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Targeting fibroblast activation protein in cancer – Prospects and caveats

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1. ABSTRACT

Fibroblast activation protein (FAP, seprase) is a serine protease with post-proline dipeptidyl peptidase and endopeptidase enzymatic activity. FAP is upregulated in several tumor types, while its expression in healthy adult tissues is scarce. FAP molecule itself and FAP+ stromal cells play an important although probably context-dependent and tumor type-specific pathogenetic role in tumor progression. We provide an overview of FAP expression under both physiological and pathological conditions with focus on human malignancies. We also review and critically analyze the results of studies which used various strategies for the therapeutic targeting of FAP including the use of low molecular weight inhibitors, FAP activated prodrugs, anti-FAP antibodies and their conjugates, FAP-CAR T cells, and FAP vaccines. A unique enzymatic activity and selective expression in tumor microenvironment make FAP a promising therapeutic target. A better understanding of its role in individual tumor types, careful selection of patients, and identification of suitable combinations with currently available anticancer treatments will be critical for a successful translation of preclinically tested approaches of FAP targeting into clinical setting.

2. INTRODUCTION

A malignant tumor can be viewed as an anomalous organ comprised of a heterogeneous mixture of several types of transformed and non-transformed elements of mesenchymal or hematopoietic origin, which has the potential of systemically influencing its host. The cellular components together with an extracellular matrix and other components of the extracellular space in the tumor form a unique tumor microenvironment (1, 2). Multidirectional interactions within this milieu significantly affect the malignant phenotype of transformed cells and the overall progression of the tumor. Therapies based on the targeting of driver mutations in transformed cells (for instance, the use of transtuzumab in tumors with amplified Her2, or imatinib in chronic myeloid leukemia with the BCR-Abl fusion protein) have been effective in

selected tumors. Their application to the majority of malignancies is, however, problematic due to a redundancy of growth-promoting signals, rapid development of resistance, as well as intra- and inter-tumoral heterogeneity (3). Identification of novel therapeutic approaches that target several aspects of the tumor microenvironment in various tumor types is therefore highly desirable.

In this review, we summarize and critically analyze the available data on the protease fibroblast activation protein (FAP, Surface Expressed Protease (seprase), antiplasmin-cleaving enzyme (APCE), (EC3.4.2.1.B28)), which is characteristically expressed by various cell types in the microenvironment of human malignancies, and which may be a promising therapeutic target in cancer treatment.

FAP is a non-classical serine protease belonging to "Dipeptidyl peptidase (DPP)-IV activity and/or structure homologues" (DASH) (4, 5). The FAP gene is highly conserved across various species. It is localized on the long arm of chromosome 2 in humans and mice (6, 7) adjacent to its closest homologue DPP-IV/CD26 (52% amino acid identity) and, similarly to DPP-IV, is organized into 26 exons. It is therefore thought to have arisen by gene duplication. The protein encoded by the human FAP gene is a 760 amino acid single pass type II transmembrane protein composed of a short cytoplasmic N terminal part (6 amino acids), a transmembrane region (amino acids 7–26), and a large extracellular domain (8). Several isoforms of FAP have been reported in the literature. A soluble form of FAP which lacks 26 amino acids of the intracellular and transmembrane portion can be detected in the plasma in various species (see also below) and is speculated to be the product of protein shedding (9). Two alternatively spliced, in-frame mRNA variants have been identified in mouse embryonic tissues. Predicted proteins encoded by these mRNAs contain a transmembrane domain and a catalytic region, but lack 33 and 5 amino acids respectively in the membrane-proximal portion of the protein (10). Goldstein *et al.* have described a shortened isoform that corresponds to the 239 carboxyterminal amino acids of a human FAP protein generated from an alternatively spliced mRNA in melanoma cells (11). It is not clear, however, whether this isoform is also expressed *in vivo*. The same group has later reported shortened forms of the human FAP/seprase produced by a proteolytic processing by EDTA-sensitive activators, especially in ovarian carcinoma. These isoforms may exhibit increased collagenolytic activity, possibly due to a reduced steric hindrance for larger substrates (12). We have recently described the existence of several molecular forms of FAP of varying pI and electrophoretic mobility in human glioblastoma tissues (13) but, like the abovementioned isoforms, their pathophysiological role is currently unknown.

FAP is enzymatically active as a homodimer. It exhibits both a post-proline dipeptidyl peptidase and endopeptidase activity (14-18), both of which are dependent on the catalytic triad comprising Ser^{624} Asp^{702} His^{734} in human and mouse FAP (7, 8). Due to the unique structure of proline, most proteases do not cleave the peptide bonds adjacent to it. In several cases, the presence of proline thus acts as a mechanism that prevents protein degradation or cleavage (19). Several bioactive peptides and structural proteins have been proposed to be FAP substrates. Of these, neuropeptide Y (NPY), Peptide YY, Substance P (SP), and B-type natriuretic peptide (BNP) are cleaved rapidly, whereas the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), as well as some other biopeptides (GLP-2, Peptide Histidine-Methionine, Growth hormone-releasing hormone) are rather poor substrates. Among structural proteins, collagen I and III have been shown to be cleaved by FAP (20-22), but efficient FAP activity seems to require denaturation or predigestion by other proteases (23, 24). Cleavage of the proteoglycan brevican has also been reported, but only after a prolonged incubation (25). Substrates which are cleaved *in vivo* include human fibroblast growth factor 21 (FGF-21), a protein involved in the regulation of energy metabolism and insulin sensitivity (26-28), human alpha2 antiplasmin (9), and probably also collagen I (24) (Figure 1). Further studies are needed to clarify the role of FAP in the proteolysis of other possible substrates. The intracellular antagonist of receptor tyrosine kinases sprouty (SPRY2) is cleaved more efficiently than alpha2 antiplasmin and has been used to study FAP substrate specificity in a study by Huang *et al.* (29). The physiological relevance, if any, of this cleavage is unclear. Similarly, the various candidate FAP substrates identified by proteomic approaches (ADAM15, interleukin 6 (IL-6), fibrillin-2, matrillin-3, serine protease 23, testican-1, and transforming growth factor beta-induced protein) (30) remain to be validated and the physiological importance of their cleavage by FAP is yet to be established.

3. FAP EXPRESSION AND FUNCTION IN HEALTH AND DISEASE

3.1. FAP expression under physiological conditions

FAP expression has been documented in some of the primitive mesenchymal cells at various stages of mouse embryonic development, but its absence did not lead to developmental defects (31, 32). Although most normal adult tissues show little or no detectable FAP expression, a soluble form of FAP is present in the blood plasma of various species (9, 33, 34). In humans, plasma concentrations of FAP measured by ELISA in healthy individuals are around 100 ng/ml or 0.6 nmol/l (median concentration reported

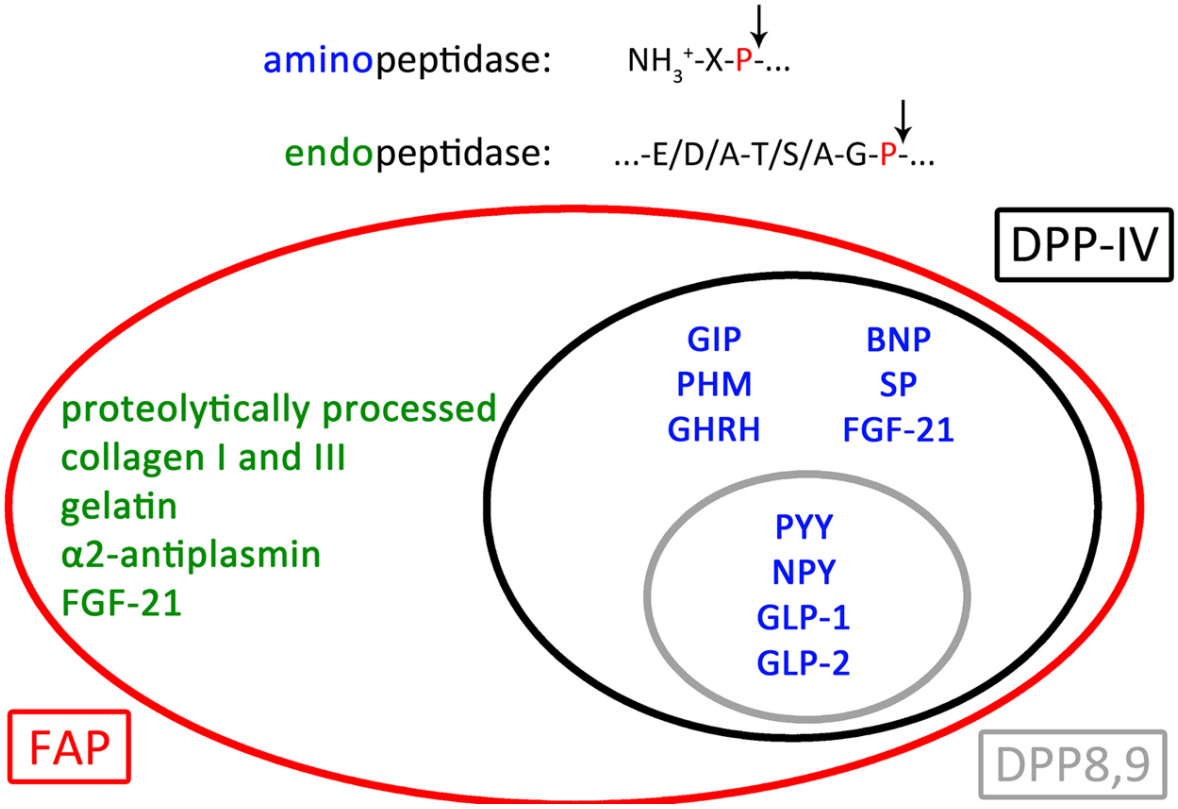

Figure 1. FAP enzymatic activity and known protein substrates. Substrates cleaved by the aminopeptidase enzymatic activity of FAP (in blue) are also substrates for the homologous exopeptidases DPP-IV, DPP8 and 9. GIP = Gastric inhibitory polypeptide/Glucose-dependent insulinotropic peptide; PHM = Peptide Histidine-Methionine; GHRH = Growth hormone-releasing hormone; BNP = Brain natriuretic peptide; SP = Substance P; GLP-1, 2 = Glucagon-like peptide-1, 2; PYY = Peptide YY; NP Y = Neuropeptide Y; FGF-21 = Fibroblast growth factor 21.

in the range of 15–500 ng/ml in various studies depending on the material and methodology used (35-41)). In mice, plasma FAP enzymatic activity is higher than in humans ((34, 42) and our unpublished data). The source of plasma FAP is at present unknown.

