

Cancer associated fibroblasts: phenotypic and functional heterogeneity

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1. ABSTRACT

Cancer associated fibroblasts (CAFs) are the most abundant stromal cell-type in solid tumor-microenvironment (TME) and have emerged as key player in tumor progression. CAFs establish communication with cancer cells through paracrine mechanisms or via direct cell adhesion as well as influence the cancer cell behaviour indirectly by remodelling the extracellular matrix. Although numerous studies have strongly suggested the tumor promoting role of CAFs, few recent reports have revealed the heterogeneity in CAFs. Here, we have summarized the recent findings on the mechanisms related to the heterogeneous behaviour of CAFs serving as positive or negative regulator of tumor progression. Further, reports related to the targeted therapy against CAF-mediated mechanisms are also summarized briefly.

2. INTRODUCTION

A growing body of evidence suggests that tumor development not only involves the malignant cancer cells but also the cells and the molecules of surrounding stroma, termed as tumor-microenvironment (TME) (1, 2). TME plays important roles in facilitating malignant cancer cells to acquire hallmarks properties through bidirectional

communication between cancer cells and the components of TME. TME is composed of cellular component and extracellular matrix (3, 4). The extracellular matrix of TME provides scaffold for its structure. The main components of this are collagens, fibronectins, proteoglycans, elastins, and laminin. Apart from these, other molecules are also trapped inside the matrix. These include matrix metalloproteinases (MMPs) secreted by transformed cancer and cells of the TME (5, 6). The cellular components of tumor microenvironment include the endothelial cells, infiltrating immune cells, pericytes and fibroblasts. In normal tissues, fibroblasts are elongated, spindle shaped cells which are present in the extracellular matrix in a suspended form (3). They provide architectural scaffold to the tissue by secreting components of the extracellular matrix. They help in regulating interstitial pressure and fluid volume and actively involved in the tissue remodelling and wound repair. Within the TME, cancer associated fibroblasts (CAFs) also known as the stromal fibroblasts or tumor associated fibroblasts are the most abundant stromal cell types. CAFs are activated mesenchymal cells present in tumor stroma (7). They are present in almost all the solid tumors in varying proportions and constitute up to 70% volume of the breast, prostate and pancreatic

tumors whereas they are present in less proportion in brain, kidney and ovarian cancers (8). These cells interact with tumor cells in a reciprocal manner and are involved in tumor development at each stage. CAFs evolve alongside tumor as it progresses and help the tumor cells to evolve (1, 5). Here, we have reviewed the recent advancement in understanding the mechanisms specifically with respect to diverse phenotypes and function of CAFs.

3. CAFs ARE DERIVED FROM DISTINCT ORIGIN AND EXHIBIT HETEROGENEITY IN IDENTIFICATION MARKERS

The origin of CAFs can be highly heterogeneous. The main source of CAFs in the TME is the resident normal fibroblasts which get converted to CAFs. Tumor cells secrete growth factors such as TGF β 1, SDF1 and PDGFR β to promote conversion of normal fibroblasts into CAFs (9-12). CAFs are recruited to the tumor site in the similar fashion as they are recruited to the site of wound healing. At the site of the wound, platelets migrate and secrete growth factors such as PDGF and TGF β 1 to recruit the normal fibroblasts at the site of injury. The fibroblasts (resident as well as distant) respond to the signals and start migrating to the injury site. After reaching to the injury site, normal fibroblasts acquire activated phenotype under the influence of various growth factors such as TGF β 1. The activated CAFs helps in wound healing process by providing growth factors, cytokines and by producing components of extracellular matrix(13, 14). Unlike the normal wound healing process where activated fibroblasts undergo apoptosis, the activated fibroblasts in tumor stroma do not follow the same fate. They continue to interact with tumor; therefore, tumors are also termed as "wound that never heals" (15, 16).

