Role of HIF-1 and NRF2 in Pancreatic Cancer

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Hypoxia inducible factor-1 and KEAP1-NRF2, stress response mechanisms for hypoxia and oxidative stress, respectively, contribute to the aggressive behaviors of pancreatic cancer. These key molecules for stress response mechanisms are activated, both in pancreatic cancer cells and in pancreatic stellate cells. Both factors are involved in the mutual activation of cancer cells and stellate cells, by inducing cancer-promoting signals and their mediators. Therapeutic interventions targeting these pathways are promising approaches for novel therapies.

HIF-1 KEAP1 NRF2

1. Dense Fibrotic Stroma in Pancreatic Cancer

Dense fibrotic stroma, known as desmoplasia, is a characteristic feature of pancreatic cancer [1]. Desmoplasia develops through the interactions between pancreatic cancer cells and stromal cells, including pancreatic stellate cells (PSCs) [2]. PSCs play critical roles in the development of pancreatic fibrosis by producing extracellular matrix (ECM) proteins, such as collagen and fibronectin [2]. Interaction between cancer cells and PSCs enhances the malignant potential of pancreatic cancer cells, such as epithelial-mesenchymal transition (EMT) [3] and cancer stem cell-related marker expression [4]. Due to the limited formation of an efficient vascular network, dense stroma forms harsh tumor microenvironments for pancreatic cancer cells and PSCs, characterized by hypoxia, few nutrients, oxidative stress, and acidic extracellular pH [5][6]. ECM proteins such as fibronectin and laminin stimulated ROS production in cancer cells, leading to an increase of oxidative stress [7]. Different concentrations of ROS exert biphasic biological effects on cancer progression [8][9]. Activation of multiple signaling pathways by adequate levels of ROS promotes cancer progression via EMT induction and growth promotion. However, a higher level of ROS triggers cell death via apoptosis, necrosis, and ferroptosis. This severe microenvironment gives continuous stresses to both pancreatic cancer cells and PSCs. Pancreatic cancer cells as well as PSCs survive in the harsh microenvironments through the altered expression of signaling molecules, transporters, and metabolic enzymes governed by various stress response mechanisms. Several stress response mechanisms enable adaptation to the microenvironment and cancer cell survival. Hypoxia and oxidative stress are two major stressors that directly lead to cell death. The adaptation mechanisms for these conditions have been studied, and the central regulators of hypoxia and oxidative stress responses have been identified: hypoxia-inducible factor-1 (HIF-1) and NRF2, respectively [10][11]. These mechanisms also yield growth advantages, metabolic reprogramming, and malignant phenotypes of cancer cells. Furthermore, these mechanisms play pivotal roles in PSCs, contributing to the formation of a cancer-promoting microenvironment.

