Expert Review of Gastroenterology & Hepatology

ISSN: 1747-4124 (Print) 1747-4132 (Online) Journal homepage: http://www.tandfonline.com/loi/ierh20

The role of hypoxia in pancreatic cancer: a potential therapeutic target?

Mert Erkan, Metin Kurtoglu & Jorg Kleeff

To cite this article: Mert Erkan, Metin Kurtoglu & Jorg Kleeff (2016) The role of hypoxia in pancreatic cancer: a potential therapeutic target?, Expert Review of Gastroenterology & Hepatology, 10:3, 301-316, DOI: 10.1586/17474124.2016.1117386

To link to this article: http://dx.doi.org/10.1586/17474124.2016.1117386

Accepted author version posted online: 11 Nov 2015.
Published online: 27 Nov 2015.

EXPERT REVIEW OF GASTROENTEROLOGY & HEPATOLOGY, 2016
VOL. 10, NO. 3, 301–316
http://dx.doi.org/10.1586/17474124.2016.1117386

Taylor & Francis
Taylor & Francis Group

REVIEW

The role of hypoxia in pancreatic cancer: a potential therapeutic target?

Mert Erkan[a‡], Metin Kurtoglu[b‡] and Jorg Kleeff [c,d]

[a]Department of Surgery, Koç University School of Medicine, Istanbul, Turkey; [b]Department of Oncology, Koç University School of Medicine, Istanbul, Turkey; [c]Department of Surgery, The Royal Liverpool and Broadgreen University Hospitals, Liverpool, UK; [d]Department of General-, Visceral- and Pediatric Surgery, University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany

ABSTRACT

One of the key factors that correlates with poor survival of patients with pancreatic cancer is the extent of hypoxic areas within the tumor tissue. The adaptation of pancreatic cancer cells to limited oxygen delivery promotes the induction of an invasive and treatment-resistant phenotype, triggering metastases at an early stage of tumor development, which resist in most cases adjuvant therapies following tumor resection. In this article, the authors summarize the evidence demonstrating the significance of hypoxia in pancreatic cancer pathogenesis and discuss the possible hypoxia-induced mechanisms underlying its aggressive nature. We then conclude with promising strategies that target hypoxia-adapted pancreatic cancer cells.

ARTICLE HISTORY
Received 14 September 2015
Accepted 4 November 2015

KEYWORDS
Pancreatic cancer; hypoxia; desmoplasia; metabolism; TH-302; PEGPH20; epithelial-to-mesenchymal transition; cancer stem cell

Introduction

The treatment of several types of cancer has benefited from recent advances in understanding cancer biology; however, pancreatic cancer has lagged significantly behind other tumors, such as breast, colon, and stomach, in terms of improvement of survival. Based on the most recent Surveillance, Epidemiology and End-Results Program from National Cancer Institute in United States data regarding gastrointestinal cancers,[1] the overall 5-year survival rate of colorectal cancer is around 65%, stomach cancer around 30% while pancreatic cancer is only about 7%. Furthermore, the deaths from the former two diseases have been declining over the past 15 years while the same is not true for pancreatic cancer. The commonly fatal outcome of pancreatic cancer can be explained by two main reasons: the fraction of people diagnosed when the disease is localized is roughly 40%, 25%, and 10% for colon, stomach, and pancreatic cancers, respectively, and thus, surgery can potentially cure only less than 10% of patients diagnosed with pancreatic cancer. Second, even in patients with localized disease, the 5-year survival rate for pancreatic cancer patients is only around 20% although it is 90% and 65% for colon and stomach cancer, respectively. While there are significant tumor-associated morbidities, e.g. obstruction (gastric outlet and bile duct), biliary sepsis, venous thromboembolism, and tumor cachexia, contributing to mortality, epidemiological studies indicate that pancreatic cancer cells spread and develop micrometastases at an early stage and that these cells do not respond well to current adjuvant chemo- and/or radiotherapies. Therefore, it is obvious that pancreatic carcinogenesis fosters an aggressive phenotype at an early stage, which seems to be distinct from other gastrointestinal tumors.

The genomic changes that drive pancreatic carcinogenesis have been analyzed in depth and mutations of KRAS, TP53, SMAD4, and CDKN2A are found to be the most prevalent.[2,3] Recently, whole genome sequencing has deepened our understanding of the mutational landscape of pancreatic cancer.[3] Patterns of chromosomal structural variation can classify pancreatic ductal adenocarcinomas (PDAC) into four subtypes with potential clinical implications: Depending on the distribution of mutations and genomic alterations, the subtypes were termed stable, locally rearranged, scattered, and unstable. A significant proportion harbored focal amplifications, many of which contained druggable oncogenes (ERBB2, MET, FGFR1, CDK6, PIK3R3, and PIK3CA), but at low prevalence. While the role of these mutations in determining the prognosis of patients with pancreatic cancer is debated, the variety and the prevalence of the mutations in a cohort of patients who are long-term survivors (>10 years) were found to be similar to patients with short survival.[4] However, these results were based on only 35 patients and did not analyze the chronological order in which the mutations occurred, which had been shown to be an important

CONTACT Jorg Kleeff kleeff@gmx.de
‡These authors contributed equally to this work.

factor.[5] Nevertheless, it indicates that processes other than mutations are vital for the prognosis. A similar conclusion can be drawn from a highly innovative study performed on autopsies of seven patients with advanced pancreatic cancer where the clones that carried the mutations found only in individual metastatic foci were in close proximity to each other within the primary tumor.[6] In contrast, the cells representing the parental clone were homogenously scattered throughout the tumor suggesting that local environmental influences may have driven the metastatic trait of the founder cells. In this regard, a fundamental player directing the aggressive behavior of pancreatic cancer is the tumor microenvironment characterized by extensive desmoplasia and hypoxia. These two biological features nurture each other favoring the progression and treatment-resistance of pancreatic cancer. In a recent report, Moffitt *et al*. studied the gene expression patterns of more than 200 PDAC and normal tissue samples as well as several cell lines by using a virtual microdissection method and identified two tumor-specific and two stroma-specific expression signatures that were independent predictors of prognosis.[7] It will be interesting to analyze in the future whether there is an association between hypoxia-specific genes and these four subtypes. In the current review article, we will focus on the role of hypoxia and its interplay with desmoplasia in promoting the progression of pancreatic cancer and reveal targets that are being exploited for the treatment of this deadly disease.

Role of hypoxia in pancreatic cancer pathogenesis

Why is PDAC a hypoxic tumor?

Clinical investigations have made it obvious that hypoxia is a common feature of several solid tumors. While normal tissue generally receives an oxygen (O_2) pressure of 30–50 mmHg, it drops to below 2.5 mmHg in up to 50–60% of locally advanced solid tumors.[8] When O_2 levels were measured in patients with various solid tumors using pO_2 histography, pancreatic cancer was found to be the most hypoxic one [9] and intraoperative pO_2 measurements of seven resectable pancreatic cancers further supported the hypoxic microenvironment.[10] A very recent study using a 2-nitroimidazole-based compound, which stains selectively the severely hypoxic areas, demonstrated that only 10% of the tumor area (range 1–26%) was marked as severely hypoxic.[11] This result is somewhat surprising as larger areas of hypoxia were expected based on previous studies, suggesting that the sensitivity and specificity of detection methods for hypoxia are different. Nonetheless, when all available data is considered, it is reasonable to conclude that pancreatic cancer tissue has limited O_2 supply.

Several mechanisms contribute to the hypoxic milieu of PDAC. The major mechanism underlying reduced tumor oxygenation is the insufficient and aberrant vasculature that cannot deliver the necessary blood supply to all sites of the tumor tissue. There is ample evidence that angiogenesis induced by the growing tumor is leaky and ineffective,[12] resulting in areas where O_2 levels cannot sustain the physiological oxidative phosphorylation. In the case of pancreatic cancer, angiographic studies demonstrated specific tumor vessels only in a minority of tumor samples,[13] a finding later confirmed by color-flow sonography.[14] In contrast to many other solid tumors, PDAC cells not only produce angiogenic substances but also produce antiangiogenic factors like angiostatin, endostatin, and pigment epithelium–derived factor that actively suppress angiogenesis resulting in reduced O_2 delivery, which leads to the development of hypoxic regions.[15–18] In addition, stromal cells in PDAC might contribute to this hypoxia both by amplifying the production of antiangiogenic substances or physically by compressing the capillaries through extracellular matrix deposition in the periacinar spaces. Importantly, hypoxia is an important activator of pancreatic stellate cells (PSC), the major fibroblastic cells of the pancreas, perpetuating the vicious cycle of hypoxia and fibrosis (Figure 1). It is probable that the already fibrotic, hypovascular microenvironment of pancreatic cancer is one of the reasons for the failure of antiangiogenic therapies in pancreatic cancer in the clinical setting. For example, a double-blind, placebo-controlled, randomized phase III trial of gemcitabine and bevacizumab (a humanized monoclonal antibody that recognizes and blocks VEGF-A) versus gemcitabine and placebo in patients with advanced pancreatic cancer, could not show any benefit for the addition of this antiangiogenic agent.[19] Furthermore, recent compelling evidence shows that the relationship between angiogenesis (indirectly hypoxia) and fibrosis is quite complex: disruption of fibrosis promoted angiogenesis in one study [20] while it inhibited angiogenesis and aggravated tissue hypoxia in another one.[21] Therefore, fibrosis, hypoxia, and impaired angiogenesis play multifaceted and not always concerted roles in pancreatic carcinogenesis.

