New insights into pancreatic cancer biology

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Pancreatic cancer remains a devastating disease. Over the last few years, there have been important advances in the molecular and biological understanding of pancreatic cancer. This included understanding of the genomic complexity of the disease, the role of pancreatic cancer stem cells, the relevance of the tumor microenvironment, and the unique metabolic adaptation of pancreas cancer cells to obtain nutrients under hypoxic environment. In this paper, we review the most salient developments in these few areas.

Key words: cancer stem cells, genomics, pancreatic cancer, stroma

introduction

Ductal adenocarcinoma of the pancreas (PDA), commonly referred to as pancreatic cancer, is a frequent and lethal disease ranking the fourth cause in cancer-related death in Western countries. Despite advances in the clinical management of the disease, including the introduction of systemic chemotherapy in the management of patients with operable disease as well as the development of novel, more effective chemotherapeutic agents, in patients with advanced disease, prognosis remains poor [1].

PDA is a genetic disease. The successive accumulation of mutations in key oncogenes and tumor suppressor genes leads to pancreatic cancer that once established is a quite complex, heterogeneous and genetically unstable disease [2–4]. In addition, PDA is composed of multiple compartments. Together with the mature and differentiated cell compartment, there is a cancer stem cell compartment that, while numerically small, is considered to be resistant to chemotherapy and radiation therapy and is involved in the process of cancer spread and treatment resistance [5]. PDA is also characterized by a dense and desmoplastic stroma composed of fibrillar elements such as collagen I and activated fibroblasts among others [6]. In this paper, we review the most salient biological features of pancreatic cancer with a focus in those that have more clear clinical applications. Critical to this progress has been the availability of preclinical models, including genetically engineered mouse models of PDA as well as patient derived xenografts that faithfully recapitulate the biology of the disease [7–9].

the genomic landscape of pancreatic cancer

Evidence suggests that pancreatic cancer results from the successive accumulation of gene mutations [2, 10]. The cancer originates in the ductal epithelium and evolves from premalignant lesions to fully invasive cancer. The lesion called pancreatic intraepithelial neoplasia (PanIN) is the best characterized histological precursor of pancreatic cancer [11]. The progression from minimally dysplastic epithelium (PanIN1A and B) to more severe dysplasia (PanIN2 and 3), and finally, to invasive carcinoma is paralleled by the successive accumulation of mutations that include the activation of the KRA2 oncogene, inactivation of the tumor suppressor gene CDKN2A/INK4A, and finally, inactivation of the tumor suppressor genes TP53 and DPCA/SMAD4 [12]. This sequence of events in pancreatic carcinogenesis is supported by studies in genetically engineered mouse models in which the targeted activation of the murine Kras2 gene with concomitant inactivation of the Trp53 or Cdkn2A/Ink4A genes results in the development of pancreatic cancer identical to the cognate human disease [9, 13, 14]. Other, less well-characterized, macroscopic, premalignant lesions of the pancreas include intrapancreatic mucinous neoplasia and mucinous cystic neoplasia [15].