There are several other sources by which CAFs are found to be originated. CAFs can be generated directly from mesenchymal stem cells (MSCs). MSCs migrate to the tumor site in the similar manner like fibroblasts migration during processes of wound healing. These migrating cells have been reported to recruit to the tumor site and differentiate into CAFs. These CAFs express activation marker α SMA, FAP, tenascin-C and thrombospondin-1 in their cytoplasm (17). CAFs can also be generated

through the process of epithelial to mesenchymal transition (EMT) from the epithelial cells. CAFs arising through EMT have also been shown to retain genetic alterations of their parental genome. Somatic mutations in the CAFs is debated (18, 19). Though, EMT-derived CAFs may contribute rarely to the total CAF population in tumor, certain reports suggest the accumulation of mutations in CAFs. Mutations in the TP53 and PTEN genes in CAFs isolated from breast cancer is demonstrated helping CAFs to acquire pro-tumorigenic behaviour (20-24). CAFs can also be generated from other cell-types such as pericytes and endothelial cells. These cells can trans-differentiate and contribute to CAFs population. Proliferating endothelial cells can undergo endothelial to mesenchymal transitions under the effect of tumor secreted TGF β 1 to give rise to CAFs (25). CAFs can also be generated from pericytes through the process of pericyte to fibroblast transition (PFT) under the influence of PDGF-BB (26). All these sources of CAFs are not mutually exclusive and may produce a vast heterogeneous population of CAFs within individual cancer-type. This could be the reason for the reported variations in the identification markers for CAFs.

Fibroblasts express various cell surface and intracellular proteins by which they are identified in different tumors. Normal fibroblasts and the CAFs, both being mesenchymal cell type, express vimentin in their cytoplasm. CAFs are identified by expression of fibroblast specific protein 1 (FSP1), also called as S100A4. However, it is also widely expressed by carcinoma cells in different tumor types (27) or due to the process epithelial to mesenchymal (EMT) transition in these cells (28). CAFs are also identified by expression of fibroblast activation protein alpha (FAP α). However, it is also not exclusively expressed only in CAFs but also reported to be expressed by normal fibroblasts and quiescent mesodermal cells (29, 30). CAFs express platelet derived growth factor receptor alpha and beta (PDGFR α/β). However, like other markers, it is also not exclusive for the CAFs as it is expressed by tumor cells undergoing EMT and by vascular smooth muscle cells, myocardial cells and skeletal muscles (31, 32). Expression of CD90/Thy1 has been reported on fibroblasts cells as cell surface marker. Fibroblasts expressing CD90 on their cell surface have been reported to function as

myofibroblastic cells compared to CD90 negative fibroblasts. Expression of CD90 can be a potential marker to identify CAFs in TME (33-36). Other markers which are expressed by CAFs are NG2 (neural glial-2), Desmin and discoidin domain receptor-2 (DDR2). CAFs are also identified by expression of stress fibres of α SMA. Activated CAFs express α SMA in their cytoplasm in most of the tumor types (3). Normal fibroblasts express α SMA during wound healing and it is also expressed by smooth muscle cells surrounding the blood vessels, pericytes, visceral smooth muscle cells and cardiomyocytes (37).

4. ROLE OF CAFs IN INVASION AND METASTASIS

Although, only a small percentage of disseminated cancer cells are capable of forming detectable metastatic tumor; it accounts for a significant number of cancer related mortality and morbidity (27). Metastasis involves a number of sequential events. For this process cancer cells must detach from the surrounding cells and intravasate into blood circulation system and lymphatic system, evade immune response, extravasate into the capillary beds of appropriate site and secondary tumor formation (38). The orchestration between tumor and stromal cells through secreted molecules and interactions with matrixes is demonstrated to facilitate the formation into metastatic tumors (1, 39).

