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The role of cancer-associated fibroblasts in renal cell carcinoma. An example of tumor modulation through tumor/non-tumor cell interactions

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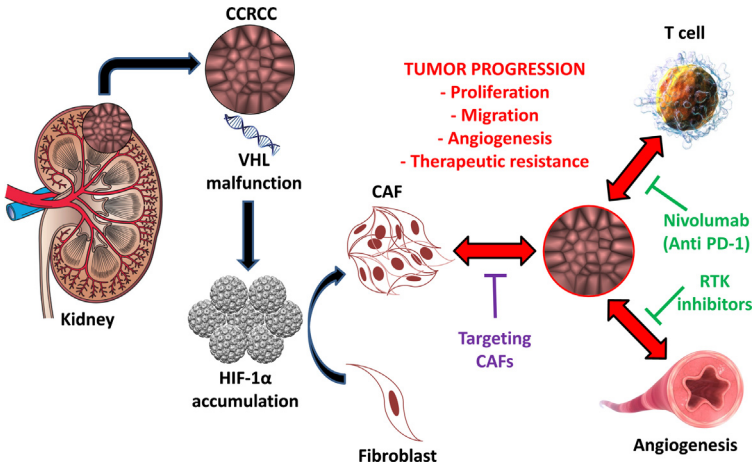
GRAPHICAL ABSTRACT

Therapeutic strategies targeting tumor cell/stroma interactions in Renal Cell Carcinoma. CAF could be activated due to the accumulation HIF-1α in tumor microenvironment, which is related with VHL gene malfunction in RCC cells. Activation of CAF is associated with RCC progression and therapeutic resistance.



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ABSTRACT

Cancer-associated fibroblasts (CAF) are a cellular compartment of the tumor microenvironment (TME) with critical roles in tumor development. Fibroblast activation protein-α (FAP) is one of the proteins expressed by CAF and its immunohistochemical detection in routine practice is associated with tumor aggressiveness and shorter patient survival. For these reasons, FAP seems a good prognostic marker in many malignant neoplasms, including renal cell carcinoma (RCC). The start point of this *Perspective* paper

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is to review the role of CAF in the modulation of renal cell carcinoma evolution. In this sense, CAF have demonstrated to develop important protumor and/or antitumor activities. This apparent paradox suggests that some type of temporally or spatially-related specialization is present in this cellular compartment during tumor evolution. The end point is to remark that tumor/non-tumor cell interactions, in particular the symbiotic tumor/CAF connections, are permanent and ever-changing crucial phenomena along tumor lifetime. Interestingly, these interactions may be responsible of many therapeutic failures.

Introduction

RCC is an aggressive disease with high impact in Western societies. Standard radio- and chemotherapy regimens are not much effective strategies in improving survival of patients with metastatic disease. A significant advance in the treatment of metastatic RCC has been made in the last decade through the inhibition of the vascular endothelial growth factor (VEGF), and its receptor (VEGFR), and the mTOR pathway [1]. Likewise, new therapeutic approaches focusing on the tumor microenvironment are being implemented in the last years. In this sense, the blockade of programmed death-1 (PD-1) and its ligand (PD-L1) in intratumor inflammatory cells is showing promising results in clear cell renal cell carcinomas (CCRCC) [2]. Following the idea of targeting not only the neoplastic cells themselves but also the accompanying elements taking part of a tumor, the focus is being also directed against other non-neoplastic cellular compartment: the cancer-associated fibroblasts (CAF).

This paper reviews the role of CAF in renal cell carcinomas and analyzes the clinical relevance of FAP expression in these neoplasms.

