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**Title: Cancer-associated Fibroblasts: Is it a key to an intricate lock of tumorigenesis?**

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**Cancer-associated Fibroblasts: Is it a key to an intricate lock of tumorigenesis?**

**Abstract:** The past few decades have witnessed a major leap in knowledge relating to the role of tumor microenvironment in carcinogenesis and evolving behaviour of the tumor. Multiple factors within the tumor microenvironment modulate the cancer cells and the associated therapies. Stephen Paget first asserted that the microenvironment plays an important role in the growth of tumor metastasis. The most important player in the tumor microenvironment is cancer-associated fibroblast which significantly participates in the proliferation, invasion and metastasis of tumor cells. Cancer-associated fibroblasts show phenotypic and functional heterogeneity. Mostly cancer-associated fibroblasts originate from quiescent resident fibroblast or mesoderm-derived precursor cells (mesenchymal stem cells), although several alternate sources of origin have been noted, however, due to a lack of specific fibroblast-restricted markers, it is very difficult to trace lineage and identify the biological origin of distinct sub-types of CAFs. CAFs are predominantly shown by several studies to mainly act as tumor-promoting agents, however, tumour-inhibiting actions are also being validated by several studies. A more objectified and comprehensive functional and phenotypic classification of CAF is required, which will help in better way for tumor management. Here, in this review, we have tried to review the current status of CAF origin, along with phenotypic and functional heterogeneity, and recent progress in cancer-associated fibroblast (CAF) research.

Keywords: CAF, TME, Fibroblast, Microenvironment

1. Introduction

It is a well-established fact that cancer is a heterogeneous disease with an aberrant mutation in tumor cells, however, data is now accumulating which indicates that tumors are also diverse by nature of their micro-environmental composition, the proportion of stromal cells, and their varied state of activation. [1,2,3] The stromal cells are non-mutant cells within tumor microenvironment (TME). [4,5] TME is in the state of kinetics throughout cancer progression, in response to changing environmental conditions and varied oncogenic signals.[5,6]

The role of TME in the initiation, progression and patient prognosis and therapeutic outcome of malignant tumors has been well-established over the past decade. [5,6,7,8] The concept that the progression of cancer is regulated by the interaction of cancer cells with the tumor micro-environment was first hypothesized by English surgeon Stephen Paget, son of the famed surgeon Sir James Paget over a century ago in 1889 when he published his “seed and soil” theory. [9,10,11,12] Since ages it has been observed that the two-way communication between cells and their stromal microenvironment is very important in normal tissue homeostasis and repair.[13] Now there is also significant accumulating evidence that tumor cells and their associated stroma have a serious cross-talk and it influences the progression and prognosis of malignant cells.[5, 14] This has been made possible because of an improved understanding of genotypic and phenotypic characteristics of malignant cells (the seed) and in-depth knowledge and stratification of a highly complex tumor microenvironment (**TME**; the soil).[9,14] Before the seed and soil theory of S Pagets, it was Rudolf Virchow in 1863, who first observed a relation between infiltrating leucocytes and tumorogenesis and proposed that chronic inflammation is the hallmark of tumors.[2]

The last decade has witnessed a huge expansion in research into the cellular component of tumor stroma, especially cancer-associated fibroblasts (CAFs).[5,15,16,17] The CAFs are shown to have an important role in the production and remodelling of extra-cellular matrix **(ECM),** and synthesis of growth factor, cytokines and chemokines which significantly influences angiogenesis, stromal plasticity, tumor mechanics, drug access and the response of cancer cells to chemotherapy.[5,17, 18,19,20,21,22] Chiefly their ability to manipulate the immune system in favour of tumors has been increasingly appreciated by researchers focussing on **TME**.[23,24,25,26]

Because of these characteristics of CAFs, targeting them by manipulating their number, phenotype or functional characteristics is being considered and translationally applied as an avenue to improve cancer therapies.[27,28,29] With the rapid evolution of precision medicine and immunology, **CAFs** have become a hot spot and prominent target in tumor management; through tumor immunotherapy and targeted therapy. However research in this area is facing numerous hurdles, not only because of a lack of precision in identifying fibroblast-specific markers that are not shared by other cell types but also because CAFs have both pro-tumorigenic and anti-tumorigenic functions.[30,31] Hui and Chen have rightly noted in their review of tumor micro-environment published in cancer letters, that it is a “sanctuary of the devil”, taming which could complement traditional treatment options and improve therapeutic outcomes.[32]

1. **The microenvironment, a very busy tram track**

Beyond the conventional understanding of the self-sufficiency of tumor cells, [33] these transformed cells do not exist as an isolated entities. Instead, they are surrounded by non-transformed cells and an extra-cellular matrix with which they closely interact (cross-talk). [34] This environment is called tumour microenvironment (TME), which is very dynamic and multiple factors including cancer cells themselves and their related therapies have been noted to modulate several of its components and their functions.[35]

The **TME** can be broadly classified into four major components **(Table 1)**, [3,5,36] as follows: (1) **stromal component** that includes non-immune/non-epithelial cells of mesenchymal origin such as mesenchymal cells (MSCs) and cancer-associated fibroblast (CAFs) [3,5]; (2) an **extracellular matrix** (ECM) component which consists of various collagen molecules, laminin, proteoglycans, polysaccharides and glycoproteins such as **tenascin-C (TNC)** and fibronectin. [37, 38] Structural support to the cell is provided by ECM and it transmits bio-mechanical signals that influence cellular functions like proliferation, differentiation and motility; (3) an immune component called **tumor immune microenvironment** **(TIME)**, composed of both innate immune cells **comprising of tumor-associated macrophages (TAMs)**, natural killer (NK) cells, neutrophils, mast cells, **dendritic cells (DCS)**, myeloid-derived suppressive cells (MDSCs), and adaptive immune cells comprising of CD4+ T helper lymphocytes (Th), CD8+ cytotoxic T cells, NK-T cells, γδ T cells, immunosuppressive regulatory T cells (Tregs) and B cells; [39] and (4) a micro vascular **endothelial/ lymphatic component** consisting of tumor micro-vessels and lymphatic endothelial cells (ECs) and pericytes. [40,41]

These components of TME interact among themselves and have a complex continuous multi-directional and multi-modal cross-talk with malignant cells which has an important role in determining the various dimensions of tumor development, progression, immune recognition metastasis and therapeutic responses. [1,2,3,4,5] It can be said that if genetic damage to the cell is the "match that lights the fire" of cancer, the TME provides the "fuel that feeds the flames". Although initially, cells in the tumor stroma possess certain tumor-suppressing capabilities, however, these cells are thought to be eventually coerced by the tumor cells and instructed to promote cancer growth, invasion, and metastasis. [4] The finest grade fuel that feeds the flame of tumour progression is CAF, as CAF progressively build and secure TME, by tilling the “soil” within which the tumor cell thrive.[42] Various studies have demonstrated that actively reshaping and modulation of the tumor microenvironment can impair tumor progression and improve the efficacy of conventional chemotherapeutic regimens.[43,44,45]

1. **“Soil and seed” theory, an early understanding of tumor microenvironment**

By analysing the autopsy findings of 735 females with carcinoma breast, Stephen Paget hypothesized that a certain group of tumor cells within the main tumor mass have metastatic potential (the “seed”) and have a preferential affinity for the growth-enhancing environment within specific organs (the “soil” i.e seeds having a growth advantage in certain specific organs). Hence his viewpoint was that metastasis occurs only if seed and soil are compatible.[9,10,11,12,46]

Paget’s autopsy analysis showed that the metastasis and spread of cancer are not random and that cancer cells exhibit selective choices when metastasizing to solid organs or bone. [47] While stressing his point he said “When a plant goes to seed, its seed is carried in all directions; but they can only live and grow if they fall on a congenial soil”, hence in his view metastasis results only when the appropriate seed is implanted in its suitable soil. [46,48]

Further, he made an important assertion at that time, that the microenvironment plays an important role in the growth of metastasis.[48] Therefore, the fate of the metastatic process is determined by a multiple complex series of inter-actions and cross-talk between metastatic cells and the microenvironment of the organ. The same is true for tumors at their primary site as we know it now and will be discussed in the following pages. However even before this, in the mid-nineteen century, Rudolph Virchow demonstrated a link between inflammation and the behaviour of the tumor, and proposed that other cells in the human body interact with tumor cells, so the tumor cells do not act as an isolated entity.[49,50] It is now clear that not only the growth of the seed is promoted by the soil, but also that the seed to support its need “educates” the soil.[51]

1. **The Fibroblast, a versatile multifunctional cell**

It was Rudolph Virchow in the 19th century, who first described fibroblast.[52] Fibroblasts are the type of cells derived predominantly from mesodermal origin following gastrulation; however, a small fraction of the fibroblast population is also derived from the neural crest. [5,53,54] In normal tissue their predominant role is in the contribution of connective tissue formation, a fibrous material that supports and connects other tissues and organs in the body. Fibroblast secretes ECM including collagen, elastin and fibronectin that help maintain the structural framework of tissue, modulation of inflammation as well as proliferation and differentiation of epithelial cells.

Hence fibroblasts have important contractile and extracellular matrix activities.[53,54,55] Existence of fibroblastic cells with contractile properties was described in 1971 by Giulio Gabbiani, in granulation tissue, and conceptualized that these cells with contractile properties have a reparative role in wound healing.[56] It was discovered later, that the contractile nature of this cell was primarily due to a splice variant of cellular fibronectin, termed ED-A FN and the actin isoform alpha-smooth muscle actin.[57,58] The important role of fibroblast is observed in inflammation, wound healing and fibrosis, and during invasive and metastatic progression of malignant cells; when they are activated and proliferate. **Nuclear factor-κβ (NF-κβ)** and **transforming growth factor β (TGF-β)** signalling pathways have been shown by several studies to have an important role in the inflammatory, fibrotic and malignant states.[5,53,59] **NF-κβ was discovered** first time by its ability to stimulate the growth of rat fibroblasts. [60]

There is a similarity in ECM deposition in both wound healing and cancer. Studies have proved that there is a certain degree of phenotypic and functional stratification during tissue repair and in chronic inflammatory conditions such as pulmonary fibrosis and colitis.[53,61,62] A study by Croft AP et al. identified a distinct fibroblast sub-set which leads to inflammation and bone damage in arthritis. They also showed that deletion of fibroblast activation protein α+ (FAP α)+ fibroblast suppressed both inflammation and bone erosion in a mouse model of resolving and persisting arthritis. They also identified two distinct sub-sets of fibroblasts within FAP α+  population - FAPαTHY1+ immune effector fibroblasts, and FAPαTHY1- destructive fibroblasts. The latter fibroblast selectively mediates bone and cartilage damage whereas the former (FAPα THY1+) mediate a more persistent inflammatory arthritis.[63] According to Kalluri R, fibroblasts could be considered the cockroaches of the human body, as they could survive severe stress otherwise lethal to the human body and could be cultured from post-mortem and decaying tissues. They have a robust intrinsic survival programme and cellular plasticity. [5] Fibroblasts can be identified and characterized by their spindle cell morphology and lack markers of epithelial, endothelial, leukocytic and malignant cells.[5,53,54]

Detailed and objectified studies of fibroblast activity/ mechanism of action have been hindered by the greatest challenge of the identification of specific markers for the identification of cell types.[64] Despite the challenge of finding a ubiquitous marker for fibroblast identification, it has been identified by using a set of commonly used mesenchymal markers and proteins associated with various degrees of activation state. Common markers routinely used to identify fibroblast include vimentin, [65] platelet-derived growth factor receptor α/β (PDGFRα/β),[66] periostin (POSTN),[64] COL1 and fibroblast-specific protein-1(FSP-1; a.k.a S100A4), [67,68] however they are not exclusive to fibroblast and may be expressed by other cells. Several markers may also identify a particular state of activation of fibroblast, hence may help in stratification into sub-population; this includes α-SMA and FAP-α [68,69]

Even within normal tissue, fibroblast heterogeneity is seen although several proteins including FAP present consistently in fibroblast from different regions of the human body and in CAFs. [70,71] Several murine model-based studies have identified different sub-set of fibroblast in normal tissue similar to heterogeneous CAFs.[72,74] This might also help in identifying different cells of origin of different CAFs which can have prognostic and therapeutic implications because distinct populations of CAFs have different roles in cancer progression., hence with lineage identification of CAFs, it will help in a better way to design potent combinatorial chemotherapeutic and immune-modulating approach for cancer treatment.[5,74,27]

