Cancer Biology & Therapy 13:3, 123–129; February 1, 2012; © 2012 Landes Bioscience

Fibroblast activation protein
A potential therapeutic target in cancer

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Keywords: fibroblast activation protein, seprase, serine protease, tumor, stroma, inhibitor, therapy

The concept of targeting antigens selectively expressed on the surface of tumor capillary endothelial cells or in tumor stroma has emerged as a promising strategy for cancer therapeutics. Identification of stromal targets for anticancer therapy and development of selective inhibitors of these targets are of great clinical interest. Fibroblast activation protein (FAP), a member of the serine protease family, selectively expressed in the stromal fibroblasts associated with epithelial cancers, whereas with low or undetectable expression in the resting fibroblasts of normal adult tissues. The proteolytic activity of FAP has been shown to support tumor growth and proliferation, making it a potential target for novel anticancer therapies, such as those by immune-based approaches.

Introduction

The clinical benefits of conventional cancer therapies to induce potent immunity in cancer patients are hampered by the genetic instability of tumor cells, leading to their escape from immune surveillance through generating drug-resistant variants. The growth of solid neoplasms beyond a diameter of 1–2 mm requires a supporting tumor stroma to ensure the supply of nutrients for tumor cells to survive and continuously grow.[1] Thus, immunization against epitopes expressed by the tumor stroma can also potentially suppress tumor growth.

FAP, formerly known as F19 cell surface antigen, is an inducible cell surface glycoprotein originally identified in 1986 in cultured fibroblasts using the monoclonal antibody (mAb) F19.[2] In 1994, the so-called F19 cell surface antigen was renamed the fibroblast activation protein (FAP).[3]

In 1990, FAP was independently identified by a different group as a gelatinase expressed by the aggressive melanoma cell line LOX and was given the name "seprase" for surface expressed protease.[4] Subsequent cloning of FAP and seprase revealed that they are the same cell-surface serine protease.[5] Expression of FAP is highly restricted to cancer-associated fibroblasts. It is not expressed in resting fibroblasts in normal tissue, but can be induced to express in nontransformed, activated stromal fibroblasts. The cancer-specific distribution of FAP makes it an emerging novel therapeutic target in cancer.

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Submitted: 08/30/11; Revised: 11/04/11; Accepted: 11/07/11
http://dx.doi.org/10.4161/cbt.13.3.18696

The Structure and Enzymatic Activity of FAP

The structure of FAP. FAP is a type II transmembrane glycoprotein consisting of 760 amino acids. It belongs to the family of post-proline dipeptidyl aminopeptidase, known as dipeptidyl peptidase-IV activity and/or structure homologs (DASH), which includes the well-studied dipeptidyl peptidase 4 (DPP4 or CD26), as well as DPP2, DPP8 and DPP9. Human FAP gene is located on chromosome 2q23, and shares similar genomic organization and 89% amino-acid-sequence identity, including a perfectly conserved catalytic triad, with the mouse FAP gene, which is also located on chromosome 2.[6] The FAP monomer has five potential N-glycosylation sites, 13 cysteine residues, three segments corresponding to the highly conserved catalytic domains of serine proteases, a hydrophobic transmembrane segment and a short cytoplasmic tail of six amino acids.[7] Homodimerization to form a 170-kDa dimer is necessary for the enzymatic activity of FAP.[8] FAP harbors 48% identical amino acid sequence with the T cell activation antigen CD26 (DPP4). The sequences of FAP and CD26 are most closely related in the putative extracellular domain, especially near the C-terminus.[5] The FAP catalytic triad is composed of residues Ser^{624}, Asp^{702}, and His^{734}, and is located at the interface of the β-propeller and α/β hydrolase domain. The eight bladed β-propeller domain is situated on the top of the catalytic triad and may serve as a gate to selectively filter protein access to the catalytic triad.[9]

Study on the transcriptional regulation of FAP gene shows that EGR1 regulates FAP expression through binding to its promoter. Downregulation of EGR1 results in a significant reduction of endogenous FAP mRNA expression.[10] Further studies are required to determine other transcription factors that may regulate FAP promoter.

