

Role of Fibroblast Growth Factor Receptor 2 in Pancreatic Cancer: Potential Target for New Therapeutic Approach?

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Abstract

Fibroblast growth factors and their receptors play a key role in cell proliferation, migration and differentiation. Fibroblast growth factor receptor 2 (FGFR2) is involved in carcinogenesis and its altered expression has been shown in several tumors, such as breast, thyroid and pancreatic cancer. The two isoforms of FGFR2 gene, FGFR2-IIIb (also known as KGF) and FGFR2-IIIc have been shown to exert differential roles in pancreatic cancer. FGFR2-IIIc supports pancreatic cell proliferation, while overexpression of FGFR2-IIIb is correlated to major invasion and metastasis formation. This review focuses on the role of FGFR2 signaling in pancreatic adenocarcinoma and the potential use of FGFR2 tissutal expression as a predictive and/or prognostic marker. Moreover, it will discuss about the potential use of strategies for FGFR2 signaling inhibition in the treatment of pancreatic cancer.

Keywords: Pancreatic adenocarcinoma; FGFR2; FGFR2-iiiib; FGFR2-Iiic; Therapeutic strategies

Introduction

FGFRs are a family of tyrosine kinase receptors consisting of four members (FGFR1-4). These receptors are characterized by two or three immunoglobulin-like extracellular domains, an intracellular tyrosine kinase domain, and a carboxyl-terminus domain [1]. They are involved in regulating cell proliferation, migration and differentiation during vertebrate development, as well as in response to injury and tissue repair [2]. In this review, we focus our attention on the FGFR2 gene, which is subjected to an alternative splicing that generates two isoforms, FGFR2-IIIb, predominantly expressed on epithelial cells, and FGFR2-IIIc, detected in mesenchymal cells. FGFR2-IIIb has a role in the control of epithelial growth and differentiation, while FGFR2-IIIc receptor is involved in the proliferation of mesenchymal cells. The two isoforms show a different affinity for ligands; in particular, FGFR2-IIIb isoform binds with high affinity to KGF (also known as FGF7), FGF10 and FGF22, and with low affinity to FGF1 and FGF3, while the FGFR2-IIIc isoform binds to FGF1, FGF2, FGF4, FGF5, FGF6, FGF8, FGF9, FGF16, FGF17, FGF18, FGF20, FGF21 and FGF23 [3].

Some studies proposed that a shift between splicing isoforms may be involved in tumor progression. For example, a switch between FGFR2-IIIb and FGFR2-IIIc isoform in rat prostate and bladder cancer models might contribute to alter the balance between epithelial and mesenchymal cells and to promote epithelial-mesenchymal transition [4,5]. However, FGFR2-IIIb isoform re-expression in prostate and bladder cancer cell lines resulted in growth suppression *in vitro* and in decreased tumor formation *in vivo* [6-8].

Up- or down-modulation of one or both FGFR2 isoforms has been shown in several types of cancer, for example in thyroid, breast, lung, stomach and pancreas cancer [9-12]. Genetic mutations of FGFR2 have been also observed, and they are correlated with cancer

susceptibility or poor prognosis. In breast cancer, single nucleotide polymorphisms (SNPs) located within intron 2 of FGFR2 gene have been associated to increased risk of cancer [13,14]. Missense mutations of FGFR2 have been observed in breast, gastric and lung cancer. Some of these mutations can alter ligand-receptor specificity or determine a ligand-independent FGFR2 activation [15]. In the present work, we discuss the role of FGFR2 signaling pathway in pancreatic cancer and the potential use of FGFR2 inhibitors as anti-cancer therapies.

Role of FGFR2 in Pancreatic Adenocarcinoma

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive human malignancy with high mortalities among all malignancies [16]. Several growth factors and their receptors are overexpressed in PDAC, such as transforming growth factor- α , epidermal growth factor receptor, FGFs, and FGFRs [17]. Several FGFs are overexpressed in pancreatic cancer [18] and their altered expression has been linked to increased pancreatic cancer cell motility, proliferation, invasion and metastatic potential [19,20]. Expression levels of FGFR1 and FGFR2 have been reported to be involved in pancreatic cancer development. FGFR1 overexpression is implicated in growth of pancreatic cancer cells [21,22], while increase of FGFR2 phosphorylation is linked with enhanced pancreatic cell proliferation, invasion and tumor angiogenesis [23].