In healthy adult humans, FAP is co-expressed with DPP-IV in the alpha cells of Langerhans islets (43). It is also present in multipotent bone marrow stromal cells (BM-MSC) in both mice and humans (44, 45). Independent of its enzymatic activity, FAP promotes the motility of human BM-MSC possibly by regulating the RhoA activity (46). FAP protein is also weakly expressed in the cervix (47) and in the uterine stroma, where it reaches highest levels during the proliferative phase. It has also been detected in the human placenta (47, 48) and in some cases in dermal fibroblasts surrounding hair follicles (49).

In mice, the highest FAP enzymatic activity was detected in the uterus, pancreas, and the submaxillary gland; some activity was also present in the skeletal muscles and the lymph nodes (34). Studies in transgenic mice allowing bioluminescent visualization and a conditional ablation of FAP expressing cells revealed the presence of FAP+ stromal cells in several tissues and suggested their important function in sustaining muscle mass and hemopoiesis. Interestingly, cancer-induced cachexia was associated with a depletion of these stromal cells from normal tissues, which further strengthens the hypothesis of their important trophic role (50). Work carried out by the same group has also revealed that FAP+ fibroblastic reticular cells in the lymph nodes play an essential role in maintaining lymph node architecture and in initiating T and B cell response to influenza A infection (51). Nevertheless, a study by Tan *et al.* (52) in FAP knockout mice suggests that FAP deficiency by itself does not cause abnormalities in immune cell subsets or abnormal anti-influenza immune response. FAP knockout mice are viable and fertile, do not show increased susceptibility to cancer, histopathology abnormalities or changes in basic metabolic and immunological parameters (32, 52). The animals are, however, protected against high-fat diet-induced obesity and metabolic changes (53), probably due to increased bioavailability of FGF-21 (54), and have an increased accumulation of collagen fragments in the lungs (24). More detailed studies closely examining changes in FAP-expressing tissues under various pathological conditions are needed to reveal possible non-redundant functions of FAP.

3.2. FAP in non-malignant diseases

Increased FAP expression is associated with several non-malignant conditions, especially those that involve tissue remodeling. Skin wound healing induces FAP expression in fibroblasts (55), and increased FAP was also reported in keloids and in scleroderma (56). Similarly, healing after myocardial infarction is accompanied by the presence of FAP+ activated fibroblasts and FAP contributes to their migratory potential (57). FAP expression has also been detected in the submucosa and muscle layer in intestinal strictured regions in Crohn's disease (58), in advanced aortic atherosclerotic plaques, and in thin-cap human coronary fibroatheromata, where it was proposed to contribute to type I collagen breakdown in the fibrous caps (59).

FAP is undetectable in a healthy liver, but markedly elevated in liver cirrhosis. FAP is expressed predominantly in the hepatic stellate cells (HSC) at the tissue remodeling interface around regenerative nodules, where it co-localizes with collagen I and fibronectin. A weaker expression has also been detected in the cells of the fibrous portal septa (60, 61). Further work has demonstrated that the intensity of FAP immunoreactivity correlates with the severity of liver fibrosis in hepatitis C infected patients (62) and its serum concentrations are elevated in patients with alcoholic liver disease (34). Functionally, FAP may, independently of its enzymatic activity, increase the adhesion, migration, and apoptosis in the HSC (61).

FAP is not detectable in normal human lung or centriacinar emphysema by immunohistochemistry. FAP is expressed in idiopathic pulmonary fibrosis, particularly in areas of ongoing tissue injury (fibroblast foci in close association with hyperplastic epithelium), but it is absent in the neighboring normal tissue (63). Interestingly, FAP seems to have a protective role in the context of idiopathic pulmonary fibrosis. Using FAP knockout mice, Fan *et al.* (24) have demonstrated that the absence of FAP led to a decrease in collagen I fragment clearance from the lungs, and thereby also an increase in the fibrotic response and decreased animal survival.

In osteoarthritis and rheumatoid arthritis, FAP expression has been demonstrated in fibroblast-like synoviocytes. In rheumatoid arthritis, higher FAP levels have been observed in connection with increased levels of other proteins involved in extracellular matrix turnover (matrix metalloproteinases (MMP) 1 and 13, and CD44 splice variants v3 and v7/8) (64). In osteoarthritis of the hip, FAP expression has been further detected in the chondrocytes in the superficial zone of the cartilage. It was induced by interleukin 1 (IL-1) and oncostatin M, cytokines which promote cartilage destruction (65). In a model of murine arthritis, radiolabeled anti-FAP antibodies accumulated in inflamed joints and signal intensity correlated with the severity of the inflammation and with response to treatment (66, 67). In addition, FAP knockout mice exhibited a decrease in cartilage destruction (68). FAP+ cells thus probably contribute to joint destruction and FAP seems to be involved in these processes.

3.3. FAP in the tumor microenvironment

FAP was originally identified as an antigen recognized by the F19 murine monoclonal antibody raised against lung fibroblasts (69). Seminal works by Rettig *et al.* suggested that this antigen was expressed in a large proportion of astrocytoma, sarcoma, and melanoma cell lines, as well as in cultured normal fibroblasts *in vitro*, whereas epithelial cells, including malignant epithelial cells and hematopoietic malignancies, were F19 negative (69-71). On the tissue level, it has been shown that the antigen is a characteristic trait of the stroma in various malignancies, while its expression under physiological conditions is very limited (55). Reflecting its predominant localization in activated fibroblasts, the abovementioned investigators coined the designation "fibroblast activation protein" (72, 73).

Further work expanded the list of malignancies that characteristically overexpress FAP and revealed that in addition to cancer-associated fibroblasts, FAP may be present in other cellular components of the tumor microenvironment. FAP has been detected in endothelial cells (74-80), in a subpopulation of CD45+ stromal cells (presumably macrophages (81, 82)), and osteoclasts in multiple myeloma (76). It has also been shown that FAP is expressed by several types of transformed cells (Table 1).

Association between FAP expression and clinicopathological variables, including patient survival, seems to be tumor type-dependent, but larger studies have not yet been undertaken. A recently published meta-analysis (83) involving 15 studies which assessed FAP expression in 11 solid cancers by immunohistochemistry concluded that FAP positivity is found in 50–100% of patients and a higher FAP expression is associated with 1) a higher local tumor invasion, 2) increased risk of lymph node metastases, 3) decreased survival, in particular in cases where FAP is expressed in the malignant cells. The association with a worse survival has been most clearly demonstrated in colorectal (80, 84) and pancreatic carcinoma (85, 86), but was also reported for hepatocellular (87), ovarian (88), non-small cell lung carcinoma (89), and osteosarcoma (90). Nonetheless, a recent retrospective study in pancreatic cancer by Park *et al.* (91) suggests that a high number of FAP+ fibroblasts is associated with increased overall survival. In breast cancer, improved prognosis in

Table 1. FAP expression in human malignancies and its association with clinicopathological variables

Tumor type	Expression detected in		Notes	Reference(s)
	malignant cells	stroma cells*		
Basal cell carcinoma, squamous cell carcinoma of the skin	-	+	Expression in fibroblasts strongest in close proximity to cancer cells. FAP expression is absent in benign epithelial tumors, its positivity in the stroma may be a useful criterion for differentiating between morpheaform/infiltrative basal cell carcinomas and FAP-negative desmoplastic trichoepithelioma.	(49, 157, 159)
Oral squamous cell carcinoma	+	+	FAP is a negative prognostic marker – elevated expression is associated with greater tumor size, lymph-node metastasis, advanced clinical stage, and worse overall survival (109).	(109, 172)
Melanoma	- (*in situ*)	+	FAP expression present in a subset of melanocytes in 30% of benign melanocytic nevi, but not detectable in malignant melanoma cells in melanoma tissues (49, 146). The quantity of FAP-positive stromal cells is positively associated with ECM content and inflammatory cell infiltration (237). Normal melanocytes express FAP *in vitro* (71). Conflicting data for FAP in melanoma cells: several human melanoma cell lines express FAP and FAP contributes to their invasiveness *in vitro* (20, 71, 96, 103, 149), but immunopositivity has not been detected in melanoma tissues. Mouse melanoma cell lines are FAP-negative and mouse FAP is a tumor suppressor independently of its enzymatic activity (147).	(20, 49, 71, 96, 103, 146, 147, 149, 237)
Esophageal cancer	+	+	FAP is expressed in cancer cells as well as in premalignant metaplastic cells of the esophagus in both adenocarcinoma and squamous cell carcinoma.	(238-241)
Gastric cancer	+	+ (including low expression in endothelial cells (77, 78)	A higher stromal FAP expression at the invasion front is associated with low tumor cell differentiation, more advanced TNM stage, serosal invasion, and poor survival (242). A higher stromal FAP is associated with worse survival (128). A higher FAP expression in intestinal-type gastric cancer (in stroma, moderately differentiated cancer cells, and endothelial cells) than in the diffuse type (mainly in cancer cells with poor cell-to-cell contacts, endothelial cells). A higher stromal FAP expression in the intestinal-type gastric cancer is associated with the presence of liver and lymph node metastases (78).	(77, 78, 128, 242-244)
Colorectal cancer	+	+	A higher stromal FAP positivity found in earlier-stage disease, but in patients with stage IV tumors high FAP is associated with worse survival (84). A higher FAP expression is associated with advanced Duke stage (79). A high FAP expression in the tumor center is a negative prognostic factor (245). Stromal FAP expression in stage II/III rectal cancer after chemoradiotherapy is associated with a worse prognosis (80). A higher FAP mRNA expression is associated with worse disease-free survival and a trend for worse overall survival (246).	(79, 80, 84, 111, 245-247)
Pancreatic adenocarcinoma	+	+	FAP expression in carcinoma cells is associated with a larger tumor size, presence of a fibrotic focus, perineural invasion, and a worse prognosis (86). Stromal FAP expression correlates with lymph node metastasis and reduced survival (85, 136, 142, 248). Nevertheless, a recent retrospective Korean study reports an association between a lower number of FAP+ fibroblasts and a decreased overall survival based on a univariate analysis (91).	(41, 85, 86, 91, 136, 248)
Hepatocellular carcinoma		+	FAP expression detected especially in tumors with abundant fibrous stroma (249). FAP mRNA expression increased in peritumoral tissue, positively correlating with the density of peritumoral activated HSCs. Higher levels are associated with more frequent early recurrence, larger tumor size, presence of vascular invasion, and an advanced TNM stage (87).	(87, 249)
Non-small cell lung cancer	-/+	+	Absence of stromal FAP expression (24% of cases) in NSCLC is associated with better survival (89). Reports regarding expression in cancer cells are inconsistent.	(55, 89, 250)
Mesothelioma	+	+	Expression, although to a variable extent, has been detected in all subtypes (47).	(47)
Breast tumors	+ (ductal adenocarcinoma)	+ (including endothelial cells (75)	FAP positivity detected mainly in the stroma (55, 81, 92); another study proposes a predominant localization in cancer cells in ductal adenocarcinoma (251). Jung *et al.* observed expression in cancer and stromal cells in 50% of cases where stroma is rich in adipose tissue (approximately 1/3 of all tumors); in these cases, FAP expression was associated with a higher tumor grade. In tumors with fibrous stroma, FAP expression was virtually absent (2/3 of all tumors) (94)	(55, 75, 81, 92-94, 154, 251-256)