Clinical significance of hypoxia and hypoxia-inducible factor-1 in pancreatic cancer

Based on the observations summarized above, it is clear that pancreatic cancer cells are forced to evolve under

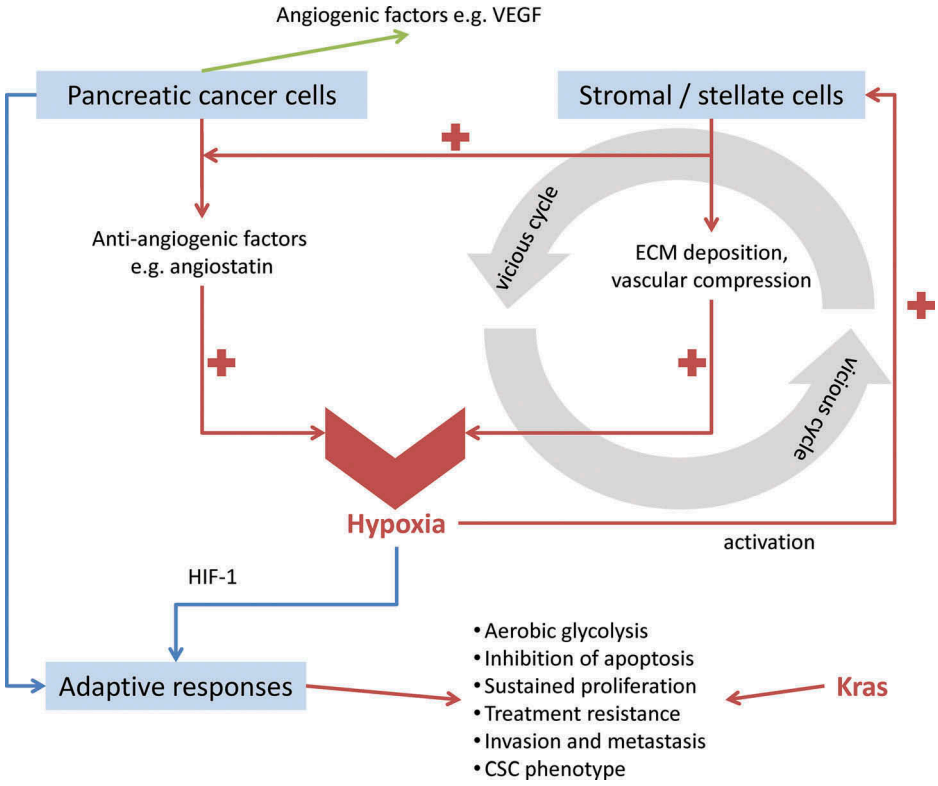

Figure 1. Hypoxia in pancreatic cancer tissue is generated via the intricate relationship between pancreatic cancer cells and the stromal/stellate cells. The crosstalk between pancreatic cancer and stromal cells initiates a cascade of events instigating the latter to deposit a dense extracellular matrix (ECM) that compromises capillary function and the former to secrete antiangiogenic factors limiting new vessel formation. The combination of these events diminishes the blood flow resulting in formation of hypoxic areas within the tumor tissue. The response to hypoxia, mainly mediated by hypoxia-inducible-factor-1 (HIF-1), further enhances the stromal/stellate cell activity creating a vicious cycle leading to severely hypoxic/anoxic areas throughout the tumor tissue. In this harsh environment, HIF-1 also acts as the master regulator to initiate adaptive responses of pancreatic cancer cells allowing them to survive by enhanced aerobic glycolysis, inhibition of apoptosis, and sustained proliferation while increasing the aggressiveness of the disease via treatment resistance, maintenance of cancer stem cell (CSC) features and invasion/metastasis.

very low O_2 levels implicating that adaptive responses to hypoxia have a prominent role in pancreatic cancer progression. Relatively novel descriptive studies have shown that in human PDAC, micro- and macronecrosis can be observed in the majority of cases and that the presence of necrosis is associated with unfavorable prognosis, implying a potential tumor-promoting role associated with the severely hypoxic/necrotic phenotype.[22]

When O_2 availability is reduced, cells respond to restore energy production, minimize the potential damage of aberrant oxidative phosphorylation, i.e. reactive oxygen species (ROS), and secrete angiogenic factors to increase O_2 delivery. This extensive re-calibration of metabolic and signaling pathways is orchestrated mainly by a transcription factor known as hypoxia-inducible factor-1 (HIF-1). When O_2 levels drop below a threshold, the prolyl residues of HIF-1α subunit cannot be hydroxylated, which prevents the binding of von Hippel–Lindau protein that normally recruits an E3-ubiquitin ligase for targeting it by proteasomal degradation.[23] The stabilized HIF-1α then dimerizes with HIF-1β, the constitutively expressed subunit, to bind to hypoxia-regulated genes.[24] Accordingly, HIF-1 is a widely used marker of hypoxia although it should be noted that the expression of this protein can also be controlled independent of O_2 levels, e.g. by α-ketoglutarate.[25]

Several studies have shown the significance of HIF-1 in predicting the poor outcome of pancreatic cancer patients. For example, HIF-1α mRNA expression was found to have a sensitivity of 87% and specificity of 56% in predicting short-term (<6 months) vs long-term (6–60 months) survival in a cohort patients whose paraffin-embedded tissues were microdissected for analysis.[26] Likewise, a meta-analysis of eight clinical studies demonstrated that expression of HIF-1α correlated with lymph node metastasis and advanced tumor stage culminating into poor survival.[27] In fact, HIF-1α may play a role even in early stages of pancreatic cancer

pathogenesis as the G1790A single-nucleotide polymorphism in the HIF-1α gene was found to be significantly more frequent in patients vs healthy volunteers.[28] In addition to HIF-1, HIF-2 has also cancer-promoting roles and these transcription factors act on overlapping and distinct targets.[29] In pancreatic cancer pathogenesis, HIF-2 has been implicated in the progression of pancreatic intraepithelial neoplasia (PanIN) to PDAC by fine-tuning the levels of β-catenin and SMAD4; however, the exact mechanism of this relationship has to be elucidated.[30] Taken together, there is a strong clinical correlation between HIF-1 expression and pancreatic cancer aggressiveness, which in fact is not surprising given the ability of HIF-1 to induce several cellular pathways that reprogram metabolism and thereby sustain proliferation under unfavorable conditions, inhibit apoptosis resulting in treatment resistance, switch on invasiveness leading to metastasis, and mediate the crosstalk with tumor stroma (Figure 1).

The role of metabolic reprogramming induced by hypoxia in pancreatic cancer pathogenesis

It appears that HIF-1 has been present since early stages of evolution including simple metazoan species to modulate the switch from oxidative phosphorylation to glycolysis depending on the availability of O_2. Several glycolytic genes are upregulated by HIF-1 and therefore conventionally it has been assumed that HIF-1 is essential for cell viability under hypoxia since it can maintain ATP production when O_2 is limited.[25] However, ATP levels were not reduced in mouse embryonic fibroblasts in which the HIF-1 gene was knocked-out challenging this hypothesis.[31] In fact, HIF-1 was essential for survival under prolonged hypoxia, not because it can sustain ATP levels, but mainly due to restraining ROS production, which occurs due to aberrant oxidative phosphorylation when O_2 is limited.[31,32] Thus, under hypoxia, glucose is actively shunted away from mitochondria to limit the potential for ROS production from the oxidation of pyruvate produced from glucose catabolism. HIF-1 also shifts the source of acetyl-coenzyme A used for the synthesis of fatty acids from glucose-derived pyruvate to glutamine-derived α-ketogluterate,[33,34] which further contributes to shunting of glucose away from mitochondria into lactate synthesis. By favoring glycolysis over oxidative phosphorylation, malignant cells can also spare their pyruvate to build the carbon skeletons necessary for the new nucleic acid and membrane synthesis required for cellular growth.[25,35]

With regard to the metabolic reprogramming of pancreatic cancer cells under hypoxia, HIF-1 expression was found to upregulate the expression of several glycolytic enzymes consistent with previous reports.[36] Upregulated glycolysis appears to protect the cells from apoptosis induced by glucose deprivation that can occur in areas of hypoxia where the perfusion by aberrant vasculature is insufficient.[37] In line with these reports, increased levels of key glycolytic enzymes were also observed in hypoxic portions of a transgenic pancreatic cancer model, although the role of HIF-1 was not investigated in this study.[38] In the same study, a comprehensive metabolic analysis revealed a symbiotic relationship between normoxic and hypoxic PDAC cells through the exchange of lactate consumed by the former and produced by the latter fractions of the tumor. This symbiosis, which was also shown in colon cancer cells,[39] uncovers the capability of adaptation to hypoxia in PDAC for sustaining the growth of not only the cells growing under low O_2 tension but also the cells with sufficient oxygenation.

Hypoxia is also shown to induce the expression of glutaminase 2, which converts glutamine to glutamate, in PDAC cells.[38] A novel hypothesis was offered to explain how high intracellular glutamine levels support the viability of hypoxic PDAC cells: Glutamine and glucose are both used in the hexosamine biosynthetic pathway, of which its final product, uridine-diphosphate-N-acetyl-glucosamine (UDP-GlcNAC), regulates the O-GlcNacylation of several proteins that are important for survival under hypoxia. Although it was not demonstrated which proteins are involved in cell viability, an O-GlcNacylation inhibitor, azaserine, was toxic to PDAC cells grown under hypoxic conditions.[38] Another reason of high glutamine uptake in hypoxic PDAC cells may be to maintain fatty acid synthesis as shown for other tumor types [33,34,40] although this phenomenon has not yet been demonstrated specifically in pancreatic cancer cells. However, a clue for increased amino-acid catabolism mediated by HIF-1 in pancreatic cancer comes from a study in which knockdown of HIF-1 inhibited the body weight loss induced by implantation of MiaPaCa-2 cells in an orthotopic xenograft mouse model.[41] Taken together, induction of both glucose and glutamine metabolism by hypoxia seems to mediate the adaptation of pancreatic tumor cells to unfavorable environmental conditions.