Cancer-associated fibroblasts. An overview

Fibroblast activation is a common process in tissues under diverse conditions, for example, in response to injury. During their activation, fibroblasts undergo a phenotypic transformation migrating to the injured area. Once they complete their mission, the degradation of the extracellular matrix (ECM) triggers their apoptosis [3]. Tumors, presumably by epigenetic mechanisms, induce the chronic activation of local fibroblasts, a subgroup of cells collectively known as CAF [4]. These cells are characterized by the expression of a subset of proteins such as α smooth muscle actin (α-SMA), the classic marker of activated fibroblasts, fibroblast specific protein-1, also known as S100A4, desmin and FAP [5], among others. To note, the expression of these proteins is not limited to CAF and their distribution is not homogeneous [6]. This heterogeneity of CAF population can be attributed, at least in part, to their different origins. The main source of CAF is the activation of resident precursors within the tumor [7]. However, endothelial and epithelial cells can undergo endothelial/epithelial-to-mesench ymal transition (EndMT/EMT) process and become CAF [8,9]. Bone marrow fibrocytes and mesenchymal stem cells are also a source of CAF [10,11]. Even pericytes, adipocytes and vascular smooth muscle cells have been described to transform in CAF under appropriate conditions [12]. Thus, although the origin of CAF has been widely reviewed in bibliography, still remains as a controversial issue [13,4,14]. Together with the origin cell type, the activation is also a complex process in which different cytokines, growth factor, miRNAs and even exosomes are involved [15,16].

Although CAF and tumor cells develop a local symbiotic relationship governed by the rules of Ecology [17], the specific functions of CAF in tumorigenesis are still not well understood. Both protumor and antitumor effects have been described in these cells supporting the idea that some type of cellular specialization must occur under pressures already unknown [18–20]. More specifically, main CAF actions have been related to hallmarks of cancer biology purposed by Hanahan and Weinberg in the beginning of the century and actualized a decade later [21,22]. For example, they regulate tumor development stages secreting cytokines and growth factors such as VEGF, FGF-2 or SDF-1α and altering the extracellular matrix composition to regulate tumor growth promoting angiogenesis and invasive phenotypes [23]. CAF also have the capacity to reprogram tumor cell metabolism and immunosuppressive effects. For example, by CAF/tumor cell contact, CAF undergo Warburg metabolism and mitochondrial oxidative stress while tumor cells reprogram toward aerobic metabolism in a process strictly regulated by the Hypoxia Inducible Factor 1 (HIF-1). This way cancer cells lose glucose dependence and increase the lactate upload to drive anabolic pathways and in consequence, cell growth [24]. Regarding immunosuppression, Harper and Sainson reviewed direct (by the creation of an inflammatory signature with immunosuppressive function on both adaptive and innate white blood cells) and indirect effects (by the regulation of the stiffness, angiogenesis, hypoxia and metabolism) of CAF to regulate the antitumor immune response [25]. Drug resistance is another crucial factor during tumorigenesis. TME and primarily CAF have a determinant role in drug resistance by both cell adhesion mediated drug resistance and soluble factor mediated drug resistance [26].

Considering the comprehensive role of CAF in tumor development described above, several attempts have been developed to target this stromal population [5]. Inhibitors of CAF specific proteins, prodrugs activated only by CAF and even vaccines to target CAF have been designed, however, all of them have been unsuccessful to date. The identification of biomarkers that may allow distinguishing different CAF subgroups with specific actions would open up new possibilities in this research area.

Renal cell carcinoma. A model of tumor/non tumor cell interaction

RCC is a complex group of tumors originating from diverse epithelial cells of the kidney tubules. The World Health Organization describes more than 15 different histologic subtypes accounting for more than 95% of tumors in the adult kidney [27]. In 2018, kidney tumors represented 2.2% of all cancers, with more than 400,000 cases diagnosed worldwide [28]. These figures make RCC a health problem of major concern. These tumors are more frequent on male population with a 2:1 ratio, and the incidence is much higher in developed countries [29]. Although nomograms and models composed by the sum of different prognostic factors like UISS (UCLA Integrated Staging System) and SSIGN (Stage, Size, Grade and Necrosis) [30] have optimized patient prognosis, only surgery impacts significantly in patient survival. However, about a third of patients who have undergone curative surgery will relapse over time. Targeted therapies such as VEGF/VEGFR and mTOR inhibitors, and immunotherapy, have had promising results improving significantly the survival in selected patients with advanced disease [1] Indeed, sunitinib became first line therapy for metastatic renal cell carcinoma (mRCC) since in 2007 was

probed that it duplicates patient progression free survival from 5 to 11 months in comparison of previous treatments [31]. mTOR inhibitors extends this period allowing disease control [32]. Recently, the resurgence of immunotherapy based on immune checkpoint inhibitors such as nivolumab or ipilimumab have changed the standard of care of mRCC. Motzer et al. demonstrated in a study with more than 1000 patients that overall survival and response rate were significantly higher in patients with nivolumab/ipilimumab treatment than with sunitinib [33]. Actually the efforts are focused in the assessment of the combination and sequence of both therapies that will optimize patient benefit [34].

