Expert Opinion

informa
healthcare

The road to integrative cancer therapies: emergence of a tumor-associated fibroblast protease as a potential therapeutic target in cancer

Ellen Puré
The Wistar Institute, Philadelphia, Pennsylvania, USA

Great inroads have been made in defining the oncogenic pathways intrinsic to neoplastic cells and the mechanisms by which they are activated in tumors. Knowledge of these pathways provides numerous opportunities that are actively being pursued to develop targeted therapies for cancer. Complementary studies, focused on the non-transformed components of the tumor microenvironment (TME), have revealed that the extrinsic cues provided by the TME are also essential for tumor cells to manifest a fully transformed phenotype, angiogenesis and metastasis. Delineation of these cues and their underlying cellular and molecular pathways will thus lead to a new era of integrative cancer therapy based on combinatorial drug regiments that act synergistically to destroy the neoplastic cells by targeting both the intrinsic and extrinsic pro-oncogenic pathways. Tumor-associated fibroblasts (TAFs) and proteases are two of the key regulators of epithelial-derived tumors that represent potential targets of such integrative therapies. Herein, we consider the potential therapeutic benefit of inhibiting the function of fibroblast activation protein (FAP), a cell surface serine protease with dipeptidyl peptidase and endopeptidase activity that is expressed on TAFs and pericytes, in an integrative approach to treating cancer.

Keywords: FAP, fibroblasts, pericytes, protease, stroma, tumor microenvironment

Expert Opin. Ther. Targets (2009) ***13****(8):1-7*

1. Introduction

Cancer mortality remains second only to cardiovascular disease. Approximately 40% of the population is predicted to be diagnosed with cancer at some point in their lifetime, and epithelial-derived solid tumors are the most prevalent form of cancer. Surgery, chemotherapy and radiation therapy remain the standard of care for the majority of solid tumor types. Although these approaches have contributed to the great inroads that have been made in the treatment of cancer, they each have their limitations; even collectively, the limitation of these modalities is evidenced by the fact that cancer remains the second most common cause of death, generally the result of metastatic disease. Although resection and local radiotherapy can be highly effective in treating primary tumors, neither surgery nor localized radiotherapy address the challenges of metastatic disease. Chemotherapy can be used to treat metastatic disease but the systemic nature and relative nonspecificity of chemotherapies are associated with serious adverse side effects. Although the abiding principle of standard radiation and chemotherapy is to preferentially inhibit proliferation and induce the death of tumor cells relative to normal cells, this ideal is difficult to achieve using current approaches.

10.1517/14728220903103841 ISSN 1472-8222

Hormone-responsive tumors offer unique opportunities for treatment, but these can also have significant side effects and, as with standard radiation and chemotherapies, tumors can develop resistance.

Several alternatives are being pursued to overcome the limitations of conventional therapies. These include therapies targeted to intrinsic tumor cell-specific oncogenic pathways. A limited number of such targeted therapies have been developed to date; they have proven to have an impact on disease progression, but less so on overall survival rates. The development of tumor resistance to drugs that act directly on tumor cell intrinsic pathways is facilitated by the inherent genetic instability of tumor cells and may be an important factor in limiting their long-term efficacy. Taken together with the growing appreciation of the critical role of extrinsic factors provided by the tumor microenvironment (TME) in tumor initiation and progression, the potential for synergistic effects of targeting both tumor cells and components of the TME has received increasing attention in recent years.

Theoretically, targeting components of the TME, has three potential advantages:

- Non-transformed cellular components of tumors are likely to be more genetically stable than tumor cells, in which genetic instability often leads to the development of drug resistance.
- The TME can promote tumorigenesis through common mechanisms in various tumor types. Therapies that target these common mechanisms therefore have the potential to be indicated in a broader population of cancer patients.
- An integrative approach to treating cancer that combines tumor cell-targeted therapies with therapies that act on components of the TME has the potential for synergistic efficacy without overlapping toxicity.

There are several interdependent compartments/processes related to the TME that merit investigation as therapeutic targets: inflammation and antitumor immunity; the vascular (blood and lymphatic vessels) compartment; and stromagenesis involving mesenchymal-derived stromal cells and extracellular matrix (ECM) remodeling. Indeed, introduction of antiangiogenic drugs, based on this paradigm, has had a clinical impact in some tumor types, as have cancer immunotherapies [1-3]. However, the emerging complexity of antiangiogenic therapies suggests that this approach may prove to be a double-edged sword in some circumstances.

