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REVIEW

Progress in researches about focal adhesion kinase in gastrointestinal tract

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Abstract

Focal adhesion kinase (FAK) is a 125-kDa non-receptor protein tyrosine. Growth factors or the clustering of integrins facilitate the rapid phosphorylation of FAK at Tyr-397 and this in turn recruits Src-family protein tyrosine kinases, resulting in the phosphorylation of Tyr-576 and Tyr-577 in the FAK activation loop and full catalytic FAK activation. FAK plays a critical role in the biological processes of normal and cancer cells including the gastrointestinal tract. FAK also plays an important role in the restitution, cell survival and apoptosis and carcinogenesis of the gastrointestinal tract. FAK is over-expressed in cancer cells and its over-expression and elevated activities are associated with motility and invasion of cancer cells. FAK has been proposed as a potential target in cancer therapy. Small molecule inhibitors effectively inhibit the kinase activity of FAK and show a potent inhibitory effect for the proliferation and migration of tumor cells, indicating a high potential for application in cancer therapy.

Key words: Focal adhesion kinase; Restitution; Survival and apoptosis; Cancer; Inhibitor

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INTRODUCTION

Focal adhesion kinase (FAK) is a 125-kDa non-receptor protein tyrosine which was originally identified in chicken embryo cells transformed by v-Src[1] and BALB/c3T3 fibroblasts[2] and was shown to localize in focal adhesions as well. FAK is a non-receptor and non-membrane associated protein tyrosine kinase (PTK), which does not contain Src homology2 (SH2) or SH3 protein interaction domains[3]. FAK contains three main domains: a centrally located catalytic kinase domain, a large N-terminal domain comprising the FERM (FAK, ezrin, radixin, moesin) region and a C-terminal domain harboring the focal adhesion targeting[3-5]. Growth factors or the clustering of integrins facilitate the rapid phosphorylation of FAK at Tyr-397 in adherent cells and this in turn recruits Src-family PTKs, resulting in the phosphorylation of Tyr-576 and Tyr-577 in the FAK activation loop and full catalytic FAK activation[3,5]; while extracellular pressure can activate the FAK in suspended cells[6,7].

FAK is associated with gastrointestinal diseases, here we will review the progresses which have been made in the researches about FAK in the gastrointestinal tract. Research data shows that FAK plays an important role in the restitution, cell survival and apoptosis and carcinogenesis of the gastrointestinal tract. Due to the crucial role of FAK in intergrin-mediated signal transduction, which affects the regulation of cell survival, proliferation, spreading and migration, FAK has been proposed to be a potential target in cancer therapy. Antisense oligonucleotides, the entire C-terminal, non-catalytic domain of FAK (FAK related non-kinase-FRNK), SiRNA

and small molecule inhibitors can affect and inhibit the activities and expression of FAK in various tumor cells. Small molecule inhibitors targeting FAK have been developed as potential cancer treatment modalities. PF-573228, PF-562271 and NVP-226 (TAE226) have already shown potent inhibitory effect for tumor cell growth *in vitro* and *in vivo*.

RESTITUTION

After intestinal superficial mucosal injuries such as erosion, ulcerations, inflammatory bowel disease and infection, the repair of epithelial injury in the gastrointestinal tract begins in a process known as restitution[8]. The restitution is established through migration of viable epithelial cells from areas adjacent to or just beneath the injured surface to cover the denuded area, independent of cell proliferation and regulated by cytokines and growth factors[9-14]. Intestinal epithelial migration, proliferation and differentiation are essential to restitution[15]. FAK has been indicated to be involved in the integrin signaling which regulates the migration, proliferation and differentiation of various normal and cancer cells[16]. Correspondingly, FAK plays an important role in the mucosal restitution of the intestine.

It is well established that intestinal epithelial cells undertake a specialized phenotype adapted to motility and mucosal healing during mucosal restitution and FAK is involved in the cell signaling which regulates the intestinal epithelial migratory phenotype[17]. The disruption of actin stress fiber formation with reduced tyrosine phosphorylation of FAK and FAK in focal adhesions can suppress the repair of gastric mucosal injury and ulcer healing[18-20]. FAK plays a critical role in lysophosphatidic acid (LPA)-induced migration, lamellipodia formation and assembly of focal adhesions in intestinal epithelial cells[21,22].