Surprisingly, to date there are no reports investigating the role of HIF-1 expression in regulating the redox homeostasis of PDAC cells. Previous studies have shown that HIF-1 shifts glucose away from mitochondria by inhibiting pyruvate dehydrogenase [32] while it optimizes the cytochrome oxidase subunits to

reduce electron leakiness, and thereby ROS production from mitochondria.[42] Furthermore, HIF-1 induces mitochondrial autophagy by increasing the expression of Bcl2/adenovirus-E1B-19-kDa-interacting protein (BNIP3) in order to remove the mitochondria damaged by excess ROS.[31] Interestingly, BNIP3 was reported to be downregulated in all pancreatic carcinoma samples via hypermethylation of its promoter, which supported the survival of pancreatic cancer cells under hypoxia.[43] Furthermore, another study demonstrated that BNIP3 was present in precursors of PDAC, PanIN grade 2 and 3, but was silenced in 59% of the carcinoma cases suggesting that silencing of BNIP3 is a late event in pancreatic cancer pathogenesis.[44] Finally, upregulation of this protein by 5-aza-2′-deoxycytidine sensitizes pancreatic cancer cells to hypoxic conditions.[45] Thus, a mechanism that contributes to protection of cells from excess oxidative damage under hypoxia also renders them sensitive to the same environmental condition, indicating that PDAC cells pay the price of adapting to survival in low O_2 tension by elevated intracellular ROS (Figure 2). Given that low ROS is associated with resistance to chemotherapy,[46] which is a common feature of pancreatic cancer, it is likely that pancreatic cancer cells may have a highly upregulated antioxidant system underlying their resistance to both hypoxia and chemotherapy simultaneously. To this end, oncogenic Kras mutations (e.g. Kras G12D), which are present in more than 90% of PDAC, have been proven to foster low intracellular ROS levels. Here, oncogenic Kras activates expression of a series of antioxidant genes via nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and also mediates an unusual metabolic pathway of glutamine to generate NADPH. This can then be used as the reducing power for ROS detoxification, leading collectively to low ROS levels in pancreatic preneoplastic cells and in cancer cells. In adult stem cells and cancer stem cells (CSCs), low ROS levels have been associated with the formation of a proliferation-permissive intracellular environment and with perseverance of self-renewal capacities. Therefore, it is conceivable that low intracellular ROS levels may contribute significantly to oncogenic Kras-mediated PDAC formation.[47] On the

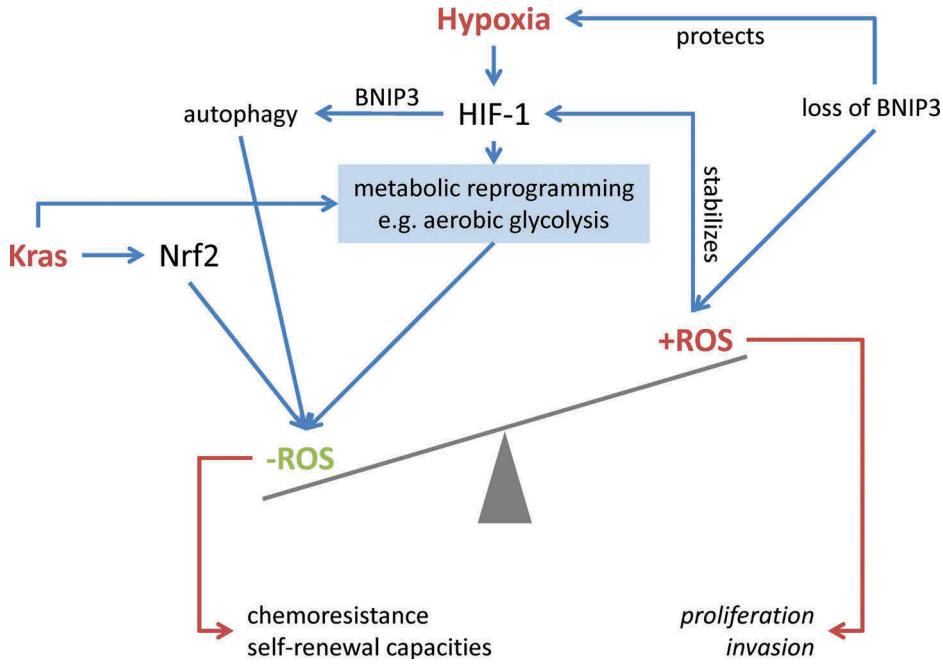

Figure 2. There is a complex relationship between hypoxia-inducible-factor-1 (HIF-1), reactive oxygen species (ROS), and Kras. When O_2 is limited, the electrons propagating through electron transport chain complexes have a tendency to leak increasing the formation of ROS. ROS, in turn, stabilizes HIF-1, which reprograms the metabolism of the cell and increases mitochondrial autophagy to limit oxidative damage. However, Bcl2/adenovirus-E1B-19-kDa-interacting protein (BNIP3), which governs mitochondrial autophagy and is a proapoptotic protein, is silenced in pancreatic cancer cells thereby preventing apoptosis in the hypoxic environment. Therefore, ROS production should be increased in these cells. However, there is a controversy whether ROS is beneficial or harmful for pancreatic cancer cells: While increased ROS sustains proliferation and invasion, it also reduces the threshold for treatment-induced cell death and prevents a cancer stem cell (CSC) phenotype. In addition, mutated Kras upregulates antioxidant pathways via nuclear factor (erythroid-derived 2)-like 2 (Nrf2), suggesting that ROS levels are fine-tuned in pancreatic cancer cells by the level of hypoxia to balance between its beneficial and harmful effects facilitating the adaptive process to fluctuating environmental conditions.

other hand, some PDAC cells are reported to produce high levels of ROS, in particular superoxide that in fact contributes to proliferation and invasiveness of these cells [48–50] and therefore the complex relationship between hypoxia, ROS, and treatment resistance in pancreatic cancer requires further research (Figure 2).

It is clear that the harsh environment created by low perfusion forces PDAC cells to reprogram their metabolism in order to survive and sustain proliferation. In general, the core of the metabolic changes appears to further exacerbate aerobic glycolysis activated by mutated oncogenes, e.g. Kras. Oncogenic Kras is known to regulate pancreatic cancer metabolism by increasing glucose uptake and channeling it into multiple anabolic pathways.[51] Furthermore, Kras-driven cells are shown to also depend on glutamine metabolism [52] resonating the metabolic changes induced by hypoxia as outlined below. Thus, the combination of Kras mutations and the hypoxic environment renders pancreatic cancer cells less dependent on glucose-mediated oxidative phosphorylation giving them the flexibility to metabolize various nutrients and thereby survive and grow in a wide spectrum of environmental conditions (Figures 1 and 2). While these metabolic changes contribute to aggressiveness of the PDAC, it also opens the gap between normal and pancreatic cancer cell metabolism which then may create a window of selectivity for targeting PDAC without causing significant toxicity.

The role of hypoxia for inhibiting apoptosis and causing chemoresistance

It is usually assumed that a major reason for why PDAC is notoriously resistant to chemotherapy is that the low perfusion due to aberrant vasculature combined with the highly desmoplastic stroma limits the amount of drug that reach the cancer cells. However, it was shown by using ^{14}C-labeled gemcitabine that there was a minimal reduction in drug levels in the hypoxic areas as compared to well-perfused areas of the tumor and that only a 4% decrease was measured even at tumor sites more than 100 μM away from the blood vessels.[53] However, there is also evidence for the inverse correlation between the extent of stroma and the concentration of a chemotherapeutic agent, i.e. gemcitabine.[54] These contradictory results can be explained by the hypothesis that chemoresistance in at least the hypoxic portions of PDAC is not simply due to poor drug delivery, but mainly a consequence of intrinsic mechanisms associated with hypoxia-inducible pathways and/or oncogenic mutations that lead to the evolution of resistant cancer clones.

Hypoxia has been associated with resistance to chemotherapy and radiotherapy for several decades with the simple notion that it slows down the cell cycle and thereby limits the number of cells that these treatment modalities can target, as their mechanism of toxicity depends on DNA synthesis and proliferation. However, more than a decade ago as the details of hypoxia-inducible pathways started to be delineated, hypoxia was found to cause chemo and/or radioresistance independent of cell cycle arrest.[55] At low O_2 levels, resistance to gemcitabine is induced via activation of phosphoinositide 3-kinase/protein-kinase B (PI3K/Akt) and nuclear-factor-kappa-light-chain enhancer of activated B-cells (NF-κB) signaling pathways [56] both of which strongly upregulate various antiapoptotic mechanisms (reviewed in [57,58]). Moreover, both of these pathways interact with HIF-1, which in turn can induce proapoptotic proteins like BNIP3,[59] Noxa [60] or NIX [61] while it can also downregulate other proapoptotic proteins like Bid and Bax [62] or upregulate antiapoptotic proteins like Bcl-X_L [63] revealing the dichotomy in its ability to modulate apoptosis. In the case of PDAC, the hypoxia-inducible proapoptotic protein BNIP3 is silenced both via epigenetic changes [43] as well as through clonal elimination of BNIP3-positive cells,[44] which supports the antiapoptotic role of HIF-1 since proapoptotic pathways such as BNIP3 are most likely downregulated. Thus, it would be interesting to investigate epigenetic changes in multiple proapoptotic genes in PDAC cells to further clarify the role of HIF-1 in apoptosis.