Enzymatic activity of FAP. In vitro studies have shown that FAP has both dipeptidyl peptidase activity that removes P2-Pro1 dipeptides from the N-terminus of the substrate and endopeptidase activity against substrates containing a Gly2-Pro1 motif, including a collagenolytic activity capable of degrading gelatin and type I collagen.[11] Both functions utilize a common active site Ser^{624}. Recently, it was reported that a2-antiplasmin is an important physiological substrate of FAP endopeptidase activity.[12] Moreover, neuropeptide Y, B-type natriuretic peptide, substance

P and peptide YY are the most efficiently hydrolysed substrates of FAP dipeptidyl peptidase activity.[13]

Based on its structure, mutations of FAP with compromised protease activities have been engineered, including FAPS624A, which lacks dipeptidyl peptidase and endopeptidase activity, as well as FAPA657S, which retains its dipeptidyl peptidase activity but lacks endopeptidase activity.[14,15] These mutant forms of FAP provide important tools for defining the in vivo relevance of the distinct activities and physiologic substrates of FAP.

Tissue Distribution of FAP

FAP is transiently expressed in some fetal mesenchymal tissues. The presence of FAP protein is normally restricted to endometrial cells. For the endometrium, FAP expression decreases during the midsecretory phase. No expression was found in samples of atrophic endometrium.[15] FAP is also expressed during diseases associated with activated stroma, including wound healing, rheumatoid arthritis, osteoarthritis, cirrhosis and pulmonary fibrosis.[16-20] Thus, the function of FAP in tissue remodeling becomes an intriguing topic. FAP is expressed selectively by cancer-associated fibroblasts (CAFs) and pericytes rather than tumor cells in more than 90% of human epithelial malignancies, including colorectal, ovarian, breast, bladder and lung. Benign and premalignant epithelial tumors, including fibroadenomas and phylloides tumors of the breast and colorectal adenomas, generally lack FAP$^+$ stromal cells.[21] As to bone and soft tissue sarcomas, expression of FAP is clearly independent of the malignant potential of the tumor, and is rather related to the histogenesis of the tumor cells.[22] Based on the highly regulated expression and restricted distribution of FAP, it has been identified as a marker of reactive tumor stromal fibroblasts. Ossama Abbas et al.[23] reported the differentiation of morpheaform/infiltrative basal cell carcinoma from desmoplastic trichoepithelioma using FAP as a marker. They also observed a gradient in the pattern of FAP staining with prominent expression noted in fibroblasts directly surrounding the tumor cells, a more diffusive pattern in the distal part of the peritumoral stroma, and minimal or absent expression in adjacent normal tissue. FAP also serves as a surface protein marker that can define MSCs from bone marrow (BM) cells, raising the possibility that MSCs may be among very few, if any, cell types in the adult human body that express FAP.[24]

A soluble form of FAP lacking the transmembrane domain has recently been discovered in human serum as antiplasmin-cleaving enzyme (APCE).[25] APCE cleaves the Pro12-Asn13 bond of Met-α2AP to a more active form, Asn-α2AP, and thus suppresses fibrinolysis.[26]

FAP in Tumorigenesis and Cancer Progression

FAP as a tumor promoter. FAP is thought to promote tumor cell growth and proliferation. The role of FAP in breast cancer has been investigated using human breast cancer cell lines that naturally express FAP (MDA-MB-435 and MDA-MB-436).[27] Suppression of FAP expression using anti-sense oligonucleotides rendered these cells sensitivity to serum starvation, whereas control transfectants with high levels of FAP expression grew well in the absence of serum. Therefore, breast cancer cells with high FAP levels are less dependent on exogenous serum factors for growth and have gained independence on normal growth regulatory controls. Independence on normal growth regulation is a key characteristic of malignantly transformed cells that distinguishes them from normal cells. It has been reported that injection with pFAP-transfected CT26 cells resulted in a larger average tumor volume than tumors formed from the control cells. This group also constructed a DNA vaccine directed against FAP. This vaccine significantly suppressed primary tumor and pulmonary metastases primarily through CD8$^+$T cell mediated killing in tumor-bearing mice.[28] Moreover, HEK293 cells transfected to constitutively express murine FAP, when xenografted into SCID mice, were 2–4 times more likely to develop tumors and showed a 10- to 40-fold enhancement of tumor growth compared with control transfectants. They also demonstrated inhibition of FAP enzymatic activity with anti-FAP antibodies was associated with growth attenuation of HT-29 xenografts.[29] This indicates that FAP has a potent effect on tumor cell growth. Chen et al.[30] reported that FAP increased the invasion, proliferation and migration of HO-8910PM ovarian cancer cells. In addition, in patients with pancreatic adenocarcinoma, higher FAP expression is associated with worse clinical outcome.[31] Taken together, a role of FAP in mediating tumor growth is becoming clearer.

