The Association Between Inflammation and Angiogenesis in Human Pancreatic Adenocarcinoma

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ABSTRACT

Pancreatic cancer represents one of the deadliest cancers with a high mortality rate, characterized by an aggressive local invasion and early metastases. This malignancy is poorly responsive to chemotherapy or chemo-radiotherapy. Several proangiogenic factors were associated with pancreatic carcinogenesis, highlighting the importance of using antiangiogenic agents as therapeutic strategy. Further studies suggest the involvement of chronic inflammation in angiogenesis processes in pancreatic carcinogenesis and inflammatory cytokines should be considered as potential biomarkers, as well as therapeutic targets in pancreatic cancer patients.

Key words: pancreatic cancer, angiogenesis, cytokines, therapy

INTRODUCTION

Pancreatic cancer represents one of the most aggressive and lethal malignancies, having an incidence closely equal to the mortality rate. This poor prognosis is mainly owed to the advanced stage of disease at the time of diagnosis. Most patients with pancreatic cancer present local invasion or micro-metastatic tumor cells at presentation (1-3). 337,872 new pancreatic cancer cases and 330,391 deaths were estimated in 2012 worldwide (4). Gemcitabine, the first-line chemotherapeutic drug for locally advanced or metastatic pancreatic cancer, improves the quality of life and prolongs survival by 5-6 months (5).

Recently completed clinical trials investigated new possible chemotherapeutic alternatives which might improve the overall survival. Some of these drugs - used alone or in combination are already approved agents for metastatic pancreatic cancer treatment: Erlotinib (a reversible tyrosine kinase inhibitor), MM-398 (nanoliposome-encapsulated irinotecan), Abraxane (protein-bound paclitaxel-amitotic inhibitor) used in combination with Gemcitabine and FLOFIRINOX (a combination of 4 drugs-oxaliplatin,

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irinotecan, fluorouracil, and leucovorin) (6-8).

Most pancreatic tumors are located in the exocrine pancreas, with pancreatic ductal adenocarcinoma (PDAC) representing approximately 95% of pancreatic cancers. The main risk factors for pancreatic cancer are smoking, type 2 diabetes mellitus, obesity and pancreatitis, and family history (9). Chronic inflammatory conditions mediated by pro-inflammatory cytokines and their receptors, reactive oxygen species, up-regulated pro-inflammatory pathways, and immune cell infiltrates are associated with pancreatic carcinogenesis (1).

Even though many studies were focused on molecular characterization of PDAC trying to identify specific therapeutic targets, the best therapeutic options for these patients are surgery and cytotoxic therapies. Nevertheless, sustained by several factors, such as tumor microenvironment, hypoxia, post-transcriptional gene regulation and somatic mutations, PDAC cells exhibit chemo-resistance (10).

ANGIOGENESIS AND INFLAMMATORY FACTORS

One of the most significant therapeutic approaches is the use of antiangiogenic agents, but even if the majority of PDAC express high levels of proangiogenic factors, the tumor vasculature is abnormal and poorly perfused with blood, restricting the chemotherapeutic penetration (11). Compared to other solid tumors, pancreatic cancers are poorly vascularized and this particularity might explain their aggressiveness and resistance to chemotherapy (12).

However, the neoangiogenesis process is amplified in pancreatic cancers. Intratumoral microvessel density is increased and seems to be an independent prognostic factor for survival in pancreatic cancer patients. Several proangiogenic factors were associated with pancreatic carcinogenesis, including EGFR (epidermal growth factor receptor), VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor) and IL-8 (13).

Angiogenesis represents a critical process for tumor development, being regulated by several different growth factors. VEGF (VEGF-A), VEGF-B, VEGF-C and VEGF-D, VEGF-F, PlGF (placental growth factor), and their receptors VEGFR-1, VEGFR-2 and VEGFR-3 are the most important angiogenic growth factors, involved in angioblast differentiation and tube formation. Expression level of VEGF represents an important marker for the detection of angiogenic diseases. VEGF aberrant expression was identified in most types of digestive cancers, including pancreatic cancer, being considered a diagnostic marker and a poor prognostic

factor of the disease (14). *In vitro* studies demonstrated that VEGF stimulation led to a metabolic transition from mitochondrial oxidative phosphorylation to glycolysis in pancreatic cancer cell lines via HIF1 α up-regulation (15).

EGFR signaling pathway plays a critical role in pancreatic cancer development; several combinations of EGFR inhibitors with other agents resulted in the inhibition of angiogenesis and cell growth. For example, combination of erlotinib with gemcitabine presented statistical significance in overall-survival (16). Moreover, inhibition of VEGF/EGFR pathway seems to reverse radio-resistance (17).

A recent study of Khan et al. revealed an indirect effect of gemcitabine treatment on angiogenesis; it demonstrated that Gemcitabine induces expression of angiogenesis-associated cytokines in pancreatic tumor cells, among which IL-8 proved the highest levels of induction. The up-regulation mechanism of IL-8 transcription in pancreatic cancer cells involves activation of NF- κ B and HIF-1 α ; IL-8 determines endothelial cell proliferation, survival, migration and angiogenesis (18).