Targeting stromal cells is the least well studied of these approaches to date. Although neither the mechanisms involved in stromagenesis nor the mechanisms by which stromal cells promote tumorigenesis are yet fully understood, evidence is mounting that tumor-associated fibroblasts (TAFs) are critical to tumorigenesis and angiogenesis, suggesting that stromal cells are fertile ground for identifying novel therapeutic targets. Herein, we consider the clinical potential of inhibiting a stromal cell-specific protease, fibroblast activation protein (FAP), in epithelial-derived tumors.

2. Building and remodeling tumors: role of stromal cells and proteases

Tumors are collections of heterogeneous populations of cells, including the transformed cancer cells themselves and a variety of non-neoplastic cells that make up the other major tumor compartments. These include the stroma, consisting of TAFs/myofibroblasts and ECM, the vasculature (blood and lymphatic vessels), as well as infiltrating inflammatory and immune cells (**Figure 1**). Although the prevalence of different cell types varies between tumors, human epithelial-derived tumors are frequently characterized by the generation of fibrous connective tissue. This desmoplastic reaction involves the recruitment, differentiation and growth of TAFs and deposition and remodeling of matrix. TAFs may be generated through differentiation of resident tissue fibroblasts, bone marrow-derived fibrocytes, or mesenchymal stem cells. TAFs may thus be heterogeneous; but, importantly, they are phenotypically and functionally distinct from resident tissue fibroblasts. This is a crucial feature if we are to consider targeting stromal cells for therapeutic purposes.

Stromal cells are essential for tumor initiation, as well as tumor progression and metastasis. Indeed, recent studies have also established that TAFs are unique in their capacity to markedly enhance tumor growth, compared to normal fibroblasts [4]. Interestingly, stromal cells have also been shown to be essential for tumor angiogenesis [4-6]. Stromal cells mediate angiogenesis through multiple mechanisms, including matrix remodeling and growth factor (VEGF) and chemokine production. In addition, the functional integrity of angiogenic vessels is dependent on the formation of a supporting layer of a subset of stromal cells, referred to as pericytes or mural cells, underlying the newly sprouting endothelial tubes [7]. Indeed, it appears that the lack of a normal layer of pericytes/mural cells accounts for the compromised function of angiogenic vessels in tumors, compared with normal tissues [7].

Stromal cells communicate among themselves, as well as with cancer cells, inflammatory and immune cells through direct cell contact and indirectly through the secretion of growth factors, chemokines, cytokines, reactive oxygen species (ROS), reactive nitrogen species (RNS), lipid mediators and proteases, and through modulation of ECM. TAFs are believed to be the major source of matrix deposition, which includes production of matrix proteins such as collagen and elastin, proteoglycans such as heparin sulfate and chondroitin sulfate, and glycosaminoglycans (particularly hyaluronan). Together, stromal cells and matrix confer many of the physical properties of tumors such as tissue compliance and stiffness, and the matrix composition is an important contributing factor in determining interstitial fluid pressure. Tissue tension regulates cell signaling, thereby regulating cell growth, differentiation and function. ECM can also function to compartmentalize tumors, regulating intercellular communication and cell behavior. Furthermore, the ECM acts as a local depot for growth factors, cytokines and chemokines.