2. Effects of Hypoxia on Pancreatic Cancer Cells

Hypoxia affects metabolism, cellular phenotype, grouwth factor production and expression of regulatory molecule in cancer cells. Glucose-deprived hypoxic conditions induce glucose transporters and the angiogenic factor VEGF in pancreatic cancer cells [12]. Another study that identified transcriptional induction of hepatocyte growth factor activator by HIF-1 led to the activation of the hepatocyte growth factor/c-Met signaling pathway and invasiveness of pancreatic cancer cells [13]. The urokinase-type plasminogen activator receptor (uPAR) plays a pivotal role in angioinvasion to establish distant metastasis. The promoter region of the *uPAR* gene contains a HIF-1 binding site, and hypoxic treatment upregulated uPAR expression and the invasive capacity of pancreatic cancer cells [14]. Hypoxia-induced repression of pyruvate dehydrogenase activity was mediated by pyruvate dehydrogenase kinase 1 in pancreatic cancer cells [15]. This metabolic reprogramming led to glycolysis dependence, and knockdown of HIF-1α or pyruvate dehydrogenase kinase 1 restored pyruvate dehydrogenase activity and repressed xenografted tumor growth in immunodeficient mice, suggesting a contribution of pyruvate dehydrogenase repression in pancreatic cancer progression [15]. Hypoxia-induced HIF-1 activation affected the migratory ability of cancer cells. Treatment of human pancreatic cancer cells with hypoxia changed cellular morphology as spindle-like cells with less cell-to-cell adhesion, compatible to EMT [16]. Along with the HIF-1α accumulation, the EMT-inducing transcriptional factor TWIST expression was observed. Knockdown of HIF-1α led to the loss of TWIST induction and EMT induction by hypoxia. Hypoxia induced pro-fibrogenic factors such as connective tissue growth factor (CTGF) via HIF-1. CTGF plays a pivotal role in renal fibrosis and skin fibrosis [17][18]. CTGF protected pancreatic cancer cells from hypoxia-mediated apoptosis [19]. CTGF also contributed to gemcitabine-resistant phenotype in cancer cells [20]. Furthermore, HIF-1 mediated immune evasion and enhanced cancer stem cell properties and autophagy in pancreatic cancer cells [21]. In addition to these conventional growth factors and signaling molecules, hypoxia also affects the expression of microRNAs such as miR-21 and miR-210 [22][23]. Elevated expression of miR-210 was associated with poor survival of patients with pancreatic cancer, suggesting its cancer-promoting role [24]. In addition to hypoxia, PSCs by themselves induced miR-210 expression in pancreatic cancer cells [25]. Inhibition of miR-210 in pancreatic cancer cells suppressed PSC-induced EMT, suggesting a role of miR-210 in the cancer-promoting interactions between PSCs and cancer cells. MiR-21 regulated migration, invasion, and chemoresistance in pancreatic cancer cells [26]. The cancer-promoting miR-21 expression was also increased by hypoxia in a HIF-1α-dependent manner [22].

3. Effects of Hypoxia on PSCs

Hypoxia also affects the cellular functions of PSCs. Hypoxia induced migration, type I collagen production, and VEGF production in PSCs [27]. Conditioned media of PSCs increased the tube formation on Matrigel in vitro and directed vessel formation in nude mice in vivo. [27]. ECM proteins, such as periostin, deposits around the capillaries of pancreatic cancer, and hypoxia increased periostin expression in PSCs [28]. Similarly, hypoxia-treated PSCs secreted significant amounts of CTGF, which promoted the invasive potential of pancreatic cancer cells [29]. Knockdown of CTGF by RNA interference blunted this effect. CTGF expression was observed in PSCs within surgically resected pancreatic cancer tissue, along with the marker of hypoxia, carbonic anhydrase 9 [29]. Hypoxia altered the ECM fiber organization produced by PSCs. A gelatin-based 3D matrix culture enabled the recapitulation of cell-free 3D matrices produced by PSCs. Hypoxia altered ECM fiber organization as a parallel pattern of

fibronectin, which promoted the directional migration of pancreatic cancer cells [30]. Despite the cancer-promoting roles of PSCs, other types of cells, such as islet cells, are damaged by PSCs. A previous study showed that PSCs reduced insulin expression and induced β-cell apoptosis [31]. Diphenylene iodonium (DPI), an inhibitor of PSC activation, protected islet cells in WBN/Kob rats, an experimental model of chronic pancreatitis [31]. Hypoxia-activated PSCs increased β-cell death via elevated ROS production in PSCs [32]. Interestingly, hypoxia also affects the cancer-suppressing effects of PSCs. PSCs produce lumican, a small leucine-rich proteoglycan, which inhibited pancreatic cancer cell growth via EGFR reduction and reduction of Akt activity [33]. Expression of stromal lumican was correlated with reduced metastatic recurrence and longer survival [33]. Hypoxia repressed lumican production from PSCs through the increased autophagic flux supported by HIF-1α and activation of AMP-regulated protein kinase. Reduction of lumican production was reversed by autophagy inhibition [34]. The hypoxic microenvironment itself recruited macrophages by chemical chemokines 2 production, which activated PSCs [35]. Chemical chemokines 2 derived from hypoxic cancer cells recruited macrophages, and these macrophages increased αSMA expression in PSCs. Compared to cancer cells, hypoxia-regulated microRNAs are few in PSCs. Hypoxia increased miR-4465 and miR-616-3p in PSCs, leading to increased proliferation, migration, and invasion of pancreatic cancer cells via exosomal transmission [36]. These effects were mediated by PTEN reduction, which was a direct target of miR-4465 and miR-616-3p [36].