The process of intravasation involves direct interactions between cancer cells, stromal cells and ECM. CAFs play significant role in tumor metastasis from the first step of breaching the basement membrane to formation of micrometastasis (40). CAFs can remodel the extracellular matrix by secreting ECM proteins such as collagens as well as ECM degrading enzymes such as matrix metalloproteinases (MMPs) leading to invasion and metastasis (41). Degradation of ECM creates a path for cancer cells to the vasculature (42).

CAFs show distinct expression of genes which are specifically involved in cell adhesion and migration. Also, through matrix remodelling, CAFs help in making the tracks in the stroma and help tumor cells to move to other sites (43). Both these

mechanisms collectively facilitate cell migration and invasion. Studies by Y Hassona *et al* suggested that senescent CAFs secrete active MMP2, which is instrumental to induce keratinocyte dis-cohesion and epithelial invasion into collagen gels in a TGF- β dependent manner (44). They express N-cadherin on their surface which binds with E-cadherin of tumor cells and pulling them along the tracks (33). This helps in directional movement of tumor cells which is necessary for successful invasion and metastasis (34). In colorectal cancer, cancer stem cells have been shown to express CD44v6 cell surface marker which facilitates cells to attach to hyaluronan which is the component of extracellular matrix (45). In case of breast tumor, increased stiffness of the matrix correlated with poor survival. Yes associated Protein (YAP) is an important player of mechanotransduction pathway. If the stiffness of ECM is high, it influences the nuclear localization of Yap1 and facilitates activation of CAFs (35). Additionally, CAFs are also shown to express factors required for neoangiogenesis and neolymphogenesis to promote metastasis (36).

CAFs are also shown to induce metastasis through paracrine signalling to induce epithelial to mesenchymal transition (EMT) (46). EMT plays an important role during the course of tumor initiation, malignant progression, metastasis and therapy resistance (47). Loss of epithelial marker E-cadherin and the expression of mesenchymal marker vimentin is a cardinal sign of EMT (48). In a study, CAFs were found to help the premalignant epithelial cells to acquire mesenchymal traits leading to invasion and metastasis whereas fibroblasts isolated from benign mammary glands failed to do so (49). In prostate cancer, IL-6 secreted by tumor cells recruited CAFs to the tumor niche which secreted metalloproteinase thereby inducing EMT and invasion in cancer cells (50). In pancreatic ductal adenocarcinoma, IL-6 secreted by CAFs helped tumor cells to undergo EMT and ultimately metastasize. When secretion of IL-6 was inhibited by retinoic acid treatment, the induction of EMT by CAFs was lost (51). In breast cancer, CAFs induce TGF β /SMAD pathway in breast cancer cells by secreting TGF β 1 leading to EMT mediated invasion and metastasis. This effect was reversed when secretion of TGF β 1 was blocked (52). Study has shown that CAFs secrete some pro-invasive

factors in hepatocellular carcinoma and activate TGF- β /PDGF signaling crosstalk to support the process of EMT and transform into an invasive phenotype. Additionally, co-transplantation of myofibroblasts with Ras-transformed hepatocytes strongly enhanced the growth of tumor. However, genetic-interference of PDGF signaling pathway reduced tumor growth and EMT (53). Another recent study suggested that CAFs secrete IL32 which promotes breast cancer cell migration by binding to integrin β 3 through RGD motif. Interaction between IL32 & integrin β 3 induced P38-MAPK signaling pathway, resulting in enhanced EMT marker expression and promote invasion (54).

The rate and type of EMT within a tumor is not differ within the population of tumor cells. Different EMT population is shown to exist in distinct tumor regions associated with a specific microenvironment in skin SCC and mammary tumors (13). Additionally, other cell types within stroma may also play crucial role during the process of EMT. *In vivo* depletion of macrophages in skin and mammary primary tumours helped in increased population of EpCAM+ epithelial tumor cells and inhibition of the EMT process (14, 15).