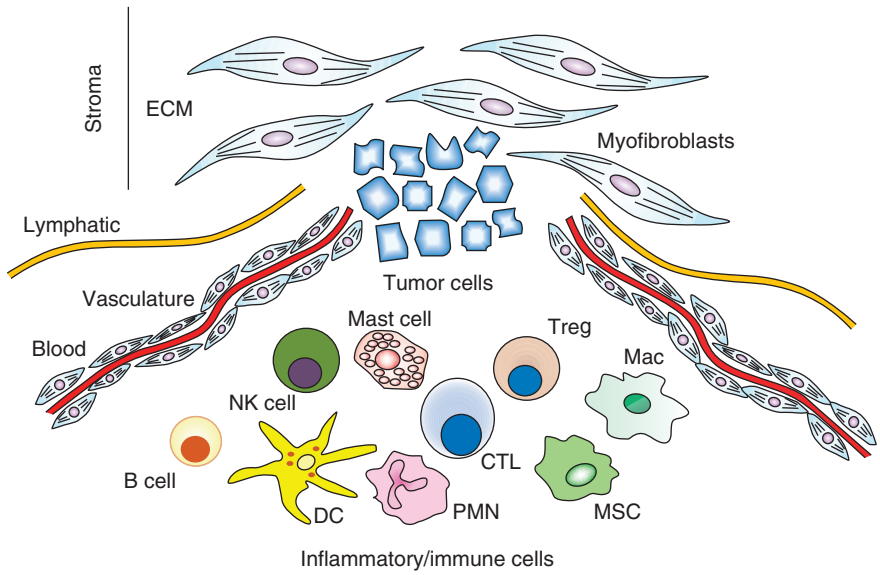

Figure 1. Major tumor compartments.

This complex communications network is pivotal to providing the appropriate microenvironment to support tumorigenesis and metastasis [8,9]. Considering the key role of the microenvironment in tumor development, the identification of stromal targets for cancer therapeutics is of great interest and could provide strategies that will complement therapies directed against cancer cells. Among these potential targets is an array of proteases [10].

Proteases are important factors in the pathophysiology of tumors, affecting tumor cell growth, migration, apoptosis and inflammation. In addition to their dependence on regulated matrix production, tumors depend on matrix turnover – that is, on proteolytic processing and degradation by a number of serine and threonine proteases such as urokinase plasminogen activator (uPA), the cathepsin cysteine proteases, aspartyl and MMPs including MMP1, MMP2, MMP3 and MMP11. Interestingly, proteolytic processing of several matrix constituents leads to the generation of biologically active fragments that act as endogenous inhibitors of angiogenesis. For example, MMP-mediated cleavage of plasminogen, collagen IV and collagen XVIII releases angiostatin, tumstatin and endostatin, respectively [11]. In addition to their role in matrix remodeling and regulating angiogenesis, proteases release matrix-associated latent signaling molecules and activate growth factors and cytokines, mediate cell invasion and cell signaling, and modify cell–cell and cell–matrix interactions.

A variety of proteases have been shown to be upregulated in many tumors. MMPs in particular, and more recently cathepsin, have been investigated as potential targets for antitumorigenic drugs [12,13]. Based on preclinical studies, MMP inhibitors were advanced to a number of clinical trials in the mid to late 1990s in a variety of cancer types. As reviewed in depth by Coussens and colleagues [12], these trials failed to demonstrate efficacy. Several properties of MMPs and the various inhibitors probably account for the disappointing performance of the latter in the clinic in cancer patients. First, dose-limiting toxicities were observed. Evidence suggested that the toxicities were due at least part in part to off-target effects, but may also reflect simply the adverse effects of inhibiting MMP activities. Secondly, the lack of fine specificity of most of these inhibitors, which each act on multiple MMPs, may be a limiting factor. Thirdly, genetic factors may impact the efficacy of MMPs. Finally – and this may be the most challenging obstacle to overcome – there is the evidence that MMPs each serve multiple functions, including functions in homeostatic processes, and can have both tumor-promoting and antitumorigenic effects, perhaps depending on tumor stage at the time of treatment [12]. In spite of these early clinical results, greater appreciation of the structures and functions of MMPs may present opportunities to develop new generations of MMP inhibitors for development in the clinic in the future. There are also important lessons from the experience with MMP inhibitors that should inform development of therapeutic strategies targeting other proteases involved in cancer. Another protease, FAP (also called FAP-α or seprase), has recently gained attention due to the fact that in adults, it is selectively expressed on reactive fibroblasts associated with tissue remodeling such as in wound healing, chronic inflammation and fibrosis, as well as in the vast majority of epithelial derived tumors [14-18]. Based on its restricted tightly regulated expression and its structurally defined enzymatic activity, FAP has recently garnered attention as a potential therapeutic target.