			FAP expression is higher in cancer cells in lobular cancer than in ductal carcinoma (252). Stromal FAP and calponin positivity may be an ancillary marker for detecting microinvasion in ductal carcinoma (253). FAP expression increases with the malignant progression of phyllodes tumors (154), but a later study detected stromal FAP expression only in 12.5% of the malignant phyllodes tumors by IHC (254). Conflicting data regarding a possible association with breast cancer survival: smaller studies have reported that a higher total FAP mRNA expression is associated with worse survival (93), while a higher stromal FAP expression detected by IHC was associated with a longer overall survival and disease-free survival (92). A recent larger study involving 939 breast cancer patients did not prove any association between FAP expression in the cancer or stromal cells and survival (94).	
Renal cancer	-	+	Stromal FAP expression (detected in 23% of cases) associated with markers of aggressiveness and worse survival in clear cell renal cell carcinoma (156). In metastatic clear cell renal carcinoma, stromal FAP expression was detected in 36% of primary and 44% of metastatic lesions, and was associated with several parameters of tumor aggressiveness and worse survival (155).	(155, 156)
Prostate cancer	-	+	Only small patient cohorts reported in literature. Expression in stromal cells detected in 7/7 cases, most intense in stromal cells adjacent to cancer cells (158).	(158, 257)
Cervical cancer	+	+	No FAP expression was detected in preinvasive cervical neoplasia (CIN1, 2), occasional positivity in stroma in CIN3 with moderate or severe inflammatory infiltrates. Enhanced expression of FAP was found in cancer cells and subepithelial stromal cells in some of the microinvasive and all of the invasive carcinomas (258).	(258)
Ovary	+	+	FAP positivity increases with tumor stage; negative FAP expression is associated with longer disease free survival (259). FAP positivity detected in cancer cells in 21% of tumors, stromal positivity in 61% (260). Another study reported stromal positivity in 92% of cancer tissues with extremely rare FAP expression in malignant cells; it also reported an association with advanced tumor stage and presence of lymph node metastases (261). FAP-positive malignant cells are present in malignant pleural and peritoneal effusions: strong positivity is associated with worse survival (88).	(55, 69, 88, 259-261)
Glioma	+	+	FAP expression increased in glioblastoma, highest expression found in the mesenchymal subtype (262) and gliosarcoma (151). Low expression in glioma stem-like cells ((25) and our unpublished data). In glioblastoma, overall FAP quantity is not associated with survival (262).	(25, 151, 262-264)
Thyroid cancer	-	+	FAP upregulated in aggressive papillary thyroid carcinomas (265). In medullary thyroid carcinoma, FAP expression in the peritumoral and intratumoral stromal compartment correlates with the degree of desmoplasia and presence of lymph node metastases (266).	(265, 266)
Parathyroid tumors	n.d.	+	FAP mRNA expression was significantly higher in parathyroid carcinomas than in adenomas (162).	(162)
Sarcomas	+ (see note)	+ (reactive fibroblasts in Ewing's sarcomas (267))	FAP expression found in malignant cells in fibrosarcomas, leiomyosarcoma, malignant fibrous histiocytoma (69), low grade myofibroblastic sarcoma, fibroblastic areas in osteosarcomas, osteoid osteoma (267), and in osteosarcoma (101). FAP is negative in malignant cells with "small round cell" phenotype (embryonal rhabdomyosarcoma, Ewing sarcoma, or mesenchymal chondrosarcoma) (69). A higher expression in osteosarcoma associated with more advanced clinical stage, presence of distant metastasis, high histological grade, and a worse progression-free and overall survival (90). FAP is expressed in both malignant and benign tumors and its positivity reflects their histogenetic origin rather than malignant potential (267).	(69, 90, 101, 267)
Myeloma	-	+	FAP expression was detected in osteoclasts, endothelial cells, adipocytes, fibrotic stroma, but not in multiple myeloma cells. FAP is upregulated in osteoclasts co-cultured with myeloma cells (76).	(76)

*Positivity in stromal cells = mesenchymal cells and/or fibroblasts (unless specified otherwise)

patients with FAP positive stroma has been reported (92), but other studies could not confirm this finding (93, 94). The cause of these somewhat discrepant results is currently unclear but may involve differences in the methodology of FAP quantification as well as differences in the FAP/seprase epitopes recognized by various antibodies (see (95) for more details), in particular in paraffin sections.

It is thought that FAP participates in several hallmarks of malignancy, including transformed cell invasiveness and proliferation, extracellular matrix

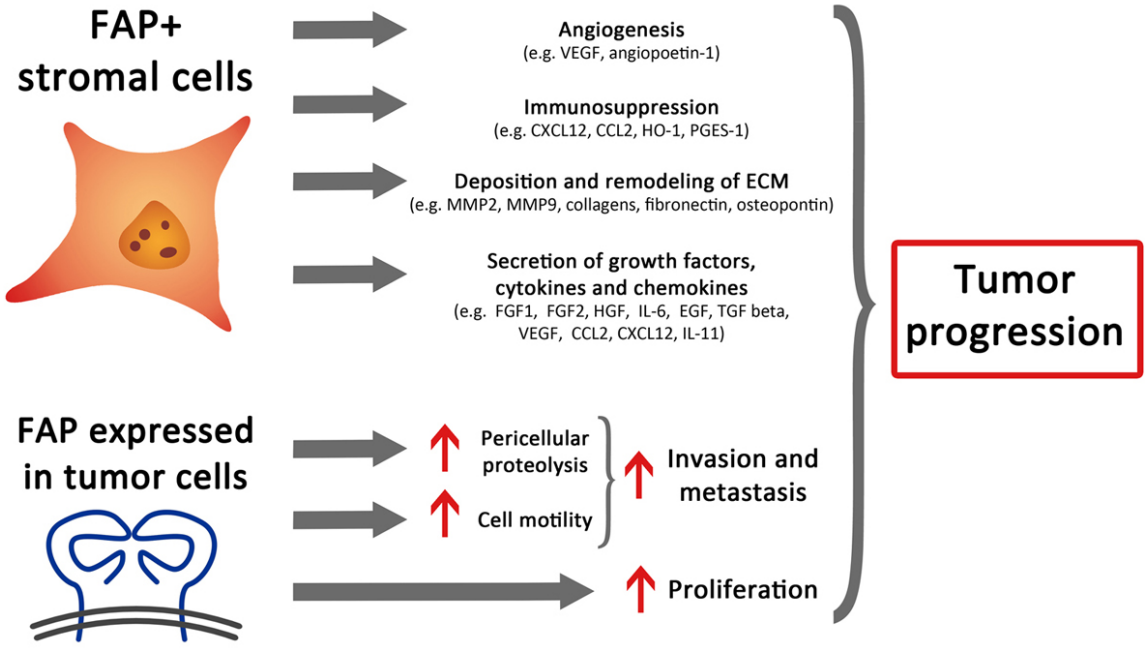

Figure 2. FAP in tumor progression. By producing a variety of structural and regulatory molecules, FAP+ stromal cells contribute to local immunosuppression, extracellular matrix remodeling, and stimulation of angiogenesis, thereby creating a protumorigenic microenvironment. Moreover, FAP expressed in certain types of transformed cells was shown to directly promote tumor cell invasiveness and proliferation. VEGF = Vascular endothelial growth factor; HO-1 = Heme oxygenase 1; PGES-1 = Prostaglandin E synthase-1; MMP2, 9 = Matrix metalloproteinase 2, 9; FGF1, 2 = Fibroblast growth factor 1, 2; HGF = Hepatocyte growth factor; IL-6, 11 = Interleukin 6, 11; TGF beta = Transforming growth factor beta.

(ECM) remodeling, tumor vascularization, and escape from immunosurveillance (Figure 2). The gelatinolytic activity of FAP contributes to ECM degradation (21-23). Several reports have shown that FAP is localized mainly in the invadopodia of migrating cells, where it forms proteolytic complexes (e.g. with DPP-IV or urokinase receptor (uPAR)) (96-99). Integrins such as alpha3 beta1 interact with FAP and are probably responsible for this specific localization of FAP (100). In line with these data, it has been shown that FAP contributes to tumor cell motility and invasiveness (e.g. (101-106)).

FAP may also contribute to tumor cell invasiveness by various non-hydrolytic mechanisms. Breast cancer cells transfected to express either enzymatically active or inactive FAP degraded extracellular matrix more extensively and had a significantly higher MMP9 expression compared to cells transfected with a control vector (107). An independent study found that human breast cancer cell lines expressing either active or inactive forms of FAP have an upregulated MMP2 and MMP9 expression (106). The mechanisms are only partly understood but seem to involve several signaling pathways that influence cell invasiveness. FAP expressed in transformed cells can activate focal adhesion kinase (FAK), integrin linked kinase, and RAC1 probably through integrins. This then promotes cell adhesion to ECM components and motility (108). Nevertheless, the data regarding FAK activation are ambiguous, since Jia *et al.* have shown that breast cancer cells which overexpress FAP have an impaired migration due to a reduced FAK activation (93). The phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway is activated in FAP-expressing cells and a downregulation of FAP then results in PTEN upregulation which inhibits PI3K/AKT signaling (106, 109). Interestingly, exposure to collagen I resulted in a FAP upregulation in ovarian cancer cells, suggesting another mechanism through which the composition of ECM could induce a pro-invasive phenotype in the transformed cells (105).