FAP as a tumor suppressor. In contrast, other studies suggest that FAP has tumor suppressive activity and show that this activity is independent of its enzymatic activity. Elevated expression of FAP in cancer causes dramatic promotion or suppression of tumor growth, depending on the model system investigated.[32] It was observed that expression of FAP, or a catalytic mutant of FAP, decreased the tumorigenicity of mouse melanoma cells in animals and restored contact inhibition and growth factor dependence.[33] A second independent observation from studies of studies of somatic cell hybrids between normal fibroblasts and HeLa carcinoma cells identified FAP as a potential inhibitor of tumorigenesis. FAP was one of eight genes differentially expressed in nontumorigenic hybrid lines; consistent with the hypothesis that FAP is involved in suppressing the tumorigenic phenotype.[34] In addition, the degree of FAP expression in breast cancer stromal cells was associated with a longer survival of patients.[35]

In summary, there is an obvious discrepancy between FAP function in tumor promotion and tumor suppression. Some researchers propose that the factor that determines this must reside in the signaling molecules that are available for interaction with FAP on the cells. Thus, FAP executes its biological functions in a cell-context dependent manner through a combination of its protease activity and its ability to form complexes with other cell-surface molecules. However, the role of FAP in tumor growth and invasion, and the exact molecular mechanisms the enzyme utilizes, still remains largely unknown.

The Role of FAP in the Tumor Microenvironment

Given the obvious discrepancy on FAP function in tumor promotion and tumor suppression, increasing attentions are being

paid to the role of the "tumor microenvironment (TME)." Tumors are composed of heterogeneous populations of cells, including infiltrating inflammatory and immune cells, endothelial cells and mesenchymal-derived smooth muscle cells, pericytes, and CAFs.[36] Stromal cells communicate with each other as well as with cancer cells and immune cells directly through cell contacts and indirectly through paracrine signaling, protease secretion, and modulation of the ECM. This complex communication network is pivotal to a supportive microenvironment for tumorigenesis, angiogenesis, and metastasis. Fibroblasts are one of the most crucial components of the tumor microenvironment, and can promote the growth and invasion of cancer cells through the synthesis, deposition and remodeling of the extracellular matrix (ECM), involved in angiogenesis and deregulation of antitumor immune responses. FAP is emerging as an important factor in the pro-oncogenic function of these stromal cells, although further studies are required to define the mechanisms involved.