5. ROLE OF CAFs IN TUMOR GROWTH AND MAINTENANCE OF STEMNESS

As discussed before, CAFs facilitate tumor growth by secreting growth factors and cytokines/chemokines and remodel extracellular matrix. Tumor cells interact with CAFs in a reciprocal manner and activate them to acquire pro-tumorigenic functions. Intriguingly, CAFs were shown to initiate malignant properties in morphologically and genotypically normal epithelial cells. Olumi *et al.*, showed that CAFs through its secreted factors could promote tumor progression in an immortalized but non-tumorigenic prostate cell whereas normal fibroblasts were failed to do so (55). CAFs secrete various factors such as hepatocyte growth factors (HGF), stromal derived growth 1 (SDF-1) and TGF β 1 which modulate the tumor progression (56-58). CAFs isolated from breast tumors could promote breast tumor growth efficiently compared to matched normal fibroblasts. This increased

tumor growth was associated with SDF1 secreting-CAF which promoted angiogenesis through recruitment of endothelial progenitor cells at tumor sites (56, 59). CAFs secrete VEGF which helps in formation of new blood vessels to supply and manage cellular metabolites (60). CAFs interact with other cells in the stroma such as endothelial and inflammatory cells. It alters their functions of secreting chemokines such as monocyte chemoattractant protein 1 (MCP1) and interleukins such as IL-1 which affect the functioning of inflammatory cells (61, 62).

CAF have been shown to affect the stem cell-like properties of tumor cells of different origins. CAFs promote lung tumor cells to undergo dedifferentiation and acquire the stem cell-like properties. To study the effect, Chen *et al.*, established a co-culture model of CAFs and lung cancer cells. CAFs were isolated from lung cancer patients and used as feeder layer. Study showed that CAFs regulate stem cell-like properties in a paracrine manner by expressing IGF-II in the TME and increase Nanog expression in tumor cells expressing IGF1R. Blocking IGF-II/IGF1R signalling affected the expression of Nanog resulting in loss of stem cell characteristics. Lung cancer cells when grown in co-culture with CAFs demonstrated enhanced capacity of self-renewal shown by sphere formation assay and expressed stem cell markers Oct4/Nanog. The effect was not seen when the tumor cells were grown with normal fibroblasts (63). Stassi *et al.*, have reported in colorectal cancer that CAFs secrete growth factors OPN, HGF, and SDF1 which helped colorectal cancer cells to acquire the CD44v6 phenotype as well as cancer stem cell-like phenotype by activating Wnt/ β -catenin pathway. CD44v6 expressing colorectal cancer stem cells showed increased migration and metastasis. Colorectal cancer patients with low CD44v6 expression predicted better survival than with high CD44v6 patients (64). In breast cancer, tumor cells educate stromal fibroblasts to express chemokine ligand 2 (CCL2). CCL2 stimulated tumor cells, expressed NOTCH1 and showed cancer stem-like cells phenotype such as increased self-renewing ability shown by sphere formation assay. In this study, patients with increased CCL2-NOTCH1 expression showed grade of poorly differentiated breast cancer tissues (65).

Burman *et al.*, have studied the role of CAF-CSC interaction in prostate cancer. They developed conditional PTEN-deleted mouse model of prostate adenocarcinoma to study reciprocal role of CAFs and cancer stem-like cells isolated from this model. The isolated epithelial cells showed the characteristics of stem-like cancer cells and expressed established markers of CSC as well as demonstrated self-renewing abilities under *in vitro* conditions. CAFs isolated from the same mouse, significantly promoted stem cell-like properties in CSC including better sphere forming ability (66). Wang *et al.*, have studied the role of CAFs in breast cancer progression. CAFs secreted chemokine (C-C motif) ligand 2 induced NOTCH1 expression in breast cancer cells and helping them to acquire cancer stem cell features. Fibroblasts co-cultured with breast cancer cells promoted stem cell like features in breast cancer cells compared to normal fibroblasts cells. Breast cancer cells secreted cytokines induced CCL2 expression in CAFs activating STAT3 in CAFs (65). In another study, cancer associated fibroblasts from esophageal squamous cell carcinoma (ESCC) secreted IL-6 which conferred chemoresistance to ESCC cells by upregulating C-X-C motif chemokine receptor 7 (CXCR7). Silencing of CXCR7 in ESCC cells significantly decreased the stem cell related gene expression suggesting the involvement of CXCR7 in stemness (67).