FAP seems to promote the migration of non-malignant stromal cells such as endothelial cells and fibroblasts (61, 99, 110) by stimulating pericellular proteolysis, and possibly by other non-enzymatic mechanisms (46). In addition to directly affecting cell motility, FAP expression in the tumor stroma is also associated with the production of motility-promoting factors (111) and an ECM organization that promotes the invasiveness of transformed cells. Lee *et al.* have demonstrated that extracellular matrix produced by FAP-positive fibroblasts stimulate beta1 integrin signaling in pancreatic cancer cells, thus promoting their motility more efficiently than their FAP-negative counterparts (112). In other models, diminished FAP enzymatic activity or protein levels resulted in a disorganized ECM that contained chaotically

accumulated collagen, glycosaminoglycans, and glycoproteins such as fibronectin, which can be an impeding factor for tumor growth and invasiveness (30, 113-115). FAP+ stromal cells seem to be a source of MMP9, since their ablation in a murine metastatic breast cancer model resulted in lower concentrations of MMP9 in the tumor mass (116).

ECM remodeling is closely linked with tumor neovascularization. Several murine models show that both FAP in the cancer and the stromal cells contributes to an increased microvascular density (114, 115, 117). Using gain- and loss of function models, Koczorowska *et al.* have demonstrated that FAP expression in fibroblasts is positively associated with the secretion of pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) or angiopoetin-1, and negatively associated with the expression of anti-angiogenic factors such as Pigment epithelium-derived factor (PEDF) (30). FAP thus seems to induce a pro-angiogenic secretome in stromal cells, a conclusion further supported by observations which demonstrate that an ablation of FAP+ cells in *in vivo* tumor models results in both a lower intratumoral VEGF concentrations and a decrease in vascular density (118, 119).

FAP-expressing stromal cells significantly contribute to the switch from a cytotoxic antitumor immunity to a tumor-promoting pro-inflammatory state characterized by an abundant presence of various immunosuppressive cells, such as macrophages or myeloid-derived suppressor cells (MDSC). In line with this observation, it has also been shown that a depletion of FAP-positive stromal cells is associated with lower counts of immunosuppresive elements in the tumor and seems to promote antitumor immune responses, mediated mostly by CD8+ cytotoxic lymphocytes (118, 120-123). FAP-expressing cells are an important source of immunosuppressive molecules such as heme oxygenase 1, prostaglandin E synthase, CXCL12, and CCL2 (82, 122, 124-126). It is possible that FAP directly contributes to the immunosuppressive activities of these stromal cells. Yang *et al.* have demonstrated that by constitutively activating the uPAR-dependent FAK–Src–JAK2–STAT3 signaling pathway, FAP is responsible for the secretion of CCL2, a chemokine which induces the infiltration of MDSC. By using a FAP and a DPP-IV inhibitor PT100 (talabostat), the authors have demonstrated that this takes place independently of FAP enzymatic activity (124).

Other works, however, highlight the importance of FAP enzymatic activity in establishing an immunosuppressive environment. Chen *et al.* have reported that CCL2 secretion by FAP+ fibroblasts could be reduced by linagliptin, a potent FAP but also DPP-IV inhibitor (127). Similarly, it has been shown that linagliptin improves the effect of Programmed cell death protein 1 (PD-1) checkpoint inhibitors in experimental gastric cancer (128). Another study suggests that FAP-expressing bone marrow mesenchymal stromal cells in multiple myeloma promote the senescence of CD4+ lymphocytes by activating the PI3K/AKT pathway, which could be inhibited by PT100 (talabostat) (129). Moreover, the enzymatic activity of FAP generates collagen I fragments which can serve as ligands for class A scavenger receptor (SR-A) expressed by macrophages, thus allowing for their increased adhesion and infiltration into the tumor stroma (130). On the other hand, it has previously been shown that highly selective DPP-IV inhibitors can, among other things, enhance the effect of checkpoint inhibitors (131). It thus still remains to be determined whether DPP-IV inhibition is the cause of or contributes to some of the observed effects.

The FAP-mediated processes described above create a “fertile soil” for the growth of transformed cells. Additionally, FAP expressed in transformed cells may directly enhance their growth. It has been shown that FAP increases both tumorigenicity and proliferation, and decreases the dependence on exogenous growth factors in several types of transformed cells including breast, oral, colorectal and ovarian carcinoma and fibrosarcoma (102, 109, 113, 117, 121, 132-134). This direct growth-promoting effect probably involves decreased PTEN activity and activation of the PI3K/AKT and Ras-ERK pathways, and it may be independent of the enzymatic activity of FAP (106, 109).

It has also been demonstrated that FAP promotes the growth of stromal fibroblasts and their transformation into cancer-associated fibroblasts (124, 135). According to several reports, FAP expressed in stromal cells contributes to the stimulatory effect of cancer-associated fibroblasts (CAF) on tumor cell proliferation (76, 114, 128). A cell cycle shift from G0/G1 to S/G2/M and inactivated retinoblastoma (Rb) protein has been reported in pancreatic tumor cells co-cultured with FAP-expressing fibroblasts, but not in co-cultures with fibroblasts devoid of FAP (136). It has been further shown that FAP-expressing stromal cells are a major source of growth factors including hepatocyte growth factor (HGF), IL-6, IL-11, epidermal growth factor (EGF), fibroblast growth factor (FGF) 1, FGF2 or transforming growth factor (TGF) beta (111, 125, 137-139). Correspondingly, a depletion of FAP and/or elimination of FAP-expressing cells in mouse models resulted in a lower proliferation of tumor cells (115, 119, 140, 141).

The complex effect of FAP on tumor progression is well documented in studies which used genetically driven mouse tumor models in FAP knockout mice. In an endogenous model of lung

adenocarcinoma, FAP deficiency was associated with a lower tumor burden, decreased tumor cell proliferation, and increased survival of the animals (114). Similarly, analysis of the development of endogenous pancreatic ductal adenocarcinoma has revealed that in FAP knockout mice, tumor occurrence was delayed, and animal survival increased. Tumors in these models showed a decreased frequency of visceral metastases and an increased necrosis, possibly due to a lower resistance to immune-mediated control of their progression. Besides stromal cells, FAP was also expressed in 4.4% of the transformed cells, which exhibited mesenchymal-like phenotype. Together, these data suggest that the complementary roles of FAP in both stromal and transformed cells jointly contribute to tumor progression (142).

Contrary to these tumor-promoting activities, in some tumor types FAP seems to act as a tumor suppressor. FAP expression was lost upon experimental oncogenic transformation induced by simian virus 40 transformation in normal fibroblasts, H-ras transformation in normal melanocytes, supertransformation of osteosarcoma cells, and enhanced N-myc expression in variant neuroblastoma cells, suggesting that FAP expression inversely correlates with growth factor independence and tumorigenicity (71). This conclusion is supported by the work of Tsujimoto *et al.*, who compared non-tumorigenic and tumorigenic hybrids originating from HeLa cells fused with fibroblasts, and identified FAP as one of the proteins downregulated in tumorigenic hybrids (143). Wesley *et al.* have demonstrated that a re-expression of DPP-IV in non-small cell lung cancer (NSCLC) and melanoma cells is accompanied by increased FAP expression and leads to a cell cycle block and increased apoptosis (144, 145). This corresponds to the observation that FAP expression is present in a subset of melanocytic cells in part of the nevi, whereas neither primary nor metastatic melanoma cells express FAP (146). Direct evidence that FAP can act as a tumor suppressor in melanoma cells has been provided by Ramirez-Montagut *et al.* who had shown that forced FAP expression in mouse melanoma cell lines abrogated their tumorigenicity, restored contact inhibition, induced cell cycle arrest, and made them more susceptible to apoptosis. Interestingly, this effect was even more pronounced when an enzymatically inactive form of FAP was used (147).

Collectively, these results indicate that FAP and FAP-expressing cells present in the tumor microenvironment significantly influence various aspects of tumor progression (Figure 2). Mechanisms by which this is achieved probably involve both the enzymatic activity and non-hydrolytic functions of FAP, whereby in transformed cells, the effects seem to be tumor type dependent.

3.4. Factors that regulate FAP expression

Regulation of FAP mRNA transcription was proposed to be the main regulatory mechanism which controls FAP expression, whereby a region of approximately 750–250bp upstream from the transcription start site was identified as the core promoter that drives the expression in various FAP-positive cell lines. It has been shown that this region contains the canonical TATA box (in humans and rats, but not in mice) and possible binding sites for transcription factors EGR1, E2F1, Sp1, and HOXA4, as well as several TGF beta-responsive cis-regulatory elements (148, 149). EGR1 (148) and Smad3/4 complex (149) binding have been experimentally verified to trigger FAP expression. Interestingly, human telomerase reverse transcriptase (hTERT), contributing to cancer development and progression through several mechanisms, might regulate FAP expression via EGR1. FAP was upregulated in hTERT-overexpressing immortalized human oral keratinocytes and hTERT knockdown in oral cancer cell lines resulted in a reduced protein expression of both EGR1 and FAP (150). Other possible FAP regulators in the context of cancer and embryogenesis include mesenchymal transcription factors TWIST1 and PARAXIS (Class A Basic Helix-Loop-Helix Protein 40, Transcription Factor 15). A correlation between TWIST1 and FAP mRNA expression has been observed in human gliomas and TWIST1 overexpression resulted in a FAP upregulation in glioma cells (151). In mice with a knockdown of PARAXIS, FAP was among the most downregulated transcripts in mouse embryo somites (152), suggesting an important role for PARAXIS in regulating FAP expression. FAP mRNA has been detected in Ago complexes isolated from pancreatic islets together with several miRNAs (153), and bioinformatics predictions (e.g. www.microrna.org) suggest that several miRNAs might regulate FAP expression post-transcriptionally. In the stromal cells of phyllodes tumors, miR21 induced the expression of FAP but the effect was indirect, mediated by PTEN downregulation (154). So FAR, no miRNA has been proven to directly regulate FAP mRNA. Factors that induce constitutive FAP expression in pancreatic alpha cells and multipotent bone marrow stromal cells (BM-MSC) are yet to be identified.

