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MINIREVIEW

Genomics of Pancreatic Cancer: Does It Make Any Improvement in Diagnosis, Prognosis and Therapy?

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Pancreatic cancer (PanC) is an extremely life-threatening neoplasm due to its late discovery, rapid progression and resistance to chemo- and radiotherapy. In the past years a significant research attention turned to this cancer. Extensive genomic analysis of PanC revealed numerous alterations, however, none of them emerged yet as a key regulator of tumor progression. Our increasing knowledge on the molecular targets in various cancer types started to change their management. Examples of success of the molecular therapies (in CML, GIST, NSCLC) may initiate more activity in pancreatic cancer as well. (Pathology Oncology Research Vol 11, No 2, 69–73)

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Due to its late discovery, rapid progression and resistance to chemo- and radiotherapy, pancreatic cancer (PanC) is an extremely life-threatening neoplasm. In statistics PanC is the fourth leading cause of cancer death in the USA, with a median survival of less than 6 months, and a 5-year survival rate of only 3%. For those patients (15-20%) that undergo potentially curative resection, the 5-year survival is only 20%. It is a real challenge to discover the reasons, including molecular mechanisms, which can govern this extremely malignant behavior. There is no doubt that some development has been made in the past years with the hope to move towards a more efficient management of cancer, based on key molecular targets. These serve as examples for such possibilities in PanC.

Molecular pattern/signature

At genetic level, numerous studies have documented an increased risk in relatives of PanC patients (approx. 3x), and it is estimated that 10% of PanC is due to inherited predisposition (involved genes: e.g. CDKN2A, BRCA2). The low penetrance of PanC associated with these germline mutations might point to a role of precursor lesions in the malignant progression rather than in the limiting events that control initiation of neoplastic growth from normal pancreatic cells.

A hallmark of solid tumors, and therefore also of PanC, is genome-wide genomic instability resulting in large chromosomal gains or losses. Comparative genomic hybridization (CGH) studies have revealed non-random chromosomal aberrations,[1] the most frequent changes were: gains of 20q, 8q, 11q, 12p, 17q or losses of 18q, 9p or 15q.[2] This method is, however, relatively insensitive, while the novel array-based CGH (matrix-CGH) offers a superior – 20-100 times higher – spatial resolution. Using this assay more than 3-fold number of DNA amplifications have been identified in widely used pancreatic cell lines and pancreatic cancer samples.[3] The most frequent amplifications mapped to 7p12.3, 8q24, 11q13 and 20q13. Two new members of the bcl family (bcl-10 and bcl-6) have also been identified, which have not been described earlier in the context of pancreatic carcinoma. In the majority of pancreatic tumor samples with 20q13 amplification, a novel gene (NFAT C2) was discovered, known for its cytokine-activation capacity.

The ability of cancer cells to adapt to hypoxic environment is increasingly recognized as an important mechanism promoting tumor growth. In general, it is thought

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that tumor cells become resistant to hypoxia during the progression of the disease by alterations in a variety of cellular mechanisms. BNIP3 (a bcl-2/adenovirus E1B 19 kDa interacting protein 3) is a hypoxia-inducible pro-apoptotic molecule. Its expression is increased under hypoxic conditions, leading to the activation of the apoptotic program in hypoxia-injured cells. However, in PanC the expression of BNIP3 is decreased compared to normal pancreatic tissue, while other hypoxia-induced genes did not change. The reason of the lower expression, which supports the survival of PanC cells, is the hypermethylation of BNIP3 promoter. The methylation-inhibitor 5-aza-2'-deoxycytidine re-established the sensitivity of PanC cells to hypoxia-induced cell death.[4]

With the advent of more and more sophisticated molecular techniques it has been (and is still) a tendency to search for correlation(s) between the morphological appearance and the type of genetic errors in human malignancies.

It is generally believed that the invasive pancreatic cancer is preceded by sequential steps of preneoplastic lesions designated PanIN-1A-1B-2 and PanIN-3. Using cDNA microarrays in microdissected early PanIN lesions Prasad et al. have identified 49 genes that were differently expressed in comparison with the normal ductal epithelium.[5] The results were confirmed by semi-quantitative and real-time PCR, immunohistochemistry or in situ hybridization. Some of these genes are not normally expressed at high levels in pancreatic tissues (e.g. KLF4, pepsinogen C, TFF1, MUC6), but they are known to be present in the epithelium of the stomach and intestine. In addition, several other foregut-markers proved to be upregulated, among them GATA4, GATA5, GATA6, gastrin, villin1, villin2, HOXA5, SOX2 or FKHR6. These findings may indicate aberrant hedgehog signaling in preneoplastic pancreatic ductal epithelium,[5] which could suggest a role of pancreatic stem cells in cancer development.