Modification of the ECM. Neoplastic cells must attach to adhesion proteins of the ECM, proteolytically degrade the ECM, and migrate to distant sites in order to invade surrounding tissues and ultimately metastasize. The remodeling of ECM and tumor invasion involves profound changes in the secretion of ECM proteins, such as collagens, fibronectin, tenascin, and laminin, as well as ECM protein degradation mediated by specific protease and protease inhibitors, such as urokinase plasminogen activator (uPA) and MMPs. FAP functions as an active serine protease capable of degrading type I collagen, and dipeptidyl-peptidase activity, thus suggesting that FAP could possibly act directly as an ECM-degrading or indirectly as regulatory protease involved in the activation/modification of other ECM-proteases/protease inhibitors. Lee et al.[37] reported that FAP remodels the ECM through modulating protein levels, as well as through increasing levels of fibronectin and collagen fiber organization. FAP-dependent architectural/compositional alterations of the ECM promote tumor invasion along characteristic parallel fiber orientations, as demonstrated by enhanced directionality and velocity of pancreatic cancer cells on FAP$^+$ matrices. This phenotype can be reversed by inhibition of FAP enzymatic activity during matrix production resulting in the disorganization of the ECM and impeded tumor invasion. Wang et al.[38] discovered that overexpression of FAP in the human hepatic stellate cell line LX-2 caused increased migration through ECM proteins and an induction of MMP-2 and adhesion proteins. In invadopodia, FAP associates with $\alpha 3\beta 1$ integrin, DPPIV, MMP-2, membrane-type 1 MMP and uPA.[39-42] The resulting complexes appear to enhance cell invasion by co-operative roles in ECM-degradation and adhesion. The exact natures of the homodimer and heterodimer complexes of FAP are poorly understood. As to astroglial tumors, FAP is highly expressed on the surface of glioma cells and contributes to diffuse glioma invasion through extracellular matrix components. In contrast, siRNA knockdown of FAP in a glioma cell line showed decreased invasion through brain extracellular matrix proteoglycan brevican and denatured collagen.[43] Additionally, William et al.[12] reported that following collagen I cleavage by MMP-1, FAP digests collagen I into smaller peptides, suggesting that FAP synergizes with other proteases to cleave partially degraded or denatured collagen I and III as ECM is excavated. Furthermore, in tumor mouse models FAP depletion increases accumulation of collagen that is not directly cleaved.[44] Waster et al.[45] also reported that UV radiation stimulated FAP-driven migration and invasion in fibroblasts, melanocytes and primary melanoma cells. Another evidence of FAP in cancer metastasis is that a significant correlation between FAP RNA expression and incidence of LN metastases was found in medullary thyroid carcinoma.[46] Interestingly, a recent study reported cancer cells expressing wild type FAP or FAPS624A degrade ECM more extensively, accumulate higher levels of matrix metalloproteinase-9 (MMP-9) in conditioned medium, are more invasive in type I collagen gels, and have altered signaling compared with control transfectants that do not express FAP and form slow growing tumors. Thus, they conclude that the proteolytic activity of FAP participates in matrix degradation, but other functions of the protein stimulate increased tumor growth.[47] Overall, these data reveal a vital role of FAP in ECM remolding.

Involvement in angiogenesis. For tumors to grow and integrate into the surrounding tissue, they must gain a blood supply for sustained growth. The first evidence for a pro-angiogenic function for FAP shows that tumors derived from FAP expressing human breast cancer cells have a 3-fold higher microvessel density as compared with tumors from cells not expressing FAP. It is thought that FAP promotes growth of breast tumors at least in part by driving angiogenesis.[48] This notion is also supported by studies showing that FAP mRNA is upregulated by endothelial cells undergoing reorganization and capillary morphogenesis.[49] In contrast, it is reported that FAP depletion decreases blood vessel density of tumor xenografts in mice.[50] In addition, depletion of FAP$^+$ cells causes rapid hypoxic necrosis of both cancer and stromal cells in immunogenic tumors through a process involving interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α), which have previously been shown to be involved in the suppression of angiogenesis.[51] In corneal stroma, FAP could express where the new vessels reached, which indicates the close correlation of FAP and angiogenesis.[52] These findings suggest that FAP can alter the tumor microenvironment at least partially by driving angiogenesis.

Clinical Applications of FAP

The enzymatic activity of FAP provides a potentially important new therapeutic target in a variety of human malignancies. Therefore, it is important to develop selective FAP inhibitors in preclinical investigation. Henry et al.[53] reported that stromal FAP is more prominent in early-stage colorectal cancer and smaller colorectal tumor xenografts. Furthermore, increased FAP is an adverse prognostic indicator in patients with advanced metastatic disease. This study also suggests that the effects of FAP inhibition should be investigated in early-stage tumors, which harbor higher levels of FAP.