In addition, CAFs have been shown to directly affect the sensitivity of cancer cells towards therapeutic agents. Golub *et al.* have reported resistance to RAF-inhibitors in BRAF-mutant melanoma cells mediated through HGF secreted from stromal microenvironment (68). Similar observations were reported by Delorenzi *et al.*, they have found that increased stromal gene expression signature confers resistance to widely used drugs such as 5-fluorouracil and other drugs (69). Karin *et al.* co-cultured CAFs with HNSCC and showed that soluble factors from CAFs help tumor cells to acquire resistance to cetuximab (70). CAFs secreted high mobility group box 1 (HMGB1) helped breast cancer cells to develop resistance against doxorubicin (71). Gemcitabine resistant CAFs in PDAC secrete exosomes with SNAIL which help tumor cells in proliferation and drug resistance (72). These studies demonstrate the potential of CAFs in the

development of drug resistance to tumor cells to most commonly used anticancer drug.

6. TUMOR RESTRAINING ROLE OF CAFs

Apart from tumor-promoting role, CAFs have also been shown to harbour tumor-restraining functions (73, 74). In pancreatic ductal adenocarcinoma (PDAC), tumor cells secrete sonic hedgehog (Shh) and direct fibroblasts cells to form a desmoplastic rich stroma. Shh-deficient tumors showed reduced stroma and aggressive, proliferating and more vascular tumors (75). In another study, Özdemir *et al.* generated transgenic mice with ability to delete α SMA-positive cells in PDAC. Depletion of α SMA-positive cells gave rise to invasive and undifferentiated tumors with increased hypoxia and EMT as well as increased cancer stem cells behaviour. Further, PDAC patients with low α SMA-positive cells showed decreased survival (76). CAFs expressing FSP1 have been shown to inhibit tumor development by encapsulating carcinogen. Here, FSP1+ve fibroblast cells helped in limiting the exposure of epithelial cells to carcinogen which could otherwise resulted in DNA damage and tumor development (43).

Further to these findings, elegant work reported by D.A. Tuveson and colleagues has demonstrated spatially separated distinct populations of inflammatory fibroblasts (iCAF) and myofibroblasts (myCAF) in PDACs. myCAF were found to be dependent on the juxtacrine interactions with cancer cells and were located in the periglandular region; whereas iCAF were distantly from cancer cells and myCAF populations in PDA and were induced by secreted factors from cancer cells through paracrine manner. iCAF produced IL6, IL11 and LIF and stimulated STAT pathway in cancer cells; whereas, myCAF were defined by high- α SMA expression. This study predicted the pro and antitumorigenic properties of CAF-subpopulations within the tumors (77). More recently, tumor secreted IL-1 is found to upregulates LIF which ultimately promote CAFs to gain inflammatory phenotype by activating JAK/STAT downstream molecules, whereas TGF β is shown to work oppositely by downregulating IL-1R1, which induces myofibroblast phenotype in CAFs in PDACs (78).

Daniela *et al.*, have shown functional heterogeneity among CAFs subpopulations. They established two types of CAFs from OSCC patients, CAF-N with transcriptome and secretome similar to normal fibroblasts and CAF-D with different expression pattern than normal fibroblasts. Both CAFs promoted tumor growth in NOD/SCID mice but CAF-N were more tumor-promoting than CAF-D. CAF-N showed more motile phenotype and inhibition of motility reduced the invasion of oral tumor cells. CAF-D were less motile and higher TGF β 1 secreting CAFs help to obtain EMT phenotype in oral tumor cells. Inhibiting TGF β 1 secretion in CAF-D, reduced keratinocyte invasion (79).