CAF have a protumor effect in RCC. In 2015, Xu *et al.* [35], showed *in vitro* that CAF are involved in tumor progression. These authors demonstrated in CAF/tumor cell co-cultures that CAF were implicated in tumor cell proliferation and migration, as well as in the development of mTOR inhibitors resistance [35]. Together with CAF, immune cells such as Tumor Associated Macrophages (TAM) have been suggested to mediate mTOR targeting resistance [36], although there is still no evidence in RCC. Also, CAF seem to have a role in early phases of CCRCC development through its relationship with hypoxia inducible factor 1 (HIF-1α) (see Graphical Abstract). The accumulation of this protein is the consequence of the Von Hippel-Lindau (*VHL*) gene malfunction, a driver event in CCRCC [37].

Accumulation of hypoxia inducible factors, induce the expression of a set of factors such as vascular endothelial growth factor (VEGF), stromal cell-derived factor-1 (SDF-1), platelet derived growth factor (PDGF), connective tissue growth factor (CTGF) and fibroblast growth factor 2 (FGF-2). Together, these factors induce the recruitment and activation of fibroblasts and other components of TME such as macrophages. Interactions between these different cell types generate the remodeling of the ECM, a key phenomenon for tumor development and metastasis [38]. Although all these specific mechanisms haven't been described in RCC yet, expression of cited cytokines has been related to worse overall survival in RCC suggesting their protumor role [39,40]. Primarily FGF-2, which's expression in the invasion front correlated with RCC aggressiveness, where CAF develop key functions [40].

Zagzag *et al.* [41] demonstrated that the loss of function of *VHL* gene induced the signaling of stromal cell derived factor-1 (SDF-1) through its receptor CXCR4 and described it as a new angiogenic pathway. The expression of SDF-1/CXCR4 by different cellular components of CCRCCs, including CAF, suggests a paracrine signaling which would increase the expression of the receptor and its ligand under hypoxic conditions [41]. SDF-1/CXCR4 signaling has been proved to affect angiogenesis, tumor cell proliferation and chemoresistance by the communication of tumor cells with TME [42]. In RCC in particular, CXCR4 upregulation, a direct effect of HIF 1α accumulation, correlated with metastatic ability and was detected in RCC circulating cells of mRCC patients. These evidences suggest that the SDF-1/CXCR4 biological axis is a main regulator of organ-specific metastases in CCRCC and set out the potential of targeting this signaling pathway [43].

However, the influence of CAF in CCRCC goes further than *VHL* malfunction (Table 1). An *in vivo* RCC model showed that the chemokine CCL3 and its specific receptor CCR5 play a key role in the intratumor accumulation of CAF and other inflammatory cells such as granulocytes or macrophages [44]. Consequently, CAF increased the expression of the hepatocyte growth factor (HGF), a major angiogenic factor, and also the MMP-9 accumulation, this way contributing to the development of tumor metastasis [44].

The symbiotic relationship between tumor cells and CAF is illustrated by the upregulation of stromal periostin (PN) detected in CCRCC [45]. This adhesion protein secreted in the ECM has been detected in many cancers and has been related to cell motility, invasion and EMT processes. *In vivo* and *in vitro* experiments have demonstrated that tumor RCC cells induce the expression of PN by stromal cells [46]. Furthermore, the expression of PN is located in the boundary region between the xenograft tumor mass and the non-tumor tissue. Such expression is coincident with α-SMA expression, the classic marker of activated fibroblasts, both in primary and metastatic CCRCC. Functionally, PN enhances significantly CCRCC cell line attachment, NIH3T3 cell proliferation, and AKT activation [45].