Inhibition of FAP protease activity. Jonathan et al.[11] reported that abrogation of FAP enzymatic activity attenuates tumor growth, indicating that the enzymatic activity of this protein plays an important role in the promotion of tumor growth, and

provides an attractive target for therapeutics designed to alter FAP-induced tumor growth through targeting its enzymatic function. The data provide a strategy for exploiting small molecule inhibitors of the protease activity of FAP and investigating their anticancer activity in preclinical models. Administration of Val-boro-Pro (PT-100; Talabostat) attenuates tumor growth in a variety of tumor models in mice.[54] In addition, PT-100 promotes the growth of primitive hematopoietic progenitor cells by increasing granulocyte-colony stimulating factor (G-CSF), interleukin-6 (IL-6) and IL-11 production by bone marrow stromal cells, making it a therapeutic candidate for the treatment of neutropenia and anemia.[55] However, this compound also inhibits multiple intracellular and extracellular dipeptidyl peptidases (e.g., FAP, CD26/DPPIV, DPP7), so that its effect cannot be directly attributed to FAP inhibition. In clinical trials, Phase II trial of Val-boro-Pro demonstrates minimal clinical activity in patients with previously treated metastatic colorectal cancer.[56] In addition, Phase II trial of talabostat and docetaxel in advanced non-small cell lung cancer shows no evidence that talabostat enhanced the clinical activity of docetaxel in patients with NSCLC.[57] PT630 is more specific than talabostat, effectively inhibiting FAP and DPPIV but not the intracellular family members. Santos et al.[44] found PT630 has potent anticancer effects in several mouse models. However, all of these methods have limitations and may be associated with a greater risk of side effects than inhibition of FAP, often arising from the fact that the DPPIV and multiple intracellular dipeptidyl peptidases have normal functions outside of the tumor. With regard to presented challenges, it is important to develop selective FAP inhibitors for the use of target validation. In addition, research with catalytic mutants suggests that at least a portion of the biological functions of FAP reside in non-proteolytic domains of FAP. Thus, inhibiting the protease activity may have profound effects on some tumors but little effect or even growth-promoting effects on others. The role of FAP in a particular tumor type must be understood in future studies.

Targeting FAP. The combinatorial approach targeting both the oncogenic pathways intrinsic to neoplastic cells and the pathways that mediate the pro-tumorigenic effects of the non-transformed stromal component is becoming a widely accepted strategy for targeted anticancer therapy.

Several groups have developed FAP-specific mAb for imaging tumors. Although a humanized anti-FAP antibody (mAb F19; sibrotuzumab) is well tolerated,[58] it shows no beneficial effect in a phase II trial for metastatic colorectal cancer,[59] and the antibodies did not inhibit FAP enzymatic activity. It was recently reported that a monoclonal anti-FAP antibody conjugated to maytansinoid, FAP5-DM1, induced long-lasting inhibition of tumor growth and complete regression in stroma-rich xenograft models of lung, pancreatic, and head and neck cancers in immune-deficient mice, with no evidence of toxicity.[60] However, further studies will be required to determine the clinical application.

Recent alternative approaches that utilize or localize FAP enzyme activity have shown potential. Potent cytotoxic agents, preferably ones that do not cause hemolysis, could be coupled to an FAP-specific peptide to generate an inactive prodrug that is selectively activated by FAP-expressing cells within the tumor stroma. Intratumoral injection of an FAP-activated protoxin produces significant lysis and growth inhibition of human breast and prostate cancer xenografts with minimal toxicity to the host animal.[61] A novel FAP-triggered photodynamic molecular beacon (FAP-PPB) comprised of a disease-specific linker, a photosensitizer (PS), and a fluorescence and singlet oxygen (O2) quencher (Q) is a potential tool for epithelial cancer detection and treatment. In vitro and in vivo experiments have validated the FAP-specific activation of FAP-PPB in cancer cells and mouse xenografts.[62] In addition, Huang et al.[63] conjugated Doxorubicin (Dox) with a FAP-specific dipeptide to develop a FAP-targeting prodrug of Dox (FTPD). They demonstrated that FAP-cleaved FTPD exhibited significantly higher cytotoxicity against 4T1 cells in vitro than the uncatalyzed prodrug. Additionally, FTPD produced similar anticancer efficacy in 4T1 tumor-bearing mice to free Dox without obvious cardiotoxic effect. These findings suggest that such FAP-based prodrug strategy is promising to achieve targeted delivery of anticancer agents.