Recently, we have demonstrated the presence of two, functionally heterogeneous subtypes of CAFs in established cell cultures and primary human tumor samples of gingivobuccal-oral cancer. The low- or high- α SMA score in tumor stroma has been shown to correlate with better or poor survival of patients respectively. Gene expression pattern based unsupervised clustering analysis resulted in identification of two subtypes of CAFs which were named as C1-type or C2-type CAFsQ. The C1-type CAFs demonstrated low- α SMA (non-myofibroblastic) phenotype compared to C2-type CAFs with myofibroblastic phenotype. Co-culture experiments between C1-type of CAFs and oral cancer cells exhibited higher percentage of proliferating cells with concomitant lower frequency of stem-like cancer cells, compared to the co-culture with C2-type CAFs. Our study has indicated that a small set of differentially expressed genes between these subtypes of CAFs may be responsible for their characteristics and distinct functions in oral tumors. Importantly, BMP4 expression by C1-type CAFs was found as one of the possible mechanisms for suppressed stemness and CAFs-mediated protective role in gingivobuccal tumors (80).

As discussed above, fibroblasts are shown to undergo myofibroblastic differentiation upon TGF β stimulation (6, 81). In our study, several genes which were differentially upregulated in C2-type CAFs were related to TGF β -pathway activation (80). Therefore, here we have examined if TGF β stimulation can induce transition of C1-type CAFs to C2-type CAFs and

the transitioned CAFs can reciprocate differently in maintaining stemness of oral cancer cells. We stimulated C1-type CAFs with 10ng/ml TGF β for 48 hours and determined the myofibroblastic differentiation of CAFs by α SMA stress fibre formation (6, 82). As expected, TGF β stimulated CAFs expressed more stress fibres suggesting that they can be activated by TGF β treatment (Figure 1A). Next, we tested whether TGF β -stimulated myofibroblastic CAFs act similarly as C2-type CAFs with increased stemness in oral cancer cells (80). TGF β -stimulated or unstimulated CAFs were co-cultured with SCC029b oral cancer cells for 4 days in low-serum media and compared for the frequency of cancer cells with high aldehyde dehydrogenase activity by Aldefluor assay. Interestingly, oral cancer cells demonstrated significantly higher frequency of ALDH-Hi cells upon co-culture with TGF β -induced myofibroblastic (C2-type) CAFs as compared to non-myofibroblastic (C1-type) CAFs (Figure 1B and C). Overall, data indicates that the microenvironmental TGF β may be one of the responsible factors for heterogeneity in stromal CAFs determining the presence of tumor suppressive or supportive type CAFs in oral tumor tissues.

7. TARGETING CAFs IN TUMOR MICROENVIRONMENT

Surgery and radiotherapy are the major treatment strategies for solid cancers. Combining both treatment modality have provided improved outcomes for patients (83). Since, TME plays crucial role in tumorigenesis, it offers a great opportunity to therapeutically target these cells. Strategies have been made to specifically target different components of TME. CAFs being the major components of TME, draws major attention in this direction. Head and neck cancer patients with higher score for α SMA expression in tumor stroma are associated with decreased disease free and overall survival; suggesting CAFs as plausible target for these patients (84). Lee and Gilboa *et al.*, have shown that targeting FAP expressing CAFs, could inhibit tumor formation ability in mice which were immunized against FAP (57). Similar approach was adopted by Loeffler and Reisfeld. They constructed

