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Received 14 July 2014

Accepted 28 July 2014

Published Online First

18 August 2014

Importance of carcinoma-associated fibroblast-derived proteins in clinical oncology

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ABSTRACT

Carcinoma-associated fibroblast (CAF) as prominent cell type of the tumour microenvironment has complex interaction with both the cancer cells and other non-neoplastic surrounding cells. The CAF-derived regulators and extracellular matrix proteins can support cancer progression by providing a protective microenvironment for the cancer cells via reduction of chemotherapy sensitivity. On the other hand, these proteins may act as powerful prognostic markers as well as potential targets of anticancer therapy. In this review, we summarise the clinical importance of the major CAF-derived signals influencing tumour behaviour and determining the outcome of chemotherapy.

INTRODUCTION

Carcinoma-associated fibroblasts in tumour stroma

In the process of tumour formation, the normal microenvironment 'niche' changes to an altered (ie, reactive or desmoplastic) stroma which is composed of non-malignant supporting cells (ie, blood vessels, infiltrating inflammatory cells and blast-like cells).^{1–2} This altered microenvironment functions as a collaborative partner in the process of tumorigenesis by influencing the homeostasis of cancer cells via paracrine regulators (eg, growth factors, cytokines and chemokines) and exosomes containing nucleic acids.^{1–3} Cancer associated fibroblasts (CAFs), prominent stromal elements in most types of human carcinomas, are α -smooth muscle actin positive, spindle-shaped, blast-like cells. Differentiation of CAFs from other cell types, such as local fibroblasts, hepatic stellate cells, mesenchymal stem cells, endothelial and epithelial cells, is mainly mediated by transforming growth factor- β 1 (TGF- β 1), but other factors, such as growth hormones (ie, epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF)), chemokines, epigenetic regulators and oxidative stress also may play a role in CAF differentiation.^{4–7} CAFs, phenotypically, closely resemble normal myofibroblasts, but they express specific markers (ie, fibroblast activation protein (FAP), fibroblast-specific protein 1, neuroglial antigen-2, vimentin, Thy-1, tenascin (TN)-C, periostin (POSTN), palladin or podoplanin (PDPN)) and display an increased proliferation and migratory behaviour *in vitro*.^{8–9} CAFs produce and secrete various extracellular matrix (ECM) proteins (ie, collagens I, III, IV), proteoglycans (ie, fibronectin, laminin, TN), chemokines (eg, CXCL and CCL), cytokines (eg, interleukin (IL)-6 and IL-8) and other tumour-promoting factors which affect vascularisation (ie, PDGF, vascular endothelial growth

factor (VEGF), stromal-derived factor-1 (SDF-1), matrix metalloproteinase (MMPs)), proliferation capacity, tumour cell invasiveness and survival (ie, TGF- β , EGF, hepatocyte growth factor (HGF) or FGF).^{1–9–11}

Regarding anticancer therapy, the frequency of genetic mutations in CAFs is one of the most important issues. Cells with genetic stability may be less prone to escape or resistance to chemotherapy than those with genomic instability.¹² Several studies demonstrated that high percentage of CAFs undergo genetic alterations, such as loss of heterozygosity or mutation of tumour suppressor genes (ie, phosphatase and tensin homolog and P53).^{13–16} The theory of genetic coevolution of CAF and the neighbouring cells (ie, random mutation of CAF generated independently from neoplastic epithelial cells that may support tumour progression) is under debate due to the potential artefacts caused by the analytical methods used for the identification of these genetic alterations.¹⁷ Other groups described that the somatic mutations of CAFs are found to be extremely rare and are unlikely to be responsible for their stable cancer-promoting attributes.^{18–19}

In this short review, we discuss those CAF-derived proteins which (1) may have an important role in the development of environment-mediated drug resistance, (2) may act as powerful prognostic markers and (3) may be promising targets of anticancer therapy.