3. Structure and function of FAP

FAP is a well-defined marker of TAFs and blood vessel-associated pericytes. FAP is a 95-kDa type II transmembrane serine protease expressed in > 90% of common human epithelial cancers [19-22]. In epithelial-derived tumors, it is expressed by cancer-associated fibroblasts, but not by the cancer cells themselves. It is also expressed during embryonic development [23], in tissues of healing wounds [24], in chronic inflammatory and fibrotic conditions such as liver cirrhosis [25,26], and in idiopathic pulmonary fibrosis [27], as well as in bone and soft-tissue sarcomas [28] and some melanomas [29]. However, expression of FAP is not detected in benign tumors or normal adult tissues [29,30]. Given the highly regulated expression and restricted distribution of FAP, the lack of overt pathology in FAP-deficient mice [31], the animal results suggesting that over-expression of FAP promotes tumorigenesis [32-34], and the correlation between high FAP expression and poor cancer prognosis [20,35], it has been suggested that FAP inhibition may be useful in cancer therapeutics.

FAP belongs to the post-proline dipeptidyl aminopeptidase family. The most closely related member of the family is dipeptidyl peptidase IV (DPPIV/CD26), which is also a cell surface protein, but more widely expressed in a variety of cell types (reviewed in [36-38]). In contrast to FAP and DPPIV, other members of the family, such as DPP7 (DPPII), DPP8 and DPP9, are cytoplasmic proteins.

The catalytic domain of FAP contains a serine protease consensus sequence in which the catalytic triad has been identified (**Figure 2**). FAP has been shown to function as a homodimer or as a heterodimer with DPPIV, although the physiologic relevance of the latter is yet unclear as only rare cells have been found to express both FAP and DPPIV. While other members of the DPP family are only known to possess dipeptidyl peptidase activity, *in vitro* studies have shown that FAP has both dipeptidyl peptidase [30,39] and endopeptidase [16,18,40] activity, including a collagenolytic activity capable of degrading gelatin [41,42] and type I collagen [43,44]. The *in vivo* substrate(s) is still not defined, although it has been alleged to degrade ECM components (collagen), thereby regulating tumor invasion [38,44,45]. Based on its structure, mutations of FAP have been engineered that compromised its protease activities, including FAPS624A, which lacks both the dipeptidyl peptidase and endopeptidase activity, as well as FAPA657S which retains its dipeptidyl peptidase activity but lacks endopeptidase activity [18,32]. These mutant forms of FAP provide important tools for defining the *in vivo* relevance of the distinct activities and physiologic substrates of FAP.

4. Role of FAP in tumorigenesis

Much of the evidence that FAP promotes tumorigenesis to date has been obtained using experimental-model systems that fail to recapitulate many of the potentially important interactions between stromal, tumor, immune and inflammatory cells. For example, a number of these studies used xenografts of human tumor cells that have been engineered to ectopically overexpress FAP or an enzymatically inactive mutant [32,33], despite the fact that in primary tumors, FAP is expressed only by the tumor-associated fibroblasts and not by the tumor cells themselves. Furthermore, by necessity these xenografts were studied in immune-incompetent mice. Understanding the molecular mechanisms by which FAP promotes tumorigenesis requires that its function be studied under experimental conditions that more faithfully depict the typical scenario seen in cancer patients. Therefore, we recently studied the impact of genetic deletion of FAP, and pharmacologic inhibition of its enzymatic activity, on the growth of colon and pancreatic tumors transplanted into syngeneic immune-competent mice, and on the development and progression of constitutively active K-ras ($K\text{-}ras^{G12D}$)-driven endogenous lung tumors in immune-competent mice [46-48]. We found that endogenous FAP expressed on tumor stromal cells promotes tumor progression in each of these models (Santos *et al.*, submitted).

5. How can FAP be exploited for therapeutic purposes?

There are several ways in which to consider exploiting FAP in the clinic. The first is to employ FAP-specific mAb for imaging tumors. Indeed, initial studies in patients demonstrated highly specific tumor targeting of the anti-FAP mAb F19 in colon cancer [49]. However, although humanized F19 (sibrotuzumab) was well tolerated, it showed no efficacy in a Phase II trial for metastatic colorectal cancer [50,51]. This is perhaps not surprising, as this mAb does not inhibit FAP activity nor does it have direct cytotoxic or cytostatic activity. On the other hand, it has recently been reported that a mAb conjugated to maytansinoid, FAP5-DM1, induced long-lasting inhibition of tumor growth and complete regressions in stroma-rich xenograft models of lung, pancreatic, and head and neck cancers in immune-deficient mice, with no evidence of toxicity [14]. Thus, future efforts to translate these findings with toxin-conjugated anti-FAP mAbs to the clinic are anticipated. Importantly, however, although these latter results indicate that eliminating FAP-expressing cells (TAFs and/or pericytes) can effectively inhibit tumor growth, thus formally establishing FAP-bearing cells as critical to tumor survival (at least in xenograft models), they do not address the importance of FAP itself.