1. **Cancer-associated fibroblast (CAF); vital fuel that feeds the flames**

Cancer-associated fibroblast (CAF), also known as tumor stromal fibroblast is a specialized group of activated fibroblast that has undergone phenotypic and functional changes, and has been shown to significantly participate in the proliferation, invasion and metastasis of tumor cells by building a unique environment called a TME **(Table 1)**; thus CAFs functions as signalling centre and remodelling machine which help in the formation of desmoplastic tumor niche.[5,75] CAFs apart from promoting cancer cell growth, also remodel the TME by the production of tensile strength within the matrix of the tumor microenvironment by collagen cross-linking making the tumor stiff and facilitating tumor invasion.[76,77,78,79,80] CAFs are phenotypically and functionally distinguishable from their normal counterpart by their increased rate of proliferation, the differential rate of expression of collagen and ECM, and upregulated secretion of pro-tumour cytokines and chemokines all of which support and promote tumorigenesis.[81,82] CAFs have a resemblance to fibroblast seen in wound healing in comparison to normal quiescent fibroblast, as tumor growth mimics the basic wound healing process and shows many resemblances, like deposition and cross-linking of fibrin and fibronectin (FN1) and the chemotaxis of immune cells. [83] CAF are present within tumor tissue samples having elongated spindle-shaped morphology, however, they tend to have slightly darker and larger nuclei and vaguely branched cytoplasm and are negative for epithelial, endothelial and leukocytic biomarkers; and express mesenchymal bio-markers such as vimentin (VIM), alpha- αSMA a.k.a ACTA2, podoplanin (PDPN), neural/glial antigen 2 (NG2), FAP, PDGF-α, COL1 including some novel markers like microfibrillar associated protein 5 (MFAP 5) and COL11A1 and, lack significant genetic mutations.[84,85,86]

Cancer cells tweak the normal wound healing properties of activated fibroblast, which follows the strict steps of homeostasis, inflammation, proliferation and remodelling/maturation, and the CAFs can outgrow disproportionately and out of context and have a potential to migrate away and invade adjacent tissue.[42]The sum effect of CAF function is the proliferation and making tumor cells more aggressive, so famously tumor is said to be “a wound that does not heal”.[87] At least this resemblance to fibroblast seen in wound healing is true for a sub-set of CAF with contractile characteristics which show expression of alpha-smooth muscle actin (α-SMA) as both of them aid in ECM protein production and extensive remodelling process.[88,89] However this is not the only phenotype of CAF, as CAF show heterogeneity and plasticity as there are a large array of functions attributed to CAFs.[90] It is seen that overall pro-tumorigenic properties of CAF predominate, and anti-tumorigenic properties are usually encountered in the early stages of cancer [91] Data is emerging at an accelerated rate that shows CAFs are specialized cells that are characterized not only by the expression of α-SMA, α-FAP, desmin and FSP-1 but also by their production of vascular endothelial growth factor (VEGF) and cytokines like IL-6 and IL-8. [5] Therefore the important question is, whether there are multiple sub-populations of CAFs or whether there is a need for the dependant and signal-dependent switching between distinct functional states.

* 1. **The cell of origin of CAF: Resident fibroblast or metamorphosis of various cells**

To propose a hypothesis regarding precise cells of origin of CAF in tumors is very challenging, as markers to flag both normal tissue resident fibroblast and CAF are ill-defined. It is seen that mostly CAFs originate from quiescent resident fibroblast or mesoderm-derived precursor cells (mesenchymal stem cells), although a full understanding of the precise origin of all CAFs in a given solid tumor type is still not fully understood.[92,93] Further research data about the precise origin of CAFs will offer a better understanding of their markers, signalling pathways that lead to their activation, and plasticity which will help in a better way to trap their pro-tumorigenic and/or intensify their anti-tumorigenic functions.[5]

There is a consensus that CAF has a varied source of origin, **(Figure 1)**, [64] besides differentiation of resident fibroblasts and mesenchymal stem cells; [94, 95] other cells like bone marrow-derived mesenchymal stem cells and myofibroblasts,[96,97,98] hematopoietic stem cells,[99] pancreatic and hepatic stellate cells,[74] or trans-differentiate from) adipocytes,[100] mesothelial cells,[101] endothelial cells (endothelial to mesenchymal transition, EndMT),[102,103] myeloid cells,[104,105] pericytes,[106] smooth muscle cells,[107] epithelial cells (Epithelial-mesenchymal transition, EMT),[108,109] myoepithelial cells in breast,[110] pericryptal myofibroblast in the gastrointestinal tract, [111] and circulating bone marrow cells known as fibrocytes or even tumor cell, [102, 109, 105,110] can differentiate into CAF. These alternative cells as precursors of CAF have been identified mostly by bone marrow transplant studies and in-vitro experiments with cancer cells, data of which should be extrapolated to human cancer tissue cautiously with further in-vivo studies.[5, 112] An in-vitro study by Öhlund D et al. identified that pancreatic stellate cells can differentiate into both inflammatory iCAFs and myofibroblastic myCAFs.[90] Mesothelial cells have been shown by sc-RNA-seq and immunohistochemical analysis to share transcriptional signature with a special sub-type of CAF, identifying an antigen-presenting CAF (apt AF).[113] Like mesothelial cells can give rise to hepatic stellate cells and myofibroblast via. mesothelial-mesenchymal transition in liver injury, similarly apCAF can derive from mesothelial cells, following their interaction with tumor cells.[113,114] Studying the morphogenesis of fibroblastic stroma as it proceeds from hyperplasia to in-situ carcinoma to florid carcinoma indicates, the origin of CAFs to be resident stromal fibroblasts. However immunohistochemical analysis and recently developed single cell-RNA-sequencing analysis of multiple tumor entities indicate several sub-population of CAFs with varied functions.[115] CAFs are present in almost all major solid tumors; however, their abundance varies between different types of cancers. For example, prostate, breast, and pancreatic cancers contain a high percentage of CAFs, whereas brain, renal, and ovarian cancers demonstrate fewer numbers.[116]

Advances in genetic lineage tracing (fate mapping), genetic labelling technologies and fluorescent tagging are beginning to shed light on the mechanism and dynamics of stem cells and progenitor cells' fate determination during development, tissue maintenance and repair as well as dysregulation in tumor formation, however, due to lack of specific fibroblast restricted markers, it is very difficult to trace lineage and identify the biological origin of distinct sub-types of CAFs.[64]

* 1. **CAF activation: is it a turning point of cancer behaviour?**

#### Tumor-derived signals and/or signals from immune cells seem to be the most significant factor that is involved in CAF recruitment and activation[117] (Figure 2). Tissue-resident fibroblast, quiescent fibroblast, mesenchymal-derived cells and some other cells can give rise to CAFs when activated by various mechanisms. Three important ways cancer cells and/or immune cells promote CAF activation include: through extracellular molecules and chemical secretion including episomes, direct malignant cell-fibroblastic interaction and cross-talk, and through various intracellular signalling pathways (Table 2) including TGF-β [118,119] (Figure 3), Nuclear factor-κB (NF-κB), [120,121] PI3K/AKT/mTOR signalling pathway,[123,124,125] Wnt signalling pathway,[133,134,135] JAK/STAT, [138, 139] or Sonic Hedgehog(SHH). [147,148]

#### Other mechanisms include physical and chemical changes in the ECM leading to CAF activation.[149] Even oncogenic and tumor suppressor mutations in the cancer cells can lead to specific activation of CAF. K-RAS and different p53 mutations have been shown to activate and sustain CAFs.[150,151]

Epigenetics also have an important role in CAF activation. The two main types of epigenetic changes include DNA methylation and histone modification via acetylation or methylation. [152] It is seen that lactate-mediated epigenetic switch,[153] and pro-inflammatory leukaemia inhibitory factor (LIF) induced epigenetic switch activates fibroblast to pro-invasive CAFs. LIF activates an epigenetic switch which leads to sustained activation of Janus kinase 1 signal transducer and activator of transcription 3 (JAK-1-STAT3) signalling, which leads to constitutive stimulation of DNA methyltransferase (DNMT1), responsible for activation of fibroblast to my-CAF subtype.[154]

Hyperproliferation of malignant cells can lead to stretching of fibroblast, this can initiate Yes-associated protein-1 (YAP-1) and SRF-driven transcription. [155,156,157,158] Heat shock factor 1, reactive oxygen species (ROS),[159] local hypoxia,[160] and metabolic stress can also lead to the formation of CAFs. Hypoxia-inducible factor-1α (HIF-1α), under the influence of lysophosphatidic acid (LPA), TGF-β1 or PDGF acquired from cancer cells can switch on aerobic glycolysis in fibroblast; this energy metabolism shift in fibroblast is thought to be an important event in its activation and conversion to CAFs.[161,162,163]

Cytokines, IL-1 and IL-6 which acts as inflammatory modulator can also activate CAF through NF-κB and, signal transducer and activators of transcription (STAT) respectively.[164,165] Tumor necrosis factor, by manipulating the immunological response of TME, have a role in activating CAFs through the NF-κB pathway. [166]

In general, TGF-β1 secreted by tumor cells and also stromal cells is found to be one of the essential factors facilitating the mobilization and activation of resident fibroblast. TGF-β1 stimulates SMAD transcription factor. SMAD-dependent pathways activate the fibroblast to CAFs **(Figure 3)**. [155,167]

The activation, recruitment and conversion of fibroblast to CAFs are also influenced by tumor-secreted exosomes (TSE), besides tumor-secreted cytokines, chemokines and growth factors.[168], Exosomes are 40-100 nm vesicles which are released into the extracellular milieu, which contain protein, lipid, genetic material and cell signal molecules. They are formed upon the fusion of intracellular multi-vesicular bodies and plasma membranes which are taken up by adjacent or distant cells (one of the modes of cell-cell crosstalk/ cell communication).[168, 169] The TSE contains various oncogenic molecules such as fusion gene mRNAs, micro RNAs (miRNAs), long non-coding RNA (lncRNA) Gm26800, fragments of mutated DNA and a variety of cell signalling molecules, all of which in concert are capable of modifying fibroblast to CAFs by NF-κB signal transducer, downstream mitogen-activated protein kinase (MAPK) and STAT3 or TGF-β signalling mechanism.[132,170,171,172] Contact signals, when the cancer cells and fibroblast come in close contact can also lead to CAF phenotype by the way of NOTCH and Eph-ephrins signalling.[173]

1. **CAF Hetrogeniety – is it the new version of Hippocrates’s original  carcinomas**

CAF represents a heterogeneous population of cells similar to cancer stem cells. Like cancer stem cells CAF is highly plastic and expresses various markers and proteins which vary over time.[5,54,174]) Initially CAF was thought to be a homogenous population of stromal cells united by their morphological architecture and spatial loc ation within TME. [5,54,74] Latter on by studying the molecular characteristics of CAFs using multi-colour flow-cytometry, immunohistochemistry as well as by recent availability of single cell-RNA-sequencing, [5,54,175] it has been noted that there is a significant degree of specialization and heterogeneity within CAF pool, giving rise to the label of different CAF phenotypes based on morphological behaviour and functional properties; and that these cells can have both pro- and anti- tumorigenic properties, although pro-tumorogenic properties overwhelm anti-tumorogenic properties.[5,54,176] Initial observations suggest particular CAF sub-populations originate from a single cellular source.[5,177] Based on single-cell RNA seq analysis, it is noted CAFs are heterogeneous, in terms of morphology, functions and markers expressed. This emergent concept of CAF subpopulations is based on several recent publications reporting the presence of several CAF subgroups in PDAC (pancreatic ductal adenocarcinoma), breast carcinoma, colon carcinoma, lung adenocarcinoma and high-grade serous ovarian cancers [178,179,180,181,182] However recently studies also indicate that depending upon episomal signals from tumor cells, therapeutic regimens and culture composition, CAFs can interconvert among sub-types after their origin from a particular cellular source; [179,183,184] ]like mouse-derived PDAC associated CAFs can be converted from α-SMA high and IL-6 producing functional state by manipulation through IL-1 and TGF-β, attesting their plasticity.[179]. This evidence suggests that CAF sub-types are not endpoints in differentiation but interchangeable cell states.[184] This characteristic of CAFs represents an innovative opportunity by focusing on shifting the sub-type of CAF towards an anti-tumorigenic or quiescent population, rather than directly targeting the particular sub-type of CAF to ablate them.[185] Biffi and Oni in their study titled “IL-1 induced JAK/STAT signalling is antagonized by TGF-β to shape CAF heterogeneity in PDAC”, have observed that it is possible to shift CAF sub-type from iCAF (inflammatory cancer-associated fibroblast) to myCAF (myofibroblastic cancer-associated fibroblast), by reprogramming the CAF subtype with the JAK inhibitor AZD1480.[179] Similarly, Ohlund and his team in 2017 demonstrated that in patients and murine-driven models of CAFs, if cells of pancreatic ductal adenocarcinoma are co-cultured there is a significant decrease in α-SMA expression by CAFs and transition from a more myofibroblast-like to a more inflammatory CAF subtype (myoCAF to iCaf).[90]

The sc-RNA-seq techniques have demonstrated that fibroblastic plasticity is not only seen in the malignant state but also in body homeostasis, chronic inflammatory conditions and wound healing, as well as ageing.[92,186,187] Observations suggested varied CAF sub-population originates from a single cellular source.[177] However alternate studies suggest that different CAF sub-populations originate from different cell types, as it is seen that there are various sources of CAF precursors and paracrine signalling.[188] Hippocrates (460-370 BC) described malignant non-ulcer-forming and ulcer-forming tumors as  “carcinomas” and “carcinoma”. The literal meaning of these words derived from the Greek language (καρκίνος) is crab, most probably applied due to the multiple finger-like spreading projections in a different directions giving the imagination of the shape of a crab.[189] The Roman physician, Celsus (25 BC - 50 AD), later translated the Greek term into cancer, the Latin word for crab.[190] CAFs because of multi-dimensional and variable phenotypic and functional heterogeneity, can be compared to the original crab or its mirror image in TME.