During the development of pancreatic carcinoma from the normal pancreas via PanIN-1-3, the sequence of genetic errors were the following: ERBB2, EGFR1, K-ras, INK4A, TP53, SMAD4/DPC4.[6] In acinar cell carcinoma the mutation in APC and β-catenin (same pathway!), and in serous adenocarcinoma the mutation of VHL was also described. Another study showed similar results: mutation in K-ras (90%) (could be present as early as in chronic pancreatitis as well!), p53 (40%), DCC loss (60%), SMAD4/DPC4 loss (50%), p16 loss (90%). The increased EGFR expression indicated a bad prognosis.[7] The difficulties to evaluate (and use!) gene expression profile in cancer management is reflected in a study where the major technologies (serial analysis of gene expression, oligonucleotide array and cDNA array) were used to compare the expressions in samples from normal pancreatic tissues, normal duodenal mucosa, chronic pancreatitis and invasive pancreatic cancer. At least 40 genes showed increased expression with two methods, but only 6 genes with all the three: keratin 19, stratifin, transglutaminase 2 (they have been observed by others as well), retinoic acid-induced 3, secreted leukocyte protease inhibitor, tetraspan 1.[8] Today, it is not known what the functional significance of these changes is, and which of them could be a potential target for therapy.

Although many genes are differently expressed between normal and tumorous pancreas, few of them are translated into proteins. Recent studies using two-dimensional gel electrophoresis and mass spectrometry revealed that many genes that were identified in previous studies have not been detected at the protein level.[9] On the other side, however, some newly detected and highly expressed proteins (cytoskeleton actin, TM2, or S100A8) may serve as diagnostic markers or may be used for differential diagnosis from chronic pancreatitis.

Chemoresistance and the NF-κB pathway

PanC is largely chemoresistant, the usual response rate to the available cytostatic drugs is generally low. It has long been known that in over 70% of the tumors the multidrug resistance gene (MDR1) and the P-glycoprotein are overexpressed,[10] but the mechanisms behind the resistant phenotype is probably much more complex.

Constitutive activation of nuclear factor-κB (NF-κB) has been described in several human malignancies including leukemias and lymphomas as well as solid tumors such as prostate, colorectal, and pancreatic carcinoma. These tumor cells express different NF-κB target genes conferring substantial growth advantage, as anti-apoptotic (e.g. cIAP, bcl-2) and cell cycle promoting (e.g. cyclin-D) genes. High NF-κB activity can lead to increased chemo- and radioresistance in pancreatic cancer cells, therefore inhibition of NF-κB activity can make them sensitive to chemotherapeutic agents (e.g. gemcitabine). Many regulators can influence the NF-κB pathway, and many genetic changes can lead to elevated NF-κB activity.

Glycogen synthase kinase-3 is a serine/threonine kinase and a regulator of glycogen synthesis. It has two isoforms: α and β. Classically GSK-3β forms complex with APC (adenomatosis polyposis coli), axin and β-catenin, and phosphorylates β-catenin targeting for degradation. In this sense GSK-3β is rather a proapoptotic gene. However, in other settings GSK-3β was found to activate NF-κB, therefore serving for cell survival.[11] It has been shown that in pancreatic cancers low β-catenin level is associated with poor prognosis. This finding is the opposite of that suggested in colon cancer. It is possible that in pancreatic cancer cells there is an active pool of GSK-3β which simulta-

neously increases β-catenin phosphorylation and degradation, and at the same time activates NF-κB, supporting survival and proliferation.[12]

Recently, it was suggested that increased βTRCP1 (β-transducin repeat-containing protein) expression in PanC cells contribute to the IL-1β-dependent activation of NF-κB as well as to chemoresistance.[13] Interleukin-1β (IL-1β) confer permanent NF-κB activation in pancreatic carcinoma along with a profound chemoresistance. An important step in the activation of NF-κB activation is the degradation of IκBα (e.g. as a response to TNF-α or IL-1β). Degradation is made by poly-ubiquitination of phospho-IκB, where the rate limiting action is the activity of E3-ubiquitin ligase (Skp1-Cullin-Fbox complex). The substrate specificity depends on the 67 kDa Fbox protein β-TRCP1 and its closely related homologue β-TRCP2. Increased activity of these Fbox proteins could act as constant derepressors of NF-κB activation, e.g. in pancreatic cancer cells.