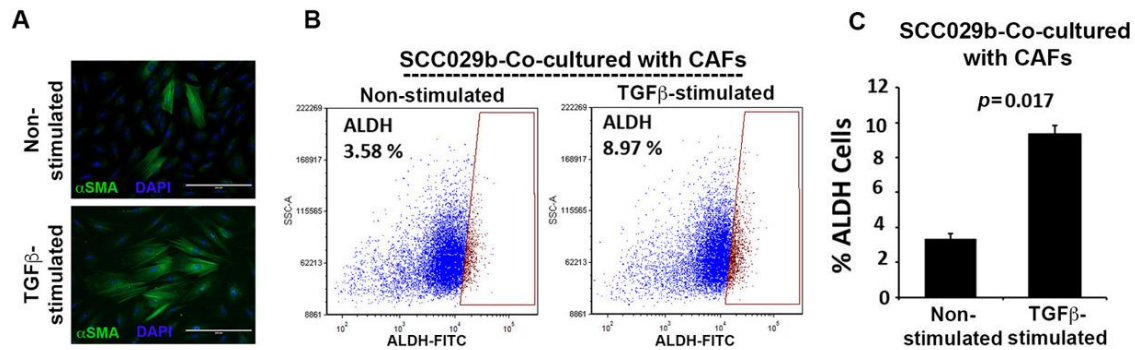


Figure 1. (A) Expression of α SMA was analyzed after treatment with TGF β . Images were taken at x200 magnification. (B) TGF β stimulated cells were co-cultured with SCC029b for 4 days in low serum media. Frequency of ALDH-Hi cells were determined by flow cytometry using Aldefluor assay and shown as dot plots. (C) Bar graph represents the average frequency of ALDH-Hi cells from three biological repeats. p value was calculated by Student's t-test.

oral DNA vaccine against FAP and demonstrated that CD8 $^{+}$ T-cell mediated targeting of FAP expressing CAFs suppressed tumor formation and metastatic ability of multidrug resistant colon and breast carcinoma (58). Wen and Nakamura, have shown that inhibition of tumor-stroma interaction by specifically targeting HGF by NK4 impaired the colon cancer growth and liver metastasis (76). Targeting HGF by monoclonal antibody could reduce glioma formation in murine models (85).

Immune evasion is one of the major hallmark characteristics of tumors. CAFs contribute in acquiring these characteristics and they could be used as a target for immunotherapy. Fujiwara *et al.*, recently reported that CAFs regulated infiltrating lymphocytes by IL-6 and blocking IL-6 or targeting CAFs could improve immunotherapy (86). FAP α is a marker of CAFs and has been utilized as target in immunotherapy directed against CAFs (87). Targeting FAP positive CAFs in PDAC helped the antitumor activity of α -CTLA-4 and α -PD-L1 which ultimately helped T cells to move to TME and act on tumor cell clearance (30). Hanks *et al.*, have shown in melanoma that inhibition of TGF β in CAFs resulted in an increase in the number of CAFs and MMP-9 secreted from CAFs cleaved PD-L1 resulting in development of anti-PD-L1 resistance (88).

Very recently, Hynes *et al.*, have shown the differential function of extracellular matrix proteins based on their source of origin in PDAC of

mouse and human tumors. Their group suggested that ECM-protein matrisome derived from tumor cells correlated with poor prognosis compared to majority of ECM-protein matrisome derived from stromal cells showed both pro- and anti-tumorigenic behaviour. The IPA analysis showed that tumor-cell ECM proteins were regulated by FGF10, FAK1, EGF and MAP2K1 while stromal-cell ECM proteins were regulated by α -catenin, AHR, BIRC5 and SMAD3 (89). Similarly, Carvalho *et al.*, has reported cancers with mutations in BRAF, SMAD4 and TP53 mutation and MYC amplification activated a distinct ECM transcription profile which correlated with poor prognosis and immunosuppressive behavior (90).