There is nonetheless evidence from three different approaches to suggest that FAP, and more specifically its protease activity, contributes to the pro-tumorigenic function of stromal cells. First, as discussed above, in our recent studies we showed that tumorigenesis was inhibited in FAP-null mice in the case of syngeneic colon and pancreatic tumor transplants, as well as endogenous K-ras-driven lung tumors (Santos *et al.*, submitted). Secondly, in the same

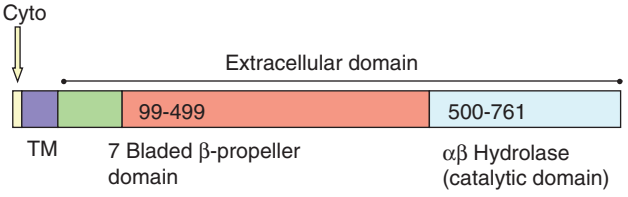

Figure 2. Schematic of FAP domain structure.

study, treatment with an inhibitor of the protease activity of FAP and its closest homologue, DPPIV, also inhibited tumorigenesis in these models. Finally, it has been reported that an antibody that inhibited the proteolytic activity of FAP inhibited the growth of human tumor xenografts [32]. Collectively, these data provide a strong rationale for developing small-molecule inhibitors of the protease activity of FAP and investigating their antitumor activity in preclinical models for potential translation to the clinic.

Several dipeptidyl peptidase inhibitors have been generated. A specific inhibitor of DPPIV, LAF237 (vildagliptin), has been developed by Novartis and approved in Europe for treatment for diabetes [37]. Compounds that inhibit FAP have also been described [15-17,52], but their potential to inhibit tumor activity *in vivo* has not yet been reported. PT100, or talabostat, an inhibitor of multiple members of the DPP family developed by Point Therapeutics, was shown to have antitumor and immune-regulatory activities in animal models [53]. Talabostat inhibits FAP and DPPIV, the two cell surface members of the DPP family, as well as several intracellular DPPs. Through an independent mechanism, talabostat also mediates immune activation. These studies indicated that the antitumor activity of talabostat was independent of DPPIV but did not define the relevant target. Furthermore, the antitumor activity of talabostat appeared to be dependent on the innate immune response, but it was not determined whether stromal cells played any role in mediating the antitumor effects. In clinical trials, talabostat also had significant activity in patients with non-small-cell lung cancer and malignant melanoma growth [53], but again the relevant target was not defined in this case.

A 'second-generation' inhibitor, PT630, also developed by Point Therapeutics, is more specific than talabostat, effectively inhibiting FAP and DPPIV but not the intracellular family members due to its lack of cell permeability. In recent studies, we found PT630 to have none of the toxicities we observed with PT100 in mice, and that this compound has potent antitumor effects in several mouse models (Santos *et al.*, submitted). Further studies will be required to determine the relative importance of the inhibition of FAP versus DDPIV to the antitumor activity of PT630. Similarly, more information about the mechanism of action of PT630 in mouse models is needed in order to guide future development of inhibitors of specific DPPs for clinical application. It is possible that in some tumor types, inhibition of either DPPIV or FAP may be efficacious, or that simultaneously inhibiting both DPPIV and FAP might be advantageous. However, it is important to keep in mind that only FAP exhibits a highly restricted tissue distribution. The broad expression pattern of DPPIV suggests that its inhibition may be associated with a greater risk of side effects than inhibition of FAP, making it a less attractive target for antitumor therapy.

6. Expert opinion

Understanding the mutually dependent roles of the multiple components of various tumors will provide the basis for integrative approaches to the treatment of cancer which combine drugs that target both the oncogenic pathways intrinsic to neoplastic cells and the pathways that mediate the pro-tumorigenic effects of the non-transformed stromal components. The projected synergy of such a combinatorial targeting approach should provide a way forward to more tumor-specific, less toxic and more efficacious cancer treatments. Cells of the stromal compartment, more specifically TAFs and pericytes, are established pillars of tumor architecture and 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. Importantly, FAP has proven amenable to the development of highly specific small-molecule and peptide inhibitors, some of which have already been shown to have favorable solubility, pharmacodynamics, toxicity and pharmacokinetic profiles for future drug development.