Several other studies have shown a negative correlation between HIF-1 expression and gemcitabine sensitivity in PDAC cells [64,65] while one study showed a direct relationship between hypoxia and multidrug resistance such that knock-down of HIF-1α downregulated the expression of multidrug resistance 1(MDR1) gene under hypoxia-mimicking conditions.[66] MDR1 expresses the P-glycoprotein, which can actively transport a number of chemotherapy drugs against their gradient reducing their intracellular concentration and thereby toxic activity.[67] Overall, there is strong evidence supporting the role of hypoxia and HIF-1 in treatment resistance of PDAC cells, but the mechanisms underlying this phenomenon appear to be complex and sometimes even contradictory. Thus, with the current data, the interaction between chemo/radiotherapy and hypoxia or HIF-1 is not completely understood. Moreover, a recent publication showing that gemcitabine-induced oxidative stress in fact stabilizes HIF-1α via NF-κB pathway further adds to the challenge of understanding the complex interaction between therapy-resistance and hypoxia-inducible pathways.[68]

Hypoxia induces epithelial-to-mesenchymal transition and CSC-like properties resulting in invasiveness and metastasis of pancreatic cancer cells

One of the major reasons for the poor prognosis of pancreatic cancer is that it is a systemic disease at an early stage and therefore surgical resection does not cure the majority of patients. Hypoxia is presumed to play a major role in causing the metastatic phenotype of PDAC since the expression of HIF-1α has been correlated with poor survival in several studies.[26–28] Metastasis is a multistep process starting with invasion followed by intravasation, survival in blood, extravasation, colonization, and finally growth of micrometastatic foci to macrometastatic disease.[69] The initial step of metastasis is the acquisition of a metastatic cell phenotype that can initiate the migration, a process known as endothelial-to-mesenchymal transition (EMT).[70] Of note, there is another type of migration called 'amoeboid leukocyte-like';[71] however, the extent of this phenotype in cancer metastasis is less studied, especially in the context of pancreatic cancer, and therefore will not be discussed in this review. Nevertheless, EMT means that cancer cells acquire mesenchymal features such as dissolution of adhesion to the extracellular matrix and loss of cell polarity, all culminating into becoming an invasive cell.[70] Moreover, EMT sustains the survival of a cancer cell in blood and metastatic sites by endowing it with stem-cell like features and inhibiting apoptosis.[72,73] Furthermore, cells that underwent EMT can also migrate collectively at the forefront of the cancer invasion creating tracks that other cancer cells can exploit.[74] Another movement type initiated by EMT is characterized by a nest of tumor cells diffusely infiltrating into a tissue, known as 'tumor budding,' which has been shown to be a negative prognostic indicator in several types of gastrointestinal cancers,[75] including pancreatic cancer.[76,77] Thus, the mesenchymal phenotype induced by hypoxia is a key step for the metastatic cascade. In addition to EMT, hypoxia-induced signaling has been shown to play a role in most, if not all, of the steps involving metastasis [78] and therefore it is not surprising to find robust evidence that pancreatic cancer, with its severely compromised oxygenation is a highly metastatic tumor type.

One of the first experimental evidence to show that tumor hypoxia correlates with the metastatic potential of pancreatic cancer was published in a study using an orthotopic xenograft of two pancreatic cancer cell lines whereby tumor hypoxia correlated well with the metastatic burden.[79] In a different model, in which orthotopic primary xenografts were used, there was a strong association between hypoxia and metastasis formation.[80] Interestingly, in this study, no correlation between hypoxia and EMT was observed although EMT has been associated with invasiveness and metastasis of almost all types of tumors,[72,73] including pancreatic cancer.[81] Furthermore, there are several reports demonstrating induction of EMT by hypoxia in PDAC cells.[82–85] One reason for these contradictory results could be the method of assessing hypoxia, which was different in these studies: In one study 2-nitroimidazole was used,[80] while HIF-1α expression was utilized in others.[64,82–85] 2-nitroimidazole becomes detectable in tissues with pO_2 less than 10 mmHg, while HIF-1α is stabilized in a wide range of oxygen levels and more importantly can even become activated by oxygen-independent mechanisms.[25] Thus, the level of hypoxia that can induce EMT requires further investigation although there is no doubt that HIF-1α plays a major role in the initiation of metastasis in pancreatic cancer.

While the relationship between HIF-1α expression and EMT in pancreatic cancer is clear, a recent article demonstrated that not every pancreatic cancer cell type acquires the same migratory potential when exposed to hypoxia.[85] Cell types that had a high CSC-like phenotype based on colony-forming capacity, aldehyde dehydrogenase activity, and tumorigenicity in mice had a higher migratory capacity under hypoxia as compared to cells with low CSC features.[85] On the other hand, CSC^{high} cells already have an upregulated migratory potential even under normoxia since they express EMT markers, low E-cadherin, and high Vimentin, which gets further augmented by hypoxia. In contrast, CSC^{low} cells display an epithelial histology, which can also get transformed into a mesenchymal type by exposure to hypoxia, albeit more slowly and less robust than CSC^{high} cells. This difference can become significant in a clinical situation since CSC^{high} cells can increase their invasiveness even in intermittent hypoxia, which occurs frequently in solid tumors, whereas CSC^{low} cells require a more prolonged time in low O_2 levels.[86] Moreover, intermittent [84,87] and sustained [88] hypoxia has been shown to induce CSC markers in pancreatic cancer cells further supporting the intricate relationship between CSC, EMT, and the oxygen levels in the tumor microenvironment.

Several other pathways have been implicated in the mechanism of hypoxia-induced invasiveness of pancreatic cancer cells. These include NF-κB,[83] membrane-type-2 matrix metalloproteinase,[89,90] quiescin-sulfhydryl-oxidase-1 (QSOX1),[91] chemokine (C-X3-C motif) receptor-1 (CX3CR1),[92] and lysyl-oxidase (LOX).[93] In

addition, the Hedgehog (Hh) signaling pathway, which has been shown to induce PanIN in addition to enhancing the tumorigenicity of PDAC [94] and which is one of the 'core' signaling pathways of pancreatic cancer,[2] can be induced by hypoxia via a ligand-independent mechanism.[95] Up-regulation of this pathway leads to EMT via direct up-regulation of the Hh pathway transcription factor Gli1, even when Hh ligands are down-regulated.[82] Finally, in a murine model of PDAC driven by oncogenic KRAS/Mek-mTOR signaling, massive tumor necrosis associated with heightened hypoxia in the primary tumor also correlated with enhanced metastatic spread.[96] One of the links between the hypoxic/necrotic cells and metastasis was found to be higher level of endoplasmic reticulum stress induction by hypoxia in these cells as compared to cell types that are non-necrotic and metastatic.[97] While an impairment in hypoxia-induced VEGF-A secretion was offered as a possible mechanism for the necrotic phenotype, a strong correlation between hypoxia-induced endoplasmic reticulum stress and the same phenotype points to a more complicated mechanism underlying the relationship between hypoxia, necrosis and metastasis.[97] In conclusion, the integration of hypoxia-inducible and several oncogenic pathways triggers and augments the invasiveness and CSC-features of pancreatic cancer cells, facilitating the metastatic potential of pancreatic cancer and current data suggests that the cross-talk between these cellular processes may be context and tumor dependent.

Hypoxia modulates the stroma of pancreatic cancer

The abundant extracellular matrix (ECM) in PDAC is produced by activated PSCs, which have a significant role in the progression of PDAC such that there is a survival difference between patients that have PSC-rich vs PSC-poor stroma.[98] This is further supported by preclinical evidence demonstrating that co-injection of PSCs and PDAC cells increases the tumor growth, metastatic foci and regional invasion.[99–101] While several pathways are offered to explain how PSCs render PDAC more aggressive, an obvious mechanism is based on the distortion of blood vessels by the fibrotic stroma resulting in decreased blood and lymphatic flow and thereby development of hypoxic regions.[102,103] In addition, PSCs have been shown to increase endostatin production of PDAC cells further resulting in diminished blood flow.[17] Although previous reports demonstrate that when cultured under hypoxia, PSCs secrete proangiogenic factors,[104] this effect is inhibited by the endostatin produced by PDAC via cleavage of collagen XVIII by matrix metalloproteinase-12.[17] It was also shown that the low O_2 levels increases the activity of PSCs creating a positive feedback loop for the propagation of the hypoxic areas in the tumor. Furthermore, hypoxic PSCs secrete connective tissue growth factor that enhances the invasiveness of PDAC cells. Finally, activation of HIF-1α induces the secretion of Sonic Hedgehog (sHH), which then stimulates fibrous tissue deposition via Hh-signaling pathway.[105] Taken together, there is a highly dynamic relationship between PSC-induced fibrotic stroma and hypoxia, amplifying each other advancing the progression of PDAC (Figure 1).

Targeting hypoxia in pancreatic cancer

Strategies to increase tumor perfusion by antifibrotic therapies

PDAC is likely the most desmoplastic epithelial tumor. It is also the most hypoxic tumor with partial tissue oxygen pressure of less than 3 mmHg.[8,9,106] Due to the strong association of hypoxia with fibrosis and presumed tumor supportive role of PSCs, antifibrotic therapy was seen as a new hope against ineffective drug delivery in PDAC. To improve tumor perfusion and hence better drug delivery, different approaches have been undertaken. Inhibition of sHH signaling to deplete the stroma initially proved to be effective in a genetic mouse model of PDAC and paved the way for a clinical trial.[54,61] This trial, however, was stopped prematurely in 2012 due to increased mortality in the therapy arm.[107] Further research revealed that the optimistic results of the first study was an artifact of a suboptimal experimental setup and chronic depletion of sHH or knock-out of this pathway increased the aggressiveness of the tumor in the same genetic mouse model of PDAC.[20]

Another attempt to deplete the stroma by using an agent to enzymatically degrade a major extracellular matrix component (Hyaluronan) seems to be more effective. In line with the experimental data prolonging survival of PDAC-bearing mouse,[108,109] a phase II clinical trial showed promising results as addition of pegylated-human-recombinant hyaluronidase (PEGPH20) to nab-paclitaxel/gemcitabine doubled the progression-free survival in a group of patients who had tumors with high hyaluronic-acid content.[110] These conflicting results show that the multifaceted role of stroma in PDAC should not be simplified in categories like tumor-supportive or tumor-restrictive. It requires a better understanding of the stromal function and more effective therapies for different cells (i.e. CSCs) that are

likely kept at bay in the stroma.[111] Interestingly, the so-called 'normal' stromal gene expression pattern of the identified four gene-expression patterns in PDAC was associated with tumor-restraining features while the 'activated' stroma included genes that promoted tumor growth, shedding some light on the contradicting roles of PDAC stroma. Thus, antistromal therapy should better be tailored to the individual patient.