The major failure of many present therapeutic regimens for the treatment of malignancy is that the primary target of these drugs is rapidly multiplying malignant cells, which very frequently develop drug resistance.[152] Since TME, especially CAFs have a major impact on the availability, absorption, distribution, penetration and pharmacological metabolism of anti-cancer drugs and have an active role in the regulation of tumour cell growth, invasion and metabolism, it is prudent that active effort should be made to target the CAFs to modify the non-tumour cell behaviour which can have a major role in tumor growth suppression.[42,191]

* 1. **Phenotypic Heterogeneity of CAF**

Different biological markers associated with CAFs are responsible for phenotypic heterogeneity. These include surface markers, intracellular cytoplasmic proteins and some extracellular markers (**Figure 4**). For a time being α-SMA (ACTA2) was the major marker used to characterize CAF, [69] although it is also expressed by normal fibroblast, myofibroblast, myocytes and pericytes.[192,193]

Latter on with more knowledge gained, CAFs were defined by expression of mesenchymal markers like vimentin, α –SMA, FSP-1 (S100A4), PDGFR- α /β, and FAP-α, transgelin (TAGLN) and by the absence of epithelial markers (cytokeratin, e-cadherin), myeloid marker (CD-45) and endothelial marker (CD31, factor VIII).[69,194] FAP-α is the most frequently expressed gene in the tumor microenvironment in the stromal cells and is up-regulated in more than 90% of epithelial. malignancy.[195] However, these markers which define CAFs are also seen in sub-group of macrophages and myeloid cells (FSP-1), pericytes (α –SMA and PDGFR- β), mesodermal cells and malignant cells undergoing epithelial-mesenchymal transition (α-FAP) and normal fibroblast (FSP-1 and PDGFR- β ),[40,196,197,198] indicating that they are non-specific markers.[40,68] Of all these markers, although PDGFRs are not relatively specific for CAFs like α –SMA and FAP, they have a widespread expression in the overall fibroblast population in tumor niche, hence they are broadly expressed in fibroblast compared to α–SMA and FAP, and since they are not up-regulated they are less responsive to environmental factors such as hypoxia. [199] Hence PDGFR can be used as a generalized fibroblast marker due to their more static expression. [40] Additionally, PDGFR shows surface expression in contrast to α–SMA, hence they can be more easily detected on flow cytometry-based sorting, especially on culture.[40]

MFAP5 and COL11A1 are novel CAF markers suggested to be extremely specific and can be used to highlight CAF lineage.[200,201] However some recent studies suggest that MFAP5 expression may vary among subtypes.[202]

There is a variable expression of these CAF markers between different CAF subtypes. [90] Recent studies in a variety of murine cancer models and human tumors have highlighted and characterized various sub-population of CAFs with specific functions based on cell surface markers, extra-cellular secreted proteins and intracellular proteins.[90,203,204] Immuno-fluorescent studies first highlighted CAF heterogeneity, however, none of the markers was exclusive for CAFs.[178,205] Latter progress in multi-colour flow cytometry and immune-histochemical methodologies along with the novel single cell-RNA-sequencing (sc-RNA-seq) techniques were able to sub-type CAFs based on the presence of markers in various combinations.[90,206] Recently proposed microfluidic platforms and a proposed method of sequential indexing are recent developments that will further help in studying CAF heterogeneity.[206,207,208] Analysis of different tumour entities, especially breast and pancreatic carcinoma and using mostly single cell-RNA-sequencing technology, CAFs have been stratified into several sub-populations and data is emerging on the front of functional heterogeneity of these cells and their impact on cancer progression[90,181,182,183,184, 213,214,215] **(Table 3)**

It was R Kalluri and the team who first highlighted CAF heterogeneity in breast and pancreatic cancer murine model, they classified them as CAF-1 and CAF-2. () CAF-1 is characterized by their expression of FSP-1 which makes tumor cells prone to distant metastasis. Further FSP-1 positive CAFs produce tenascin which stimulates VEGA-A mediated neo-vascularization and metastasis. The second subtype CAF-2 show positivity for α –SMA, neural/glial antigen 2 (NG2) and PDGFR-β expression and production of type I collagen promoting a barrier of fibrotic connective tissue which immunomodulates the TME by preventing tumor infiltration by cytotoxic T-lymphocytes.[5,225]

However, the **most studied and recurrent subtypes** of CAF seen in several malignant tumors are inflammatory CAF (iCAF) and myofibroblasts (myCAF).[209, 226, 227] **iCAF** have an immune-modulating function and is characterized by the secretion of numerous inflammatory cytokines including CXCL-1, CXCL-12, IL-6, IL-11 and leukaemia inhibitory factor (LIF) that promote tumor evasion by immunosuppression. [25, 26] IL-1β-led activation of NF-κB is shown to promote iCAF phenotype. [210] iCAF also secretes CCL-17 and CCL-22 which promote the immunosuppressive microenvironment by recruiting Regulatory T cells  (Tregs) in the malignant lesion.[228] Also, iCAF has promoted the recruitment and stimulation of monocytes that have an inhibitory effect on CD8+ lymphocytes.[229,230] Overall iCAF has an immunomodulatory function and promotes angiogenesis and metastasis. In pancreatic cancer, Elyada and colleagues demonstrated the synthesis of hyaluronan as a component of ECM under the influence of HAS1 and HAS2 expressed by iCAF which has been demonstrated as a protective barrier for cancer cells against treatment. [183] **myCAF** has cytoskeleton remodelling properties and is characterized by the production of TGF- β and collagen [51, 90] TGF- β led signalling pathway leads to transcriptional activation of myCAF and they are identified by high-level expression of α-SMA. [206, 231]

There are several other descriptions of sub-types of CAFs in different types of cancers by various researchers. Costea et al. described two CAF sub-type with differential tumor-promoting abilities in oral squamous cell carcinoma, termed CAF-N (normal) and CAF-D (divergent). They noted that CAF-D switches EMT in cancer cells and sequentially aids in cell migration, which is due to TGF-β production by this CAF sub-type. While CAF-N promotes tissue invasion by malignant cells and creates a hyaluronic acid-rich ECM which has immunosuppressive properties by acting as a barrier, this is due to the secretion of matrix metalloproteinase and hyaluronic acid by this subset of CAFs.[232] Using single cell-RNA-sequencing, Lambrechts and his group revealed a highly complex TME that profoundly moulds stromal cells and described five distinct subtypes of CAFs in lung cancer tumour microenvironment by comparing them with matching non-malignant lung samples based on unique collagen types and ECM proteins.[233] Davidson and his colleagues working on murine melanoma and draining lymph nodes (LNs), described three sub-populations of CAF- immune or S1, desmoplastic or S2 and contractile stromal cells or S3 using sc-RNA-sequencing. S1- immune sub-type showed overexpression of PDGFR-α, PDPN and CD-34 and by expression of pro-inflammatory cytokines like CSF-1 or SDF-1 andIL-6 receptor-promoted recruitment of immune cells. S2- Desmoplastic type showed low expression of α-SMA and intermediate expression of PDGFR- α and showed the increased synthesis of COL1A1, COL1A2 and COL6A2 collagen, Tenascin C (TNC) and periostin (POSTN) a protein-coding gene, leading to an intense desmoplastic reaction. S3- contractile stromal subtype showed over-expression of α-SMA due to increased activity of genes involved in rearrangement and regulation of actin cytoskeleton, this subtype of stromal CAF was noted to be most proliferative. They also noted that, whereas "immune" stromal cells are observed in early tumors, "contractile" cells become more prevalent at the later stage of the tumor. [234]

Gong L et al., by applying single-cell RNA sequencing to 66,627 cells from 14 patients with nasopharyngeal carcinoma identified and characterized five major stromal clusters and 36 distinct subpopulations based on genetic profiling.[235]

**6.2 Functional heterogeneity of CAF- a Philharmonic Orchestra?**

The functions of CAFs are as diverse as their cellular origin, ways by which they originate and phenotypic sub-populations. It would be early to state specific and reproducible functions of CAFs. Inhibition of cancer progression is seen with normal fibroblast,[236,237,238] however CAFs are predominantly shown by several studies to mainly act as tumor promoting agents, however, tumor inhibiting actions are also being validated by several studies.[5,14,16,18,20, 42, 54, 239] Fibroblasts in TME establish a self-activating and perpetual feedback loop, which forms the basis of their tumorigenic capacities.[240] A multi-pronged approach is needed to discover the precise roles of CAF in tumors, by overcoming the experimental difficulties and limitations and making the picture clear regarding the phenotypic heterogeneity of CAF markers. Current limitations include a lack of methodologies to track the phenotypic and heterogeneous variability during tumor progression, inability to consistently observe the molecular determinants and signalling pathway over time and to objectify their physiological and pathological relationship with other members of TME, such as immune cells including macrophages and lymphocytes, extracellular matrix components, and intra-tumoral angiogenesis and hypoxia.[5,34, 54,64,185, 225, 241] Data is emerging that reveals that CAFs not only directly influence the cancer cells, but their impact also extends to various cellular components of the TME that regulate the function of angiogenesis and the tumor immune system.[164, 242,243,244,245] There are several ways and mechanisms by which CAF have been shown to modify cancer growth. **(Table 1; Figure 5).** Important ones include:

**6.2.1 Secreting ECM and deposition:** CAF initiates perpetual remodelling of ECM, making ECM stiffer by excessive deposition of type I and III collagen and degradation of type IV collagen.[5,64] A distinct ECM is required for tumor progression, invasion and metastasis, for which CAF is shown to have an active role ranging from production, secretion and deposition of ECM to ECM proteolysis, cross-linking and assembly.[42,246] This ECM deposit acts as a protective barrier against drug delivery by inherent physical properties of the collagen matrix,[247,248] and also due to elevated interstitial fluid pressure and hypoperfusion by compressing lymphatics and blood vessels respectively.[249, 250, 251] Constitutive collagen cross-linking is mediated by CAFs under the influence of enzyme lysol oxidase (LOX) in early breast carcinogenesis and hypoxic cancer cells in the latter stages, prompting invasiveness.[78, 252]

ECM also provide active support to growing tumor cells with biochemical and mechanical cues,[253,254] and nutrients[80] The “inside-out” and “outside-in” mechanical cues termed “mechanoreciprocity ” with the interplay of Integrin-α11β1 stromal cell receptor leads to even more stiff stroma by remodelling, helping in cancer cell invasion.[255, 256, 257]

A more stiffened ECM is generated due to the secretion of matrix metalloproteinase and excessive deposition of ECM that generate permissive tracks to promote motility and invasiveness of cancer cells to increase their metastatic potential.[64, 258, 259] Cell invasion is also promoted in a stiff ECM through focal adhesion kinase (FAK) phosphorylation, integrin clustering and Rho GTPase activation. [260]

Acerbi I et al. demonstrated that human breast cancer cell invasion and aggressiveness correlate with extracellular stiffness.[261] Dupont S and his team studied the role of YAP/TAZ signalling in mechanotransduction and demonstrated that YAP/TAZ signalling disturbs the actin cytoskeleton when it is activated by stiff ECM leading to mechanical stress, which further promotes tumour aggressiveness.[262] Therapeutic targeting of ECM composition has been tried to increase the efficacy of immunotherapy and chemotherapy.[246, 247,248,263]

**6.2.2 Angiogenic activity of CAFs:** The term “angiogenesis” was originally introduced by the British surgeon John Hunter in 1787, to describe the formation of new vessels in the process of wound healing.[264] Almost two centuries after this term was proposed, angiogenesis and lymphangiogenesis are found to be a very essential factors for cancer cells to sustain and proliferate.[265, 266] Proliferation of endothelial cells (EC) and recruitment of pericytes and endothelial cells in the tumor is promoted by CAFs which secretes many angiogenic factors like PDGF, VEGF, FGF, HAF or CXCL.[20, 267, 268, 269]

Remarkably there is the production of other pro-angiogenic factors like IL-6, IL-8 and placental growth factor, through the stimulatory effect of PDGF and VEGF by taking the CAFs into the autocrine loop.[270, 271] A study by Huang B et al. identified that the CAFs of hepatocellular carcinoma regulate the EZH2/VASH1 pathway via VEGF secretion, thereby promoting the proliferation of endothelial cells and angiogenesis.[272]

