncogenic crosstalk may be advantageous for breast cancer patients [30].

The relationship between obesity and PC involves a pleiotropic network of regulatory factors that have not yet been fully identified. An early study suggested that leptin inhibits the PC cell growth, but enhanced cell migration and invasion [34]. In contrast, in a PC xenograft mouse model, the overexpression of leptin promoted tumor growth and lymph node metastasis [35]. We found that leptin, at concentrations similar to those found in overweight patients (20 ng/ml), increased proliferation of several PC cell lines (BxPC-3, Panc-1, MiaPaCa-2 and AsPC-1). Furthermore, leptin induced PCSC and tumorsphere formation, as well as Notch expression. Inhibition of Notch signaling diminished the effect of leptin, suggesting leptin-induced Notch signaling is involved in obesity-enhanced PC progression. The specific inhibition of leptin signaling (via leptin peptide antagonist bound to iron oxide nanoparticles, IONP-LPrA2) significantly delayed onset and decreased growth of PC xenografts in immunodeficient mice. IONP-LPrA2 treatment also reduced the expression of OB-R, Notch and PCSC markers (Table 1). These data suggest that leptin-Notch axis is involved in PCSC maintenance that could lead to PC progression and chemoresistance [31].

	Growth	PCSC	Notch expression	ATP-binding cassette	IONP-LPrA2 effects
BxPC-3	increased	increased	increased	-	decreased
MiaPaCa-2	increased	increased	increased	increased	decreased
Panc-1	increased	increased	-	-	decreased
AsPC-1	increased	increased	-	-	decreased
PC xenograft mouse model	increased	increased	increased	increased	decreased

Table 1: Effects of leptin on pancreatic cancer. Note: Data show results from investigations on the effects of leptin signaling in vitro (PC cell lines) and in vivo (heterotopic mouse model: MiaPaca-2 derived PC xenografts). Leptin increased PC growth, PCSC, the expression of Notch receptors and ligands, and ABCB1. Specific inhibition of leptin signaling using IONP-LPrA2 diminished leptin's effects.

Activation of Notch occurs through different proteases that lead to the formation of an intracellular truncated Notch receptor (NICD), which is transported to the nucleus where it binds a repression factor: RBP-Jk (CBF1/CSL). Eventually, NICD-RBP-Jk complex acts as a transcriptional activator inducing the expression of Notch targeted genes [36]. RBP-Jk is an important transcriptional regulator that is essential in Notch canonic signaling. Notch independent RBP-Jk regulation results in DNA-transcriptional activation, while Notch dependent RBP-Jk regulation leads to DNA-transcriptional repression of Notch target genes. The mechanism through which this gene facilitates transcription is thought to involve chromatin remodeling via epigenetic regulation using histone acetylase or histone deacetylase proteins [37]. The blockade of Notch-mediated repression of E-

cadherin reduced catenin activation, and resistance to anoikis, and induced a series of downstream mechanisms leading to tumor evasion of apoptosis, reduced tumor growth and diminished metastasis [38].

While it is known that Notch signaling pathway is directly involved in an oncogenic signaling mechanism that ultimately serves to promote tumor development, the connection between obesity in pancreatic cancer and Notch/RBP-Jk remains somewhat of a mystery [39]. Our data suggests that leptin induces Notch and RBP-Jk, which was linked to increased migration and proliferation of breast cancer cells [30]. Additionally, the loss of RBP-Jk significantly increased the expression of factors directly associated with cancer growth, metastasis and CSC, such as Notch3/Notch4, N-cadherin and CD24 and CD44.

These data are relevant considering the crucial role that CSC play in tumor initiation, metastasis and therapeutic resistance. In light of data reported by Kulic et al. [37], showing that RBP-Jk is downregulated as tumors grow, more research must be implemented to uncover details of its role in PC. Once this signaling pathway is fully elucidated, the effects of obesity (leptin)-mediated regulation of RBP-Jk, and its impact on cancer progression may have clinical implications. It is envisaged that meticulous investigations in adipocyte biology, tumor microenvironment and obesity-related cancer will lead to the identification of new therapeutic targets for cancer and metabolic diseases [40].

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