Paracrine or juxtacrine mediators seem to play an important role in inducing FAP expression. Indeed, it has been demonstrated in a broad range of tumor types (41, 49, 85, 86, 111, 155-159) that stromal FAP expression is strongest in close proximity to transformed cells. Moreover, conditioned media or direct contact with transformed cells in co-culture frequently leads to upregulation of FAP in stromal cells (111, 160-166). In line with these observations, numerous growth factors, cytokines, and signaling molecules have been identified as possible regulators

of FAP expression in various cell types. In FAP-negative fetal leptomeningeal fibroblasts, FAP expression is upregulated by TGF beta1, phorbol ester, retinol, or retinoic acid (71, 72). FAP is also upregulated by Wnt5a, platelet-derived growth factor (PDGF-BB), and TGF in adult human fibroblasts (104, 160) and by fibroblast growth factor and phorbol ester in melanocytes (71). Several other studies report an induction of FAP expression via the TGF beta signaling pathway (46, 57, 58, 72, 111, 149, 167-169). Exposure of metastatic melanoma cells to TGF beta1 induced a fast and significant upregulation of FAP mRNA, accompanied by a proteasomal degradation of the Smad transcriptional repressor c-Ski and an increase in the binding of Smad3/4 to the FAP promoter (149). Similarly, Tillmanns *et al.* observed an induction of FAP expression in human cardiac fibroblasts by TGF beta1 via the Smad2/Smad3 pathway *in vitro* (57). FAP mRNA levels were significantly decreased by a blocking of the TGF beta signaling in invasive melanoma cells by an antibody against TGF beta, a TGF receptor inhibitor, or by an overexpression of c-Ski (149). Interestingly, compared to invasive melanoma cells, TGF beta induces FAP expression neither in normal melanocytes nor in non-invasive melanoma cells (149). Similarly, TGF beta seems to have no effect on FAP expression in fibroblasts derived from non-strictured, as compared with strictured, intestinal regions in Crohn's disease patients (58). These observations suggest the importance of additional factors for the induction of FAP by TGF beta. In various cell types, FAP expression was also induced by inflammatory cytokines, such as tumor necrosis factor (TNF) alpha (58, 59), IL-1 (46, 65, 168), and oncostatin M (56, 65). Enhanced expression of FAP has also been observed in endometrial fibroblasts after their activation elicited by estrogen or lipopolysaccharide stimulation, the latter being accompanied by an increase in TGF beta expression (170, 171). A recently described mechanism of fibroblast activation by cell clustering in spheroids (nemosis) resulted in an increased FAP expression in fibroblasts isolated from oral squamous cell carcinoma (172). A recent study further suggests that chronic stress contributes to an upregulation of FAP and other CAF markers in the cancer microenvironment via adrenergic stimulation which induces the release of inhibin beta A from transformed cells (173). Furthermore, FAP expression was upregulated by exposure to low concentrations of type I collagen in ovarian cancer cells (105), by UV radiation in melanoma (104, 160), and by direct contact with cancer-associated fibroblasts in non-small cell lung cancer cells (165).

To conclude, the frequently observed upregulation of FAP in cancer tissue seems to be the result of a complex paracrine and/or juxtacrine communication between the transformed cells and their microenvironment, of the recruitment of bone marrow-derived mesenchymal stem cells (174), and of an activation of the epithelial-mesenchymal transition (EMT) and corresponding transcription factors in transformed cells.

4. THERAPEUTIC APPROACHES TO FAP TARGETING

A utilization of FAP as a therapeutic target in cancer was proposed soon after its discovery. Indeed, its relatively selective expression in tumors, its unique enzymatic activity, the importance of FAP-positive stromal cells in shaping the tumor microenvironment, and the presumed direct role of FAP in various aspects of cancer progression make it an attractive target. To date, various strategies have been explored, including inhibition of FAP enzymatic activity, ablation of FAP-positive cells, or the exploitation of selective expression of FAP in the activation or targeted delivery of cytotoxic compounds in the tumor microenvironment.

4.1. FAP inhibition using low molecular weight inhibitors

Several preclinical studies indicate that inhibition of FAP enzymatic activity by low molecular weight compounds could decrease the invasiveness of malignant cells and reduce tumor growth. The effect of Gly-ProP(OPh)2, a dipeptide proline diphenyl phosphonate FAP/DPP-IV inhibitor, has been investigated in an *in vitro* melanoma model. UV irradiation increased FAP expression in melanocytes, primary melanoma cells, and fibroblasts. Concomitantly, the migration and invasion potential of these cells was increased, an effect reduced by Gly-ProP(OPh)2 (160), albeit at relatively high concentrations (100µM). Santos *et al.* have studied the impact of a pharmacologic inhibition of FAP enzymatic activity by PT630 (GluBoroPro dipeptide) which is known to inhibit not only FAP but also DPP-IV. In mice, the compound efficiently reduced tumor growth both in an endogenous lung tumor model driven by a conditional activation of the oncogenic allele of K-ras^{G12D} and in a syngeneic colorectal carcinoma model that used transplanted CT26 cancer cells. Administration of the compound resulted in a decrease in tumor cell proliferative index, a lower number of intratumoral myofibroblasts, and the inhibition of angiogenesis (114). M83, a highly effective FAP and prolyl oligopeptidase (POP) pseudopeptide inhibitor suppressed the growth of human lung cancer H441 and HCT116 colon cancer xenografts in immunodeficient mice, possibly by inhibiting angiogenesis and promoting collagen accumulation (175). PT-100 (Val-boro-Pro, talabostat), which competitively inhibits dipeptidyl peptidase activity of FAP, CD26/DPP-IV, and DPP8/9, reduced tumor growth in a multiple myeloma model by inhibiting the pro-survival effects of osteoclasts on the transformed cells (176). Similarly, PT-100 slowed

the growth of tumors derived from fibrosarcoma, lymphoma, melanoma, and mastocytoma cell lines in syngeneic mouse models, but had no effect on tumor cell growth or viability *in vitro* and only limited effect in immunodeficient mice. The mechanism of action probably involved the production of cytokines and chemokines capable of promoting antitumor immune responses (177). Correspondingly, an independent study in immunodeficient mice and xenotransplanted breast tumor cells failed to demonstrate the antitumor effects of PT-100 and PT630 (107), a finding which further supports the importance of the immune system in mediating the effects of these compounds. PT100 has been shown to improve the response to chemotherapy in a colon cancer model. Intratumoral levels of TGF beta3, FGF2, and osteopontin, as well as the number of immature dendritic cells and M2 macrophages were lower in PT100-treated animals, and PT100 mitigated the oxaliplatin-induced accumulation of FAP+ cancer-associated fibroblasts. Vascularity and tumor growth were lower and mouse survival longer in mice that received both oxaliplatin and PT100 than in animals that received single treatments (178). PT100 in combination with dendritic cell (DC) vaccines led to a complete tumor regression in mice challenged with urothelial carcinoma cells MB49, acting as an effective adjuvant accelerating the expansion of tumor-specific T cells. Its inefficacy in immunosuppressed mice and in mice with CD4+ and/or CD8+ T cells depletion demonstrated that the antitumor activity of PT100 was indeed mediated by the immune system (179).

The notion of possible beneficial effects of FAP enzymatic activity inhibition in alleviating immunosuppression is supported by recent studies with linagliptin, a dual FAP and DPP-IV inhibitor (180). In a CT26 colon cancer syngeneic model, cancer-associated fibroblasts (CAF) with high, as opposed to low, FAP expression induced resistance to an anti-PD-1 immune checkpoint antibody, possibly due to a decreased T cell infiltration, recruitment of myeloid-derived suppressor cells (MDSCs), and a higher PD-L1 expression. Treatment with linagliptin reversed the immunosuppression and in combination with anti-PD-1 antibody led to a decrease in tumor growth (127). In another study, Wen *et al.* have reported that in mice injected with a gastric cancer cell line together with FAP-positive CAF, treatment with an anti-PD-1 antibody had no effect. In combination with linagliptin, a mild inhibition of tumor growth was observed, while the greatest effects were achieved when FAP expression in CAF was abrogated using shRNA. This was associated with an increased T cell activation and infiltration in the tumor, upregulation of IL-2, IL-4, IL-10, IFN gamma, and TNF alfa, and a downregulation of PD-L1 and PD-L2 (128).

PT100 (talabostat) is the only FAP inhibitor tested so far in clinical trials in cancer patients. It was well tolerated in a phase I study in solid tumor patients who were receiving myelosuppressive chemotherapy and it accelerated neutrophil recovery (181). However, despite the promising results in preclinical tests, it failed in phase II clinical trials. Patients with metastatic colorectal cancer who had previously received systemic chemotherapies were treated with talabostat. The treatment significantly, though incompletely, inhibited FAP enzymatic activity in plasma, but had a minimal clinical effect (182). In another study, a combinatorial treatment of talabostat and docetaxel was tested in 42 patients with stage IIIB/IV NSCLC. Patients experienced an increase in serum IL-6 and IL-8, but the study was unable to show that talabostat enhanced the clinical activity of docetaxel (183). A phase II trial, where talabostat and cisplatin were administered to metastatic melanoma patients, has also failed to show any significant improvement over cisplatin alone (184). A major limitation of the studies with FAP inhibitors performed so far is the possible off-target effect on related proteases. In this respect, a parallel inhibition of DPP-IV and/or DPP8/9 may significantly contribute to the observed immunomodulatory effects (131, 185, 186).

4.2. FAP-mediated activation of prodrugs

The unique proline-specific proteolytic activity of FAP has been exploited in the design of several FAP-activated prodrugs. Upon a cleavage by FAP, a non-toxic prodrug is selectively activated to a highly potent cytotoxin in the peritumoral fluid. This leads to the killing of both FAP-positive and neighboring FAP-negative cells. In one of the approaches, melittin, a well-characterized cytolytic toxin produced in honey bees, was modified by adding a peptide sequence into the prodomain of the peptide. This engineered form of promelitin could be hydrolyzed only by FAP to generate a toxic form of the venom. The protoxin was more active towards FAP-transfected than FAP-non-transfected MCF-7 cells and inhibited tumor growth when injected intratumorally. Nevertheless, its use for a systemic delivery is limited because even the unprocessed protoxin causes hemolysis in experimental animals (187). Similarly, attachment of a benzyloxy-blocked Gly-Pro (Z-Gly-Pro) to the amino group of doxorubicin produced a FAP-activatable doxorubicin prodrug. This prodrug's antitumor effect was comparable to the parental drug in a mouse model. Although a slight nonspecific hydrolysis in the normal organs and plasma was observed, toxic side effects were markedly suppressed in comparison with the original drug (188). In a follow-up study, the authors developed a nanomicellar system to overcome the low water solubility of the Z-Gly-Pro doxorubicin prodrug, whereupon they were able to demonstrate an enhanced accumulation of the drug in the tumor and its faster clearance from plasma (189). These authors have also recently reported a Z-Gly-Pro epirubicin