Strategies to inhibit HIF-1 pathway

The reports summarized above provide a clear rationale for targeting hypoxia-inducible pathways in pancreatic cancer. Since the master regulator of these pathways is HIF-1, agents that can inhibit its function should demonstrate clinical activity in PDAC. An antisense oligodeoxynucleotide that specifically targets HIF-1α (EZN-2968) was tested in a pilot clinical trial, which was unfortunately closed prematurely when the sponsor of the trial halted further development of this agent. [112] 10 patients were treated and grade 3 or 4 toxicities were negligible while two out of six patients biopsied had significant decreases in HIF-1α protein levels although none of the patients had a decrease in the HIF-1-target genes lessening the interest in further development of this agent. Another compound derived from melphalan, PX-478, which inhibits HIF-1α expression in pancreatic cancer leading to reduced tumor growth preclinical,[113] demonstrated limited clinical activity in a phase I study (published in abstract form, [114]) and currently this agent is not being tested in another trial. An interesting compound that may be efficacious in pancreatic cancer is the well-known cardiac glycoside, digoxin, which is shown to inhibit HIF-1α synthesis effectively at a relatively low concentration, but there is no registered clinical trial testing this hypothesis.[115] An agent that has been shown to decrease HIF-1 as well as the CSC features of pancreatic cancer is a plant-derived agent, triptolide,[116,117] and its derivative is now being tested in advanced gastrointestinal cancers including pancreatic cancer (NCT01927965).

Besides targeting directly HIF-1, another strategy is to eliminate the signals that upregulate HIF-1α expression. Oncogenic KRAS activates robust Mek/Erk signals that phosphorylate the tuberous sclerosis complex and activates the mammalian target of rapamycin (mTOR) pathway in PDAC. In turn, mTOR mediates the translation of HIF-1α [118] and therefore mTOR inhibitors may have a role in pancreatic cancer. However, despite its success in pancreatic neuroendocrine tumors,[119] single-agent everolimus had minimal clinical activity in gemcitabine-refractory pancreatic cancer.[120] Two other small studies confirmed this disappointing result.[121] However, recently Kong *et al*. have shown that while single inhibition of mTOR was ineffective, dual inhibition of Mek (downstream of mTOR) and PI3K effectively induced cancer cell apoptosis.[96] Furthermore, in a separate study in which everolimus was combined with capecatibine, moderate clinical activity was observed,[122] indicating that combination of mTOR inhibition with another targeted therapy or cytotoxic agent that is active in pancreatic cancer may show clinical benefit.

As mentioned above, one of the major events that protect the cells from damage under hypoxia is the limitation of ROS production by HIF-1-mediated metabolic reprogramming. Thus, it is not surprising that elevated ROS, in addition to low O_2 levels, can also stabilize HIF-1α as a protective mechanism from excess oxidative damage. Based on this contention, it has been reported that attenuation of ROS by either antioxidants [123] or extracellular superoxide dismutase [124] suppresses HIF-1α activity leading to reduced tumor growth or hepatic metastasis. However, currently there is no clinical application in pancreatic cancer based on this intriguing preclinical data.

Evidently, despite the data revealing the significant role of HIF-1 activity in PDAC, compounds that can inhibit its activity or expression in patients is still eagerly awaited. Nonetheless, there is a strong possibility that the selective inhibition of this ubiquitously expressed transcription factor that has multiple roles in human physiology is toxic. Furthermore, there is limited but sufficient evidence implicating alternative hypoxia-inducible pathways modulating the progression of pancreatic cancer. As mentioned above, a mechanism by which HIF-2α promotes the progression of PanIN lesions to PDAC via upregulation of β-catenin and SMAD4 has been reported.[30] Therefore, methods that can exploit the decreased oxygen availability in PDAC cells as a whole, rather than targeting individual pathways, should demonstrate greater clinical efficacy.

Strategies to exploit the metabolic reprogramming in PDAC due to hypoxia

The metabolic alterations induced by hypoxia further enhance the glycolytic pathway, which is already turned on by the oncogenic transformation regardless of the oxygen level. While in the presence of O_2, inhibition of this pathway does not appear to produce sufficient energy depletion that can kill the cell, in an environment that limits O_2 availability glycolytic inhibitors are shown to produce significant toxicity.[125] In

preclinical studies, 2-deoxy-D-glucose (2-DG) demonstrated promising results in killing hypoxic portions of several types of tumors [126] although there was limited activity in a phase I clinical trial, which combined it with docetaxel in refractory solid tumors.[127] However, pancreatic cancer was not present in this cohort of patients and therefore further studies are required to assess the clinical potential of 2-DG in PDAC. Furthermore, a close analog of 2-DG, 2-fluoro-deoxy-D-glucose was shown to be an even more potent glycolytic inhibitor,[128] which may also be efficacious in pancreatic cancer treatment. 3-bromopyruvate is another compound which can target hypoxic tumor cells via selective uptake through monocarboxylate-transporter-1,[129] but has been halted in its development due to unexpected toxicity via its alkylating properties.[130] However, it has been reengineered by microencapsulating in a complex with β-cyclodextrin and has demonstrated a strong antitumor effect with no discernible toxicity in an orthotopic pancreatic cancer model.[131] Collectively, these articles provide a rationale for further developing glycolytic inhibitors for clinical use in PDAC patients either alone or most likely with cytotoxic therapy as they can target the treatment-resistant portions of the tumor while chemo- or radiotherapy can eliminate the well-oxygenated areas.

Direct targeting of hypoxic PDAC cells via compounds that get activated under low O_2 tension

When O_2 is limited, certain chemical moieties have the potential to be metabolized by enzymatic reduction. Based on this concept, several compounds have been synthesized, which undergo reductive reaction to produce cytotoxic agents (reviewed in [132]). Among these compounds, TH-302 has been tested in pancreatic cancer extensively and there is already clinical evidence for its activity. It is a 2-nitroimidazole-based prodrug and after undergoing redox cycling, it produces ifosfamide, which is a well-established DNA cross-linking toxin.[133] In a randomized phase II trial, combination of TH-302 with gemcitabine significantly prolonged progression-free survival as compared to gemcitabine alone (5.6 vs 3.6 months) without any toxicity resulting in treatment discontinuation in patients with advanced pancreatic cancer.[134] A phase III trial is currently being conducted (NCT01746979) to explore the effect of TH-302 on overall survival. Furthermore, it is being tested also as a single agent (NCT01833546) and in combination with nab-paclitaxel (NCT02047500). Thus, TH-302 appears to be a promising agent to selectively target the hypoxic areas of PDAC and paves the way for developing other hypoxia-activated prodrugs, i.e. agents that can induce toxicity via mechanisms other than DNA damage. It is important to note that this drug should continue to be used in combination with other chemotherapeutic agents as circulating cancer cells or newly developing hematogenous metastasis may – for a period – have a higher oxygen concentration in their vicinity preventing conversion of TH-302 to its active form by hypoxia.

Conclusions

There is no doubt that oncogenic mutations in pancreatic cancer are one of the major obstacles in successful treatment since the ones that occur at the earliest stages of cancer development (PanIN) like KRAS and p53 are associated with aggressiveness and therapy resistance. The contribution of the microenvironment to the fatal outcome of PDAC is also substantial. One of the tumor-promoting factors in the PDAC microenvironment is hypoxia, which arms the cancer cells with several mechanisms to overcome intrinsic and extrinsic damage generated by rapid tumor growth in unfavorable conditions. Furthermore, hypoxia promotes the invasiveness and metastatic potential of PDAC via inducing EMT and CSC pathways, which all culminate into the aggressive phenotype of this disease. Finally, while the desmoplastic stroma appears to be one of the major reasons for the generation of hypoxic areas in PDAC, hypoxia in turn can induce further fibrosis creating an amplifying loop between these two central components of pancreatic cancer pathogenesis. These diverse pathways are governed by various hypoxia-inducible pathways although HIF-1 has been studied most. It is important to note that HIF-1 can also be induced by oxygen-independent mechanisms and therefore studies that correlate HIF-1 expression with hypoxia needs to be interpreted with caution. Nevertheless, our better understanding of how hypoxia is generated in PDAC and strategies to target them should translate into significant clinical improvements in the future.

Expert commentary

Pancreatic cancer has one of the worst prognoses of all human malignancies. One of the key features responsible for its aggressive phenotype is the severely hypoxic/anoxic microenvironment covering a significant portion of the tumor. Adaptation to hypoxia renders pancreatic cancer resistant to conventional chemo- and/or radiotherapy and promotes its early metastatic phenotype.

Thus, targeting the hypoxic microenvironment of pancreatic cancer should significantly impact on clinical outcome. However, this is not a straightforward task as the complex relationship between the cancer cells, stromal components, hypoxia, and adaptive responses to limited oxygen availability results in unpredictable outcomes when one of these elements is targeted. Thus, intelligently designed drugs that are active under hypoxic conditions and/or drugs that target the stroma of pancreatic cancer appear to be promising in the future.