Fully established pancreatic cancer almost universally carries one or more of four genetic defects [16]. Ninety percent of tumors have activating mutations in the KRA2 oncogene. Transcription of the mutant KRAS gene produces an abnormal Ras protein that is ‘locked’ in its activated form, resulting in the aberrant activation of proliferative and survival signaling pathways. Likewise, 95% of tumors have inactivation of the CDKN2A gene with the resultant loss of the p16 protein, a regulator of the G1-S transition of the cell cycle, and a corresponding increase in cell proliferation. TP53 is abnormal in 50%–75% of tumors, permitting cells to bypass DNA damage control checkpoints and apoptotic signals and contributing to genomic instability. The deleted in pancreatic carcinoma 4 gene (DPCA—also known as SMAD4/MADH4) is lost in ~50% of pancreatic cancers, resulting in aberrant signaling by the transforming growth factor-β (TGFβ) cell surface receptor. In a study using human pancreatic cancer samples from primary and metastatic lesion, mutations in the DPC4 gene has been associated with higher metastatic potential.
The full mutational landscape of PDA was determined in a study that reported an extensive and comprehensive genetic analysis of 24 pancreatic cancers. The results from this study showed that PDA is a extremely complex and heterogeneous. The average number of likely relevant genetic abnormalities per tumor was 63 in that study, mainly point mutations, which may be organized in 12 functional cancer relevant pathways (Figure 1). However, not all tumors have alterations in all pathways, and the key mutations in each pathway appear to differ from one cancer to another [2]. While no frequent drug targets were encountered, individual patients harbored mutations in genes that could be therapeutically counterbalance. Thus, a mutation in the PALB2 gene found in one cancer suggested that this cancer could be sensitive to DNA damaging agents. Indeed, treatment of this individual with alkylating agents resulted in marked tumor regression and long survival [17]. However, in most patients, no drug targets were found. More recently, two studies analyzed the genomic characteristics of primary and paired metastatic lesions [3, 4]. These studies showed that the clonal populations that originate distant metastasis are present in the primary tumor. This clones evolved genetically to give raise to metastatic clones over a long period of time. Genomic instability frequently persists after cancer dissemination, resulting in ongoing, parallel and even convergent evolution among different metastases. There is a genetic heterogeneity among metastasis-initiating cells that may require additional driver mutations beyond those required for primary tumors.

The genetic diversity of pancreatic cancer is also reflected in different patterns of gene expression. A recent study has proposed three subtypes of pancreatic cancer based on analysis of gene expression profiles that, in addition to variable prognosis, appears to be linked to differences in drug response. These data may have implications to patient stratification in clinical trials and for personalize medicine [18].

The tumor microenvironment

A characteristic of pancreatic cancer is the formation of a dense stroma, termed a desmoplastic reaction (Figure 1) [6, 19]. The pancreatic stellate cells (also known as myofibroblasts) play a critical role in the formation and turnover of the stroma. Upon activation by growth factors such as TGFβ-1, PDGF (platelet derived growth factor), and FGF (fibroblast growth factor), these cells secrete collagen and other components of the extracellular matrix; stellate cells also appear to be responsible for the poor vascularization characteristic of pancreatic cancer [20, 21]. Further, stellate cells regulate the reabsorption and turnover of the stroma, mainly though the production of matrix-metalloproteinases [22]. The stroma is not just a mechanical barrier but constitutes a dynamic compartment critically involved in the process of tumor formation, progression, invasion, and metastasis [6, 19]. Stromal cells express multiple proteins such as Cox-2, PDGF receptor, vascular endothelial growth factors (VEGF), stromal derived factor (SDF), chemokines, integrins, SPARC (secreted protein-acid rich in cysteine), and hedgehog pathway elements, among others, which have been associated with worse prognosis and resistance to treatment.

The better understanding of the PDA stroma is starting to have therapeutic implications. Preclinical studies using genetically engineered mouse models of PDA have shown that one of the characteristics of PDA is its poor vascularization. Disruption of the stroma with agents such as inhibitors of the hedgehog resulted in increased vascular supply and heightened drug delivery [23–25]. Unfortunately, the clinical development of hedgehog inhibitors in PDA has not been, for reasons that are not yet fully understood, too promising so far.

One interesting stromal target in PDA is SPARC, also known as osteonectin, which binds albumin. SPARC is though to be the target of Nab-paclitaxel (Celgene, NJ, USA), an albumin coated nanoparticle of paclitaxel. In preclinical studies, this agent binds SPARC and accumulates in tumor tissues. In patient derived xenograft of pancreas cancer, Nab-paclitaxel eliminates the pancreas cancer stroma, increases the delivery of gemcitabine and is associated with high antitumor activity [26]. Additional studies in GEMM of PDA have shown that Nab-paclitaxel blocks the catabolism of gemcitabine leading, by this mechanism, to high intratumor concentration of the agent. In agreement with this preclinical studies, a phase I/II study of gemcitabine in combination with nab-paclitaxel has shown promising activity results and is now in phase III studies.