4. Effects of NRF2 Activation in Pancreatic Cancer Cells

NRF2 activation contributes to malignant phenotype of pancreatic cancer cells. The human pancreatic cancer cell line MIAPaCa-2 was exposed to low-dose gemcitabine for 6 months, and a gemcitabine-resistant cell line was established. This cell line showed increased intracellular ROS and NRF2 accumulation and elevated the expression of NRF2 target genes [37]. NRF2 knockdown by RNA interference sensitized pancreatic cancer cells to gemcitabine, suggesting that NRF2 activation is essential for acquiring resistance. Crosstalk between NRF2 and other cancer-promoting signals also contributes to the malignant phenotype. The inducer of EMT, transforming growth factor-β1 signaling, was attenuated by knockdown of NRF2 in pancreatic cancer cells [38]. PanIN lesions of surgically resected human pancreas tissue showed increased expression of nuclear NRF2 and decreased expression of E-cadherin, compared to normal pancreatic duct epithelium [38]. Accumulation of p62 also activated NRF2 in pancreatic cancer, leading to accelerated carcinogenesis. Pancreas-specific mutant *K-ras* expression and deletion of *IκB kinase α* promoted pancreatic cancer by increasing inflammation. The inflamed pancreatic parenchyma revealed p62 accumulation, and the deletion of p62 attenuated cancer progression [39]. The major oncogene *K-ras*, frequently mutated in pancreatic cancer, also activated Nrf2. Pancreas-specific expression of *K-ras*, together with other oncogenic *B-raf* mutations or *Myc* overexpression, resulted in the activation of Nrf2, which reduced intracellular ROS and increased cellular proliferation [40]. Introduction of *Nrf2-null* background into the KPC mouse, a pancreatic cancer model driven by pancreas-specific mutant *K-ras*/*p53* expression [41], delayed pancreatic cancer development via the attenuation of mRNA translation [42]. In this study, pancreatic organoids from KPC mice and *Nrf2-null* KPC mice were established, and *Nrf2-null* organoids showed vulnerability to AKT inhibition. Another study compared the development of precancerous lesions, pancreatic intraepithelial neoplasm (PanIN), and progression to invasive cancer between KPC mice and *Nrf2-null* KPC mice [43].

5. Oxidative Stress and PSC Activation

In addition to hypoxia, oxidative stress activates PSCs. Stimulation of isolated PSCs with inducers of oxidative stress, such hydrogen peroxide, activated multiple signaling pathways [44]. This treatment increased collagen production, thereby promoting fibrosis. The key components of the ROS-producing enzyme NADPH oxidase were expressed in PSCs. DPI treatment attenuated platelet-derived growth factor-BB, interleulin-1β, and angiotensin II-induced ROS production, leading to the inhibition of PSC activation [45]. A wide variety of stimuli increase oxidative stress in PSCs, leading to their activation. Oxidative stress-inducing treatments such as ethanol, acetaldehyde, and high glucose activated PSCs [46][47], which were blocked by N-acetylcysteine treatment, suggesting that ROS plays a central role in PSC activation. Another free radical scavenger, edaravone, decreased inflammatory cytokine production and PSC activation in a dibutylin dichloride-induced chronic pancreatitis rat model [48]. PSCs also affected the oxidative stress response of cancer cells. PSC-derived interleukin-6 and stromal-derived factor-1 α activated NRF2 in pancreatic cancer cells, leading to increased proliferation and ROS detoxification [49]. These lines of evidence suggested that oxidative stress responses in PSCs substantially contribute to cancer progression. In a previous study, a global *Nrf2* knockout was introduced into KPC mice [43]. The *Nrf2-null* KPC mouse also lacked *Nrf2* in PSCs. There were less stromal cells surrounding PanINs in *Nrf2-null* KPC mice, suggesting attenuation of the cancer-promoting effects of PSCs. Indeed, PSCs isolated from *Nrf2-null* mice showed less proliferation, migration, and activation by serum stimulation [50]. *Nrf2-null* PSC-derived conditioned medium did not increase cancer cell proliferation in vivo. Furthermore, co-injection of *Nrf2-null* PSCs with cancer cells into the dorsal flank of immunodeficient mice failed to increase subcutaneous tumor size, compared to wild-type PSCs.

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