Five-year view

No significant progress has been achieved in the systemic treatment of pancreatic cancer since FOLFIRINOX and nab-Paclitaxel/Gemcitabine have been established as first-line palliative therapy. Therefore, the results of several ongoing trials targeting the tumor microenvironment and/or hypoxia, such as the phase II trial testing the hypoxia-activated agent, TH302 in combination with gemcitabine, are eagerly awaited. A positive result of the latter will accelerate the development of other hypoxia-activated prodrugs that are in the pipeline. It is clear, however, that targeting the severely hypoxic areas of pancreatic cancer is not the final answer for improving the dismal outcome of this disease. Further, hypoxia-activated drugs do not circumvent all the obstacles for killing cancer cells in hypoxic areas of the tumor such as the delivery of the agent to an area with diminished blood flow (although this assumption is challenged by some evidence) or drug-resistant mechanisms induced by hypoxia-inducible pathways. Once these pathways supporting the treatment-resistant and metastatic phenotype of pancreatic cancer are induced by hypoxia, they may be retained independent of the oxygen levels creating a natural resistance to hypoxia-targeting agents. Thus, it is important to characterize the complex mechanisms underlying the formation of hypoxic areas for avoiding the induction of hypoxia-inducible pathways as well as for enhanced drug delivery. To achieve this task

Key issues

- Pancreatic cancer is significantly more hypoxic when compared to other solid tumors. However, while there are several methods to measure the extent of hypoxia in pancreatic cancer tissues *in vivo*, their sensitivity and specificity differ significantly compromising the assessment of the amount and localization of hypoxic areas.
- When oxygen is limited, hypoxia-inducible pathways become activated, which in turn initiate a cascade of events that reinforces the aggressiveness of pancreatic cancer.
- Among hypoxia-activated pathways, the most studied is hypoxia-inducible factor-1 (HIF-1), which governs metabolism, apoptosis, and invasion-related mechanisms. Thus, the outcome of high HIF-1 is poor survival of pancreatic cancer patients; however, it should be noted that its expression can be up-regulated also by oxygen-independent pathways.
- Adaptation of hypoxia forces pancreatic cancer cells to acquire metabolic alterations that allow them to utilize various types of nutrients. On the other hand, these metabolic alterations may also be vulnerable for targeting, and agents for this purpose are under development.
- A key feature of hypoxia adaptation is epithelial-to-mesenchymal transition, which is one of the initiating factors of the metastatic cascade. Several signaling pathways have been implicated in epithelial-to-mesenchymal transition; however, the exact mechanisms remain elusive.
- While HIF-1 is responsible for several of the hypoxia-related features of pancreatic cancer that underlie its fatal clinical course, currently there is no promising clinical agent that can effectively target this transcription factor.
- A unique feature of pancreatic cancer histology is the extensive desmoplasia that surrounds the tumor tissue. There is a highly complex relationship between fibrosis and hypoxia amplifying each other and thereby reinforcing the metastatic and treatment-resistant phenotype of pancreatic cancer.
- There are contradictory reports regarding the role of components of oncogenic pathways, extracellular matrix, and the tumor microenvironment in pancreatic cancer progression and therefore clinical application of any promising target requires extensive preclinical investigation to avoid unexpected outcomes. The failure of the Hedgehog signaling inhibitor trial is a good example regarding this issue.
- There are several strategies to target the hypoxic portions of pancreatic cancer that are under preclinical and clinical development.

targeting the tumor stroma may be a promising strategy, which is supported by the results of the phase II trial testing recombinant hyalurinodase. Therefore, other agents with antistromal activity should be expected in the next few years. However, attacking tumor stroma may also not significantly alter the clinical course of the disease as the abundant fibrosis may have additional roles other than generation of hypoxia and supporting tumor cell survival, i.e. limiting the growth and invasion of cancer cells. Taken together, we will most likely not witness a big breakthrough in the treatment of pancreatic cancer within next 5 years. However, there will be better understanding of the complex association between the tumor microenvironment and pancreatic cancer pathogenesis with major advances in identification of tumor initiating or CSCs that are one of the key reasons for treatment-failure after current therapy (surgery, chemotherapy, and radiation). Identification of genomic, proteomic or metabolic alterations of these cells in the next few years should inspire novel strategies to treat this deadly disease.

Financial & competing interests disclosure

M Erkan and J Kleeff are members of the Expert Tumour Hypoxia Steering Committee and have acted as consultants for Merck-Serono, Merck KGaA, Darmstadt, Germany. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

ORCID

Jorg Kleeff http://orcid.org/0000-0003-3432-6669

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Surveillance, epidemiology, and end results program (based on 2008-2012 cases and deaths). Available from: http://seer.cancer.gov.
2. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008;321(5897):1801–1806.
3. Waddell N, Pajic M, Patch AM, et al. Whole genomes redefine the mutational landscape of pancreatic cancer. Nature. 2015;518(7540):495–501.
4. Dal Molin M, Zhang M, de Wilde RF, et al. Very long-term survival following resection for pancreatic cancer is not explained by commonly mutated genes: results of whole-exome sequencing analysis. Clin Cancer Res. 2015;21(8):1944–1950.
5. Whittle MC, Izeradjene K, Rani PG, et al. RUNX3 controls a metastatic switch in pancreatic ductal adenocarcinoma. Cell. 2015;161(6):1345–1360.
6. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature. 2010;467(7319):1114–1117.

• **This article demonstrates that tumor cells that carry mutations found in the metastatic foci are nested together in the primary tumor.**

7. Moffitt RA, Marayati R, Flate EL, et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. 2015;47(10):1168–1178.
8. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev. 2007;26(2):225–239.
9. Vaupel P, Hockel M, Mayer A. Detection and characterization of tumor hypoxia using pO2 histography. Antioxid Redox Signal. 2007;9(8):1221–1235.

•• **This article compares the oxygenation of several solid tumors and identifies pancreatic cancer as the most hypoxic.**

10. Koong AC, Mehta VK, Le QT, et al. Pancreatic tumors show high levels of hypoxia. Int J Radiat Oncol Biol Phys. 2000;48(4):919–922.
11. Dhani NC, Serra S, Pintilie M, et al. Analysis of the intra- and intertumoral heterogeneity of hypoxia in pancreatic cancer patients receiving the nitroimidazole tracer pimonidazole. Br J Cancer. 2015;113(6):864–871.
12. Vaupel P. Tumor microenvironmental physiology and its implications for radiation oncology. Semin Radiat Oncol. 2004;14(3):198–206.
13. Ranniger K, Saldino RM. Arteriographic diagnosis of pancreatic lesions. Radiology. 1966;86(3):470–474.
14. Yassa NA, Yang J, Stein S, et al. Gray-scale and color flow sonography of pancreatic ductal adenocarcinoma. J Clin Ultrasound. 1997;25(9):473–480.
15. O'Mahony CA, Albo D, Tuszynski GP, et al. Transforming growth factor-beta 1 inhibits generation of angiostatin by human pancreatic cancer cells. Surgery. 1998;124(2):388–393.
16. Kisker O, Onizuka S, Banyard J, et al. Generation of multiple angiogenesis inhibitors by human pancreatic cancer. Cancer Res. 2001;61(19):7298–7304.
17. Erkan M, Reiser-Erkan C, Michalski CW, et al. Cancer-stellate cell interactions perpetuate the hypoxia-fibrosis cycle in pancreatic ductal adenocarcinoma. Neoplasia. 2009;11(5):497–508.

•• **In this article, the intricate relationship between pancreatic cancer cells and pancreatic stellate cells leading to an antiangiogenic phenotype is shown.**

18. Samkharadze T, Erkan M, Reiser-Erkan C, et al. Pigment epithelium-derived factor associates with neuropathy and fibrosis in pancreatic cancer. Am J Gastroenterol. 2011;106(5):968–980.
19. Kindler HL, Niedzwiecki D, Hollis D, et al. Gemcitabine plus bevacizumab compared with gemcitabine plus

placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). J Clin Oncol. 2010;28(22):3617–3622.

20. Rhim AD, Oberstein PE, Thomas DH, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell. 2014;25(6):735–747.

•• **The results of this article challenges the supposition that the fibrotic stroma of pancreatic cancer only supports its growth and invasion.**

21. Ozdemir BC, Pentcheva-Hoang T, Carstens JL, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell. 2014;25(6):719–734.

• **This article demonstrates that inhibiting some of the stromal elements may unexpectedly worsen the outcome of pancreatic cancer.**

22. Hiraoka N, Ino Y, Sekine S, et al. Tumour necrosis is a postoperative prognostic marker for pancreatic cancer patients with a high interobserver reproducibility in histological evaluation. Br J Cancer. 2010;103(7):1057–1065.
23. Kaelin WG Jr., Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell. 2008;30(4):393–402.
24. Wang GL, Semenza GL. Purification and characterization of hypoxia-inducible factor 1. J Biol Chem. 1995;270(3):1230–1237.
25. Semenza GL. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest. 2013;123(9):3664–3671.
26. Hoffmann AC, Mori R, Vallbohmer D, et al. High expression of HIF1a is a predictor of clinical outcome in patients with pancreatic ductal adenocarcinomas and correlated to PDGFA, VEGF, and bFGF. Neoplasia. 2008;10(7):674–679.
27. Ye LY, Zhang Q, Bai XL, et al. Hypoxia-inducible factor 1alpha expression and its clinical significance in pancreatic cancer: a meta-analysis. Pancreatology. 2014;14(5):391–397.
28. Wang X, Liu Y, Ren H, et al. Polymorphisms in the hypoxia-inducible factor-1alpha gene confer susceptibility to pancreatic cancer. Cancer Biol Ther. 2011;12(5):383–387.
29. Gordan JD, Simon MC. Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr Opin Genet Dev. 2007;17(1):71–77.
30. Criscimanna A, Duan LJ, Rhodes JA, et al. PanIN-specific regulation of Wnt signaling by HIF2alpha during early pancreatic tumorigenesis. Cancer Res. 2013;73(15):4781–4790.
31. Zhang H, Bosch-Marce M, Shimoda LA, et al. Mitochondrial autophagy is an HIF-1-dependent adaptive metabolic response to hypoxia. J Biol Chem. 2008;283(16):10892–10903.
32. Kim JW, Tchernyshyov I, Semenza GL, et al. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006;3(3):177–185.
33. Wise DR, Ward PS, Shay JE, et al. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of alpha-ketoglutarate to citrate to support cell growth and viability. Proc Natl Acad Sci U S A. 2011;108(49):19611–19616.
34. Metallo CM, Gameiro PA, Bell EL, et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature. 2012;481(7381):380–384.
35. Parks SK, Chiche J, Pouyssegur J. Disrupting proton dynamics and energy metabolism for cancer therapy. Nat Rev Cancer. 2013;13(9):611–623.
36. Chaika NV, Gebregiworgis T, Lewallen ME, et al. MUC1 mucin stabilizes and activates hypoxia-inducible factor 1 alpha to regulate metabolism in pancreatic cancer. Proc Natl Acad Sci U S A. 2012;109(34):13787–13792.
37. Akakura N, Kobayashi M, Horiuchi I, et al. Constitutive expression of hypoxia-inducible factor-1alpha renders pancreatic cancer cells resistant to apoptosis induced by hypoxia and nutrient deprivation. Cancer Res. 2001;61(17):6548–6554.
38. Guillaumond F, Leca J, Olivares O, et al. Strengthened glycolysis under hypoxia supports tumor symbiosis and hexosamine biosynthesis in pancreatic adenocarcinoma. Proc Natl Acad Sci U S A. 2013;110(10):3919–3924.
39. Sonveaux P, Vegran F, Schroeder T, et al. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest. 2008;118(12):3930–3942.
40. Fendt SM, Bell EL, Keibler MA, et al. Reductive glutamine metabolism is a function of the alpha-ketoglutarate to citrate ratio in cells. Nat Commun. 2013;4:2236.
41. Wang F, Li SS, Segersvard R, et al. Hypoxia inducible factor-1 mediates effects of insulin on pancreatic cancer cells and disturbs host energy homeostasis. Am J Pathol. 2007;170(2):469–477.
42. Fukuda R, Zhang H, Kim JW, et al. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell. 2007;129(1):111–122.
43. Okami J, Simeone DM, Logsdon CD. Silencing of the hypoxia-inducible cell death protein BNIP3 in pancreatic cancer. Cancer Res. 2004;64(15):5338–5346.
44. Erkan M, Kleeff J, Esposito I, et al. Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. Oncogene. 2005;24(27):4421–4432.
45. Abe T, Toyota M, Suzuki H, et al. Upregulation of BNIP3 by 5-aza-2′-deoxycytidine sensitizes pancreatic cancer cells to hypoxia-mediated cell death. J Gastroenterol. 2005;40(5):504–510.
46. Cuezva JM, Krajewska M, de Heredia ML, et al. The bioenergetic signature of cancer: a marker of tumor progression. Cancer Res. 2002;62(22):6674–6681.
47. Kong B, Qia C, Erkan M, et al. Overview on how oncogenic Kras promotes pancreatic carcinogenesis by inducing low intracellular ROS levels. Front Physiol. 2013;4:246.
48. O'Leary BR, Fath MA, Bellizzi AM, et al. Loss of SOD3 (EcSOD) expression promotes an aggressive phenotype in human pancreatic ductal adenocarcinoma. Clin Cancer Res. 2015;21(7):1741–1751.
49. Cullen JJ, Weydert C, Hinkhouse MM, et al. The role of manganese superoxide dismutase in the growth of