However, there have also been obstacles that have stood between the initial description of FAP as a marker of TAFs in carcinomas > 20 years ago and its exploitation as a potential therapeutic target. First, many of the tools, reagents and mice required to study the role of the tumor microenvironment were generated in the past decade. With regard to FAP itself, since its discovery in the 1980s some effort has been devoted to exploiting FAP as a target of antibody-based therapies. However, as discussed above, although such antibodies exhibit exquisite tumor targeting, they are not cytotoxic; and the author is not aware of antibodies that inhibit the enzymatic activity of FAP being available yet for translation to the clinic. Others have focused on the development of small-molecule inhibitors of FAP but these pharmacologic approaches have also presented challenges. First, the endogenous substrates of FAP have not yet been definitively determined. Second, as for other enzymatic targets such as kinases, the highly conserved structure of the catalytic domains of serine proteases, in particular the structural relatedness of FAP to other members of the DPP family proteases, can defy the rationale of the highly specific small-molecule inhibitors required to avoid off-target effects. But as summarized above, significant progress has been made in this regard.

Yet there are challenges to be met in the future preclinical studies required to pave the way to developing FAP inhibitors as cancer therapies and to their clinical application. First, the *in vivo* animal model studies must be extended to the more specific inhibitors becoming available to determine the relative importance of FAP and DDPIV to the observed antitumorigenic activity of PT630 in different tumor types. It will be important in future studies to deliberately focus on animal models that most faithfully recapitulate the properties of human tumors. 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. The development of additional models that recapitulate this important feature of human tumors is therefore also crucial.

Finally, although the expression of FAP in tumors is highly selective compared with normal tissue, induction of FAP is also a feature of wound healing and other pathologies associated with chronic inflammation, tissue remodeling and fibrosis. Thus, a deliberate effort must be made to use the same tools as those used to study the role of FAP in tumors, to define the role of FAP in other conditions such as atherosclerosis, pulmonary fibrosis, liver cirrhosis and arthritis, to determine whether or not the use of FAP inhibitors is likely to have beneficial or deleterious effects on these conditions. With this information, it will possible to define the potential risks and design clinical trials of FAP inhibitors in an appropriate subset of cancer patients.

Acknowledgements

The author thanks Angelica Santos and Michele Jacob for many helpful discussions and review of the manuscript, and Stephanie Berliner for assistance in preparation of the manuscript. The FAP studies in the author's laboratory were supported by The Ludwig Institute for Cancer Research and Point Therapeutics, who also generously provided inhibitors for these studies.

Declaration of interest

The author states no conflict of interest and has received no payment in preparation of this manuscript.