CAFs have been shown to promote neovascularization largely by NF-κB activation, in early breast cancer xenograft models.[273] “Late-stage mammary tumor virus-polyoma middle tumor-antigen” (MMTV-PyMT) mouse model of breast cancer, displayed an increased population of CAFs with vascular and pro-metastatic functions compared to early-stage, which was dependent on factors other than NF-κB signalling.[274] Using a 3D *in vitro* tissue model of vasculogenesis, Mary Kathryn Sewell-Loftin and her team noted increased vascularization in the presence of breast cancer CAFs compared to normal breast fibroblasts.  CAFs generated larger deformations within the ECM, compared to normal breast fibroblasts, which stimulate the self-assembly of ECs into a vascular network. They further observed this process is mediated via mechano-transductive pathways in the CAF including ROCK2, YAP, and SN1. They also noted that although VEGF signalling is important, the inhibition of VEGF receptors only partially inhibits the CAF-promoted growth.[275] It is also seen in a study that Hypoxic cancer-associated fibroblasts increase NCBP2-AS2/HIAR to promote endothelial sprouting through enhanced VEGF signalling.[160] Harrera A. and his team in their study noted that the SNAI1 receptor on fibroblast in 3D matrices derived from PDGF-BB-stimulated fibroblasts through the FAK pathway, as an important factor for the unique organization of ECM fibres. These fibres which showed a parallel orientation acts as a “tract” for endothelial cells, helping stimulation of endothelial cells and creation of tubular structures resembling in-vivo capillary formation. Further Snail1 expression in fibroblast was noted as a requirement for the co-adjuvant effect of the cell on matrix remodelling and neo-angiogenesis when co-xenografted in nude mice. Remarkably, they also noted a direct correlation between SNAI1 and the endothelial marker (CD34) n tumor samples from colorectal cancer patients.[270]

In a study by Albertini and colleagues, they co-injected patient-derived CAFs and human Merkel cell carcinoma MKL-1 cells into **severe combined immunodeficiency trait**  **(**SCID**)** mice and found that CAFs exert a pro-angiogenic activity in Merkel cell carcinoma mediated by the aminopeptidase A (APA)/Ang II-III/Angiotensin-II Type I Receptor**(**AT1R**)** axis, with the expression of APA in CAFs being the upstream triggering event.[276]

**6.2.3 Immunosuppression by CAFs:** The cell population within the tumor immune microenvironment (TIME) is monitored by CAFs, including its polarization, recruitment and functions of immune cells.[23, 277] CAFs have a major role in immune regulation, either directly by secretion of different molecules (chemokines or cytokines), or indirectly through the remodelling of ECM that **rations** intra-tumoral infiltration.[265] Diverse CAF subtypes have an essential role in regulating the immune landscape in cancer, sustaining an overall immune-suppressive, and therefore tumor-promoting status [278,279,280] CAF contributes to immunosuppression by upregulation of immune suppressive cytokines synthesis and immune checkpoint ligands.[241] Chemokines like CCL2, CCL22, CXCL-1, CXCL-5, CXCL-12, IL-1β and IL-8 produced by activated fibroblasts are involved in recruitment of monocytes, myeloid cells and macrophages in tumor.[228,281,282 ]

CAF enhances the activity of T regulatory cells (Tregs) and M2 macrophages via TGF-β, IL-15, CCL2, CCL-17, CXCL2-2 and CXCL-8 which have an overall immunosuppressive effect on tumor microenvironment. [28, 283,284,285] Further they inhibit the activity of CD8+ T cells, dendritic cells, NK cells, Th1 lymphocytes and M1 macrophages further contributing to immune escape. [286]

The activity of cytotoxic T lymphocytes is also inhibited by the destruction of CD8+ T cells by a CAF-assisted immune-mediated mechanism. PD-L2 and FASL, so that the overall T-cell role is directed to tumor promotion.[287] T-regulatory cells (Tregs), an immune suppressive T-lymphocyte characterized by expression of transcription factor forkhead box 3 (FOXP3) and IL-2 receptor α chain are especially recruited by FAP and αSMA positive sub-population of CAFs.[215, 278, 280]

A unique way of immune suppression by CAF was noted in pancreatic ductal adenocarcinoma by Gorchs et al. involving direct cell to cell contact. Expression of immune checkpoint ligand on CD4+ and CD8+ T-cell was induced by CAFs, and these immune checkpoint expressing T-cells produced less IFN-γ, TNF-α and CD107a leading to an immunosuppressive environment. [288]

**6.2.4 Promoting epithelial-mesenchymal transition and invasion:** Epithelial cancer cells are well known to show epithelial-mesenchymal transition (EMT), and CAFs are the predominant cell type among few other stromal cells that have been shown to initiate and sustain EMT.[289]

CAFs were demonstrated to promote cancer cell EMT via TGF-β secretion and induction of the TGF-β/SMAD signaling pathway in the cancer cells in a study by Yu Y et al. they co-cultured fibroblasts isolated from breast cancer patients with a panel of breast cancer cell lines. [289] Jia C et al. did a study with an aim to explore the role of CAFs in HCC epithelial-mesenchymal transition (EMT) and its mechanism. They found that liver cirrhosis, inferior clinicopathologic characteristics, elevated EMT-associated markers, and poorer survival in human HCC was associated with high density of CAF in tumor. They demonstrated that EMT was induced within HCC tumor cells after they were co-cultured with HCC associated CAFs (H-CAFs). H-CAFs secreted  IL-6 and hepatocyte growth factor (HGF) which stimulated EMT. Proteomic analysis revealed that overexpression of transglutaminase 2 (TG2) a multi-functional enzyme which catalyzes the formation of inter-molecular isopeptide bonds between glutamine and lysine side-chains, on HCC cells promoted EMT and there was remarkable attenuation of H-CAF induced EMT with knockdown of TG2. TG2 expression on HCC cells was increased after cells were stimulated by IL-6.[224] A Study by Fiaschi T et al. observing the effect of Carbonic anhydrase IX on cancer-associated fibroblasts in prostatic carcinoma noted that acidification of the microenvironment by CA IX can also directly promote an EMT program in prostate carcinoma cells. [290] They also noted that cancer cell derived IL-6 generated a CAF phenotype with increased level of MMP2 and MMP9 which can lead to a favourable environment for EMT.

Goulet CR et al. studied conditioned medium from iCAFs (CM iCAF) on EMT markers expression of non-invasive RT4 bladder cancer cell line. They observed that CM iCAF induces the up-regulation of mesenchymal markers, such as N-cadherin and vimentin, while supressing epithelial markers p-β-catenin and E-cadherin expression in non-invasive RT4 cells. Further EMT transcription factors TWIST1, SNAIL1 and ZEB1 were up-regulated in CM iCAF-cultured RT4 cells compared to control.[291] It is also observed that Snail1-dependent cancer-associated fibroblasts induce epithelial-mesenchymal transition in lung cancer cells via exosomes. [292]

Most carcinoma cells have weak invasive potential in vitro and in vivo, denoting that constituents of their microenvironment may help in transition from in situ to invasive stages during progression. Facilitation of tumor cell invasion is aided by CAFs, by its ability to modify the orientation of collagen fibres through Cdc42-dependent reorganization of collagen fibers. [293] Invasive propertu is provided to insitu ductal carcinoma breast (DCIS) by a more perpendicular alignment of collagen fibres near the potential invasive front; compared to the normal collagen organization parallel to the circumference of the duct. This was identified using laser-scanning multiphoton and second harmonic generation microscopy.[294,295 ] It is also noted that IL-6 expression by CAFs promote DCIS microinvasion through activation of Cathepsin-B expression in tumor cells. [296] CAF population of growing tumor also facilitates invasion of tumor cells via. CAF chemokine C-XC motif ligand 1(CXCL-1) secretion and its interaction with C-XC motif chemokine receptor 2 (CXCR2) in the tumor cells.[297]

**6.2.5 Promoting Metastasis:** The evolving concept that similar to the cancer cells, CAF can disseminate into the blood and lymphatic circulation and travel to distant metastatic sites, suggests that CAFs have additional complex role in metastasis.[298,299] However fibroblasts at metastatic site can also be activated by metastatic cancer cells.[300]

Stromal fibroblasts at metastatic sites can be termed metastasis-associated fibroblasts (MAFs).[301] Generally, the source of origin of MAFs is similar to the source of CAFs in the primary tumor at that site and similar to sources of fibroblast at primary site varies considerably.[300, 302]

A study by Joyce T O'Connell et al.,  on transgenic mice that express viral thymidine kinase under control of the S100A4 promoter to specifically ablate S100A4(+) stromal cells which are likely fibroblasts in this setting, showed that ablation of S100A4(+) stromal cells significantly reduced metastatic colonization without affecting primary tumor growth.[301] A study by Kong et al. to interrogate the role of intercellular communication between MAFs and secondary organs via. extracellular vesicles (EVs)  in salivary adenoid cystic carcinoma (SACC) metastasis, they observed that MAF’s EVs participated in the pre-metastatic niche formation in the lung.[302]  A study on colorectal cancer (CRC) model by Qing Ji et al. shows that cancer cells by secreting integrin beta-like 1 (ITGBL1)-rich extracellular vesicles (EVs) mediated activation of fibroblasts which induces the pre-metastatic niche formation and promote metastatic cancer growth by secreting pro-inflammatory cytokine, such as IL-6 and IL-8. The finding of this study reveals a tumor-stromal cross-talk in the metastatic tumor micro-environment and uncovers a novel signaling communication between primary tumors and metastases through the ITGBL1-loaded EVs.[300] Another study also reveals signaling communication between primary tumors and metastases, which shows  in the lung metastatic niche, high-metastatic potential hepatocellular carcinoma (HCC) cells exhibit a greater capability to convert resident fibroblasts to cancer-associated fibroblasts (CAFs), these high-metastatic HCC cells secrete exosomal miR-1247-3p that directly targets B4GALT3, leading to activation of β1-integrin–NF-κB signaling in fibroblasts.[303]

**6.2.6 Stemness of tumor neiche:** It has been observed in recent studies that cancer stem cell (CSC) niche is maintained by ECM remodeling components and secretion of inflammatory cytokines by CAFs in several tumour types.[304,305,306] For example, periostin (POSTN), a component of the extracellular matrix, is expressed by fibroblasts in the stroma by primary tumour stimulation. POSTN is required for maintenance of cancer stem cells, and blocking its function prevents metastasis. POSTN recruits Wnt ligands and thereby increases Wnt signalling in cancer stem cells.[307] An in-vitro study by Vaziri N et al. showed leukemia Inhibitory Factor (LIF) is produced by CAF when co-cultivated with breast cancer cells, which significantly promoted stemness through the dedifferentiation process and regaining of stem-cell-like properties. There was up-regulation of LIFR signalling in malignant cells of breast cancer in the presence of CAF secreted LIF which promotes Nanog and Oct4 expression and the stem cell markers are increased in breast cancer cells.[308] A recent study noted and shed light on the fact that tumor-initiated Hedgehog signalling leads to CAF phenotype changes which facilitates stemness in breast cancer in both human breast cancer patients and mouse models, and that negative modulation of hedgehog signalling in CAF can be a useful therapeutic modality to decrease breast cancer cell plasticity.[309,310] CAF-derived growth factors like HGF (via WNT signalling) and insulin-like growth factor-II (IGF-II) are also responsible for promoting stemness of tumor niche. [311, 312] In addition to the epithelial-to-mesenchymal transition, global plasticity programmes affecting cell stemness and behaviour is also controlled by  transcriptional factors of the Snail, ZEB and Twist families (EMT-TFs). A study reported that, a sub-type of CAF positive for cell surface markers  GPR77 and CD10 (GPR77+CD10+ CAF), sustain cancer stemness and chemo-resistance in breast cancer  through high secretion of IL-6 and IL-8 due to sustained NFκB signalling.[219] This finding is attested by another study which revealed that increased NFκB signalling in p53mut PDAC cells was responsible for an increase in pro-invasive perlecan production in adjacent CAFs via TNF-α secretion from the cancer cells.[164]

**6.2.7 Metabolic adaptations:** Cancer cells are under a metabolic stress with low oxygen tension and nutrition deficient unfavourable environment; and strive to produce energy to overcome nutrient deprivation via different survival pathways so that high proliferation rate of tumor cells can be maintained and it can have survival edge.[265] Tumor microenvironment (TME) is believed to be the most important factor leading to metabolic reprogramming of cancer cells.[314] Bi-directional metabolic communications between tumor cells and stromal cells contribute to tumor growth and invasiveness while affecting therapeutic responses.[315]