The expression and activation levels of FAK protein are linked to phenotypic changes which affect cell differentiation, function, adhesion and migration in various tissues[23-29]. Activated FAK^{397} levels vary with differentiation and cell migration in Caco-2 and HT-29 human colon cancer cells[30]. The expression level of activated FAK is related to gastric wound healing *in vivo*[19,31]. Intestinal epithelial cell motility regulates FAK protein abundance at the mRNA level in both human Caco-2 and rat non-transformed IEC-6 intestinal epithelial cells[32]. It has been shown that immunoreactivity to FAK is decreased in cells migrating across matrix protein compared to static Caco-2 cells[33] and immunoreactivity to FAK and FAK^{397} were lower in epithelial cells at the migrating edge of the ulcer[34].

FAK mediates the mitogenic response to repetitive deformation in intestinal epithelial cells. Two deformation-activated signal pathways that converge upon FAK have been proposed: one is Src- and Rac1-independent-which stimulates FAK-Tyr397 phosphorylation, and the other is Src- and Rac1-dependent, which is required to further activate FAK by phosphorylation at FAK-Tyr576 (within the FAK kinase activation loop)[28]. Repetitive deformation stimulates intestinal epithelial motility across fibronectin, which requires both Src activation and a novel Src-independent FAK-Tyr 925-dependent pathway activating extracellular signal-related kinase (ERK)[29]. Smad3-dependent disruption of the transforming growth factor-β (TGF-β) signaling pathway impairs the healing of murine intestinal mucosal ulcers, which is followed by altering patterns of activated FAK and ERK immunoreactivity important for cell migration at the ulcer edge[15].

Recently, the relationship between TGF-β and FAK has been studied. TGF-β was found to enhance FAK protein, mRNA levels and FAK promoter activity in human and rat intestinal epithelial cells[34]. TGF-β also affected the restitution and proliferation partly mediated through its induction of FAK expression[35]. It is considered to play an essential role in embryogenesis, host response to tumors, and the repair response damaging the tissues by immune and non-immune reactions[36].

Taken together, the interaction between inflammatory cells, the extracellular matrix, locally released cytokines and growth factors guarantee efficient ulcer healing[31]. Tissue injury and wound healing spatially and temporally activate several growth factors and extracellular matrix facilitates the rapid phosphorylation of FAK at Tyr-397 and this in turn recruits Src-family PTKs, resulting in the phosphorylation of Tyr-576 and Tyr-577 in the FAK activated loop and other focal adhesive proteins including talin, α-actinin, vinculin, paxillin and p130Cas (Figure 1)[3,5,16,37]. Activated FAK and cell adhesive protein transduct the signal to the Mek/Erk to down-regulate proliferation, differentiation and migration in the process of restitution. However, the detailed mechanism for the role of FAK in the process of restitution is still unknown.

SURVIVAL AND APOPTOSIS

Programmed cell death, or apoptosis, is a complex and tightly regulated process that executes crucial roles in tissue homeostasis and repair[38,39]. It is well established that the Bcl-2 family of proteins plays a major role in cell survival and apoptosis[38,40,41]. Extracellular signals can affect the expression and/or functions of the Bcl-2 family by signaling events to determine if a cell lives or dies[42]. FAK is the canonical mediator of such extracellular signals which originate from intergrin and growth factors[5]. Thus, FAK is related to cell survival and apoptosis in the gastrointestinal epithelium.

The detachment of intestinal epithelial cells from matrix induces apoptosis through the disruption of anti-apoptotic signals transduced by integrin/FAK/Src[43]. Induced FAK suppresses apoptosis by activating nuclear factor κB (NF-κB) signaling in intestinal epithelial cells[44]. FAK inhibition in human intestinal epithelial cells produces anoikis while FAK induction in rat intestinal IEC-6 cells suppresses apoptosis[44,45].

Recent studies in the function of FAK in survival and apoptosis of intestinal epithelial cells have focused on integrin/Fak/Src, PI3K/Akt and MEK/Erk pathways