NF-κB is responsible to the deregulation of many anti-apoptotic factors, including bcl-2, bcl-XL, IAPs, FAP1 (Fas-associated phosphatase 1), cFLIP, DcRs (decoy death receptors). It has been found that although death receptors (TNFR1, TRAILR, Fas) are coexpressed in pancreatic cell lines, the cancer cells remained resistant to apoptosis triggered by these receptors. The resistance is caused mainly by the overexpression of the decoy receptors (DcR2 and 3), and of bcl-XL and bcl-2. The inhibition of bcl-XL dramatically increased the response to some antitumor agents (e.g. Geldanamycin, PS-341, doxorubicin) and TRAIL. Geldanamycin (heat shock protein 90 inhibitor) and PS-341 (Velcade, proteasome inhibitor) synergistically inhibit NF-κB and the AKT pathway, downregulate antiapoptotic agents, as bcl-2, bcl-XL, cIAP-1 and cyclin-D1. It is suggested that a Geldanamycin, PS-341 and TRAIL triple combination could be a novel therapeutic approach for PanC.[14]

Monitoring of PanC progression

For diagnostic purposes the best serum markers are CA19-9, CA24-2, CA51 and CA72-4, while AFP and CEA are much less sensitive and specific.[15] Another study on 160 patients found that hCG and CA72-4 are the most useful serum prognosticators of PanC.[16] It is known that PanC is frequently associated with thrombotic complications. That is why thrombocytosis is a very good clinical marker. Retrospective analysis of PanC patients indicated that the average survival of thrombocytotic patients is much less than that of patients without this symptome.[17] Although the genetic background of this phenomenon is unknown, a recently identified megakaryocytic mimicry (ectopic expression of megakaryocytic genes) may explain the increased number of platelets.[18]

Genomics of PanC progression

Pancreatic adenocarcinoma has been characterized by a 120-gene signature containing the metastasis suppressor NME4, the apoptosis regulator galectin-3, the metastasis-associated S100P and S100A6/11, as well as the basement membrane proteoglycan, perlecan and the β4 integrin. The signature contains proteolytic systems too, as uPA/uPAR and the inhibitor, Maspin.[19] It is extremely interesting that the gene signature of the progressing PanC is entirely different from this: it contains several matrix proteins (chondroitin sulfate proteoglycan, lumican, collagen I and IV and osteonectin), the integrin α5, and MMP-2, -11 and -14 metalloproteases. The loss of cell adhesion molecules E- and N-cadherin is associated with a change in β-catenin function, and with an increased risk for lymphatic and hepatic metastatization.[20] It is of note that in progressing PanC the expression of inducible adhesion molecules (e.g. ICAM1 and VCAM1) is upregulated. The only overlapping signature between primary and progressing PanC is uPA/uPAR which is a marker of poor prognosis.[21]

Classical progression markers

Proliferation markers such as DNA ploidy, S-phase fraction, or Ki-67 labeling index are not useful in PanC.[22] However, the protein expression of the tumor suppressor, p27, was proved to be a marker of favorable prognosis, based on a cohort of 60 patients,[23] and supported by another larger cohort as well.[24] Pancreatic cancers frequently produce significant amount of mucin and express MUC5A and MUC6 genes. While overexpression of MUC5A is associated with increased risk of lymphatic and hematogenous dissemination, overexpression of MUC6 supported longer survival, suggesting that the MUC genes are involved in the regulation of the progression of PanC.[25] PanC may express EGFR and HER2. Immunohistochemistry indicated that EGFR could be found at the cell membrane in the intraductal component (non-invasive form), while it became cytoplasmic in the invasive part of the cancer. Furthermore, cytoplasmic expression of EGFR is more pronounced in grade 3 tumors, and is associated to poor prognosis.[26] Expression of HER2 alone is not a sensitive marker of prognosis in PanC, but together with AKT activation it could be responsible for dysregulation of the apoptotic machinery.[27] Beside „conventional" proteases, some others are also involved in the progression of PanC. It was shown that expression of ADAM9, a membrane protease with dysintegrin function, is associated with an unfavorable prognosis.[28]