prodrug which exhibits a diminished cardiotoxicity and a similar antitumor effect as the free epirubicin in a breast cancer mouse model (190). FAP is present predominantly in stromal cells, whose proliferation is in general slower than the proliferation of transformed cells. Thapsigargin, a highly toxic natural plant product which inhibits the endoplasmic reticulum calcium ATPase pump and is toxic even for non-proliferating cells, was therefore chosen to prepare FAP-activatable prodrugs (42). The thapsigargin prodrug generated by coupling a FAP-cleavable peptide to a thapsigargin analog was less toxic and was not nonspecifically taken into the cells. In a MCF-7 breast adenocarcinoma and a LNCaP prostate carcinoma xenograft model, the prodrug specifically targeted stromal cells and inhibited tumor growth (42). Compared to docetaxel, the prodrug exhibited a similar efficacy but lower toxicity in a prostate cancer model (191). Nevertheless, the prodrug had a limited efficacy in a tumor model with a low stromal content and was cleaved and thereby activated by blood plasma FAP, which together with the lipophilic character of the molecule might possibly lead to a partial deposition of the active drug in healthy organs (191). The same investigators have also synthesized a prodrug based on emetine, a highly toxic protein synthesis inhibitor. This prodrug is selectively activated by a sequential cleavage by FAP and DPP-IV. Nevertheless, it was found to be stable in human plasma and 200x times less toxic than free emetine. In the presence of FAP and DPP-IV, the prodrug was shown to inhibit the growth of prostate and breast cancer cell lines *in vitro* (192). Recently, arenobufagin, a Na+/K+ ATPase inhibitor, was used to prepare a FAP-activated prodrug. It showed a specific hydrolysis by recombinant human FAP and by homogenates from FAP-expressing tumor tissue, and moreover, activation of the prodrug was effectively blocked by PT100 (talabostat). The prodrug reduced tumor size in mice bearing MDA-MB-231 xenografts and exhibited less cardiac toxicity than the original compound (193). Recently, a FAP-activated vascular disrupting agent derived from vinblastine was described. A Z-Gly-Pro dipeptide was attached to desacetyl-vinblastine monohydrazide, creating a FAP-cleavable prodrug. By selectively targeting FAP-positive pericytes, the compound overcame the resistance of the highly pericyte-covered vessels, characteristically found at tumor periphery, to vascular disrupting agents. Its application therefore led to a disruption of blood vessels not only in the core of the tumors, but also around the periphery, and resulted in tumor regression in several mouse xenotransplantation models (194).

Ji *et al.* designed FAP-cleavable amphiphilic peptide (CAP) monomers, which due to their amphiphilicity self-assembled into nanofibers and had the ability to encapsulate hydrophobic drugs. When hydrolyzed by FAP, the nanoparticles rapidly disassembled and released their cargo. This was demonstrated *in vitro* by the selective toxicity of doxorubicin-loaded nanoparticles towards FAP+ cancer-associated fibroblasts. Moreover, the CAP-encapsulated doxorubicin inhibited tumor growth in mouse xenotransplantation models, displaying better tumor penetration and a stronger anticancer effect than the free drug. Importantly, the therapeutic effect was observed even in tumors originating from FAP-negative cancer cells due to a strong bystander effect (195).

4.3. Immune-based therapies targeting FAP

4.3.1. FAP antibodies and their conjugates

The F19 murine antibody, which was utilized in the original studies that identified FAP as a cancer-associated antigen (55, 70), was used in proof-of-concept studies to demonstrate the ability of FAP antibodies to accumulate in the tumor tissue after intravenous application. In patients with metastatic colorectal cancer, a purified ^{131}I-labeled F19 antibody accumulated in liver metastases. The methodology allowed for a detection of metastases as small as 1cm using Single-photon emission computed tomography (SPECT), and suggested a possible diagnostic and therapeutic potential of FAP antibodies (196). The elimination half-life of the antibody was similar to other mouse antibodies that do not bind to specific tissue antigens, which further supports its selectivity for tumor tissues (197).

Several humanized versions of the F19 antibody have been prepared and tested. Using phage display technology and human V-repertoires, the V_L and V_H regions of F19 flanking the mouse CDR3 region were replaced by analogous human V-regions in order to reduce its xenoantigenic potential while maintaining the parental epitope specificity (198). A technique called “guided selection”, which involves a sequential display of human antibody light and heavy chains compared with a known murine variable region, was used to develop several fully human single-chain variable fragments (scFv) based on the F19. To increase their affinity, the scFvs were further converted to bivalent minibodies, which were then successfully used for immunohistochemical detection of FAP in human carcinoma samples (199). Various approaches were subsequently deployed to evaluate the antibodies in mouse models. Biodistribution of a humanized monoclonal anti-human FAP antibody (BIBH-7) labeled with ^{131}I and ^{125}I was tested in a novel breast cancer tumor model containing human stroma. Female SCID mice were grafted with human skin and tumor xenografts were then established using MCF-7 cells. The stroma expressed human FAP and the BIBH-7 antibody preferentially accumulated in this compartment (200). Another approach involved the generation of high-affinity, species cross-reactive,

FAP-specific scFvs converted into a bivalent derivative (minibody MO36). The minibody was shown to detect FAP in the stromal cells of different human carcinomas as well as in murine host stroma in a tumor xenograft model by immunohistochemistry (201). Sibrotuzumab, a humanized version of the F19 antibody, was tested in a phase I clinical trial in metastatic cancer patients; it was demonstrated that it is not toxic, does not accumulate in healthy tissues, and it successfully targets the tumor stroma (202). Nevertheless, no therapeutic response was observed in either this phase I or the subsequent phase II trial in metastatic colorectal cancer, and some of the patients developed anti-human antibodies (203).

It is largely unknown whether the abovementioned antibodies inhibit FAP enzymatic activity. Zhang *et al.* have recently prepared scFv antibodies capable of inhibiting FAP enzymatic activity: the scFv antibody modified the FAP-mediated rearrangement of the fibronectin fibers *in vitro*, but its effect on cancer growth is currently undetermined (204). It has been shown that polyclonal antibodies that inhibit the enzymatic activity of FAP do slow tumor growth in a mouse xenotransplantation model in which FAP overexpressing HEK293 were used, but their effect is modest (132).

It thus seems that anti-FAP antibodies, even those that inhibit FAP enzymatic activity, have a limited or even no antitumor activity *per se*. Nevertheless, their excellent tumor stroma targeting properties offer a possibility of designing conjugates with e.g. toxins, radioisotopes, or immunomodulatory cytokines for localized delivery. Ostermann *et al.* have prepared and tested a monoclonal FAP antibody conjugated with the highly toxic maytansinoids DM1 and DM4 through a cleavable linker. The FAP mAb used, FAP5, cross-reacted with human, monkey, and mouse FAP, which made it suitable for preclinical testing in animal models. The conjugated antibodies inhibited tumor cell proliferation *in vitro* and tumor growth *in vivo* in the pancreas, lung, and head and neck squamous cell carcinoma mouse models. The mAb FAP5-DM1 had excellent tolerability and exerted its effect by disrupting the stromal compartment and by mitotic arrest and apoptosis of malignant FAP-negative epithelial cells, which suggests a strong bystander effect (205). Another study used an anti-FAP scFv linked to the heavy and light chain of the pseudomonas exotoxin A (PE38). The immunotoxin selectively elicited cytolysis in 293T human embryonic kidney cells transfected with mouse or human FAP and, as shown by PET imaging, accumulated in experimental tumors. When used alone, the immunotoxin retarded tumor growth in a mouse breast carcinoma model only to a modest degree; this was accompanied by a decrease in TAM (tumor-associated macrophages) infiltration and by altered intratumoral concentrations of various cytokines and chemokines. Nevertheless, it significantly improved the antitumor activity of paclitaxel (116). This immunotoxin also enhanced the effect of a trivalent tumor cell antigen directed vaccine in a melanoma model. In this case, a combination treatment substantially reduced proliferation and increased apoptosis in the tumor tissue, thus impeding tumor growth and improving animal survival. The synergistic mechanism involved a stimulation of the antitumor immune response as evidenced by the greatly increased intratumoral CD8+/Treg, CD4+/Treg, CD8+/MDSC, and CD4+/MDSC ratios and a local cytokine profile promoting immune responses (141). In another study, two monoclonal human-mouse cross-reactive antibodies selected by phage display (ESC11 and ESC14) were labeled with the beta-emitting radiolanthanide ^{177}Lu. Both antibodies accumulated specifically in FAP-positive human melanoma xenografts and delayed tumor growth. The ^{177}Lu-ESC11 exhibited the most pronounced growth delay while maintaining a relatively low presence in the blood and organs (206).

To selectively activate apoptotic pathways in tumor cells, a bispecific antibody (RG7386) binding FAP and death receptor 5 (DR5) was prepared. The binding of this antibody to FAP on stromal cells led to a hyperclustering of DR5 and thereby to an induction of apoptosis in tumor cells, as shown in co-culture systems *in vitro*, and reduced tumor growth *in vivo*. Importantly, the bispecific antibody had more than additive effects in combination with irinotecan: their joint action led to acomplete tumor eradication in a colorectal cancer mouse model (207). Another approach used TNF-based immunocytokines which activated TNF receptor signaling only when bound to the FAP molecule, thus reducing the systemic toxic effects of the TNF. A dimeric anti-FAP-TNF protein was found to be most effective. It caused a delay in tumor growth accompanied by macrophage recruitment in immunodeficient mice xenografted with FAP-transfected fibrosarcoma HT1080 cells (208, 209).

Other approaches aimed at a specific stimulation of local antitumor immune response are currently being developed. They utilize various bispecific antibodies that crosslink FAP+ cells with T cells, as well as immunocytokines containing variant IL-2 (IL-2v) and IL-15. In a 3D heterotypic spheroid model containing colon cancer cells, fibroblasts, and peripheral blood mononuclear cells, a bispecific antibody targeting FAP and CD3 epsilon depleted FAP-positive fibroblasts; this effect was more pronounced when IL-2v was simultaneously applied. A FAP-targeted version of IL-2v (the anti-FAP-IL-2v immunocytokine) accumulated specifically in fibroblast-containing regions; it is currently evaluated in a phase I clinical trial under identification number NCT02627274 (210). Using the FAP targeting antibodies MO33, MO36 (201), Hornig *et al.* have constructed a bispecific

FAP-CD3 antibody and fusion protein consisting of a FAP antibody and a costimulatory ligand B7.2. Its combination with another costimulatory ligand 4-1BBL fused to an anti-endoglin antibody led to a T cell activation and a IL-2 and IFN gamma release in the presence of target cells expressing both FAP and endoglin (211). In another study, a FAP-targeted version of IL-15 fused with an extended sushi domain of the IL-15R alpha was effective in mediating trans-presentation of IL-15, and activating T cell proliferation and cytotoxicity. Application of this FAP-targeted form improved the antitumor effect of IL-15 in a mouse model that utilized FAP-transfected B16 melanoma cells (212).