Differential expression of FGFR2-IIIb and its ligand has been shown in different pancreatic cell types, such as acinar, ductal and islet cells [24]. We also confirmed these results by immunohistochemical staining on a tissue array of 40 cases of ductal adenocarcinoma, grade 1-3, with a home-made monoclonal antibody against FGFR2-IIIb [25]. Furthermore, several studies show concomitant overexpression of FGFR2-IIIb and its ligand KGF in PDAC [26,27], which is correlated with high expression of vascular endothelial growth factor-A (VEGF-A), venous invasion, and poor prognosis [28]. In metastatic pancreatic cancer cell lines, the upregulation of KGF secretion is linked with pancreatic progression and metastatic formation, suggesting that the

altered expression of KGF and FGFR2-IIIb may be involved in early signals in the progression of pancreatic cancer [29].

As previously reported, normal pancreatic cells lack FGFR2-IIIC, while enhanced expression of the latter has been detected in pancreatic cancer cell lines and PDAC tissues, boosting proliferative ability *in vivo* [30].

Several studies have shown a correlation between FGFR2 gene amplification or single nucleotide polymorphism in intron 2 with cancer progression [11,12,31]. FGFR2 amplification has been associated to poorly differentiate gastric cancer [32]. Notably, an amplification of FGFR2 was also detected in pancreatic cancer based on cell line and xenograft aberration profiles originated from genome-wide analysis [33]. Most importantly, FGFR2 inhibition by means of specific shRNA, leads to cell proliferation, migration and invasion [34].

Soluble factors released from tumor stroma have been shown to play a role in tumor growth. For example, cell-derived factor-1 (SDF-1/CXCL12) released by fibroblasts can promote cancer cells proliferation, while CXCR4 is released in several types of malignancies, such as breast cancer [35] and pancreatic cancer [36,37]. Also FGF10, produced by fibroblasts, has been demonstrated to induce cell migration and invasion in pancreatic cancer cells [19], while FGF7 is able to promote pancreatic cell proliferation. Nomura et al., have investigated the role of FGF10/FGFR2 pathway in pancreatic cancer. Their results demonstrated that FGF10 induces cell migration and invasion, thus influencing the expression of genes related to cell mobility, such as membrane type 1-matrix metalloproteinase (MT1-MMP) and transforming growth factor beta (TGF- β 1). In particular, mRNA expression of MT1-MMP and TGF- β 1 were upregulated by FGF10 in CFPAC-1 cells.

FGF/FGFR Signaling as a Therapeutic Target

Pancreatic adenocarcinoma is characterized by high mortality, incidence of metastatic disease and rapid progression. It is very difficult to obtain an early diagnosis, and in the majority of cases at the time of diagnosis patients just show several metastases, with an unfavorable prognosis. Therefore, new therapies for pancreatic cancer are extremely necessary. To detect new molecular mechanisms at the basis of the onset and progression of this cancer might improve its diagnosis and therapy.

Growth factors and their receptors have a role in several events that occur in tumorigenesis [38]. Since FGFR, in particular, is involved in cancer progression and invasion, its signaling may represent a therapeutic target for anticancer therapy. Several therapeutical approaches against FGFR are now available. Small molecular tyrosine kinase inhibitors with anti-angiogenic activity against VEGFR and PDGFR are currently in preclinical or clinical trial. Dovitinib (TKI-258), an inhibitor of FGFR1, FGFR2 and FGFR3, showed antitumor activity in FGFR-amplified breast cancer [39]. This inhibitor has been also evaluated in bladder cancer and relapsed multiple myeloma cases. An alternative therapeutical strategy is based on the use of neutralizing antibodies. This method promises a high specificity against the molecule of interest, thus allowing the development of targeted therapies. Some of these monoclonal antibodies, just approved by FDA, are nowadays used as anticancer monotherapy or in combination with standard chemotherapy. For example, anti-EGFR antibodies, such as Cetuximab and Panitumumab, are now used in the treatment of colon cancer, while the anti-HER2 antibody Trastuzumab is currently administered to breast cancer and gastric cancer patients

in combination with chemotherapy. Also FGFs and FGFRs became a relevant target for the development of specific neutralizing antibodies. Specific antibodies against FGFR1/FGFR3 were tested in bladder and myeloma cancer with successful results [40,41]. Another approach consists in the use of FGF ligand traps that prevent FGF binding with its receptor.