pancreatic adenocarcinoma. Cancer Res. 2003;63 (6):1297–1303.
50. Teoh ML, Sun W, Smith BJ, et al. Modulation of reactive oxygen species in pancreatic cancer. Clin Cancer Res. 2007;13(24):7441–7450.
51. Ying H, Kimmelman AC, Lyssiotis CA, et al. Oncogenic Kras maintains pancreatic tumors through regulation of anabolic glucose metabolism. Cell. 2012;149(3):656–670.
52. Son J, Lyssiotis CA, Ying H, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature. 2013;496(7443):101–105.
53. Kramer RM, Russell J, Humm JL. Distribution of gemcitabine is nearly homogenous in two orthotopic murine models of pancreatic cancer. Cancer Biother Radiopharm. 2015;30(7):299–304.
54. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009;324(5933):1457–1461.
55. Seta KA, Spicer Z, Yuan Y, et al. Responding to hypoxia: lessons from a model cell line. Sci STKE. 2002;2002(146): re11.
56. Yokoi K, Fidler IJ. Hypoxia increases resistance of human pancreatic cancer cells to apoptosis induced by gemcitabine. Clin Cancer Res. 2004;10(7):2299–2306.
57. Nakanishi C, Toi M. Nuclear factor-kappaB inhibitors as sensitizers to anticancer drugs. Nat Rev Cancer. 2005;5 (4):297–309.
58. Longo VD, Lieber MR, Vijg J. Turning anti-ageing genes against cancer. Nat Rev Mol Cell Biol. 2008;9(11):903–910.
59. Sowter HM, Ratcliffe PJ, Watson P, et al. HIF-1-dependent regulation of hypoxic induction of the cell death factors BNIP3 and NIX in human tumors. Cancer Res. 2001;61 (18):6669–6673.
60. Kim JY, Ahn HJ, Ryu JH, et al. BH3-only protein Noxa is a mediator of hypoxic cell death induced by hypoxia-inducible factor 1alpha. J Exp Med. 2004;199(1):113–124.
61. Bruick RK. Expression of the gene encoding the proapoptotic Nip3 protein is induced by hypoxia. Proc Natl Acad Sci U S A. 2000;97(16):9082–9087.
62. Erler JT, Cawthorne CJ, Williams KJ, et al. Hypoxia-mediated down-regulation of Bid and Bax in tumors occurs via hypoxia-inducible factor 1-dependent and -independent mechanisms and contributes to drug resistance. Mol Cell Biol. 2004;24(7):2875–2889.
63. Chen N, Chen X, Huang R, et al. BCL-xL is a target gene regulated by hypoxia-inducible factor-1{alpha}. J Biol Chem. 2009;284(15):10004–10012.
64. Wang R, Cheng L, Xia J, et al. Gemcitabine resistance is associated with epithelial-mesenchymal transition and induction of HIF-1alpha in pancreatic cancer cells. Curr Cancer Drug Targets. 2014;14(4):407–417.
65. Cheng ZX, Wang DW, Liu T, et al. Effects of the HIF-1alpha and NF-kappaB loop on epithelial-mesenchymal transition and chemoresistance induced by hypoxia in pancreatic cancer cells. Oncol Rep. 2014;31(4):1891–1898.
66. Yang SY, Song BQ, Dai SL, et al. Effects of hypoxia-inducible factor-1alpha silencing on drug resistance of human pancreatic cancer cell line Patu8988/5-Fu. Hepatogastroenterology. 2014;61(136):2395–2401.
67. Szakacs G, Paterson JK, Ludwig JA, et al. Targeting multidrug resistance in cancer. Nat Rev Drug Discov. 2006;5 (3):219–234.
68. Arora S, Bhardwaj A, Singh S, et al. An undesired effect of chemotherapy: gemcitabine promotes pancreatic cancer cell invasiveness through reactive oxygen species-dependent, nuclear factor kappaB- and hypoxia-inducible factor 1alpha-mediated up-regulation of CXCR4. J Biol Chem. 2013;288(29):21197–21207.
69. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009;9(4):274–284.
70. Yilmaz M, Christofori G. Mechanisms of motility in metastasizing cells. Mol Cancer Res. 2010;8(5):629–642.
71. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009;9(4):239–252.
72. Thiery JP, Acloque H, Huang RY, et al. Epithelial-mesenchymal transitions in development and disease. Cell. 2009;139(5):871–890.
73. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol. 2003;15 (6):740–746.
74. Friedl P, Wolf K. Tube travel: the role of proteases in individual and collective cancer cell invasion. Cancer Res. 2008;68(18):7247–7249.
75. Prall F, Ostwald C. High-degree tumor budding and podiaformation in sporadic colorectal carcinomas with K-ras gene mutations. Hum Pathol. 2007;38(11):1696–1702.
76. Ohike N, Coban I, Kim GE, et al. Tumor budding as a strong prognostic indicator in invasive ampullary adenocarcinomas. Am J Surg Pathol. 2010;34(10):1417–1424.
77. Karamitopoulou E, Zlobec I, Born D, et al. Tumour budding is a strong and independent prognostic factor in pancreatic cancer. Eur J Cancer. 2013;49(5):1032–1039.
78. De Bock K, Mazzone M, Carmeliet P. Antiangiogenic therapy, hypoxia, and metastasis: risky liaisons, or not? Nat Rev Clin Oncol. 2011;8(7):393–404.
79. Buchler P, Reber HA, Lavey RS, et al. Tumor hypoxia correlates with metastatic tumor growth of pancreatic cancer in an orthotopic murine model. J Surg Res. 2004;120(2):295–303.
80. Chang Q, Jurisica I, Do T, et al. Hypoxia predicts aggressive growth and spontaneous metastasis formation from orthotopically grown primary xenografts of human pancreatic cancer. Cancer Res. 2011;71(8):3110–3120.
81. Song Y, Washington MK, Crawford HC. Loss of FOXA1/2 is essential for the epithelial-to-mesenchymal transition in pancreatic cancer. Cancer Res. 2010;70(5):2115–2125.
82. Lei J, Ma J, Ma Q, et al. Hedgehog signaling regulates hypoxia induced epithelial to mesenchymal transition and invasion in pancreatic cancer cells via a ligand-independent manner. Mol Cancer. 2013;12:66.
83. Cheng ZX, Sun B, Wang SJ, et al. Nuclear factor-kappaB-dependent epithelial to mesenchymal transition induced by HIF-1alpha activation in pancreatic cancer cells under hypoxic conditions. PLoS One. 2011;6(8):e23752.
84. Zhu H, Wang D, Zhang L, et al. Upregulation of autophagy by hypoxia-inducible factor-1alpha promotes EMT and metastatic ability of CD133+ pancreatic cancer stem-like cells during intermittent hypoxia. Oncol Rep. 2014;32 (3):935–942.