There are various chemotherapeutic drugs are being tested for targeting stromal compartment. Sibrotuzumab is antagonist of FAP and functions by inhibiting CAF differentiation (91). AMD-3100 and IPI-926 target SDF1/CXCL2 and smoothen of sonic hedgehog pathway, respectively and demonstrated to impair the tumor-stroma crosstalk in multiple myeloma, Non-Hodgkin's lymphoma and pancreatic cancer (1, 92). Specifically targeting the stromal and its derived components such as PDGF-C, Tenascin-C, and COX-2 has been tested in model systems of multiple myeloma, PDAC, and astrocytoma and Non-Hodgkin's lymphoma with exciting results (67). Targeting NOX4 by RNA interference or by pharmacological inhibition impairs the trans-differentiation of CAFs with reduced tumor growth (93).

Table 1. Functions of CAFs in tumor microenvironment

Sl. No.	Functions	References
1	Invasion and Metastasis	(33, 34, 40, 43)
2	Extracellular matrix remodeling	(41, 42)
3	Secretion of MMP	(44)
4	Attachment to the matrix	(45)
5	Angiogenesis	(36, 60)
6	Epithelial to mesenchymal transition (EMT)	(49-54)
7	Stemness	(1, 63-67, 85, 92)
8	Growth Factor secretion	(55-59, 61, 62, 64, 65)
9	Drug Resistance	(68, 69, 70, 71, 72)
10	Anti-tumorigenic	(43, 73, 75, 76, 74)

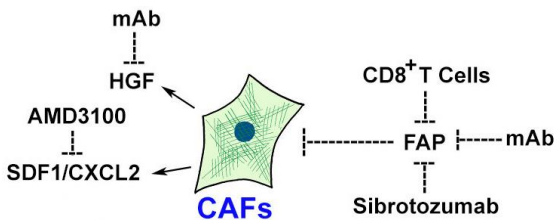


Figure 2. Targets against CAFs in tumor microenvironment: Direct depletion of cancer associated fibroblasts (CAFs) via immunotherapies / chemotherapies or targeting crucial signals responsible for CAFs-mediated function can be adapted as approach in CAFs-directed anticancer strategies. FAP, fibroblast activation protein; mAb, monoclonal antibody; HGF, hepatocyte growth factor; SDF1, stromal-derived factor1; CXCL-2 (C-X-C motif) ligand 2.

The clinical trials to target CAFs have been attempted with few degree of success. The iodine 131-labeled monoclonal antibody F19 (131I-mAbF19) which targets FAP in colon cancer has proved to be useful in diagnostics therapeutics (94). The phase III trial has been done for Bevacizumab against malignant pleural mesothelioma and it has shown improvement in overall survival of the patients (95). A phase II trial of Ruxolitinib, an inhibitor of myelofibrosis, was done for PDAC patients. The results suggest that it affects directly to tumors and it is also effective in those patients who have systemic inflammation (96).

These studies provide an opportunity to intervene stromal fibroblasts leading to cancer therapy, although they present a great challenge

to carefully design the patient trails (97, 98). Various strategies to target CAFs in TME is depicted in Figure 2.

8. CONCLUDING REMARKS

Collectively, we have highlighted the recent findings on the mechanisms of CAFs mediated role in tumor progression (Table 1). Due to their pro-survival or pro-metastatic functions, CAFs have become an attractive target for achieving more effective response of standard treatment. However, caution has to be applied in targeting CAFs as uniform cell type. we discussed that the stromal components of the tumor may also evolve side by side along with the cancerous cells. The stromal cells upon getting distinct instructions from other components of tumor in the form of cytokines, chemokines or growth factors may give rise to heterogeneous population of CAFs with distinct phenotype and functions. The traditional view of considering the CAFs as pro-tumorigenic niche has been recently challenged in some tumor types. Clearly, more basic research is needed in comprehending the role of heterogeneous subpopulations of CAFs. Reciprocation between various other cellular and non-cellular components during the course of tumor evolution may lead to high degree of dynamic complex interactions. Therefore, deeper molecular characterization specifically from the patient samples may lead to define the cellular subsets of CAFs. Overall, understanding the heterogeneity in CAFs

subpopulations and related complexity in reciprocal cross-talk within TME may possibly provide best treatment advantage to cancer patients.

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