Relation of CAFs to microenvironment-mediated drug resistance

Emerging data suggests that several factors of the tumour microenvironment play a critical role in determining therapy response.¹⁰ CAF-derived factors may contribute to the development of a protective milieu via influencing the following: (1) cell–cell/cell–matrix interactions, (2) cancer cell survival, (3) interstitial fluid pressure (IFP) within the tumour and (4) suppression of antitumoural immune responses.^{2–20}

Cell adhesion-mediated drug resistance (CAM-DR) is mediated by the adhesion of tumour cells to stromal fibroblasts or to ECM components.²¹ Physical contact between host fibroblasts and tumour cells (eg, melanoma and non-small cell lung cancer (NSCLC) cells) supports tumour cell survival via activation of antiapoptotic pathways or inducing epithelial-to-mesenchymal transition (EMT).^{10–22–24} EMT-originated blastoid cells can acquire cancer stem cell-like traits and drug resistance against conventional chemotherapeutics (eg, taxol, vincristine, oxaliplatin or gemcitabine), as well as anti-EGFR therapy.^{7–24–25} Adhesion of cancer cells to ECM proteins, such as CAF-derived



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To cite: Valcz G, Sipos F, Tulassay Z, et al. *J Clin Pathol* 2014;**67**:1026–1031.

laminin, collagen, fibronectin and POSTN also results in CAM-DR.^{10 20} These proteins can bind to integrin receptors (eg, $\alpha 1$ - $\alpha 6\beta 1$ and $\alpha v\beta 5$) located on the surface of tumour cells and cause protection against drug-induced apoptosis via activation of the phosphatidylinositide 3-kinase (PI3K)/AKT pathway, which inhibits the release of drug-induced apoptotic factors, like cytochrome-c.^{26 27}

CAF-derived soluble regulators, such as hormones, chemokines and cytokines are also able to mediate the therapy resistance in different ways. Activation of receptors by prostaglandin E2 (PGE₂), sphingosine-1-phosphate and PDGF-C ligand promotes tumour cell survival by activation of PI3K/AKT and Hedgehog signals *in vitro*.²⁰ DNA damage-induced nuclear factor- κ B (NF- κ B) dependent wntless-type MMTV integration site family member 16B (WNT16B) protein expression in fibroblast can lead to mitoxantron therapy resistance in prostate cancer cells through T cell factor/lymphoid enhancer binding factor activation.²⁸

IFP and transvascular transport of anticancer drugs also may influence the success of chemotherapy. Inhibition of CAF-derived VEGF, PDGF or their receptors results in lower IFP within tumours, hence, improves chemotherapy efficacy.^{29 30} For example, treatment with anti-PDGF antibody may regulate IFP by the relaxation of cells constituting connective tissues, integrin-mediated contacts with ECM fibres, or ECM rebuilding into a less dense structure.²⁹

CAFs are also involved in tumour-mediated immunosuppression by the expression of immunomodulating ECM proteins, such as TN-C, inhibiting the migration of monocytes and adhesion of T lymphocytes to fibronectin, and thrombospondin-1, regulating cellular phenotype of antigen-presenting cells/APCs and T cells.^{31 32} CAF-derived chemokines, such as monocyte chemoattractant protein-1/CCL2, modulate monocyte migration and release of IL-4 and interferon- γ by CD4 T lymphocytes.³² The chemokine SDF-1 promotes monocyte transdifferentiation to M2 polarised macrophages.³³ M2-like tumour-associated macrophages help to protect cancer cells from the effect of anticancer drugs (eg, paclitaxel and cisplatin) via releasing survival signals (ie, MFG-E8, milk fat globule-EGF 8 protein), proteases (eg, cathepsins) and supporting angiogenesis by secreted factors compensating the effect of anti-VEGF therapy.^{34–36} CAF-derived ECM modulator proteinases, such as MMPs, FAP, urokinase-type plasminogen activators and proinflammatory cytokines (ie, IL-6 and IL-8) may also influence the relation of tumour cells to immune responses.³²

Practical role of CAF-derived factors in clinical oncology

As we summarised above, CAFs are actively involved in tumourigenesis and in formation of environment-mediated drug

resistance. Based on these properties of CAFs, their changed protein expression may be used as a prognostic marker. The abundance and molecular repertoire of CAFs, such as FAP, PDGF/PDGFR (platelet-derived growth factor receptor), TN-C, PDPN, secreted protein acidic and rich in cysteine, hepatocyte-derived growth factor receptor (HGFR, Met), TGF- β and TGF β Rs, carry significant prognostic information about the clinical behaviour of a given tumour (table 1).