All in all, the studies analyzed above demonstrate the implication of CAF in proliferation, angiogenesis, metastasis development and drug resistance during RCC tumorigenesis. This fact has postulated CAF as potential clinical tools for RCC diagnosis, prognosis and treatment. Several interstitial collagenases, which are mainly secreted by CAF [47], have demonstrated to have prognostic relevance in some neoplasms. The expression levels of MMP-2 and MMP-9, for example, have been correlated with tumor progression in a wide variety of neoplasms, included RCC [48,49].

On the other hand, the expression of Fer, a non-receptor tyrosine kinase, in stromal cells, including fibroblasts and immune cells, correlated with a better prognosis in RCC [50]. This finding contrasts with Fer expression in tumor cells, where it has been linked with tumor aggressiveness and shorter survivals [51]. Fer expression in the stroma has been associated with a lower intratumor macrophage density (measured by $CD68^+$ expressing cells) and correlated positively with $CD57^+$ cell density, a common marker of NK cells in humans. Overall, these results suggest that stromal Fer may act as a suppressor of tumor progression in RCC although the clarification of the specific mechanisms involved in this process is unclear.

Gupta *et al.* [52] developed a fibroblast-derived ECM based on a 3D culture model to assess the clinical relevance of stromal markers in combination with their analysis in pathologic specimens. The authors concluded that palladin was a useful biomarker of poor prognosis in non-metastatic RCC [52]. In addition, they suggested that the assessment of stromal progression could be added to tumor stage as a useful clinical prognostic variable since stromal transformation not always

Table 1
Summary of interactive signaling pathways between CAF and tumor cells in RCC and their specific actions in tumor development.

Authors	Interactive signaling pathway between CAF and tumor cells	Effect
Zagzag et al. 2005 [41]	VHL-HIF axis malfunction induces SDF-1/CXCR4 pathway overexpression which presumably increases angiogenesis by paracrine signaling	Protumor
Wu et al. 2008 [44]	CCL3/CCR5 axis paracrine signaling recruits fibroblasts to the tumor environment where they induce tumor progression by HGF and MMP-9 overexpression	Protumor
Bakhtyar et al. 2013 [45]	CCRCC cells induce periostin expression by CAF which induce tumor cell proliferation and attachment and CAF proliferation	Protumor
Xu et al. 2015 [35]	CAF/RCC *in-vitro* co-culture promotes tumor progression by MAPK/Erk and Akt pathway activation	Protumor
Chuanyu et al. 2017 [46]	Periostin promotes migration and invasion of renal cell carcinoma through the integrin/focal adhesion kinase/c-Jun N-terminal kinase pathway	Protumor

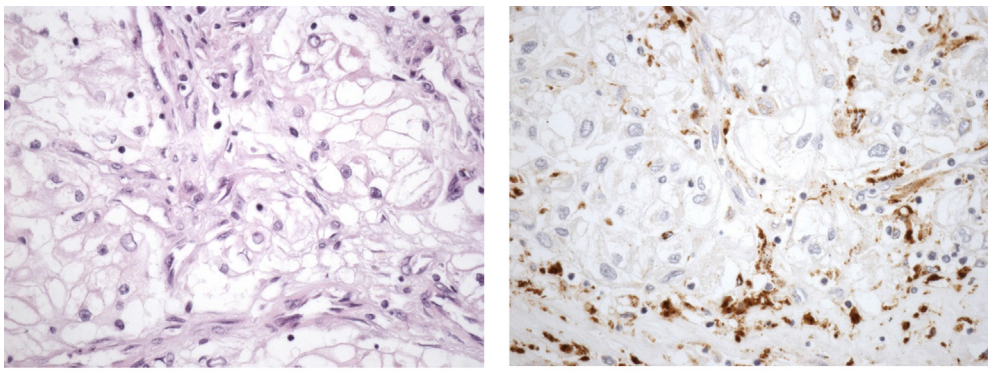

Fig. 1. High power view of a high grade clear cell renal cell carcinoma (A) showing positive immunostaining with fibroblast activation protein restricted to the cancer-associated fibroblasts within the tumor (B) (original magnification, ×400).

correlates with tumor stage [52]. The authors remarked the usefulness of 3D culture models as surrogates of *in vivo* models, considering their capacity to mimic them by the increase of stromal markers as α-SMA, palladin and urokinase receptor associated protein [52].