Host reactions in progressing PanC did not attracted much attention. In a study analyzing tumor infiltrating lymphoid subsets, the presence of intratumoral $CD4^+$ and $CD8^+$ T-cells was connected to better prognosis.[29]

Tumor-stroma relationships in pancreatic cancer

Excessive production of connective tissue (desmoplastic reaction) is a common finding in pancreatic adenocarcinoma. *In vitro* and *in vivo* studies have provided evidence that the main source of fibrogenesis in pancreas is the pancreatic stellate cell (PSC) distributed in periacinar location. These myofibroblast-like cells share some similarities with the hepatic Ito cells: they can store vitamin A, express α-smooth muscle actin and GFAP, and (upon activation) produce extracellular matrix (ECM) components such as collagen I, III, fibronectin, or laminin. Many different cytokines were shown to activate the quiescent cells: platelet-derived growth factor (PDGF), TGF-α, TGF-β1, activin A, basic fibroblast growth factor, tumor necrosis factor-α (TNF-α), or interleukins (IL-1, IL-6). Interestingly enough, a positive feedback was observed, because PSCs themselves are able to produce TGF-β1, activin A, or IL-1.[30] Recent studies have shown that the interaction between the carcinoma cells and the stromal components directs gene expression and determines the spreading of the tumor. Moreover, the altered microenvironment may partly be responsible also for the acquired drug resistance.[31] Several extracellular matrix proteins (galectin-1, TM2) are highly expressed in pancreatic cancer.[9] Galectin-1, for example, is absent in the normal pancreas and chronic pancreatitis cases, and seems to regulate the ras-signaling pathway upon activating H-ras and K-ras in their GTP-bound state.[32] Thus, galectin-1 may serve as a new diagnostic marker or a therapeutic target.

Angiogenesis

Hypoxia can exist in a significant portion of PanCs (25-90%), inducing metabolic changes and activating angiogenesis. A characteristic feature of PanC is the overexpression of HIF1α gene and protein, and the stimulation of the respective target genes (e.g. VEGF and IL-8).[33] In the hypoxic PanC tissue expression of DEC1, hexokinase II and phospho-glucose isomerase (autocrine motility factor, AMF) can also be detected.[34] AMF and its cognate receptor, gp78/AMFR are involved in the progression of several cancer types and thought to be responsible for the autocrine regulation of cancer cell motility in melanoma, squamous cell-, urinary bladder- and prostate cancers.[35] It is a question whether this pathway is under the control of the HIF1α. PanCs are characterized by a relatively heterogeneous density of microvessels. The high density of vessels is frequently associated with the risk of hepatic metastases and poor survival.[36] The dominant angiogenic cytokine of PanC is VEGF-A. Expression of bFGF is also relatively frequent in PanC but its expression alone is not a prognostic factor.[37] PanC often expresses VEGFR1 and VEGFR2, suggesting the formation of a unique autocrine mitotic regulatory loop, which could be a target for novel antiangiogenic therapy. The observation that VEGF is mitogenic not only for endothelial cells but for PanC cells as well supports this notion.[34] Intratumoral blood vessels of PanC may express activation markers such as aminopeptidase/CD13. Interestingly, the presence of this activation marker on tumoral blood vessels is associated with microvessel density and proved to be a marker of poor prognosis.[38] There is a continuously growing list of natural negative regulators of angiogenesis, one of these agents being PEDF (pigment epithelium-derived factor). Around 25% of PanC are positive for angiosuppressor PEDF, which is associated with lower microvessel density and longer survival, suggesting the importance of the net outcome of positive and negative angiogenesis regulatory factors in a given cancer in respect to the intensity of neoangiogenesis, and its effect on prognosis.[39]

Conclusion

Extensive genomic analysis of PanC revealed numerous genetic alterations, however, none of them emerged yet as a key regulator in the development and progression of this cancer type. Therefore, it is still a hard challenge how these genetic changes can be related to the classical biological and clinical features of PanC. With the improvement of our understanding, more and more genetic alterations could be considered as diagnostic or therapeutic targets. The reality of this approach is firmly supported by results of the introduction of such therapies in an increasing number of malignancies (e.g. CML, GIST, NSCLC).

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