Model has been proposed termed as “Reverse Warburg” pathway (via Lactate schuttle etc), which supports massive and uncontrolled proliferation and nutrients demand of tumor cells, it occurs due to metabolic coupling of cancer cells with CAFs i.e.  cancer cells highjack CAFs and reprogram their metabolism. In normal fibroblast, glucose is metabolized through oxidative phosphorylation.[314] In this “Reverse Warburg” pathway oxidative stress is induced on CAFs by cancer cells, so that CAFs switches to **glycolysis** providing energy-rich lactate, pyruvate, glutamate and fatty acids to metabolically support adjacent cancer cells as new energy fuels for cancer cells to maintain macromolecular biosynthesis and energy requirements.[118]  Several studies have provided evidence to support the reverse “Warburg Effect” hypothesis. Cancer cell derived lysophosphatidic acid (LPA), platelet derived growth factor (PDGF) and TGF-β1 act on CAFs and is able to switch on aerobic glycolysis in CAFs via. hypoxia inducible factor-1α (HIF-1α); also aerobic glycolysis in CAF can directly be induced in by tumor cell derived mitochondrial transfer or caveolin-1 (CAV-1).[161,162,163] Zhang D et al. also reported that down-regulation of α subunit of the isocitrate dehydrogenase 3 complex (IDH3α) leads to metabolic switch to glycolysis from oxidative phosphorylation in CAFs, and that overexpression of IDH3α prevents this switch in fibroblast.[316] A study by Shan T et al. also attested the “Reverse Warburg” theory in CAF; they showed that mRNA and protein expression of pancreas associated CAF’s glycolytic enzymes, lactate dehydrogenase and pyruvate kinase m2 (PKM2) as well as, monocarboxylate transporter 4 (MCT4) was raised and it was responsible for lactate secretion.[317]

Previously, “Warburg effect” (aerobic glycolysis) discovered by O. Warburg was thought as a predominant source of energy for cancer cells, in which cancer cells were believed to adapt to a low-oxygen situation consume high levels of glucose and produce ten time more lactic acid than normal cells rather than pyruvate.[318,319]

**6.2.8 Conferring therapy resistance and radioprotection:**

In the past few decades, the role of cancer-associated fibroblasts (CAFs) in resistance to therapies has emerged. Cytokines, chemokines and growth factors in the TME secreted by CAFs leading to paracrine signaling between CAFs and cancer cells are significantly related to chemoresistance and poor prognosis in cancer patients.[320,321,322,323,324] Recent studies suggests that IL-6 derived from CAFs can induce a poor response to chemotherapy in gastrointestinal cancers, including colorectal carcinoma (CRC), esophageal cancer (ESOC), and Gastric carcinoma.[325,326,327] CAF can also mediate drug resistance by transcriptional and non-transcriptional mechanisms. [5,328] Hepatocyte growth factor (HGF), TGF-β and IL-6 secreted by CAFs can bind to receptors on cancer cells, leading to transcriptional modulation in favour of drug resistance.[5,328] Non-transcriptional mechanisms which can lead to cancer drug resistance include inactivation of activator of apoptosis (eg. Bim, a pro-apoptotic Bcl-2 member),[329] or increased activation of suppressor of apoptosis or cell cycle regulator (eg. P27Kip-1 associated cell cycle arrest). [330]

Metabolic coupling of energy between anabolic cancer cells and catabolic CAFs can confer multidrug resistance by increasing mitochondrial activity.[331] In a study by Taveres-Valente et al. regarding cancer cell bioenergetics and pH regulation, it was seen that in “reverse Warburg pathway” lactate released by CAFs, provide lower extracellular pH in the TME, which confers paclitaxel and doxorubicin resistance and provide a migratory medium for cancer.[332] Observing changes in TME and gene expression profiling of CAFs by Taxotere chemotherapy treatment, Rong Guohua and his team proposed that CXCR7 contributes to chemotherapy resistance in breast cancer and that, decreased expression of fibroblast growth factor -1 (FGF-1) is also involved in chemotherapy resistance.[333]

Regarding radio-resistance, in vivo and in-vitro based studies have revealed that either by direct or paracrine interactions, CAF shows inhibitory cancer radiation response. A recent study by Jason D. Domogauer et al.  revealed that CAFs are radio-resistant and experience significant changes in indices of oxidative metabolism. The fate of residual tumor cells is modulated by remaining viable CAF population.[334] A study by [Kenoki Ohuchida](https://pubmed.ncbi.nlm.nih.gov/?term=Ohuchida+K&cauthor_id=15126362) and team demonstrated that radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions, they concluded that CAFs under influence of radiotherapy treatment lead to sustained hepatocyte growth factor (HGF) secretion, however exposure of pancreatic cancer cells to supernatant from irradiated fibroblasts resulted in increased phosphorylation of c-Met (HGF receptor) and mitogen-activated protein kinase activity, facilitating the proliferation and metastasis of pancreatic cancer cells. They also demonstrated that  enhanced invasiveness of pancreatic cancer cells induced by co-culture with irradiated CAFs was largely blocked by NK4, a specific antagonist of HGF.[335] Another study confirmed raised secretion of CXCL12 by CAFs under influence of radiotherapy, which led to increased pancreatic cancer cell migrationand EMT associated drug resistance.[336]

Desmoplastic response by CAFs in addition to making cancer cells chemoresistant, also makes them radioresistant through via integrin β1 and the downstream FAK and MAPK-AKT signaling pathways in cancer cells.[337] Horsman and his team demonstrated that desmoplastic response creates a hypoxic TME. This will further lead to radioresistant property of cancer cells.[338]

**6.2.9 Anti-tumorigenic activities of CAFs**

Contrary to their predominant tumor promoting functions, CAFs can also have anti-tumor activities with respect to, tumor growth and invasion especially in early stages of malignancy.[5] Park and colleagues by observing prognostic significance of CAFs in TME of 155 surgically resected pancreatic ductal adenocarcinomas noted that low FAP+ CAF sub-population significantly correlated with reduced overall survival as compared with high FAP+  counts. Authors stated that the CAFs present in TME are not always an indicator of worse prognosis, and concluded that CAFs have a potential tumor restraining role also.[239] In a study by Özdemir et al, and another study by Rhim et al., it was noted that α-SMA expressing CAFs shows enhanced activation of Shh signalling that decreases the synthesis of CXCL-12, VEGF and IL-8, which negatively impacts the tumor growth, angiogenesis an immunosuppression. Again these authors concluded   that at least a sub-populations of the stromal compartment, including CAFs, can act to restrain tumor growth.[287,339] Chen K et al. by scRNA-seq in PDAC patients identified a new CAF sub-type, termed complement secreting CAF (csCAF) which is characterized by high expression of complement proteins such as C3 and C7 and shows immune/inflammatory response and have a potential tumor inhibitory activities especially in early stages.[340] Another subset of CAFs (CD146+ and Slit+ CAF) have been identified which has antitumoregenic properties and increases the chemosensitivity of tumor cells.[203] In breast cancer recently three subsets of tumor suppressive CAFs have been identified, termed CD146 + CAFs, CAV1high CAFs, and PDGFRα + Saa3- CAFs.[341] Mesenchymal stem cell marker, Meflin is recently discovered as an important functional activator of cell restraining CAFs ( rCAF) that inhibit the activity of cancer promoting CAFs in PDAC.[214]

Inhibition of tumor cell proliferation is seen in sarcoma and prostatic adenocarcinoma due to activation of NF-κB and YAP1/TAZ signalling because of CAF secretome TGF-β, TNF-α and IL-6.[236, 342, 343, 344] Narra k et al. stated that atleast tumor-inhibitory CAFs are present in the early stages of tumor development, driving from the fact that tumor-supportive α-SMA+ CAFs have been reported to arise from tumor-inhibitory Meflin+ cells upon PDAC progression. [345]

1. **Conclusion**

From the conceptualization of the crude “seed and soil theory” to a metamorphic picture of “CAFs in TME” we have covered a long scientific journey of carcinogenesis with multiple halts from signals by translational research. It is becoming increasingly clear that CAF has a major role in persistant “education” of cancer cells, its integrity and tumor dynamics; changing the behaviour of cancer cells from non-aggressive or indolent to a invasive and metastatic phenotype. Since the initial description of CAFs in early 1990s there have been dramatic changes in our understanding pertaining to its role in cancer progression and prognosis. With reapidly accumulating research data and advances in molecular biology we now have somewhat clear picture regarding heterogeneity and the varied multi-dimensional functions and phenotypes of CAF; although much more objectified and comprehensive functional and phenotypic classification of CAF is required. Multiple sub-populations of CAFs have been marked out, and further pathological and molecular autopsy will lead to discovery of more practical markers and functional states of CAF. This will help in a big way for combinatorial strategies targeting mutated cancer cells and not-mutated CAFs in the accompanying microenvironment that can be translated into a meaningful and clinically relevant approach for cancer treatment. This is important as it is frequently seen that cancer cells develop resistance if they are targeted by chemotherapy alone. As understanding as well as methods and techniques to classify CAFs is undergoing more refinement, a much better multipronged and “molecularly informed” therapies can be tailored, targeting both the tumor cells and the heterogenous population of CAFs. Achille’s tendon of cancer therapy failure, i.e. metastasis and chemotherapy resistance can be strengthened, if CAFs reseacrh is directed in a proper direction. One of the major cavet which have to be addressed is that CAF display temporal as well as spatial heterogeneity in terms of phenotype and function, with progression of tumor