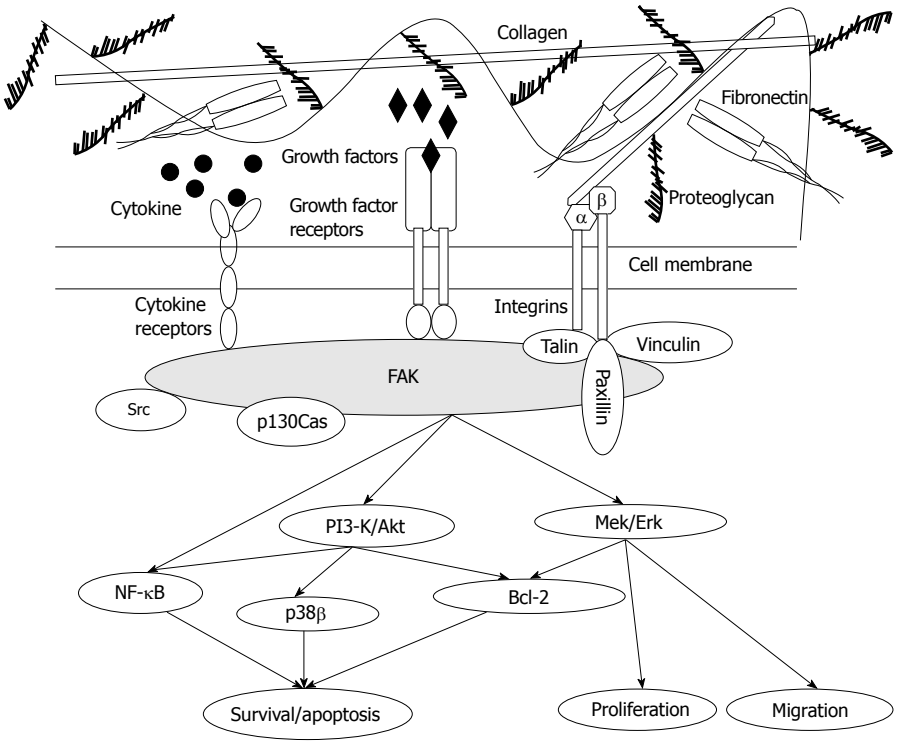

Figure 1 FAK mediates the extracellular signaling to regulate the proliferation, migration and survival/apoptosis of the cells. NF-κB: Nuclear factor κB; FAK: Focal adhesion kinase.

which are all presumed to modulate the expression and function of multiple Bcl-2 homologs[42,44-46]. Many studies showed that integrin/FAK/Src modulate the PI3K/Akt and MEK/Erk pathways individually or in combination in different cell lines[5,42,47-55]. The Bcl-2 family is the central regulator of caspase activation which executed the cell-suicide program and play an anti- or pro-apoptotic role in cell apoptosis[56]. Butyrate-induced apoptosis of Caco-2 cells might occur *via* NF-κB activation together with a defective b1 integrin-FAK-PI3-kinase pathway signaling[47]. A study showed that integrins, FAK, PI3-K/Akt-1, MEK/Erk, and p38 isoforms play distinct roles in the regulation of HIEC-6 cell survival and/or death, accompanied by modulating individual Bcl-2 homologs[46]. β1 integrins/Fak/Src signaling down-regulated PI3-K/Akt-1 and MEK/Erk pathways in the suppression of anoikis, which play a role in the survival of differentiated cells, whereas the PI3-K/Akt-1 pathway is crucial for cell survival regardless of the state of differentiation[45]. β1 integrins/Fak/Src signaling translates into integrated, complex regulatory functions by PI3-K/Akt-1 and MEK/Erk in the expression/activity of Bcl-2 homologs, as well as in the specific activation of the pro-apoptotic p38b SAPK isoform, thus determining their own requirement (or not) in the suppression of HIEC (Human Intestinal Epithelial Crypt) apoptosis/anoikis[42].

Extracellular/Fak/Src signaling down-regulates PI3-K/Akt and Mek/Erk and further regulates the expression and activity of Bcl-2, and finally control the survival and apoptosis. PI3-K/Akt also specifically activates the apoptosis/anoikis driving p38β SAPK, and regulates the survival and apoptosis. Besides, extracellular/Fak/Src signaling has a new pathway to control the survival and apoptosis *via* regulating the NF-κB.

CANCER

FAK is closely associated with cancer. Many studies have shown FAK over-expression in various tumor cells and its expression correlate with increased tumor malignancy. The alteration of FAK function in normal cells causes tumor progression.

FAK has been indicated to over-express at mRNA and protein levels in various tumors including gastrointestinal tumors. As early as in 1993, researchers found increased levels of FAK in 1 of 8 adenomatous tissues, in 17 of 20 invasive tumors, and in all 15 of 15 metastatic tumors, which suggests that FAK over-expression may result in changes in the signaling pathways involved in tumor cell invasion[57]. In human colon cancer cells, increased dosage of the FAK may contribute to the elevated protein expression during conversion from adenoma to carcinoma[58]. Quantitative realtime RT-PCR of gene expression levels in all gastrointestinal stromal tumors (GIST) indicated that FAK was over-expressed in malignant GIST[59]. Immunohistochemical analysis also demonstrated that FAK is over-expressed in colorectal, esophageal, pancreatic and mammary cancers, which indicated that FAK and P-FAK are involved in the carcinogenesis of digestive organs[60,61]. Another research group got similar results *via* immunohistochemistry, which showed that high levels of FAK and Src were predictive for recurrence of colorectal cancer[62]. The FAK expression level might be a valuable marker for the carcinogenesis and progression of some types of carcinoma[63,64].