Bibliography

1. Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer 2008;8:592-603
2. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity 2008;29:372-83
3. Melief CJ, van der Burg SH. Immunotherapy of established (pre) malignant disease by synthetic long peptide vaccines. Nat Rev Cancer 2008;8:351-60
4. Orimo A, Gupta PB, Sgroi DC, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005;121:335-48
5. Koyama H, Kobayashi N, Harada M, et al. Significance of tumor-associated stroma in promotion of intratumoral lymphangiogenesis: pivotal role of a hyaluronan-rich tumor microenvironment. Am J Pathol 2008;172:179-93
6. Noma K, Smalley KSM, Lioni M, et al. The essential role of fibroblasts in esophageal squamous cell carcinoma-induced angiogenesis. Gastroenterology 2008;134:1981-93
7. Gerhardt H, Betsholtz C. Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res 2003;314:15-23
8. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol 2006;1:119-50
9. Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. Nature 2004;432:332-7
10. Lopez-Otin C, Matrisian LM. Emerging roles of proteases in tumour suppression. Nat Rev Cancer 2007;7:800-8
11. Mueller MM, Fusenig NE. Friends or foes? Bipolar effects of the tumour stroma in cancer. Nat Rev Cancer 2004;4:839-49
12. Coussens LM, Fingleton B, Matrisian LM. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295:2387-92
13. Palermo C, Joyce JA. Cysteine cathepsin proteases as pharmacological targets in cancer. Trends Pharmacol Sci 2008;29:22-8
14. Ostermann E, Garin-Chesa P, Heider KH, et al. Effective immunoconjugate therapy in cancer models targeting a serine protease of tumor fibroblasts. Clin Cancer Res 2008;14:4584-92
15. Edosada CY, Quan C, Tran T, et al. Peptide substrate profiling defines fibroblast activation protein as an endopeptidase of strict Gly2-Pro1-cleaving specificity. FEBS Lett 2006;580:1581-6
16. Edosada CY, Quan C, Wiesmann C, et al. Selective inhibition of fibroblast activation protein protease based on dipeptide substrate specificity. J Biol Chem 2006;281:7437-44
17. Hu Y, Ma L, Wu M, et al. Synthesis and structure-activity relationship of N-alkyl Gly-boro-Pro inhibitors of DPP4, FAP, and DPP7. Bioorg Med Chem Lett 2005;15:4239-42
18. Meadows SA, Edosada CY, Mayeda M, et al. Ala657 and conserved active site residues promote fibroblast activation protein endopeptidase activity via distinct mechanisms of transition state stabilization. Biochemistry 2007;46:4598-605
19. Goscinsky MA, Suo Z, Florenes VA, et al. FAP-alpha and uPA show different expression patterns in premalignant and malignant esophageal lesions. Ultrastruct Pathol 2008;32:89-96
20. Henry LR, Lee HO, Lee JS, et al. Clinical implications of fibroblast activation protein in patients with colon cancer. Clin Cancer Res 2007;13:1736-41
21. Scanlan MJ, Mohan Raj BK, Calvo B, et al. Molecular cloning of fibroblast activtion protein a, a member of the serine protease family selectively expressed in stromal fibroblasts of epithelial cancers. Proc Natl Acad Sci USA 1994;91:5657-61
22. Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers.