CAFs are key modulators of the immune TME and that their elimination in vivo has profound effects on immune polarization in the TME.[64] Importantly, with highly restricted of FAP to CAFs, the development FAP-based immunotherapy could be optimized for anticancer therapy in a clinical setting. Sequential application of scFv-IL-872 and dimeric IgG1-TNF fusion proteins significantly enhanced antitumor activity in mice when compared either to a single-agent treatment or sequential application of non-targeted cytokines, indicating that the tumor-restricted sequential application of IL-872 and TNF is a promising approach for cancer therapy.[65] A group recently developed a new tumor vaccine, FAPtau-MT, which was produced by conjugating 1-methyl-tryptophan (1-MT), a specific inhibitor of Indolamine2, 3-dioxygenase (IDO) to FAP. The vaccine breaks tumor immune tolerance as a local IDO inhibitor. Most importantly, administration of the FAPtau-MT vaccine did not lead to pregnancy failiure in mice carrying allogeneic fetuses.[66] A bispecific fusion protein, AntiFAP-mGITRL (murine glucocorticoid-induced tumor necrosis factor-related receptor), is able to costimulate CD8+ and CD4+ effector T cells resulting in increased proliferation, IFN-gamma and IL-2 production. In suppression assays, membrane-bound antiFAP-mGITRL is 100-fold more effective in overcoming Treg-mediated suppression than unbound fusion protein. These studies suggest that targeted tumor therapy with antiFAP-mGITRL fusion protein could induce tumor rejection while minimizing autoimmune side effects.[67] Future studies need to address the mechanism how a FAP-directed immune response affects tumor growth, whether by directly affecting the tumor-associated fibroblast or via collateral damage inducing a local inflammatory response.

FAP can serve as a novel target for active vaccination against cancer, especially if combined with chemotherapy. Tumor tissue of FAP-vaccinated mice revealed markedly decreased collagen type I expression and up to 70% greater uptake of chemotherapeutic drugs. Most importantly, pcDNA3.1/V5-His-TOPO-Fap-vaccinated mice treated with chemotherapy show a 3-fold prolonged survival and marked suppression of tumor growth, with 50% of

the animals completely rejecting a tumor cell challenge.[68] This strategy opens a new venue for the combination of immunotherapies and chemotherapies.

Yet there are challenges to be met in the future preclinical studies required to develop FAP inhibitors to their clinical application. Although these studies provide the proof of principle, the development of additional animal models that recapitulate the properties of human tumors will be important in future studies. This includes endogenous tumor models in immune-competent mice, even though the endogenous tumor models currently available in mice may not typically exhibit the dramatic desmoplastic response seen in many epithelial-derived tumors in patients.[69]

Importantly, however, besides the involvement of FAP in tumorigenesis, it also plays a role in biological function such as embryonic development and tissue remodeling. Thus, a deliberate effort must be made to use to determine whether or not the use of FAP inhibitors is likely to have beneficial or deleterious effects on normal tissue. Further clinical trials of FAP inhibitors will be required to design to define the potential risks in cancer patients.

Conclusion

It is increasingly recognized that the stromal compartment plays a crucial role in tumorigenesis and invasion. FAP is a product overexpressed by CAFs, the predominant component of cancer stoma in most types of cancer. In comparison to tumor cells, FAP may represent a more viable therapeutic target for cancer immunotherapy. Since its discovery in 1986, a great amount of research has been performed on the localization and expression of this protease. However, the role of FAP in tumor growth and invasion, as well as the exact molecular mechanisms the enzyme utilizes remain unknown. FAP does seem to have an important role in malignant cell invasion and metastasis through participating in angiogenesis, deregulation of antitumor immune responses and synthesis, deposition and remodeling of ECM. The availability of a potent and selective, in vitro and in vivo applicable FAP inhibitor opens new perspectives for further studies of the physiological function of FAP. Future studies on the contribution of FAP to tumor growth and invasion will constitute an essential step toward stroma-targeted anticancer therapy.

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Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.