CAFs overexpress a wide range of factors which are critical to surrounding neoplastic cell growth. Based on these features, the clinical application of these CAF-related ligands seems to be logical as the targets of anticancer therapies. One possible candidate among the CAF-derived factors is TGF- β , which acts as a suppressor of tumour formation in premalignant conditions and promotes tumour growth, invasion, angiogenesis and metastasis formation in advanced tumours ('TGF- β Paradox').^{57 58} It has a basic role in the development of abnormal niche as a master regulator of trans-differentiation of CAFs from its precursors and with other regulators (ie, EGF, FGF and PDGF) of invasive and metastatic phenotype formation via EMT.^{59 60} It also influences the expression of invasion-associated proteins (including $\alpha_{III}\beta 1$ integrin and fibulin-5) as well as cellular adhesion via decreasing the expression of E-cadherin.⁶¹ By the effect of TGF- β , the fibroblasts actively produce collagen, laminin, TN and fibronectin, all of which may also contribute to a decreased cytotoxicity of anticancer agents via CAM-DR.^{10 20 62} Upon treatment with TGF- β , mammary fibroblasts also causes an upregulation of SDF-1 chemokine, which modulates tumour cell proliferation, angiogenesis, apoptosis and antitumoural immune responses.^{33 63 64} TGF- β inhibits the proliferation, differentiation and the tumour-targeting activities of natural killer (NK) and T cells, as well as influencing the migration of monocytes and macrophages into the tumour microenvironment.^{58 61} Targeted therapy against TGF- β and its receptors includes neutralising monoclonal antibodies (mAb), antisense oligonucleotides (ASO; blocking of activation of TGF- β ligands synthesis via inhibiting TGF- β mRNA expression), small molecule receptor kinase inhibitors (arresting downstream canonical and non-canonical signalling by inhibiting the kinase activity of TGF β RI or TGF β RII) are discussed in table 2.

PDGF and VEGF, basically, play a role in induction and progression of angiogenesis as well as regulation of IFP in tumour interstitium.^{29 30} PDGF acts as a proliferative and chemotactic factor to CAFs, as well as enhances their growth factor expression including insulin-like growth factor-1 (IGF-1), HGF, FGF and VEGF influencing tumour growth, invasion and angiogenesis.^{8 69} PDGF-D ligand may cause the failure of gemcitabine treatment (via acquisition of a chemoresistant EMT phenotype)

Table 1 Function and prognostic role of CAF-related proteins

Protein	Role in tumour biology	Disease and references
FAP	Tumour cell growth, proliferation, ECM remodelling, metastasis formation and angiogenesis ³⁷	DCIS, NSCLC, CRC ^{38–40}
PDGF/PDGFRs	Angiogenesis, regulation of interstitial fluid pressure in tumours ⁴¹	Pancreas and breast carcinoma, NSCLC ^{42–44}
TN-C	Hypoxia-driven angiogenesis, proliferation, migration, escape from immune surveillance ⁸	CRC, breast cancer, melanoma ⁸
PDPN	Cancer cell invasion ⁴⁵	LACC, CRC (good prognosis) breast cancer, oesophagus adenocarcinoma ^{45–48}
SPARC	Cell migration, proliferation, matrix cell adhesion and tissue remodelling ⁴⁹	Pancreatic cancer, NSCLC ^{49 50}
Met	Receptor of HGF, it plays a role in the tumour cell invasion and CAF proliferation ⁵¹	LACC ⁵²
TGF- β /TGF β Rs	Tumour growth, proliferation, invasiveness, angiogenesis, immunosuppression. ^{53 54}	CRC and breast cancer ^{55 56}

CAF, cancer associated fibroblast; CRC, colorectal carcinoma; DCIS, ductal carcinoma in situ; ECM, extracellular matrix; FAP, fibroblast activation protein; HGF, hepatocyte growth factor; LACC, lung adenocarcinoma; NSCLC, non-small cell lung cancer; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PDPN, podoplanin; TGF- β , transforming growth factor- β ; TN-C, tenascin-C; SPARC, secreted protein acidic and rich in cysteine; Met, hepatocyte-derived growth factor receptor.

Table 2 Types and clinical use of anti-TGF- β /TGF- β R targeted therapies

Drug	Type, target and refs	Disease, stage and refs
Fresolimumab/GC-1008	mAb against TGF- β 1,-2,-3 ⁶⁵	Breast cancer (with radiotherapy, Phase I), RCC and malignant melanoma (Phase II) ^{65 66}
Trabedersen/AP12009	ASO against TGF- β 1 mRNA ⁵³	CRC, pancreatic carcinoma and malignant melanoma, NSCLC (Phase I/II) ⁵³
Belagenpumatucel-L/Lucanix;	ASO (antisense gene-modified allogeneic tumour cell vaccine) against TGF- β 2 mRNA ^{53 66}	NSCLC (Phase II) ⁶⁷
LY2157299	TGF β 1 serine/threonine kinase inhibitor ⁶⁸	HCC (Phase II), pancreatic cancer (with gemcitabine, Phase I/II) ^{66 68}