4.3.2. Immunoliposomes targeting FAP

Anti-FAP antibody fragments were immobilized on liposomes in order to create nanocarriers for therapeutically active agents which would specifically target tumor tissue. Immunoliposomes presenting single-chain Fv molecules targeting FAP (scFv 36) were able to bind to FAP-expressing cells and were internalized (213). These immunoliposomes, loaded with the fluorescent dye DY-676-COOH, were used to visualize FAP+ cells *in vitro* and in a mouse xenograft model where they accumulated in FAP-overexpressing tumors (214). In a follow-up study, the immunoliposomes were used to detect the metastatic spread of tumors (215). Bispecific immunoliposomes recognizing FAP and endoglin (CD105) exhibited a higher binding to the target stromal cells than monospecific liposomes which targeted FAP or endoglin separately (216). To allow the targeting of both stromal and transformed cells, bispecific liposomes containing single-chain antibody fragments specific for FAP and HER2 (trastuzumab) were prepared. In a mouse breast cancer model, these bispecific immunoliposomes accumulated in fibroblasts, perivascular cells, as well as in HER2-positive tumor elements (217). Messerschmidt *et al.* have engineered a composite targeted delivery system containing single-chain (sc) TNF-functionalized polystyrene particles, lipid coating, and scFv 36 for FAP targeting. Encapsulation of TNF-containing particles reduced their nonspecific binding and scTNF-mediated cytotoxicity, and resulted in a moderate improvement in selective toxicity towards FAP+ target cells (218). In summary, several types of FAP-targeting immunoliposomes have been studied but their therapeutic potential remains to be assessed.

4.3.3. FAP vaccines

Several approaches including DNA, protein, and dendritic cell vaccines have been utilized to elicit effective immune responses that would eliminate FAP-positive cells. An orally administered DNA vaccine (*S. typhimurium* transformed with a vector encoding mouse FAP) decreased tumor growth by CD8+ T cell-mediated killing of the target cells in mouse CT26 colon carcinoma and D2F2 breast carcinoma models. In the treated animals, intratumoral collagen I levels decreased and doxorubicin uptake increased, which resulted in a significantly improved effect of the combination of chemotherapy and FAP vaccination on survival over individual treatments alone (219). A similar synergistic effect was observed when the two treatments were tested in a 4T1 murine model of metastatic breast cancer. A more detailed analysis of immunomodulatory effects of this combination treatment revealed a shift in the Th2 to Th1 polarization of the immune system, decreased infiltration of tumor-associated macrophages, myeloid derived suppressor cells, and T regulatory lymphocytes, as well as an increase in the recruitment of dendritic cells and CD8+ T cells. Moreover, angiogenesis and lymphangiogenesis was reduced in the treated tumors due to a reduction of stroma-derived angiogenic growth factors and cytokines (118). The results produced in a CT26 mouse colon carcinoma model were independently verified using a liposomal DNA vaccine injected subcutaneously in a prophylactic setting. The authors observed that CD8+ T cell-mediated antitumor immune response inhibited tumor growth and metastasis, consequently prolonging the lifespan of the mice. This was accompanied by a reduction of the FAP+ cells in the stroma and by reduced intratumoral collagen expression (121). In another study (123), an adenoviral vector-based vaccine with full-length mouse FAP induced an effective immune response and prolonged survival in a genetically engineered as well as a syngeneic mouse melanoma model. It had also improved the effect of an anti-tumor adenoviral vaccine expressing multiple epitopes from melanoma-associated antigens. Similar to other studies, a stimulation of CD8+ T cells, leading to a depletion of FAP+ stromal cells, was identified as the mechanism responsible for this effect. Importantly, the authors demonstrated that the reduced content of FAP+ stromal cells, achieved by a combined vaccination targeting both stroma and tumor cells, is associated with decreased STAT6 activation, lowered number and effector functions of tumor-infiltrating immunosuppressor cells, as well as complex changes of the cytokine and chemokine profile in the tumor microenvironment. These changes result in a decreased metabolic stress of the tumor-infiltrating CD8+ T lymphocytes and in their improved antitumor activity (123). Xia *et al.* reported in a series of papers the efficacy of several DNA vaccines tested in a mouse breast cancer model in prophylactic and therapeutic settings. The vaccines elicited a specific antitumor immune response and reduced tumor growth. Nevertheless, when used alone, their effect was limited due to immunosuppressive mechanisms including a high expression of IL-10. A combination with a low-dose cyclophosphamide resulted in a significantly improved therapeutic response characterized by an increase

in the number of effector T cells, reduced Treg, IL-10, CXCL12, VEGF, intratumoral collagen I, and FAP expression. This suggests a more effective ablation of FAP+ stroma by a combination treatment (220-223). A peptide FAP vaccine based on the catalytic domain of mouse FAP and CpG as an adjuvant was reported: it reduced the growth of experimental B16 melanoma tumors, but only in combination with curcumin, possibly due to curcumin's suppressive effect on indolamine-2,3-dioxygenase (IDO) expression (224). A mild antitumor effect was also observed in mice vaccinated with FAP-overexpressing fibroblasts concurrently with an implantation of 4T1 syngeneic breast cancer cells. In this case, CD8+ and CD4+ T cell-mediated immune response led to a decrease in tumor vascularization and collagen I content, and to increased apoptosis in transformed cells (135).

Using dendritic cell vaccines, Fassnacht *et al.* have tested the ability of several antigens overexpressed in the tumor microenvironment to induce T lymphocyte responses. Unlike the MMP9 or MMP14, FAP-transfected dendritic cells consistently elicited human cytotoxic T lymphocyte responses *in vitro*. To enhance MHC class II presentation, a lysosomal targeting signal derived from the lysosomal protein LAMP-1 was attached to the FAP carboxyterminal, which led to a stronger *in vitro* response that involved the activation of CD4+ Th cells (225). This concept was extended to *in vivo* assays in which mice were immunized using dendritic cells transfected to express mouse FAP. In several subcutaneous syngeneic tumor models, this led to delayed tumor growth. In a B16/F10.9 melanoma model, the effect was comparable to the targeting of the tumor cell-expressed antigen tyrosinase-related protein 2 (TRP-2). A lysosomal targeting signal–modified FAP antigen as well as co-vaccination against TRP-2 further increased the effect of stroma-targeted dendritic cell vaccination in this model. With the exception of a slight negative effect on wound healing, no FAP vaccination-associated mortality or morbidity was observed in this study (226). The effectiveness of this dual transformed cell and FAP+ stroma-targeting approach was independently confirmed by Gottschalk *et al.* (227). A single dose of a dendritic cell vaccine which co-targeted tumor cells (through TRP2) and CAFs (through FAP) and silenced A20, a negative regulator of NF kappa B-mediated dendritic cell activation, resulted in a fourfold increase in the percentage of infiltrating CD8+ T cells, reduced tumor growth, and increased animal survival in a B16 melanoma model (227).

4.3.4. Chimeric antigen receptor (CAR) T cells targeting FAP

Genetically engineered chimeric antigen receptor (CAR) T cells that specifically recognize a selected tumor-associated antigen have recently expanded the armamentarium of immunotherapy. In 2013, a FAP-CAR based on an anti-FAP antibody MO36 was constructed by Kakarla *et al.* (140). *In vitro*, T cells expressing this CAR recognize mouse and human FAP-transfected cells, as well as cell lines which endogenously express FAP, as demonstrated by a release of proinflammatory cytokines and a cytotoxicity assay. In mice injected with lymphoblastoid and A549 lung cancer cells, FAP-specific CAR T cells decreased tumor growth by eliminating FAP+ stromal cells. In the A549 lung cancer model, improved survival has been observed especially when the treatment was combined with cancer cell antigen (EphA2) targeting CAR T cells. Due to the limited persistence of the CAR T cells, however, the tumors did eventually progress (140). In another study, CD8+ human T cells were retrovirally transduced with a CAR based on the anti-FAP F19 antibody. In contact with FAP-positive mesothelioma cells, these CAR T cells released IFN gamma and specifically lysed the target cells *in vitro*. Consistent with this result, the CAR T cells delayed tumor growth and increased mice survival in an *in vivo* mesothelioma model (47). In an independent study, it has been demonstrated that CAR T cells targeting mouse FAP secrete IFN gamma and kill FAP-transfected 3T3 cells *in vitro*. *In vivo*, these CAR T cells reduced the number of FAP+ stromal fibroblasts and leukocytes, thus slowing tumor growth in several syngeneic mouse models. Interestingly, the effects were to a large extent dependent on augmentation of the endogenous CD8+ T cell antitumor response, since the effects were not observed in immunodeficient mice. In line with this observation, FAP-CAR T cells significantly improved the antitumor effect of a tumor cell vaccine, most likely by alleviating the intratumoral immunosuppression (228). A follow-up study focused on the impact of FAP-CAR T cells on tumor-induced desmoplasia as a possible driver of tumor progression. In highly desmoplastic tumor models of lung and pancreatic adenocarcinoma, FAP-CAR T cells reduced tumor growth independently of the endogenous immune response. This was accompanied by a disruption of the desmoplastic stroma, reduced angiogenesis, decreased cancer cell proliferation, and by an increase in apoptosis. The effects were not observed in FAP-null mice, which confirms that the effects are mediated by FAP+ stromal cells (119). In contrast to these encouraging studies, the work by Tran *et al.* had shown that FAP-targeting CAR T cells may lead to severe side effects. Two CAR constructs were generated using the scFv from FAP-specific monoclonal antibodies FAP5 and sibrotuzumab. The resulting CAR T cells were capable of specific degranulation and could produce effector cytokines in the presence of FAP-expressing cell lines. Nonetheless, their effect on tumor growth in a broad panel of mouse models was limited, probably due to the tumors' relatively low stromal content. More importantly, the injection of FAP5-CAR-transduced T cells led to morbidity and mortality in most of the mice.