An increased expression of FAK is associated with the invasive potential of colon and breast tumors[65]. Immunohistochemical analysis of gastric cancer and colorectal cancer showed that the expression of FAK is more significantly associated with carcinogenesis,

differentiation and metastasis, and furthermore FAK may not only be a transformation-linked enzyme but also a progression-linked enzyme[63]. FAK over-expression of esophageal squamous cell carcinoma was related to cell differentiation, tumor invasiveness, and lymph node metastasis[66]. The expression of gastrin-releasing peptide (GRP) and its cognate receptor critically mediates a GRP-dependent phase of cell motility by phosphorylating FAK at multiple specific sites in colon cancer cells[30]. Gastrin can evidently promote invasiveness of Colo320 cells *via* the gastrin-gastrin receptor-FAK signal transduction pathway[67].

Not only the expression level but also the activities of FAK are essential for the motility and invasion of cancer cells. Colon carcinomas exhibited a marked elevation in FAK tyrosine kinase activity and phosphotyrosine content and the catalytic activity of FAK is enhanced by its phosphotyrosine content[68]. The amount of total FAK and FAK phosphorylated at Y397 and Y407 correlates closely with the differentiation of human colon cancers[69]. The migratory phenotype of colon cancer cells is controlled by the combined activities of Src and FAK, and the recruitment of FAK to adhesive sites results in its phosphorylation by Src and other peripheral tyrosine kinases[70].

The over-expression and elevated activities of FAK are associated with motility and invasion of cancer cells, however the exact mechanism is still unknown. Integrinα2/FAK/ERK/μ-calpain signaling pathway plays a critical role in tumor cell motility and these results would cause the interruption of FAK function at the early stages of colon tumorigenesis[71]. In a colon adenocarcinoma, cell proliferation and differentiation can occur concomitantly and these deregulated processes are controlled by autocrine secretion through the ErbB1/ERK1, 2 and FAK pathways[72]. The Cholecystokinin-2 receptor regulates the invasion and motility of colon cancer cells, and supports the role of CCK2R in the progression of colon cancer through the activation of FAK[73]. EGFR pathway substrate 8 could modulate the expression of FAK *via* mTOR/STAT3, which enable the cells to proliferate and migrate[74]. The mechanism of the increasing invasion of colon cancer cells by gastrin17 is probably that gastrin17 makes FAK-Tyr397 phosphorylate and localize to lamellipodia, causing the formation of FAK-Src-p130(Cas)-Dock180 signaling complex when it is bound to its receptor CCK-2 and the activation of Rac[75]. The engagement of α1-integrins with functional molecular scaffolds using FAK/Src and p130Cas/JNK is involved in human colon cancer cell invasion through the induction and activation of the MMP-2 and MMP-9 matrix metalloproteinases[76]. A model has been proposed to indicate how the interaction of FAK and SFKs down-regulate the MAPK/Erk1/2 and PI3K/Akt pathways in the early process of cell adhesion in SW480 colon cancer cells: Integrin engagement induces quick FAK-Y397 autophosphorylation and subsequent translocation of a fraction of FAK in raft compartments, FAK interacts only with Fyn in lipid domains, while it interacts with c-Src and Fyn in non-raft fractions. In parallel, PI3K/Akt signaling is quickly activated which is dependent on lipid domain integrity, while MAPK/Erk1/2 signaling is activated with longer kinetics which is not dependent on lipid domain integrity. Both signaling pathways contribute to the adhesive process of SW480 cells[77].

These data show the strong relationship between the expression and activity level of FAK and the generation and progression of gastrointestinal tumors, however the exact mechanism needs further studies.

Inhibitor

Due to the crucial role of FAK in integrin-mediated signal transduction, which affects the regulation of cell survival, proliferation, spreading and migration, FAK has been considered a potential target in cancer therapy. There are many ways to suppress the activity and expression of FAK, thereby inhibiting the growth of tumor cells. The attenuation of FAK expression *via* antisense oligonucleotides induces detachment and apoptosis in tumor cells[78]. The entire C-terminal, non-catalytic domain of FAK (FAK related non-kinase-FRNK) is autonomously expressed in some cell types, and has been used as a dominant negative mutant to elucidate FAK function[79-82]. Specific short interfering RNA is often used to reduce the expression of FAK. Knockdown of FAK protein through FAK-SiRNA significantly inhibited LPA-induced migration of both IEC-18 and IEC-6 cells[22].