85. Salnikov AV, Liu L, Platen M, et al. Hypoxia induces EMT in low and highly aggressive pancreatic tumor cells but only cells with cancer stem cell characteristics acquire pronounced migratory potential. PLoS One. 2012;7(9): e46391.
86. Durand RE. Intermittent blood flow in solid tumours–an under-appreciated source of 'drug resistance'. Cancer Metastasis Rev. 2001;20(1–2):57–61.
87. Zhu H, Wang D, Liu Y, et al. Role of the hypoxia-inducible factor-1 alpha induced autophagy in the conversion of non-stem pancreatic cancer cells into CD133+ pancreatic cancer stem-like cells. Cancer Cell Int. 2013;13(1):119.
88. Hashimoto O, Shimizu K, Semba S, et al. Hypoxia induces tumor aggressiveness and the expansion of CD133-positive cells in a hypoxia-inducible factor-1alpha-dependent manner in pancreatic cancer cells. Pathobiology. 2011;78(4):181–192.
89. Zhu S, Zhou Y, Wang L, et al. Transcriptional upregulation of MT2-MMP in response to hypoxia is promoted by HIF-1alpha in cancer cells. Mol Carcinog. 2011;50 (10):770–780.
90. Zhu SK, Zhou Y, Cheng C, et al. Overexpression of membrane-type 2 matrix metalloproteinase induced by hypoxia-inducible factor-1alpha in pancreatic cancer: implications for tumor progression and prognosis. Mol Clin Oncol. 2014;2(6):973–981.
91. Shi CY, Fan Y, Liu B, et al. HIF1 contributes to hypoxia-induced pancreatic cancer cells invasion via promoting QSOX1 expression. Cell Physiol Biochem. 2013;32(3):561–568.
92. Zhao T, Gao S, Wang X, et al. Hypoxia-inducible factor-1alpha regulates chemotactic migration of pancreatic ductal adenocarcinoma cells through directly transactivating the CX3CR1 gene. PLoS One. 2012;7(8): e43399.
93. Miller BW, Morton JP, Pinese M, et al. Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: inhibition of LOX abrogates metastasis and enhances drug efficacy. EMBO Mol Med. 2015;7(8):1063–1076.
94. Thayer SP, Di Magliano MP, Heiser PW, et al. Hedgehog is an early and late mediator of pancreatic cancer tumorigenesis. Nature. 2003;425(6960):851–856.
95. Onishi H, Kai M, Odate S, et al. Hypoxia activates the hedgehog signaling pathway in a ligand-independent manner by upregulation of Smo transcription in pancreatic cancer. Cancer Sci. 2011;102(6):1144–1150.
96. Kong B, Wu W, Cheng T, et al. A subset of metastatic pancreatic ductal adenocarcinomas depends quantitatively on oncogenic Kras/Mek/Erk-induced hyperactive mTOR signalling. Gut. 2015. doi:10.1136/gutjnl-2014-307616. [Epub ahead of print].
97. Kong B, Cheng T, Wu W, et al. Hypoxia-induced endoplasmic reticulum stress characterizes a necrotic phenotype of pancreatic cancer. Oncotarget. 2015;6 (31):32154–32160.
98. Erkan M, Michalski CW, Rieder S, et al. The activated stroma index is a novel and independent prognostic marker in pancreatic ductal adenocarcinoma. Clin Gastroenterol Hepatol. 2008;6(10):1155–1161.
99. Bachem MG, Schunemann M, Ramadani M, et al. Pancreatic carcinoma cells induce fibrosis by stimulating proliferation and matrix synthesis of stellate cells. Gastroenterology. 2005;128(4):907–921.
100. Hwang RF, Moore T, Arumugam T, et al. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. Cancer Res. 2008;68(3):918–926.
101. Vonlaufen A, Joshi S, Qu C, et al. Pancreatic stellate cells: partners in crime with pancreatic cancer cells. Cancer Res. 2008;68(7):2085–2093.
102. Neesse A, Michl P, Frese KK, et al. Stromal biology and therapy in pancreatic cancer. Gut. 2011;60(6):861–868.
103. Mahadevan D, Von Hoff DD. Tumor-stroma interactions in pancreatic ductal adenocarcinoma. Mol Cancer Ther. 2007;6(4):1186–1197.
104. Masamune A, Kikuta K, Watanabe T, et al. Hypoxia stimulates pancreatic stellate cells to induce fibrosis and angiogenesis in pancreatic cancer. Am J Physiol Gastrointest Liver Physiol. 2008;295(4):G709–G717.
105. Spivak-Kroizman TR, Hostetter G, Posner R, et al. Hypoxia triggers hedgehog-mediated tumor-stromal interactions in pancreatic cancer. Cancer Res. 2013;73(11):3235–3247.
106. McKeown SR. Defining normoxia, physoxia and hypoxia in tumours-implications for treatment response. Br J Radiol. 2014;87(1035):20130676.
107. Amakye D, Jagani Z, Dorsch M. Unraveling the therapeutic potential of the Hedgehog pathway in cancer. Nat Med. 2013;19(11):1410–1422.
108. Provenzano PP, Cuevas C, Chang AE, et al. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012;21(3):418–429.
109. Jacobetz MA, Chan DS, Neesse A, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut. 2013;62(1):112–120.
110. Hingorani SR, Harris WP, Hendifar AE, et al. High response rate and PFS with PEGPH20 added to nab-paclitaxel/gemcitabine in stage IV previously untreated pancreatic cancer patients with high-HA tumors: interim results of a randomized phase II study. J Clin Oncol. 2015;33(suppl):Abstr 4006.

•• **The agent tested in this clinical trial provides evidence for the benefit of stroma-targeting in the treatment of pancreatic cancer.**

111. Erkan M. The role of pancreatic stellate cells in pancreatic cancer. Pancreatology. 2013;13(2):106–109.
112. Jeong W, Rapisarda A, Park SR, et al. Pilot trial of EZN-2968, an antisense oligonucleotide inhibitor of hypoxia-inducible factor-1 alpha (HIF-1alpha), in patients with refractory solid tumors. Cancer Chemother Pharmacol. 2014;73(2):343–348.
113. Zhao T, Ren H, Jia L, et al. Inhibition of HIF-1alpha by PX-478 enhances the anti-tumor effect of gemcitabine by inducing immunogenic cell death in pancreatic ductal adenocarcinoma. Oncotarget. 2015;6(4):2250–2262.
114. Tibes R, Falchook GS, Von Hoff DD, et al. Results from a phase I, dose-escalation study of PX-478, and orally available inhibitor of HIF-1 alpha. J Clin Oncol. 2010;28(suppl): Abstr 3076.
115. Zhang H, Qian DZ, Tan YS, et al. Digoxin and other cardiac glycosides inhibit HIF-1alpha synthesis and block tumor growth. Proc Natl Acad Sci U S A. 2008;105(50):19579–19586.

116. Ding X, Zhou X, Jiang B, et al. Triptolide suppresses proliferation, hypoxia-inducible factor-1alpha and c-Myc expression in pancreatic cancer cells. Mol Med Rep. 2015;12(3):4508–4513.
117. Liu L, Salnikov AV, Bauer N, et al. Triptolide reverses hypoxia-induced epithelial-mesenchymal transition and stem-like features in pancreatic cancer by NF-kappaB downregulation. Int J Cancer. 2014;134(10):2489–2503.
118. Abraham RT. mTOR as a positive regulator of tumor cell responses to hypoxia. Curr Top Microbiol Immunol. 2004;279:299–319.
119. Yao JC, Shah MH, Ito T, et al. Everolimus for advanced pancreatic neuroendocrine tumors. N Engl J Med. 2011;364(6):514–523.
120. Wolpin BM, Hezel AF, Abrams T, et al. Oral mTOR inhibitor everolimus in patients with gemcitabine-refractory metastatic pancreatic cancer. J Clin Oncol. 2009;27 (2):193–198.
121. Javle MM, Shroff RT, Xiong H, et al. Inhibition of the mammalian target of rapamycin (mTOR) in advanced pancreatic cancer: results of two phase II studies. BMC Cancer. 2010;10:368.
122. Kordes S, Klumpen HJ, Weterman MJ, et al. Phase II study of capecitabine and the oral mTOR inhibitor everolimus in patients with advanced pancreatic cancer. Cancer Chemother Pharmacol. 2015;75(6):1135–1141.
123. Shimojo Y, Akimoto M, Hisanaga T, et al. Attenuation of reactive oxygen species by antioxidants suppresses hypoxia-induced epithelial-mesenchymal transition and metastasis of pancreatic cancer cells. Clin Exp Metastasis. 2013;30(2):143–154.
124. Sibenaller ZA, Welsh JL, Du C, et al. Extracellular superoxide dismutase suppresses hypoxia-inducible factor-1alpha in pancreatic cancer. Free Radic Biol Med. 2014;69:357–366.
125. Kurtoglu M, Maher JC, Lampidis TJ. Differential toxic mechanisms of 2-deoxy-D-glucose versus 2-fluorodeoxy-D-glucose in hypoxic and normoxic tumor cells. Antioxid Redox Signal. 2007;9(9):1383–1390.
126. Xi H, Kurtoglu M, Lampidis TJ. The wonders of 2-deoxy-D-glucose. IUBMB Life. 2014;66(2):110–121.
127. Raez LE, Papadopoulos K, Ricart AD, et al. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. Cancer Chemother Pharmacol. 2013;71(2):523–530.
128. Lampidis TJ, Kurtoglu M, Maher JC, et al. Efficacy of 2-halogen substituted D-glucose analogs in blocking glycolysis and killing "hypoxic tumor cells". Cancer Chemother Pharmacol. 2006;58(6):725–734.
129. Birsoy K, Wang T, Possemato R, et al. MCT1-mediated transport of a toxic molecule is an effective strategy for targeting glycolytic tumors. Nat Genet. 2013;45(1):104–108.
130. Kunjithapatham R, Geschwind JF, Rao PP, et al. Systemic administration of 3-bromopyruvate reveals its interaction with serum proteins in a rat model. BMC Res Notes. 2013;6:277.
131. Chapiro J, Sur S, Savic LJ, et al. Systemic delivery of microencapsulated 3-bromopyruvate for the therapy of pancreatic cancer. Clin Cancer Res. 2014;20(24):6406–6417.
132. Wilson WR, Hay MP. Targeting hypoxia in cancer therapy. Nat Rev Cancer. 2011;11(6):393–410.

• **This review summarizes the agents that are in clinical trials or currently being developed for targeting the hypoxic portions of solid tumors.**

133. Duan JX, Jiao H, Kaizerman J, et al. Potent and highly selective hypoxia-activated achiral phosphoramidate mustards as anticancer drugs. J Med Chem. 2008;51 (8):2412–2420.
134. Borad MJ, Reddy SG, Bahary N, et al. Randomized phase II trial of gemcitabine plus TH-302 versus gemcitabine in patients with advanced pancreatic cancer. J Clin Oncol. 2015;33(13):1475–1481.

•• **The results of the phase II clinical trial show a benefit of the hypoxia-activated prodrug TH302 for delaying the progression of the disease.**