**Alphabetical list of Abbreviations:**

**α-SMA**: alpha -smooth muscle actin

**ACTA 2**: Smooth muscle alpha (α)-2 actin

**AKT**:Ak strain transforming

**ApCAF**: Antigen presenting CAF

**CAF**-Cancer associated fibroblast

**CAV**-1: Caveolin-1

**CCL**: C–C motif chemokine ligand

**CD**: Cluster of Differentiation

**CSC:** Cancer stem cell

**CSF-1**: Colony stimulating factor-1

**CXCL-1:** c-xc motif chemokine ligand 1

**CXCL-12:** c-xc motif chemokine ligand 12

**CXCR:** c-xc motif chemokine receptor

**DNMT**: DNA methyltransferase

**ECM:** Extracellular matrix

**EC:** Endothelial cells

**ED-A FN**: Spliced domain-A fibronectin

**EMT**: Epithelial-to-mesenchymal transition

**Eph**: Erythrophoyetin-producing human hepatocellular

**EVs**: Extracellular vesicles.

**FAK**: Focal adhesion kinase

**FAP**:  Fibroblast-activated protein

**FAPα**: Fibroblast-activated protein α

**FGF-2**: Fibroblast growth factor-2

**FN-1**: Fibronectin-1

**FSP-1**: Fibroblast specific protein-1

**GDF**: Growth differentiation factor

**GLI2**: GLI family zinc finger 2

**HGF**: Hepatocyte growth factor

**HIF-1α**:Hypoxia inducible factor-1α

**iCAF**: Inflammatory CAF

**IL-6**: Interleukin 6

**IL-8**: Interleukin 8

**LIF**: leukemia inhibitory factor

**LOX**: Lyxol oxidase

**JAK-STAT pathway**: Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway

**LncRNA**: Long non-coding RNA

**LPA**: Lysophosphatidic acid LIF-

**MAP-K**: Mitogen-activated protein kinases

**MDSC**: Myeloid derived suppressive cell

**MFAP 5:** Microfibrillar associated protein 5

**miRNAs**: micro RNAs (

**mTOR**: Mammalian target of rapamycin

**myCAF**: Myofibroblastic CAF / Myofibroblast

**NF-kB** : Nuclear factor kappa-light-chain-enhancer of activated B cells

**NG2**: Neural/glial antigen 2

**NK-T cell**: Natural Killer T-cell

**PDAC**: Pancreatic ductal adenocarcinoma

**PDGF**: Platelet derived growth factor

**PDGFR**: Platelet derived growth factor receptor

**PDPN**: Podoplanin

**PI3K**: Phosphoinositide 3-kinases

**PK-M2**: Pyruvate kinase M2

**POSTN**: Periostin

**ROCK**: Rho associated protein kinase

**SCID**: Severe combined immune deficiency

**scRNA-seq**: Single cell RNA sequencing

**SDF-1**: Stromal derived factor-1

**Shh signalling**: Sonic Hedgehog signalling

**Shh/Smo signalling pathway**:  Sonic Hedgehog /seven-transmembrane protein Smoothened pathway

**SMAD pathway**:  Small mothers against decapentaplegic pathway

**TAGLN**: Transgelin

**TAM:** Tumor associated macrophages

**TIME**: Tumor immune microenvironment

**TME**: Tumor micro-environment

**TNC**: Tenacin-C

**Treg**: Regulatory T-cell

**TSE**: Tumor secreted exome

**TGF-β**: Tumor growth factor -beta

**VEGF**: Vascular endothelial growth factor

**WNT signalling**: Wingless-related integration site signalling

**YAP1/TAZ**: Yes-associated protein 1 (YAP1) and PDZ-binding motif (TAZ)

**References:**

1. Chung KPS, Leung RWH, Lee TKW. Hampering Stromal Cells in the Tumor Microenvironment as a Therapeutic Strategy to Destem Cancer Stem Cells. Cancers (Basel). 2021; 13(13):3191 doi: 10.3390/cancers13133191
2. Quail DF, Joyce JA. Micro-environmental regulation of tumor progression and metastasis. Nat Med. 2013; 19(11): 1423-37. doi: 10.1038/nm.3394
3. Yuan Y, Jiang YC, Sun CK, Chen QM. Role of the tumor microenvironment in tumor progression and the clinical applications (Review). Oncol Rep. 2016; 35(5): 2499-515. doi: 10.3892/or.2016.4660
4. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. Nat Rev Clin Oncol. 2018 Jun;15(6):366-381. doi: 10.1038/s41571-018-0007-1
5. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016; 16(9):582-598. doi: 10.1038/nrc.2016.73
6. Whiteside TL. The tumor microenvironment and its role in promoting tumor growth. Oncogene. 2008 Oct 6;27(45):5904-12. doi: 10.1038/onc.2008.271
7. Neophytou CM, Panagi M, Stylianopoulos T, Papageorgis P. The Role of Tumor Microenvironment in Cancer Metastasis: Molecular Mechanisms and Therapeutic Opportunities. Cancers (Basel). 2021 Apr 23;13(9):2053. doi: 10.3390/cancers13092053
8. Giraldo, N.A., Sanchez-Salas, R., Peske, J.D. et al. The clinical role of the TME in solid cancer. Br J Cancer **120**, 45–53 (2019). doi: 10.1038/s41416-018-0327-z
9. Akhtar M, Haider A, Rashid S, Al-Nabet ADMH. Paget's "Seed and Soil" Theory of Cancer Metastasis: An Idea Whose Time has Come. Adv Anat Pathol. 2019 Jan;26(1):69-74. doi: 10.1097/PAP.0000000000000219. PMID: 30339548
10. Langley RR, Fidler IJ. The seed and soil hypothesis revisited--the role of tumor-stroma interactions in metastasis to different organs. Int J Cancer. 2011 Jun 1;128(11):2527-35. doi: 10.1002/ijc.26031
11. Ribatti D, Mangialardi G, Vacca A. Stephen Paget and the 'seed and soil' theory of metastatic dissemination. Clin Exp Med. 2006 Dec;6(4):145-9. doi: 10.1007/s10238-006-0117-4
12. Mendoza M, Khanna C. Revisiting the seed and soil in cancer metastasis. Int J Biochem Cell Biol. 2009 Jul;41(7):1452-62. doi: 10.1016/j.biocel.2009.01.015
13. Gomes, R.N., Manuel, F. & Nascimento, D.S. The bright side of fibroblasts: molecular signature and regenerative cues in major organs. npj Regen Med. 2021; **6**: 43. doi: 10.1038/s41536-021-00153-z
14. Ping, Q., Yan, R., Cheng, X. et al. Cancer-associated fibroblasts: overview, progress, challenges, and directions. Cancer Gene Ther. 2021; **28**: 984–999. https://doi.org/10.1038/s41417-021-00318-4
15. Denton AE, Roberts EW, Fearon DT. Stromal Cells in the Tumor Microenvironment. Adv Exp Med Biol. 2018;1060:99-114. doi: 10.1007/978-3-319-78127-3\_6)( Räsänen K, Vaheri A. Activation of fibroblasts in cancer stroma. Exp Cell Res. 2010; 316(17): 2713-22. doi: 10.1016/j.yexcr.2010.04.032
16. Räsänen K, Vaheri A. Activation of fibroblasts in cancer stroma. Exp Cell Res. 2010; 316(17): 2713-22. doi: 10.1016/j.yexcr.2010.04.032
17. Zhang J, Liu J. Tumor stroma as targets for cancer therapy. Pharmacol Ther. 2013; 137(2): 200-215. doi: 10.1016/j.pharmthera.2012.10.003
18. Erdogan B, Webb DJ. Cancer-associated fibroblasts modulate growth factor signaling and extracellular matrix remodeling to regulate tumor metastasis. Biochem Soc Trans. 2017; 45(1):229-236. doi: 10.1042/BST20160387
19. Xing F, Saidou J, Watabe K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. Front Biosci. 2010; 15(1): 166-79. doi: 10.2741/3613
20. Tang D, Gao J, Wang S, Ye N, Chong Y, et al. Cancer-associated fibroblasts promote angiogenesis in gastric cancer through galectin-1 expression. Tumour Biol. 2016; 37(2): 1889-99. doi: 10.1007/s13277-015-3942-9
21. Grauel AL, Nguyen B, Ruddy D, Laszewski T, Schwartz S, et al. TGFβ-blockade uncovers stromal plasticity in tumors by revealing the existence of a subset of interferon-licensed fibroblasts. Nat Commun. 2020; 11(1): 6315. doi: 10.1038/s41467-020-19920-5
22. Karagiannis GS, Poutahidis T, Erdman SE, Kirsch R, Riddell RH, et al. Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. Mol Cancer Res. 2012; 10(11): 1403-18. doi: 10.1158/1541-7786.MCR-12-0307
23. Ziani L, Chouaib S, Thiery J. Alteration of the Antitumor Immune Response by Cancer-Associated Fibroblasts. Front Immunol. 2018; 9: 414. doi: 10.3389/fimmu.2018.00414
24. Mhaidly R, Mechta-Grigoriou F. Fibroblast heterogeneity in tumor micro-environment: Role in immunosuppression and new therapies. Semin Immunol. 2020; 48: 101417. doi: 10.1016/j.smim.2020.101417.
25. Mao X, Xu J, Wang W, Liang C, Hua J, et al. Crosstalk between cancer-associated fibroblasts and immune cells in the tumor microenvironment: new findings and future perspectives. Mol Cancer. 2021; 20(1):131. doi: 10.1186/s12943-021-01428-1
26. Barrett RL, Puré E. Cancer-associated fibroblasts and their influence on tumor immunity and immunotherapy. Elife. 2020; 9: e57243. doi: 10.7554/eLife.57243
27. Hanley CJ, Thomas GJ. Targeting cancer associated fibroblasts to enhance immunotherapy: emerging strategies and future perspectives. Oncotarget. 2021; 12(14): 1427-1433. doi: 10.18632/oncotarget.27936
28. Liu, T., Han, C., Wang, S. *et al.* Cancer-associated fibroblasts: an emerging target of anti-cancer immunotherapy. *J Hematol Oncol* 2019; **12**, 86. doi: 10.1186/s13045-019-0770-1
29. Saw PE, Chen J, Song E. Targeting CAFs to overcome anticancer therapeutic resistance. Trends Cancer. 2022; 8(7): 527-555. doi: 10.1016/j.trecan.2022.03.001
30. Shah K, Mallik SB, Gupta P, Iyer A. Targeting Tumour-Associated Fibroblasts in Cancers. Front Oncol. 2022 J; 12: 908156. doi: 10.3389/fonc.2022.908156)
31. Baghban R, Roshangar L, Jahanban-Esfahlan R, Seidi K, Ebrahimi-Kalan A, et al. Tumor microenvironment complexity and therapeutic implications at a glance. Cell Commun Signal. 2020; 18(1): 59. doi: 10.1186/s12964-020-0530-4
32. Hui L, Chen Y. Tumor microenvironment: Sanctuary of the devil. Cancer Lett. 2015; 368(1): 7-13. doi: 10.1016/j.canlet.2015.07.039
33. Fouad YA, Aanei C. Revisiting the hallmarks of cancer. Am J Cancer Res. 2017; 7(5): 1016-1036
34. De P, Aske J, Dey N. Cancer-Associated Fibroblast Functions as a Road-Block in Cancer Therapy. Cancers (Basel). 202; 13(20): 5246. doi: 10.3390/cancers13205246
35. Kiaris H, Chatzistamou I, Kalofoutis Ch, Koutselini H, Piperi Ch, et al. Tumour-stroma interactions in carcinogenesis: basic aspects and perspectives. Mol Cell Biochem. 2004; 261(1-2):117-22. doi: 10.1023/b:mcbi.0000028746.54447.6c
36. Kobayashi H, Enomoto A, Woods SL, Burt AD, Takahashi M, Worthley DL. Cancer-associated fibroblasts in gastrointestinal cancer. Nat Rev Gastroenterol Hepatol. 2019; 16(5): 282-295. doi: 10.1038/s41575-019-0115-0
37. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. Curr Opin Cell Biol. 2010 Oct;22(5):697-706. doi: 10.1016/j.ceb.2010.08.015.)
38. Ozbek S, Balasubramanian PG, Chiquet-Ehrismann R, Tucker RP, Adams JC. The evolution of extracellular matrix. Mol Biol Cell. 2010; 21(24): 4300-5. doi: 10.1091/mbc.E10-03-0251
39. Binnewies M, Roberts EW, Kersten K, Chan V, Fearson DF *et al.* Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med* 2018; **24**: 541–550. doi: 10.1038/s41591-018-0014-x
40. Nurmik M, Ullmann P, Rodriguez F, Haan S, Letellier E. In search of definitions: Cancer-associated fibroblasts and their markers. Int J Cancer. 2020 Feb 15;146(4):895-905. doi: 10.1002/ijc.32193)
41. Hanahan D, Coussens LM. Accessories to the crime: functions of cells recruited to the tumor microenvironment. Cancer Cell. 2012 Mar 20;21(3):309-22. doi: 10.1016/j.ccr.2012.02.022)
42. Liu T, Zhou L, Li D, Andl T, Zhang Y. Cancer-Associated Fibroblasts Build and Secure the Tumor Microenvironment. Front Cell Dev Biol. 2019; 7: 60. doi: 10.3389/fcell.2019.00060.
43. Vennin C, Murphy KJ, Morton JP, Cox TR, Pajic M, Timpson P. Reshaping the Tumor Stroma for Treatment of Pancreatic Cancer. Gastroenterology. 2018 Mar;154(4):820-838. doi: 10.1053/j.gastro.2017.11.280
44. Yeo D, Phillips P, Baldwin GS, He H, Nikfarjam M. Inhibition of group 1 p21-activated kinases suppresses pancreatic stellate cell activation and increases survival of mice with pancreatic cancer. Int J Cancer. 2017 May 1;140(9):2101-2111. doi: 10.1002/ijc.30615
45. Steele CW, Karim SA, Leach JDG, Bailey P, Upstill-Goddard R, et al. CXCR2 Inhibition Profoundly Suppresses Metastases and Augments Immunotherapy in Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2016; 29(6): 832-845. doi: 10.1016/j.ccell.2016.04.014
46. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989; 8(2): 98-101
47. Seyfried TN, Huysentruyt LC. On the origin of cancer metastasis. Crit Rev Oncog. 2013;18(1-2):43-73. doi: 10.1615/critrevoncog.v18.i1-2.40
48. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer. 2003 Jun;3(6):453-8. doi: 10.1038/nrc1098
49. Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS, Pujari VB. Inflammation and cancer. Ann Afr Med. 2019 Jul-Sep;18(3):121-126. doi: 10.4103/aam.aam\_56\_18