The usefulness of FAP as a biomarker in CCRCC has been described recently [53,54]. FAP is a transmembrane serine protease expressed by CAF in epithelial tumors originated in a wide variety of tissues [55]. Although the specific actions of this protease remain unclear, a relationship with the urokinase-mediated plasminogen activation system has been observed. Actually, FAP may form protein complexes with the urokinase plasminogen activator receptor (uPAR), its main substrate being α2-antiplasmin [56]. uPAR has been closely related to an aggressive behavior in cancer and has been proposed as a potential new therapeutic target [57].

The immunohistochemical expression of FAP in formalin-fixed paraffin embedded tumor samples (see Fig. 1) correlated with high tumor diameter, high grade and high stage in a series of 208 CCRCC [53]. Furthermore, this protein was a strong predictor of aggressiveness, the survival rate of patients with FAP positive tumors being significantly lower [53]. Another study analyzing 59 CCRCC and their paired metastases showed a correlation between FAP expression in CAF and histological parameters of aggressive behavior like necrosis and sarcomatous phenotype [54]. Furthermore, FAP expression in primary CCRCC was associated with the development of synchronous lymph node metastases [54].

FAP positive cells have been described as SDF-1 synthesizers. In fact, the induction of SDF-1 expression by FAP+ CAF has been described to promote tumorigenesis and the escape of immune surveillance in melanoma and pancreatic ductal carcinoma [58,59]. Moreover, targeting this SDF-1 resulting from FAP+ CAF has been proved to synergize with immunotherapy [60]. Unravelling if this effect occurs in RCC would have a direct impact in the era of immunotherapy resurgence.

CAF are main responsible of ECM remodeling in TME by the production and secretion of proteases [61,62] Fibroblast activation protein has a unique dual enzymatic activity (both collagenase and serine-peptidase), that enables the reorganization of collagen and fibronectin fibers to promote tumor cell invasion in pancreatic cells [63]. Strong relationship of FAP with RCC aggressiveness suggests a similar role for CAF and FAP in this tumor [54].

Conclusions and future perspectives. Targeting CAF, a new front against tumor microenvironment

In the era of personalized medicine and targeted therapies the comprehension of the tumor as a *society* composed by different elements extends the scope of action of anti-cancer drugs. This aspect acquires special relevance in RCC due to its radio- and chemo-resistant identity. In fact, tyrosine-kinase receptor inhibitors-based antiangiogenic treatment and PD-1/PD-L1 targeting immunotherapy are the main treatments that significantly improve patients' survival. An effective targeting of CAF, one of the most important cell population in tumor microenvironment, seems the ideal complement considering their protumor role.

The fact that FAP is exclusively expressed in CAF, makes this protein an attractive target to develop CAF-mediated anti-tumor drugs. Different strategies have been designed such as inhibitors of its enzymatic activity [64,65], prodrugs activated by its activity [66], FAP targeting antibodies [67], vaccines and even CAR-T cell technology [68]. Some of them are still being tested in clinical trials.

Although these strategies seem promising, targeting CAF in an effective manner appears much more complex than inhibiting the function of a specific protein. Unraveling if CAF conform subgroups specialized in different actions marks a milestone in the comprehension of the role of CAF in cancer in general and in RCC in particular. Similarly, understanding the impact of FAP expression by a subset of CAF will measure the usefulness of FAP targeting strategies. Removing the protumor cohort of CAF and potentiating the effect of those with antitumor activity is still utopic. However, reeducating those foes to friends as recently was proposed by Chen *et al.* would undoubtedly suppose a step forward in the war against cancer [69].

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Compliance with ethics requirements

This article does not contain any studies with human or animal subjects

Declaration of Competing Interest

The authors have declared no conflict of interest

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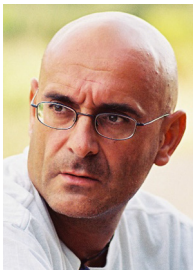

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