As described earlier in this article, phosphorylation of Tyr-397 at FAK is essential to the phosphorylation of Tyr-576 and Tyr-577 in the FAK activation loop, full catalytic FAK activation, the activity of other adhesive protein and its downstream molecules which all play important roles in integrins or growth factors initiated signaling pathways. So targeting the phosphorylation of FAK seems to be promising for the cancer therapy. Small molecule inhibitors targeting FAK as potential cancer therapies have been developed. Sulindac sulfide (NSAID) and the phenolic antioxidant caffeic acid phenethyl ester were used to reduce the phosphorylation of FAK and cell invasion in human colon carcinoma cells[83]. Butyrate treatment results in a significant down-regulation of c-Src and FAK in human colon cancer cells and finally inhibits tumor growth and invasion[84]. Exposure of HT-29 cells to 10 mmol/L garcinol inhibited cell invasion and decreased the dose-dependent tyrosine phosphorylation of FAK, which suggests that garcinol reduces cell invasion and survival through inhibiting the downstream signaling of FAK[85].

Recently, compounds PF-573228, PF-562271 and NVP-226 (TAE226) have been generated by two groups. These compounds are ATP analogs and effectively inhibit the kinase activity of FAK[86,87]. PF-573228 inhibited phosphorylation of FAK and its downstream effector paxillin, and affected cell migration and adhesion turnover[86]. But PF-573228 had little inhibitory effect on the growth and apoptosis of normal and cancer cells possibly because the FAK kinase activity is not essential for cell growth-proliferation mediated through FAK FERM regulation of p53[88].

PF-562271 is a newly developed diaminopyrimindine-type compound that inhibits FAK and Pyk2 and shows a high degree of selectivity in the inhibition of PTKs[89]. PF-562271 have inhibited the tumor growth of prostate, pancreatic, colon, glioblastoma, and H460 lung xenotropic tumor models[89] and blocked bFGF-stimulated blood vessel angiogenesis as shown in chicken chorioallantoic membrane assays. Low dosage of PF-262271 potently blocked blood vessel sprouting without detectable changes in vascular leakage[88]. The oral administration of PF-562271 suppressed the growth and local spread of intratibial tumors and restored tumor-induced bone loss[90]. The combination of PF-562271 and sunitib could effectively block the growth and recovery of human hepatocellular carcinoma in a rat xenograft model[91]. PF-562271 has since moved to clinical trials, and has shown minimal toxicity along with tumor regression[92].

TAE226 is a novel ATP-competitive tyrosine kinase small-molecule inhibitor designed to target FAK, and can effectively prevent FAK phosphorylation, ERK, S6 ribosomal protein phosphorylation and downstream signal transduction, as determined by decreased AKT. TAE226 inhibits insulin receptor (InsR) and insulin-like growth factor-I receptor (IGF-IR), albeit, 10 fold less potently (IC50 = 44 nmol/L for InsR and IC50 = 140 nmol/L for IGF-IR), and is a potent inhibitor of FAK (IC50 = 5.5 nmol/L)[87,93]. TAE226 was shown to induce apoptosis in breast cancer cell lines[94]. Furthermore, TAE226 can significantly prolong the survival of animals bearing intracranial glioma xenografts and ovarian tumor cells orthotopic implantation[86,95]. TAE226 also showed a potent inhibitory effect of tumor cell growth in gastrointestinal tract. When esophageal adenocarcinoma cells were treated with TAE226, cell proliferation and migration were greatly inhibited with an apparent structural change of actin fiber and a loss of cell adhesion, which suggest that TAE226, a dual tyrosine kinase inhibitor for FAK and IGF-IR, might become a new remedy for Barrett's esophageal adenocarcinoma[96]. Furthermore, TAE226 has shown significant inhibitory effects on mTOR signaling and the esophageal cancer cell growth[97]. TAE226 can effectively suppress the growth of imatinib-resistant GIST cells, indicating its potential application for treating the imatinib-resistant GISTs[98]. So the small molecule inhibitors show a significant promise for cancer therapy.

CONCLUSION

FAK plays a critical role in the biological processes of normal and cancer cells including the gastrointestinal tract. Research data shows that FAK plays an important role in the restitution, cell survival and apoptosis and carcinogenesis of the gastrointestinal tract, however the exact mechanism needs further studies. FAK is over-expressed in cancer cells and over-expression and enhanced activities of FAK are associated with motility and invasion of cancer cells. So FAK has been proposed as a potential target in cancer therapy. Small molecule inhibitors effectively inhibit the kinase activity of FAK and show a potent inhibitory effect in the proliferation and migration of tumor cells, indicating a high potential for future application in cancer therapy.

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