ASO, antisense oligonucleotides; CRC, colorectal carcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; TGF- β , transforming growth factor- β ; mAb, monoclonal antibody.

of hepatocellular carcinoma (HCC) cells; furthermore, PDGF-C mediates resistance to antiangiogenic therapy with VEGF inhibitors.^{43 70} VEGF influences tumour perfusion, vascular volume, permeability activity, microvascular density and the number of circulating endothelial and progenitor cells.^{30 71} VEGF/VEGFR (vascular endothelial growth factor receptor) signalling protects endothelial, ovarian and NSCLC cells against chemotherapeutic injury via the activation of PI3K/AKT pathway and induction of antiapoptotic proteins including Bcl-2 and survivin.^{20 72–74} Inhibition of PDGFRs and VEGFRs by multikinase inhibitors and anti-VEGF mAbs seems to be effective anticancer strategies (table 3).

Carcinoma cells induce HGF secretion of CAFs by different regulator secretion, such as IL-1 β , bFGF, PGE₂, PDGF and TGF- β .^{8 84} CAF-secreted HGF signal influences the invasion of transformed epithelial cells (including enhanced dissociation and stromal migration of cancer cells) and stimulates CAF proliferation as autocrine loop via Met-tyrosine kinase receptor activation.^{51 52 85} HGF protects cancer cells from chemotherapeutic agents via enhancing DNA repair by reactivation of Met/PI3K/extracellular-signal-regulated kinase (ERK) cascades and downregulation of the expression of antiapoptotic proteins (ie, Bcl-XL).^{86 87} HGF is also involved in the mechanisms of resistance development to BRAF and human epidermal growth factor receptor 2 inhibitors as well as selective EGFR- tyrosine kinase inhibitors (TKIs) (ie, gefitinib and erlotinib).^{2 88 89} It is a chemotactic factor for T cells and a negative regulator of the cytotoxic activity of NK cells furthermore modulates the immunoglobulin production and maturation of B-cells.³² In table 4, we summarise the most important HGF mAbs and selective/non-selective Met-TKIs.

Stromal IGF-1 increases the invasive capacity of cancer cells, influences the proliferation of epithelial cells (via upregulation of mitogen-activated protein kinase (MAPK)/AKT, Cyclin D1 and downregulation of p27) and upregulates the proliferation-associated genes in stromal fibroblasts.^{107 108} IGF-1/IGF-1R signal mediates the therapeutic response to conventional chemotherapeutic agents, such as 5-fluorouracil and gemcitabine, through increased survivin stability.^{109 110} CAFs secreted IGF-2 also can influence the survival of cancer cells induced via acquisition of stem cell-like properties.¹¹¹ Blocking of IGF signalling with anti-IGF-1R mAbs, such as AMG 479, B11B022 and cixutumumab/IMC-A12 together with IGF-1R kinase inhibitors (eg, AXL 1717, BMS-754807 and linsitinib/OSI-906) proved clinical benefit in the treatment of HCC, SCLC, NSCLC, breast and pancreatic cancers (phase I/II stage).⁸

FAP/F19 belonging to the family of plasma membrane-bound serine proteases promoting tumour cell growth and proliferation plays a role in ECM remodelling (ie, degrading type I collagen and influencing MMPs' expression), metastasis formation, angiogenesis and deregulation of antitumoural immune responses.¹¹² The use of humanised mAb F19 (sibrotuzumab) and small-molecule FAP enzyme-inhibitor (talabostat) showed no therapeutical benefits alone or in combination in case of metastatic colorectal carcinoma, NSCLC, stage IV melanoma and chronic lymphocytic leukaemia.^{112 113} FAP-based target therapies were found to be promising in preclinical studies, such as doxorubicin-combined FAP-targeting prodrugs and DNA vaccines.^{114 115} FAP radioimmunotherapy (targeted delivery of therapeutic radioisotopes to the tumour site by the developed sensitive FAP mAbs) and short heparin RNA vector-based therapies (RNA interference) also seems to be a promising anticancer treatment in the future.^{116 117}