Previous studies evaluated circulating concentration of two proangiogenic factors VEGF and bFGF in pancreatic cancer patients and healthy controls (19-22). Increased serum levels of VEGF and bFGF in cancer patients significantly correlate with tumor diameter, stage of disease, standard proliferative marker, Ki67, and conventional markers CA 19-9 and CEA. A possible explanation for the simultaneous increase of these two molecules could be their closely related downstream signaling pathways. EGFR pathway activation increases the production of angiogenic proteins in tumor cells VEGF and bFGF (23,24). Although they are not specific to pancreatic cancer, soluble VEGF and bFGF might be included in the pancreatic cancer biomarker panel as a valuable tool for detection of early recurrence and monitoring of therapy. In an attempt to find a relevant serum marker/set of markers for pancreatic cancer, several other angiogenic molecule and growth factors have been proposed. Among them EGFR (25), IL-8 (26), the ratio of VEGF /VEGFR-1 (27), TGF- β (28) and MIC-1 (29,30) showed a significant association with pancreatic cancer. Extensive research have been performed regarding the local/tissue biomarkers: angiogenic factors [e.g. EGF, VEGF, heparanase, cathepsins (31,32)], matrix metalloproteinase 7, fibroblast activation protein (33), caveolin-1 (34,35) are only a few proteins whose increased expression in pancreatic cancer tissue correlates with an unfavorable prognostic.

Recent studies reported increased circulating levels of proangiogenic factors VEGF and bFGF as potential prognostic biomarkers in PDAC patients compared with

healthy individuals. bFGF, VEGF, PDGF-A, and Ang-1 levels were also associated with lymph node metastasis in PDAC (22,36).

Nevertheless, clinical trials on bevacizumab, a monoclonal antibody against VEGF and Cetuximab, a monoclonal antibody against EGFR, failed to establish a survival advantage of anti-angiogenic therapy for patients with pancreatic cancer. Inflammatory factor overexpression seems to represent one of the mechanisms by which pancreatic tumors progress and become refractory to cytotoxic and antiangiogenic therapies (fig. 1)(36). Recent studies reported a significant correlation between pro-inflammatory cytokines IL-6, TNF- α , IL-1 β , IL-10 and serum concentration of VEGF and bFGF. IL-6, IL-1 β , and TNF- α levels were also significantly correlated with circulating levels of CA 19-9 while IL-8, IL-10, TNF- α levels were significantly correlated with CEA levels in the patients with PDAC. Tumor volume was significantly correlated with IL-1 β , IL-10 and VEGF levels; all the inflammatory markers analyzed, as well as VEGF and bFGF levels, were correlated with tumor stage (1). Another biomarker with potential prognostic value in PDAC patients seems to be circulating IL-8. IL-8 levels were significant associated with worse survival, being linked with carcinogenesis by promotion of angiogenesis and metastasis, *via* mitogen-activated protein kinase pathway; IL-8 could be considered a potential target for combination therapy in PDAC (37-39). TNF- α level was found to be increased in the sera of patients with PDAC compared with those of healthy individuals. Moreover, elevated serum levels of TNF- α were correlated with tumor grade and overall survival in patients with PDAC, probably facilitating recurrent tumor growth (40). TNF- α is also involved in metastasis process, serum TNF- α being elevated in patients with metastatic PDAC compared with non-metastatic disease (41). Interestingly, anti-TNF- α treatment (Infliximab) resulted in a decreased serum VEGF concentration in patients with rheumatoid arthritis (42). In PDAC patients a positive correlation between VEGF and bFGF serum levels was identified; these results suggest that future therapies can target TNF- α in combination with other treatments in order to delay tumor progression in PDAC (1). Serum IL-1 β was also associated with circulating VEGF and bFGF levels in PDAC patients, acting as promoter of tumor angiogenesis, invasiveness and metastasis (43).

Many studies reported the potential role of inflammatory cytokines in tumor resistance to therapy suggesting that blocking cytokines may sustain the efficacy of anticancer agents (44). Therefore, the levels of many inflammatory cytokines are significantly increased

Figure 1 - Interrelationship between tumor microenvironment and tumor angiogenesis

in the circulation of patients with PDAC. Moreover, the concentrations of inflammatory cytokines are associated with proangiogenic factor levels and both are correlated with poor outcome in PDAC patients (45). An increasing number of studies sustain that inflammatory cytokines can directly promote angiogenesis, as well as induce expression of proangiogenic factors in PDAC (39,46).

FUTURE PERSPECTIVES

Angiogenesis has a significant contribution in pancreatic cancer aggressiveness; evaluation of inflammatory cytokine levels combined with angiogenic biomarkers may improve early diagnosis performance, may predict recurrence and survival rate and also tailor the management of personalized therapy in pancreatic cancer patients.

Author's contribution

All authors have contributed equally to this paper.

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