One of the characteristics of the pancreatic cancer stroma is that contributes to create an immunosuppressive tumor microenvironment that can restrain antitumor immunity. There has been interest in reversing this phenomenon therapeutically. Because CD40 activation can reverse immune suppression and drive antitumor T-cell responses, clinical studies have tested the combination of an agonist CD40 antibody with gemcitabine chemotherapy in patients with surgically incurable PDA and have shown tumor regressions. Mechanistic studies showed that this agent works by stimulating the infiltration of tumor macrophages that deplete the cancer stroma [27].

Pancreatic cancer stem cells

Several parallel studies, using primary tumor xenograft models, have identified a subset of pancreas cancer cells with stem cell properties [5, 28]. These cells, which phenotypic identification is still a matter of debate, have different biologically important characteristics such as the capacity to self-renewal and asymmetric division. In PDAC, early data have suggested that the identification of CSCs in primary tumors is associated with shorter overall survival, resistance to the standard cytotoxic agent gemcitabine and enhanced metastatic potential [28, 29]. CSCs are also heterogeneous with different cell populations that may have different functional properties. One important recent observation is that CSC are very plastic with transition among different states including from epithelial to mesenchimal state which may be involved in the metastatic spread of pancreatic cancer [30].

One interesting observation is that PDA CSC have unique therapeutic targets [31–34]. These include developmental pathways such as Hedgehog, Wnt and Notch, apoptotic pathway targets such as DR5 and novel pathways such as nodal-activin. In preclinical models of human pancreatic
cancer, targeting these pathways results in prolonged tumor control while chemotherapy alone induces only short-lived tumor regressions. This interesting hypothesis, however, has not been yet translated in clinical trials because the studies with Hedgehog inhibitors, so far, have been only conducted in patients with advanced disease in whom probably the cancer stem cells are not the principal tumor compartment.

**nutritional requirements of PDA**

Like other cancers, pancreatic cancer cells rely on fuel sources for homeostasis and proliferation; as such, interrupting the use of two major nutrients, glucose and glutamine, may provide new therapeutic avenues. In addition, cancer cell are adapted to hypoxic environment and able to extract energy for survival and growth under these conditions. Blocking enzymes involved in these processes offers the opportunity for therapeutic targeting. Examples of such targets includes LDHA which regenerate nicotinamide adenine dinucleotide (NAD+) that is required for the further glycolytic conversion of glucose to pyruvate to generate ATP. Another interesting metabolic target is glutaminase which seems to be particularly effective in cells with mutant IDH1. Two recent studies targeted glutaminolysis in cancer by a specific glutaminase inhibitor, BPTES (bis-2-[5-(phenylacetamido)-1,3,4-thiadiazol-2-ethyl sulfa) [35]. In addition it has been noted that pancreatic cancers display substantial autophagic activities for survival. In fact, Ras transformed cells depend on autophagy for survival [36]. Hence, inhibition of autophagy with the anti-malarial agent chloroquine has resulted in substantial preclinical responses of
pancreatic cancer xenographs and allografts in treated mice as compared to control. Chloroquine also diminishes pancreatic tumorigenesis in a transgenic model and is currently been tested in clinical studies.

**summary**

Over the last few years our molecular understanding of pancreatic cancer has advanced importantly. These included the genetic understanding of the disease, the notion that metastasis are genetically instable and heterogeneous and occur early in the disease. Another important development is the realization of the importance of the stroma for both cancer development and progression and as a barrier to the optimal delivery of chemotherapy. A group of pancreatic cancer cells have stem cell properties and have been found to be resistant to chemotherapy and radiation therapy. Finally, insights into the mechanism responsible for cancer adaptation to hypoxic environments and nutrient procurements are also providing new therapeutic targets. Some of these findings are already resulting in new therapeutic targets and treatment strategies that are showing promising results in early clinical trials.

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**references**