50. Zhao, H., Wu, L., Yan, G, Chen U, Zhou M *et al.* Inflammation and tumor progression: signaling pathways and targeted intervention. *Sig Transduct Target Ther* 2021; **6**: 263.
51. Belhabib I, Zaghdoudi S, Lac C, Bousquet C, Jean C. Extracellular Matrices and Cancer-Associated Fibroblasts: Targets for Cancer Diagnosis and Therapy? Cancers (Basel). 2021 Jul 11;13(14):3466. doi: 10.3390/cancers13143466
52. Breathnach CS. Rudolf Virchow (1821-1902) and Die Cellularpathologie (1858). J Ir Coll Physicians Surg. 2002; 31(1): 43-6. PMID: 11908520
53. Plikus MV, Wang X, Sinha S, Forte E, Thompson SM, et al. Fibroblasts: Origins, definitions, and functions in health and disease. Cell. 2021 Jul 22;184(15):3852-3872. doi: 10.1016/j.cell.2021.06.024
54. LeBleu VS, Neilson EG. Origin and functional heterogeneity of fibroblasts. FASEB J. 2020; 34(3):3519-3536. doi: 10.1096/fj.201903188R
55. Lendahl, U., Muhl, L. & Betsholtz, C. Identification, discrimination and heterogeneity of fibroblasts. *Nat Commun. 2022;*  **13**: 3409. https://doi.org/10.1038/s41467-022-30633-9
56. Gabbiani G, Ryan GB, Majne G. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia. 1971; 27(5): 549-50. doi: 10.1007/BF02147594
57. Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, et al. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. J Cell Biol. 1998 Aug 10;142(3):873-81. doi: 10.1083/jcb.142.3.873
58. Santi A, Kugeratski FG, Zanivan S. Cancer Associated Fibroblasts: The Architects of Stroma Remodeling. Proteomics. 2018; 18(5-6): e1700167. doi: 10.1002/pmic.201700167
59. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009; 1(6):a001651. doi: 10.1101/cshperspect.a001651
60. Frolik CA, Dart LL, Meyers CA, Smith DM, Sporn MB. Purification and initial characterization of a type beta transforming growth factor from human placenta. Proc Natl Acad Sci U S A. 1983; 80(12):3676-80. doi: 10.1073/pnas.80.12.3676
61. Wilson MS, Wynn TA. Pulmonary fibrosis: pathogenesis, etiology and regulation. Mucosal Immunol. 2009; 2(2): 103-21. doi: 10.1038/mi.2008.85
62. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. Mayo Clin Proc. 2019; 94(1): 155-165. doi: 10.1016/j.mayocp.2018.09.013
63. Croft AP, Campos J, Jansen K, Turner JD, Marshall J, et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. Nature. 2019; 570(7760): 246-251. doi: 10.1038/s41586-019-1263-7
64. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020; 20(3):174-186. doi: 10.1038/s41568-019-0238-1
65. Stock K, Estrada M, Vidic S. et al. Capturing tumor complexity in vitro: Comparative analysis of 2D and 3D tumor models for drug discovery. Sci Rep **6**, 28951 (2016). doi: 10.1038/srep28951
66. Yeo SY, Ha SY, Lee KW, Cui Y, Yang ZT, et al. Twist1 is highly expressed in cancer-associated fibroblasts of esophageal squamous cell carcinoma with a prognostic significance. Oncotarget. 2017; 8(39): 65265-65280. doi: 10.18632/oncotarget.17941
67. Strutz F, Okada H, Lo CW, Danoff T, Carone RL, et al. Identification and characterization of a fibroblast marker: FSP1. J Cell Biol. 1995; 130(2): 393-405. doi: 10.1083/jcb.130.2.393
68. Österreicher CH, Penz-Österreicher M, Grivennikov SI, Guma M, Koltsova EK, et al. Fibroblast-specific protein 1 identifies an inflammatory subpopulation of macrophages in the liver. Proc Natl Acad Sci U S A. 2011; 108(1): 308-313. doi: 10.1073/pnas.1017547108
69. Desmoulière A, Guyot C, Gabbiani G. The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. Int J Dev Biol. 2004;48(5-6):509-17. doi: 10.1387/ijdb.041802ad
70. Garin-Chesa P, Old LJ, Rettig WJ. Cell surface glycoprotein of reactive stromal fibroblasts as a potential antibody target in human epithelial cancers. Proc Natl Acad Sci U S A. 1990 Sep;87(18):7235-9. doi: 10.1073/pnas.87.18.7235
71. Kendall RT, Feghali-Bostwick CA. Fibroblasts in fibrosis: novel roles and mediators. Front Pharmacol. 2014; 5:123. doi: 10.3389/fphar.2014.00123
72. Driskell RR, Watt FM. Understanding fibroblast heterogeneity in the skin. Trends Cell Biol. 2015; 25(2):92-9. doi: 10.1016/j.tcb.2014.10.001
73. Rinkevich Y, Walmsley GG, Hu MS, Maan ZN, Newman AM, et al. Skin fibrosis. Identification and isolation of a dermal lineage with intrinsic fibrogenic potential. Science. 2015; 348(6232): aaa2151. doi: 10.1126/science.aaa2151
74. Manoukian P, Bijlsma M, van Laarhoven H. The Cellular Origins of Cancer-Associated Fibroblasts and Their Opposing Contributions to Pancreatic Cancer Growth. Front Cell Dev Biol. 2021 Sep 27;9:743907. doi: 10.3389/fcell.2021.743907
75. Morsing M, Klitgaard MC, Jafari A, Villadsen R, Kassem M, et al. Evidence of two distinct functionally specialized fibroblast lineages in breast stroma. Breast Cancer Res. 2016 Nov 3;18(1):108. doi: 10.1186/s13058-016-0769-2
76. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007 Dec;9(12):1392-400. doi: 10.1038/ncb1658
77. Goetz JG, Minguet S, Navarro-Lérida I, Lazcano JJ, Samaniego R, et al. Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis. Cell. 2011 Jul 8;146(1):148-63. doi: 10.1016/j.cell.2011.05.040
78. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009 Nov 25;139(5):891-906. doi: 10.1016/j.cell.2009.10.027
79. Bechtel W, McGoohan S, Zeisberg EM, Müller GA, Kalbacher H, et al. Methylation determines fibroblast activation and fibrogenesis in the kidney. Nat Med. 2010 May;16(5):544-50. doi: 10.1038/nm.2135
80. Olivares O, Mayers JR, Gouirand V, Torrence ME, Gicquel T, et al. Collagen-derived proline promotes pancreatic ductal adenocarcinoma cell survival under nutrient limited conditions. Nat Commun. 2017 Jul 7;8:16031. doi: 10.1038/ncomms16031
81. Bauer M, Su G, Casper C, He R, Rehrauer W, Friedl A. Heterogeneity of gene expression in stromal fibroblasts of human breast carcinomas and normal breast. Oncogene. 2010 Mar 25;29(12):1732-40. doi: 10.1038/onc.2009.463.
82. Pidsley R, Lawrence MG, Zotenko E, Niranjan B, Statham A, et al. Enduring epigenetic landmarks define the cancer microenvironment. Genome Res. 2018 May;28(5):625-638. doi: 10.1101/gr.229070.117.
83. Schäfer M, Werner S. Cancer as an overhealing wound: an old hypothesis revisited. Nat Rev Mol Cell Biol. 2008 Aug;9(8):628-38. doi: 10.1038/nrm2455.
84. Qiu W, Hu M, Sridhar A, Opeskin K, Fox S, et al. No evidence of clonal somatic genetic alterations in cancer-associated fibroblasts from human breast and ovarian carcinomas. Nat Genet. 2008 May;40(5):650-5. doi: 10.1038/ng.117
85. Walter K. N., Omura S.M., Hong M. G., and Goggins M. 2008. Pancreatic cancer associated fibroblasts display normal allelotypes. Cancer Biol. Ther. 7:882–888. doi: 10.4161/cbt.7.6.5869
86. Hosein AN, Wu M, Arcand SL, Lavallée S, Hébert J, et al. Breast carcinoma-associated fibroblasts rarely contain p53 mutations or chromosomal aberrations. Cancer Res. 2010 Jul 15;70(14):5770-7. doi: 10.1158/0008-5472.CAN-10-0673
87. Dvorak H. F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N. Engl. J. Med. 315 1650–1659. doi: 10.1056/NEJM198612253152606
88. Foster DS, Jones RE, Ransom RC, Longaker MT, Norton JA. The evolving relationship of wound healing and tumor stroma. JCI Insight. 2018 Sep 20;3(18):e99911. doi: 10.1172/jci.insight.99911
89. Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H. Cancer-Associated Fibroblasts: Their Characteristics and Their Roles in Tumor Growth. Cancers (Basel). 2015 Dec 11;7(4):2443-58. doi: 10.3390/cancers7040902.
90. Öhlund D, Handly-Santana A, Biffi G, Elyada E, Almeida AS, et al. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J Exp Med. 2017 Mar 6;214(3):579-596. doi: 10.1084/jem.20162024
91. Maman S, Witz IP. A history of exploring cancer in context. Nat Rev Cancer. 2018 Jun;18(6):359-376. doi: 10.1038/s41568-018-0006-7.)
92. Rognoni E, Pisco AO, Hiratsuka T, Sipilä KH, Belmonte JM, et al. Fibroblast state switching orchestrates dermal maturation and wound healing. Mol Syst Biol. 2018 Aug 29;14(8):e8174. doi: 10.15252/msb.20178174
93. Madar S., Goldstein I. and Rotter V. (2013). ‘Cancer associated fibroblasts’--more than meets the eye. 19, 447-453. doi: 10.1016/j.molmed.2013.05.004
94. Jung Y, Kim JK, Shiozawa Y, Wang J, Mishra A, et al. Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. Nat Commun. 2013;4:1795. doi: 10.1038/ncomms2766
95. Fukino K, Shen L, Matsumoto S, Morrison CD, Mutter GL, Eng C. Combined total genome loss of heterozygosity scan of breast cancer stroma and epithelium reveals multiplicity of stromal targets. Cancer Res. 2004; 64(20): 7231-6. doi: 10.1158/0008-5472.CAN-04-2866
96. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, et al. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. Cancer Cell. 2011 Feb 15;19(2):257-72. doi: 10.1016/j.ccr.2011.01.020
97. Worthley DL, Si Y, Quante M, Churchill M, Mukherjee S, Wang TC. Bone marrow cells as precursors of the tumor stroma. Exp Cell Res. 2013; 319(11): 1650-1656. doi: 10.1016/j.yexcr.2013.03.006
98. Ishii G, Sangai T, Oda T, Aoyagi Y, Hasebe T, Kanomata N, Endoh Y, Okumura C, Okuhara Y, Magae J, Emura M, Ochiya T, Ochiai A. Bone-marrow-derived myofibroblasts contribute to the cancer-induced stromal reaction. Biochem Biophys Res Commun. 2003 Sep 12;309(1):232-40. doi: 10.1016/s0006-291x(03)01544-4
99. McDonald LT, Russell DL, Kelly RR, Xiong Y, Motamarry A, et al. Hematopoietic stem cell-derived cancer-associated fibroblasts are novel contributors to the pro-tumorigenic microenvironment. Neoplasia. 2015 May;17(5):434-48. doi: 10.1016/j.neo.2015.04.004
100. Kidd S, Spaeth E, Watson K, Burks J, Lu H, et al. Origins of the tumor microenvironment: quantitative assessment of adipose-derived and bone marrow-derived stroma. PLoS One. 2012; 7(2): e30563. doi: 10.1371/journal.pone.0030563
101. Rynne-Vidal A, Jiménez-Heffernan JA, Fernández-Chacón C, López-Cabrera M, Sandoval P. The Mesothelial Origin of Carcinoma Associated-Fibroblasts in Peritoneal Metastasis. Cancers (Basel). 2015; 7(4): 1994-2011. doi: 10.3390/cancers7040872
102. Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res. 2007; 67(21): 10123-10128. doi: 10.1158/0008-5472.CAN-07-3127
103. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med. 2007 Aug; 13(8): 952-961. doi: 10.1038/nm1613
104. Ino K, Masuya M, Tawara I, Miyata E, Oda K, Nakamori Y, Suzuki K, Ohishi K, Katayama N. Monocytes infiltrate the pancreas via the MCP-1/CCR2 pathway and differentiate into stellate cells. PLoS One. 2014; 9(1): e84889. doi: 10.1371/journal.pone.0084889
105. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. Nat Rev Immunol. 2011 ; 11(6): 427-35. doi: 10.1038/nri2990
106. Hosaka K, Yang Y, Seki T, Fischer C, Dubey O, et al. Pericyte-fibroblast transition promotes tumor growth and metastasis. Proc Natl Acad Sci U S A. 2016 Sep 20; 113(38):E5618-27. doi: 10.1073/pnas.1608384113
107. McAnulty RJ. Fibroblasts and myofibroblasts: their source, function and role in disease. Int J Biochem Cell Biol. 2007; 39(4): 666-71. doi: 10.1016/j.biocel.2006.11.005
108. Iwano M, Plieth D, Danoff TM, Xue C, Okada H et al. Evidence that fibroblasts derive from epithelium during tissue fibrosis. J Clin Invest. 2002; 110(3): 341-350. doi: 10.1172/JCI15518
109. Petersen OW, Lind Nielsen H, Gudjonsson T, Villadsen R, Rønnov-Jessen L, Bissell MJ. The plasticity of human breast carcinoma cells is more than epithelial to mesenchymal conversion. Breast Cancer Res. 2001; 3(4): 213-217. doi: 10.1186/bcr298
110. Petersen OW, Nielsen HL, Gudjonsson T, Villadsen R, Rank F, Niebuhr E, Bissell MJ, Rønnov-Jessen L. Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am J Pathol. 2003; 162(2): 391-402. doi: 10.1016/S0002-9440(10)63834-5
111. Kikuchi Y, Kashima TG, Nishiyama T, Shimazu K, Morishita Y, et al. Periostin is expressed in pericryptal fibroblasts and cancer-associated fibroblasts in the colon. J Histochem Cytochem. 2008 Aug;56(8):753-64. doi: 10.1369/jhc.2008.951061