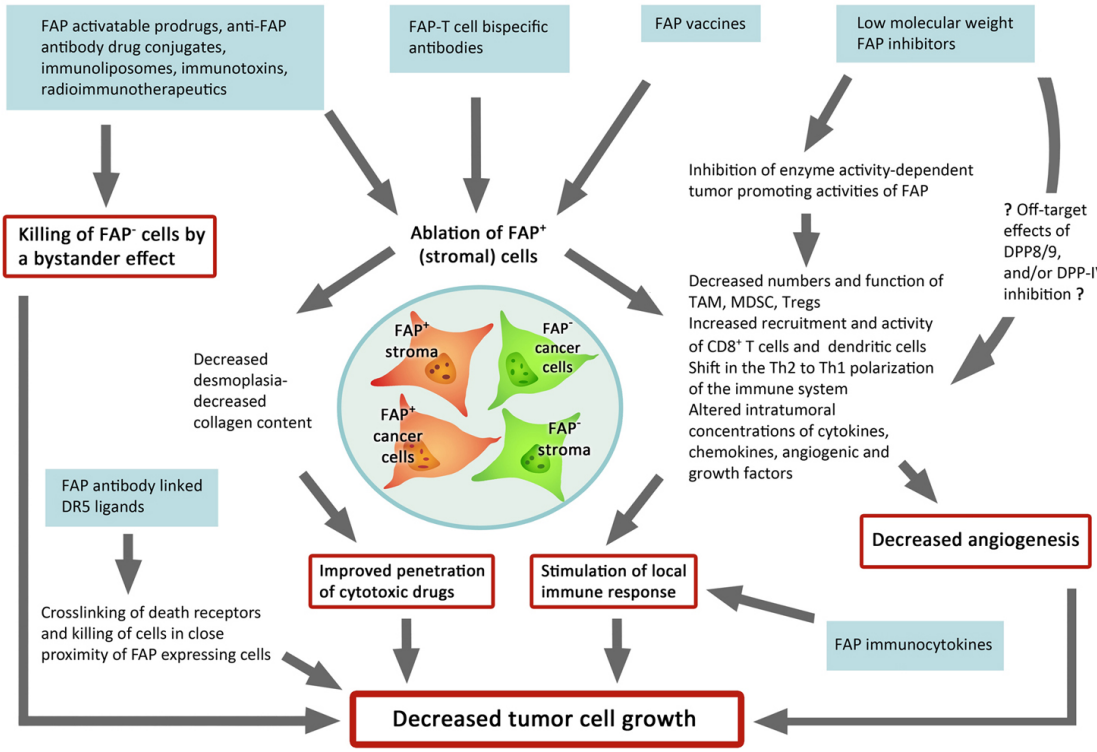

Figure 3. FAP-targeting therapies and their expected impact on tumors composed of transformed and stromal cells with heterogeneous FAP expression. Both FAP enzyme activity inhibition and ablation of FAP-positive cells lead to changes in the tumor microenvironment, including alleviation of immunosuppression, decreased neovascularization, and changes in extracellular matrix content and composition. This improves the effect of standard chemotherapeutics and potentiates endogenous and/or the therapeutically induced antitumor immune responses. FAP-activatable prodrugs, anti-FAP immunotoxins, and radioimmunotherapeutics can also achieve the killing of FAP-negative cancer cells by a bystander effect. DR5 = death receptor 5; DPP = dipeptidyl peptidase; TAM = tumor-associated macrophages; MDSC = myeloid-derived suppressor cells

Severe bone marrow hypocellularity and cachexia caused by the targeting of FAP+ osteogenic cells, including bone marrow stromal cells (BMSC) and possibly mesenchymal stromal cells in other organs (44, 50), were observed in FAP5-CAR-T cell treated animals. Similar toxic effects were seen in a pancreatic adenocarcinoma model rich in FAP expressing stroma, which suggests that they were not caused merely by a low representation of FAP-positive stroma. It is therefore currently unclear why these side effects have not been observed in other studies that utilize FAP-targeting CAR-T cells (44).

5. CONCLUSION

Over the past three decades, research had shown that FAP is associated with the pathogenesis of various tumors. It is a robust marker of functionally important stromal cells in the tumor microenvironment and it quite possibly contributes to their tumorigenic effects (30). FAP may even directly contribute to the malignant phenotype of transformed cells via both enzyme activity dependent and non-hydrolytic mechanisms. Nevertheless, its function seems to be context dependent and at least in part tumor type specific.

Various FAP-targeting modalities have been tested (Figure 3), mostly in preclinical cancer models. In general, the available data suggest that this stroma-targeting approach may be feasible and useful, especially when combined with additional anticancer treatments. Importantly, most studies indicate that the FAP-targeting approaches are not associated with serious toxicity. The particular strategies of FAP targeting differ in their potential to affect cancer progression. The use of low molecular weight FAP inhibitors seems to have several limitations. First of all, these compounds can only affect the enzyme activity-dependent functions of FAP. They thus do not interfere with the numerous non-hydrolytic protumorigenic effects that FAP exerts in malignant and stromal cells. Secondly, their effectiveness critically depends on the role(s) of FAP in the pathogenesis of particular tumor types. And finally, both PT100 and PT630 cyclize at physiological pH (229), whereupon they lose inhibitory activity. These factors may have contributed to the failure of clinical trials with PT100 (talabostat) (182-184). Moreover, most studies that worked with low molecular weight FAP inhibitors have used rather non-specific ones. It is therefore unclear to what extent the inhibition of other related proteases, namely DPP-IV (127, 128, 177), prolyl oligopeptidase (PREP) (175),

and DPP8/9, may have contributed to the observed effects. Indeed, recent studies which show that talabostat (PT100) triggers a proinflammatory form of cell death (pyroptosis) in monocytes and macrophages by inhibiting DPP8 and 9 (185, 186) strongly suggest that talabostat's immunomodulatory effects observed in cancer studies may be independent of FAP. Highly specific FAP inhibitors became available only recently (230-232). These compounds are non-toxic and their effect on cancer growth *in vivo* is yet to be determined. Nonetheless, available preclinical data suggest that these highly specific inhibitors ought to be tested as immunotherapeutic adjuvants rather than in combination with cytotoxic agents, because their main effect may be the enhancement of the antitumor immune response.

Unlike the low molecular weight FAP inhibitors, FAP-targeting approaches exploiting a preferential expression of the protease, rather than directly interfering with its function in the tumor microenvironment seem promising especially due to their potential use in a variety of neoplasms. Importantly, a broad safety evaluation in preclinical mouse models using FAP vaccines and FAP-CAR T cells revealed either minimal (140, 228) or no changes (219) in healthy organs. In the case of FAP-activatable advanced delivery systems (195) and prodrugs, the effect is not limited to FAP-expressing cells. Similarly, FAP-targeting immunotoxins, antibody conjugates, immunoradioisotopes, and immunoliposomes offer a possibility of targeting FAP-negative tumor cells through a bystander effect. In preclinical models, ablation of a FAP-positive tumor stroma by FAP vaccines or CAR T cells, for example, did not usually result in a permanent cure, but alleviated tumor immunosuppression, decreased angiogenesis, and disrupted the tumorigenic desmoplasia (119). By exerting complex effects on the tumor microenvironment, these FAP-targeting approaches lead to the disruption of several hallmarks of cancer and hold the promise of significantly improving the effects of other cancer therapies (Figure 3). Moreover, depletion of the tumor-supporting stroma may be an important factor in preventing tumor recurrence.

6. PERSPECTIVES

FAP expression varies even in tumor types known to be generally FAP-positive. It is thus highly desirable for future clinical studies to assess therapeutic effectiveness of FAP-targeting approaches in patients selected for high intratumoral FAP. PET and SPECT probes for the detection of FAP have recently become available (66, 67, 233) but data on their use in cancer patients are as yet limited (196). Given the intratumoral heterogeneity of FAP expression, these probes could be more accurate in estimating FAP quantity in the tumor tissue than an examination of biopsy samples. Nevertheless, a correlation between the imaging data and FAP quantity determined by commercially available ELISAs or specific enzymatic assays (34, 234) remains to be verified.

Optimal timing and combination of FAP-targeting approaches with current therapeutic paradigms is yet another challenge. Data on FAP expression in recurrent and residual disease are so far missing. Eradication of individually dispersed transformed cells that have not induced a strong stromal reaction is less likely to be achieved by FAP targeting. Implementation of these approaches shortly after a surgical removal of the tumor with curative intent may therefore be problematic. On the other hand, FAP targeting may help suppress the fibrotic processes and stromal activation evoked by radiotherapy, which can somewhat paradoxically create a supportive microenvironment for cancer progression (235). Further studies are, however, needed to resolve the issue of possible antifibrotic effects of FAP, especially in the context of lung diseases (24).

Based on preclinical evidence, FAP-targeting therapies may significantly enhance the response to immunotherapy approaches that exploit, for example, PD-1 and CTLA4 blockade and immunization against cancer cells (141). Especially in highly desmoplastic tumors, FAP targeting may also improve the efficacy of conventional cytostatic agents by increasing their penetration into the tumor tissue (219) and reducing CAF functions (178). FAP-targeting therapies, including redirected T cells (NCT01722149 at *clinicaltrials.gov*), an immunocytokine containing interleukin 2 variant (IL-2v, NCT02627274), and a bispecific FAP-DR5 antibody (NCT02558140), are currently being tested in Phase I clinical trials. These studies should help us better understand the possible on-target toxicity associated with FAP-based therapies in the clinical setting.

Patients with liver fibrosis/cirrhosis (60), advanced atherosclerosis (59), Crohn's disease (58), idiopathic pulmonary fibrosis (63), recent myocardial infarction (57), keloids, scleroderma (56), or rheumatoid arthritis (64) should not be involved in trials with anticancer FAP treatments. Careful monitoring of side effects should include assessments of glycemic control (43), hematotoxicity (44, 45), muscle wasting (50), gynecological (47, 48) and skin toxicities (49), as well as possible musculoskeletal side effects which may appear especially in older patients with osteoarthritis (65).

Future studies should investigate the proposed physiological functions of FAP in metabolic regulation (54) and turnover of collagen (24), as well as the importance of FAP+ stromal cells in maintaining normal tissue homeostasis (44, 50). Cautious

extrapolation of data obtained in mouse cancer models to human subjects is advisable. Circulating levels of FAP are in general substantially higher in mice (at least based on FAP enzymatic activity measurements), and it remains to be determined whether the physiological expression pattern and role of FAP found in mouse models correspond to humans. Additionally, stroma development and importance in traditionally used xeno- or syngeneic-transplantation models may not accurately reflect the situation in human tumors (236), including FAP expression (124) and function.

A better insight into the physiological and pathological functions of the FAP molecule itself and FAP+ cells will be critical for a successful translation of preclinically tested approaches to its targeting into clinical setting.

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