Proc Natl Acad Sci USA 1990;87:7235-9

23. Niedermeyer J, Garin-Chesa P, Kriz M, et al. Expression of the fibroblast activation protein during mouse embryo development. Int J Dev Biol 2001;45:445-7
24. Mathew S, Scanlan MJ, Mohan Raj BK, et al. The gene for fibroblast activation protein [alpha] (FAP), a putative cell surface-bound serine protease expressed in cancer stroma and wound healing, maps to chromosome band 2q23. Genomics 1995;25:335-7
25. Wang XM, Yao TW, Nadvi NA, et al. Fibroblast activation protein and chronic liver disease. Front Biosci 2008;13:3168-80
26. Levy MT, McCaughan GW, Abbott CA, et al. Fibroblast activation protein: a cell surface dipeptidyl peptidase and gelatinase expressed by stellate cells at the tissue remodelling interface in human cirrhosis. Hepatology 1999;29:1768-78
27. Acharya PS, Zukas A, Chandan V, et al. Fibroblast activation protein: a serine protease expressed at the remodeling interface in idiopathic pulmonary fibrosis. Hum Pathol 2006;37:352-60
28. Rettig WJ, Garin-Chesa P, Beresford HR, et al. Cell-surface glycoproteins of human sarcomas: differential expression in normal and malignant tissues and cultured cells. Proc Natl Acad Sci USA 1988;85:3110-4
29. Huber MA, Kraut N, Park JE, et al. Fibroblast activation protein: differential expression and serine protease activity in reactive stromal fibroblasts of melanocytic skin tumors. J Invest Dermatol 2003;120:182-8
30. Aertgeerts K, Levin I, Shi L, et al. Structural and kinetic analysis of the substrate specificity of human fibroblast activation protein alpha. J Biol Chem 2005;280:19441-4
31. Niedermeyer J, Kriz M, Garin-Chesa P, et al. Targeted disruption of mouse fibroblast activation protein. Mol Cell Biol 2000;20:1089-94
32. Cheng JD, Valianou M, Canutescu AA, et al. Abrogation of fibroblast activation protein enzymatic activity attenuates tumor growth. Mol Cancer Ther 2005;4:351-60
33. Cheng JD, Dunbrack RL Jr, Valianou M, et al. Promotion of tumor growth by murine fibroblast activation protein, a serine protease, in an animal model. Cancer Res 2002;62:4767-72
34. Huang Y, Wang S, Kelly T. Seprase promotes rapid tumor growth and increased microvessel density in a mouse model of human breast cancer. Cancer Res 2004;64:2712-6
35. Cohen SJ, Alpaugh RK, Palazzo I, et al. Fibroblast activation protein and its relationship to clinical outcome in pancreatic adenocarcinoma. Pancreas 2008;37:154-8
36. Havre PA, Abe M, Urasaki Y, et al. The role of CD26/dipeptidyl peptidase IV in cancer. Front Biosci 2008;13:1634-45
37. Brandt I, Joossens J, Chen X, et al. Inhibition of dipeptidyl-peptidase IV catalyzed peptide truncation by Vildagliptin ((2S)-{[(3-hydroxyadamantan-1-yl)amino]acetyl}-pyrrolidine-2-carbonitrile). Biochem Pharmacol 2005;70:134-43
38. Niedermeyer J, Scanlan MJ, Garin-Chesa P, et al. Mouse fibroblast activation protein: molecular cloning, alternative splicing and expression in the reactive stroma of epithelial cancers. Int J Cancer 1997;71:383-9
39. Levy MT, McCaughan GW, Marinos G, Gorrell MD. Intrahepatic expression of the hepatic stellate cell marker fibroblast activation protein correlates with the degree of fibrosis in hepatitis C virus infection. Liver 2002;22:93-101
40. Lee KN, Jackson KW, Christiansen VJ, et al. Antiplasmin-cleaving enzyme is a soluble form of fibroblast activation protein. Blood 2006;107:1397-404
41. Aggarwal S, Brennen WN, Kole TP, et al. Fibroblast activation protein peptide substrates identified from human collagen I derived gelatin cleavage sites. Biochemistry 2008;47:1076-86
42. Pineiro-Sanchez ML, Goldstein LA, Dodt J, et al. Identification of the 170-kDa melanoma membrane-bound gelatinase (seprase) as a serine integral membrane protease. J Biol Chem 1997;272:7595-601
43. Park JE, Lenter MC, Zimmerman RA, et al. Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J Biol Chem 1999;274:36505-12
44. Christiansen VJ, Jackson KW, Lee KN, McKee PA. Effect of fibroblast activation protein and [alpha]2-antiplasmin cleaving enzyme on collagen types I, III, and IV. Arch Biochem Biophys 2007;457:177-86
45. Kelly T. Fibroblast activation protein-alpha and dipeptidyl peptidase IV (CD26): Cell-surface proteases that activate cell signaling and are potential targets for cancer therapy. Drug Resist Updat 2005;8:51-8
46. Kissil JL, Walmsley MJ, Hanlon L, et al. Requirement for Rac1 in a K-ras-induced lung cancer in the mouse. Cancer Res 2007;67:8089-94
47. Grimm J, Kirsch DG, Windsor SD, et al. Use of gene expression profiling to direct in vivo molecular imaging of lung cancer. Proc Natl Acad Sci USA 2005;102:14404-9
48. Tuveson DA, Shaw AT, Willis NA, et al. Endogenous oncogenic K-ras(G12D) stimulates proliferation and widespread neoplastic and developmental defects. Cancer Cell 2004;5:375-87
49. Welt S, Divgi CR, Scott AM, et al. Antibody targeting in metastatic colon cancer: a phase I study of monoclonal antibody F19 against a cell-surface protein of reactive tumor stromal fibroblasts. J Clin Oncol 1994;12:1193-203
50. Scott AM, Wiseman G, Welt S, et al. A phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin Cancer Res 2003;9:1639-47
51. Hofheinz RD, Al-Batran SE, Hartmann F, et al. Stromal antigen targeting by a humanised monoclonal antibody: an early phase II trial of sibrotuzumab in patients with metastatic colorectal cancer. Onkologie 2003;26:44-8
52. Gilmore BF, Lynas JF, Scott CJ, et al. Dipeptide proline diphenyl phosphonates are potent, irreversible inhibitors of seprase (FAPalpha). Biochem Biophys Res Commun 2006;346:436-46
53. Adams S, Miller GT, Jesson MI, et al. PT-100, a small molecule dipeptidyl peptidase inhibitor, has potent antitumor effects and augments antibody-mediated cytotoxicity via a novel immune mechanism. Cancer Res 2004;64:5471-80

Affiliation

Ellen Puré PhD
The Wistar Institute,
3601 Spruce Street,
Philadelphia, PA 19104-4268, USA
Tel: +1 215 898 1570; Fax: +1 215 898 3937;
E-mail: pure@wistar.org