FGFR2 has a key role in the establishment of cancer, so it represents a potential target for cancer treatment. Monoclonal antibodies for this receptor have been produced. Bai et al., created a specific antibody against FGFR2-IIIb isoform that is able to suppress ligand induced phosphorylation [42]. As concerning pancreatic cancer therapy, the use of monoclonal antibodies against growth factors has been evaluated. Phase I clinical trials showed the feasibility of Panitumumab, a monoclonal antibody against epidermal growth factor (EGFR), for the treatment of patients with locally advanced pancreatic cancer (LAPC). The combined use of panitumumab, at a maximum tolerated dose of 1.5 mg/kg, with gemcitabine-based chemoradiotherapy, can be safely and effectively used in LAPC patients [43]. In PDAC, not only cancer cells but also tumor microenvironment contributes to the initiation, progression, and metastasis of tumor. Therefore, pancreatic cancer is extremely complex, and the use of single monoclonal antibody therapy is not a successful therapeutic strategy. Nevertheless, the discovery of new predictive and response biomarkers is necessary to develop new therapeutic approaches. In light of these considerations, FGFR2 might be a marker for pancreatic cancer and its investigation is needed to evaluate its potential use as a target for therapy.

Limitations of anti-FGFR2 Therapeutic Approaches

FGFR2 plays a relevant role in pancreatic cancer and it might represent a potential marker for new therapeutic strategies. In particular, in this review we focused our attention on FGFR2-IIIb/KGF signaling, which is involved in pancreatic cancer progression, invasion and metastasis formation. A therapeutic approach against FGFR2-IIIb isoform could be useful, but the lack of highly specific drug against FGFR2-IIIb is the major limitation of this approach. In fact, the use of pan-FGFR inhibitors might influence also other signaling. Nowadays, clinical trials on tyrosine kinase inhibitors direct against FGFR1/2/3 are available, but these inhibitors do not target all activating mutation. Therefore, studies to determine which aberration plays a role in tumorigenesis are required in order to understand the implication of genomic alteration.

GP369 is the only antibody used *in vitro* and *in vivo* to suppress IIIb isoform in amplified human breast and gastric cancer [42]. This antibody is efficient only in cancers with FGFR2 amplification, so it would be necessary to evaluate FGFR2-IIIb expression and type of mutation before administration of FGFR2-IIIb inhibitor to pancreatic cancer patients. Therefore, FGFR2 evaluation should be introduced in routine diagnosis. For this reason, the use of therapeutic approach base on FGFR2 might have several limitations due to the need for expensive genetic analyses.

Conclusions

Pancreatic adenocarcinoma is characterized by high mortality and poor prognosis due late diagnosis. The lack of predictive and prognostic markers supports the aggressive nature of pancreatic tumors. Several growth factors and their receptors have been shown to be involved in cancer onset and progression, and the investigation of

their potential as predictive and prognostic markers might be useful to ameliorate cancer diagnosis.

FGFRs family has an important role during physiological and pathological processes, and also in carcinogenesis. FGFR2 altered expression was observed in different types of cancer, including pancreatic ductal adenocarcinoma. The overexpression of this receptor is associated to major invasion, cell proliferation and metastasis formation; therefore FGFR2 up regulation might be considered an early step in the development of pancreatic cancer. For this reason, FGFR2 might represent a potential marker for diagnostic and prognostic evaluation of pancreatic cancer.

The identification of novel molecules involved in pancreatic tumor progression might result in the development of promising therapeutic strategies, thus increasing the available anticancer agents and treatment opportunities. Nowadays, different therapeutic approaches have been produced against FGFR2, and the current information about the relationship between this receptor and pancreatic cancer clearly indicate that the inhibition of this signalling pathway could find an application also in the treatment of PDAC.

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