Table 3 Types and clinical use of anti-VEGF mAbs and multi-kinase inhibitors

Drug	Type and target	Disease, stage and references
Avastin/Bevacizumab	mAb against VEGF ⁷⁵	Multiple tumour types including: CRC, NSCLC, and breast cancer (FDA app)
Pazopanib/GW786034	TKI of VEGFR-1,-2,-3, PDGFR α - β , Kit, FGFR-1,-3, Itk, Lek, c-Fms ⁷⁶	Multiple tumour types including: solid tumours (with paclitaxel/carboplatin, Phase I), RCC (FDA app) ⁷⁷
Imatinib/STI157	TKI of PDGFR β , Bcr-Abl, Kit ⁷⁸	Multiple tumour types including: GIST (FDA app) ⁷⁸
Sunitinib/SU11248	TKI of VEGFR1,-2,-3, PDGFR α - β , Kit, FLT-3, CSF1R, RET ⁷⁸	GIST (imatinib resist; FDA app), RCC (FDA app), NSCLC (Phase III) ^{78 79}
Motesanib	TKI of VEGFR1,-2, PDGFR β , Kit, RET ⁸⁰	NSCLC (with carboplatin/paclitaxel, Phase III), breast cancer (with docetaxel/paclitaxel, Phase I/II) ^{80–82}
Sorafenib	TKI of VEGFR2,-3, PDGFR β Kit, RET, B-Raf, FLT-3 ^{78 83}	Multiple tumour types including: RCC (FDA app), HCC (FDA app) ⁸³

CSF1R, colony-stimulating factor 1 receptor; CRC, colorectal carcinoma; FDA app, Food and Drug Administration approved; FLT-3, FMS-like tyrosine kinase-3; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; Itk, interleukin-2 receptor inducible T-cell kinase; Lek, leucocyte-specific protein tyrosine kinase; NSCLC, non-small cell lung cancer; PDGFR/PDGFR- α / β , platelet-derived growth factor receptor- α / β ; RCC, renal cell carcinoma; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; mAb, monoclonal antibody; TKI, tyrosine kinase inhibitors; FGFR, fibroblast growth factor receptor; RET, rearranged during transfection.

Table 4 Types and clinical use of anti-HGF mAbs and multi-kinase inhibitors

Drug	Type, target and references	Disease, stage and references
Ficlatuzumab/AV-299	mAb against HGF ⁹⁰	Solid tumours (Phase I/II), NSCLC (combined with gefitinib, Phase II). ⁹⁰
Rilotumumab/AMG 102	mAb against HGF ⁹⁰	Multiple tumour types including: RCC (Phase II), prostate cancer (combined with mitoxantrone and prednisone, Phase II). ^{91 92}
Onartuzumab/PRO143966	mAb against Met ⁹³	NSCLC (combination with erlotinib, Phase II), solid tumours (Phase I) ^{94 95}
Cabozantinib/BMS-907351	TKI of Met, VEGFR2, RET, Kit, AXL and FLT3 ⁹⁶	Multiple tumour types including: prostate, breast cancer, NSCLC (Phase II) ^{97 98}
Crizotinib/PF-02341066	TKI of Met, ALK and ROS1 ⁹⁹	NSCLC (Phase III), GEC (Phase I) ^{99 100}
Tivantinib/ARQ 197	TKI of Met ¹⁰¹	HCC (Phase II), NSCLC (Phase II), gastric cancer (Phase II) ^{102–104}
Foretinib/GSK1363089	TKI of Met, VEGFR2, RON, Kit and AXL ¹⁰⁵	Gastric cancer (Phase II), solid tumours (Phase I) ^{105 106}

ALK, anaplastic lymphoma kinase; FLT3, FMS-like tyrosine kinase-3; GEC, gastroesophageal cancer; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; RON, Recepteur d'Origine Nantais; VEGFR, vascular endothelial growth factor receptor; mAb, monoclonal antibody; TKI, tyrosine kinase inhibitors; RET, rearranged during transfection; ROS1, c-ros oncogene 1; Met, hepatocyte-derived growth factor receptor.

CONCLUSION

CAF-induced signalling pathways function as key factors in stroma-supported cancer progression, and can be therapeutically targeted against microenvironment-mediated drug resistance. Focusing the attention on these microenvironmental cells may help us to better understand their role in tumour pathogenesis and, moreover, may help to better predict the clinical outcome of disease, and to make patient-tailored anticancer treatments.

Take home messages

- ▶ CAF-derived factors may provide pro-tumorigenic effects via altering the tumorous microenvironment.
- ▶ Targeting CAF-derived factors may act as potential anti-cancer therapeutic strategies.
- ▶ CAF-derived factors may be clinically used as prognostic factors of different tumorous diseases.

Contributors GV: wrote the manuscript; FS, ZT, BM, YY: corrected and made critical review of the manuscript.

Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

Correction notice A sentence under the section 'Practical role of CAF-derived factors in clinical oncology' has been corrected since published Online First.

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