112. Kurashige, M., Kohara, M., Ohshima, K. *et al.* Origin of cancer-associated fibroblasts and tumor-associated macrophages in humans after sex-mismatched bone marrow transplantation. *Commun Biol* **1**, 131 (2018). doi: 10.1038/s42003-018-0137-0
113. Huang H, Wang Z, Zhang Y, Pradhan RN, Ganguly D et al. Mesothelial cell-derived antigen-presenting cancer-associated fibroblasts induce expansion of regulatory T cells in pancreatic cancer. Cancer Cell. 2022; 40(6):656-673.e7. doi: 10.1016/j.ccell.2022.04.011
114. Li Y, Wang J, Asahina K. Mesothelial cells give rise to hepatic stellate cells and myofibroblasts via mesothelial-mesenchymal transition in liver injury. Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):2324-9. doi: 10.1073/pnas.1214136110
115. Baslan T, Hicks J. Unravelling biology and shifting paradigms in cancer with single-cell sequencing. Nat Rev Cancer. 2017 Aug 24;17(9):557-569. doi: 10.1038/nrc.2017.58
116. Neesse A, Michl P, Frese KK, Feig C, Cook N, et al. Stromal biology and therapy in pancreatic cancer. Gut. 2011 Jun;60(6):861-8. doi: 10.1136/gut.2010.226092
117. Kuzet SE, Gaggioli C. Fibroblast activation in cancer: when seed fertilizes soil. Cell Tissue Res. 2016 Sep;365(3):607-19. doi: 10.1007/s00441-016-2467-x
118. Pavlides S, Whitaker-Menezes D, Castello-Cros R, Flomenberg N, Witkiewicz AK et al. The reverse Warburg effect: aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle* 2009; **8**: 3984–4001 doi: 10.4161/cc.8.23.10238
119. Karagiannis, G. S. et al. Collective migration of cancer-associated fibroblasts is enhanced by overexpression of tight junction-associated proteins claudin-11 and occludin. *Mol. Oncol.* 2014; **8**: 178–195.
120. Hayden, M. S. & Ghosh, S. Shared principles in NF-kappaB signaling. *Cell* **132**, 344–362 (2008).
121. Perkins, N. D. & Gilmore, T. D. Good cop, bad cop: the different faces of NF-kappaB. *Cell Death Differ.* 2006; 13, 759–772.
122. Sun, S. C. Non-canonical NF-κB signaling pathway. *Cell Res*. 2011; 21: 71–85.
123. Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004; 18(16): 1926-45. doi: 10.1101/gad.1212704
124. Lien, E. C., Dibble, C. C. & Toker, A. PI3K signaling in cancer: beyond AKT. *Curr. Opin. Cell Biol.* 2017; 45: 62–71.
125. Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. Cell. 2017 Mar 9;168(6):960-976. doi: 10.1016/j.cell.2017.02.004
126. Zhou Y, Ren H, Dai B, Li J, Shang L, et al. Hepatocellular carcinoma-derived exosomal miRNA-21 contributes to tumor progression by converting hepatocyte stellate cells to cancer-associated fibroblasts. J Exp Clin Cancer Res. 2018;37(1):324. doi: 10.1186/s13046-018-0965-2
127. Ning, X., Zhang, H., Wang, C. & Song, X. Exosomes released by gastric cancer cells induce transition of pericytes into cancer-associated fibroblasts. *Med. Sci. Monit.* **24**, 2350–2359 (2018).
128. Zhang, S., Zhou, C., Zhang, D., Huang, Z. & Zhang, G. The anti-apoptotic effect on cancer-associated fibroblasts of B7-H3 molecule enhancing the cell invasion and metastasis in renal cancer. OncoTargets Ther. 2019; 12: 4119–4127. doi: 10.2147/OTT.S201121
129. Wang, Y. M., Wang, W. & Qiu, E. D. Osteosarcoma cells induce differentiation of mesenchymal stem cells into cancer-associated fibroblasts through Notch and Akt signaling pathway. *Int. J. Clin. Exp. Pathol.* 2017; **10**; 8479–8486.
130. Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta. 2010 Apr;1802(4):396-405. doi: 10.1016/j.bbadis.2009.12.009
131. Lee S, Rauch J, Kolch W. Targeting MAPK Signaling in Cancer: Mechanisms of Drug Resistance and Sensitivity. Int J Mol Sci. 2020 Feb 7;21(3):1102. doi: 10.3390/ijms21031102
132. Dror S, Sander L, Schwartz H, Sheinboim D, Barzilai A et al. Melanoma miRNA trafficking controls tumour primary niche formation. Nat Cell Biol. 2016; 18(9): 1006-1017. doi: 10.1038/ncb3399
133. Latres E, Chiaur DS, Pagano M. The human F box protein beta-Trcp associates with the Cul1/Skp1 complex and regulates the stability of beta-catenin. Oncogene. 1999 Jan 28;18(4):849-54. doi: 10.1038/sj.onc.1202653
134. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009 Jul;17(1):9-26. doi: 10.1016/j.devcel.2009.06.016
135. Gao C, Chen YG. Dishevelled: The hub of Wnt signaling. Cell Signal. 2010 May;22(5):717-27. doi: 10.1016/j.cellsig.2009.11.021
136. Kikuchi A, Yamamoto H, Sato A, Matsumoto S. New insights into the mechanism of Wnt signaling pathway activation. Int Rev Cell Mol Biol. 2011;291:21-71. doi: 10.1016/B978-0-12-386035-4.00002-1
137. Yu B, Wu K, Wang X, Zhang J, Wang L, et al. Periostin secreted by cancer-associated fibroblasts promotes cancer stemness in head and neck cancer by activating protein tyrosine kinase 7. Cell Death Dis. 2018 Oct 22;9(11):1082. doi: 10.1038/s41419-018-1116-6
138. Delgoffe GM, Murray PJ, Vignali DA. Interpreting mixed signals: the cell's cytokine conundrum. Curr Opin Immunol. 2011 Oct;23(5):632-8. doi: 10.1016/j.coi.2011.07.013
139. Ina K, Kusugami K, Kawano Y, Nishiwaki T, Wen Z, et al. Intestinal fibroblast-derived IL-10 increases survival of mucosal T cells by inhibiting growth factor deprivation- and Fas-mediated apoptosis. J Immunol. 2005 Aug 1;175(3):2000-9. doi: 10.4049/jimmunol.175.3.2000
140. Fan J, Xu G, Chang Z, Zhu L, Yao J. miR-210 transferred by lung cancer cell-derived exosomes may act as proangiogenic factor in cancer-associated fibroblasts by modulating JAK2/STAT3 pathway. Clin Sci (Lond). 2020; 134(7):807-825. doi: 10.1042/CS20200039. Erratum in: Clin Sci (Lond). 2020 Jul 17;134(13):1801-1804.
141. Teramoto K, Igarashi T, Kataoka Y, Ishida M, Hanaoka J, et al. Clinical significance of PD-L1-positive cancer-associated fibroblasts in pN0M0 non-small cell lung cancer. Lung Cancer. 2019 Nov;137:56-63. doi: 10.1016/j.lungcan.2019.09.013
142. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol. 2006 Jul; 7(7):b505-16. doi: 10.1038/nrm1962
143. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol. 2001 Feb;2(2):127-37. doi: 10.1038/35052073
144. Yang CC, Graves HK, Moya IM, Tao C, Hamaratoglu F, Gladden AB, Halder G. Differential regulation of the Hippo pathway by adherens junctions and apical-basal cell polarity modules. Proc Natl Acad Sci U S A. 2015 Feb 10;112(6):1785-90. doi: 10.1073/pnas.1420850112
145. Bao M, Xie J, and Piruska A.  3D microniches reveal the importance of cell size and shape. *Nat Commun* 2017; 8: 1962 (2017). doi:10.1038/s41467-017-02163-2
146. Kakarla M, Challa Siva Kanaka S, Dufficy MF, Gil V, Filipovich Y, et al. Ephrin B Activate Src Family Kinases in Fibroblasts Inducing Stromal Remodeling in Prostate Cancer. Cancers (Basel). 2022 May 9;14(9):2336. doi: 10.3390/cancers14092336
147. Yauch RL, Gould SE, Scales SJ, Tang T, Tian H, Ahn CP, Marshall D, Fu L, Januario T, Kallop D, Nannini-Pepe M, Kotkow K, Marsters JC, Rubin LL, de Sauvage FJ. A paracrine requirement for hedgehog signalling in cancer. Nature. 2008 Sep 18;455(7211):406-10. doi: 10.1038/nature07275.
148. Walter K, Omura N, Hong SM, Griffith M, Vincent A, Borges M, Goggins M. Overexpression of smoothened activates the sonic hedgehog signaling pathway in pancreatic cancer-associated fibroblasts. Clin Cancer Res. 2010 Mar 15;16(6):1781-9. doi: 10.1158/1078-0432.CCR-09-1913
149. Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res. 2010; 316(8):1324-31. doi: 10.1016/j.yexcr.2010.02.045
150. Tape CJ, Ling S, Dimitriadi M, McMahon KM, Worboys JD, et al. Oncogenic KRAS Regulates Tumor Cell Signaling via Stromal Reciprocation. Cell. 2016 May 5;165(4):910-20. doi: 10.1016/j.cell.2016.03.029
151. Schmid JO, Dong M, Haubeiss S, Friedel G, Bode S, et al. Cancer cells cue the p53 response of cancer-associated fibroblasts to cisplatin. Cancer Res. 2012; 72(22):5824-32. doi: 10.1158/0008-5472.CAN-12-1201
152. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, et al. Drug resistance in cancer: an overview. Cancers (Basel). 2014 Sep 5;6(3):1769-92. doi: 10.3390/cancers6031769
153. Bhagat TD, Von Ahrens D, Dawlaty M, Zou Y, Baddour J, et al. Lactate-mediated epigenetic reprogramming regulates formation of human pancreatic cancer-associated fibroblasts. Elife. 2019 Nov 1;8:e50663. doi: 10.7554/eLife.50663
154. Albrengues J, Bertero T, Grasset E, Bonan S, Maiel M, et al. Epigenetic switch drives the conversion of fibroblasts into proinvasive cancer-associated fibroblasts. Nat Commun. 2015 Dec 15;6:10204. doi: 10.1038/ncomms10204
155. Calvo F, Ege N, Grande-Garcia A, Hooper S, Jenkins RP, et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. Nat Cell Biol. 2013 Jun;15(6):637-46. doi: 10.1038/ncb2756
156. Ao M, Brewer BM, Yang L, Franco Coronel OE, Hayward SW, et al. Stretching fibroblasts remodels fibronectin and alters cancer cell migration. Sci Rep. 2015 Feb 9;5:8334. doi: 10.1038/srep08334
157. Foster CT, Gualdrini F, Treisman R. Mutual dependence of the MRTF-SRF and YAP-TEAD pathways in cancer-associated fibroblasts is indirect and mediated by cytoskeletal dynamics. Genes Dev. 2017 Dec 1;31(23-24):2361-2375. doi: 10.1101/gad.304501.117
158. Cui Y, Hameed FM, Yang B, Lee K, Pan CQ, et al. Cyclic stretching of soft substrates induces spreading and growth. Nat Commun. 2015 Feb 23;6:6333. doi: 10.1038/ncomms7333
159. Chan JS, Tan MJ, Sng MK, et al. Cancer-associated fibroblasts enact field cancerization by promoting extratumoral oxidative stress. Cell Death & Disease. 2017 Jan;8(1):e2562. DOI: 10.1038/cddis.2016.492. PMID: 28102840; PMCID: PMC5386391
160. Kugeratski FG, Atkinson SJ, Neilson LJ, Lilla S, Knight JRP, et al. Hypoxic cancer-associated fibroblasts increase NCBP2-AS2/HIAR to promote endothelial sprouting through enhanced VEGF signaling. Sci Signal. 2019 Feb 5;12(567):eaan8247. doi: 10.1126/scisignal.aan8247
161. Zhang Z, Gao Z, Rajthala S, Sapkota D, Dongre H, et al Metabolic reprogramming of normal oral fibroblasts correlated with increased glycolytic metabolism of oral squamous cell carcinoma and precedes their activation into carcinoma associated fibroblasts. Cell Mol Life Sci. 2020 Mar;77(6):1115-1133. doi: 10.1007/s00018-019-03209-y
162. Yoshida GJ. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. J Exp Clin Cancer Res. 2015 Oct 6;34:111. doi: 10.1186/s13046-015-0221-y)
163. Radhakrishnan R, Ha JH, Jayaraman M, Liu J, Moxley KM, et al. Ovarian cancer cell-derived lysophosphatidic acid induces glycolytic shift and cancer-associated fibroblast-phenotype in normal and peritumoral fibroblasts. Cancer Lett. 2019 Feb 1;442:464-474. doi: 10.1016/j.canlet.2018.11.023
164. Erez N, Truitt M, Olson P, Arron ST, Hanahan D. Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-kappaB-Dependent Manner. Cancer Cell. 2010 Feb 17;17(2):135-47. doi: 10.1016/j.ccr.2009.12.041
165. Sanz-Moreno V, Gaggioli C, Yeo M, Albrengues J, Wallberg F, et al. ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. Cancer Cell. 2011 Aug 16;20(2):229-45. doi: 10.1016/j.ccr.2011.06.018
166. Laha D, Grant R, Mishra P, Nilubol N. The Role of Tumor Necrosis Factor in Manipulating the Immunological Response of Tumor Microenvironment. Front Immunol. 2021 Apr 27;12:656908. doi: 10.3389/fimmu.2021.656908
167. De Wever O, Nguyen QD, Van Hoorde L, Bracke M, Bruyneel E, et al. Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. FASEB J. 2004 Jun;18(9):1016-8. doi: 10.1096/fj.03-1110fje
168. Kalluri R, LeBleu VS. The biology**,** function**,** and biomedical applications of exosomes. Science. 2020 Feb 7;367(6478):eaau6977. doi: 10.1126/science.aau6977
169. Hofmann L, Ludwig S, Vahl JM, Brunner C, Hoffmann TK, Theodoraki MN. The Emerging Role of Exosomes in Diagnosis, Prognosis, and Therapy in Head and Neck Cancer. Int J Mol Sci. 2020 Jun 6;21(11):4072. doi: 10.3390/ijms21114072
170. Ringuette Goulet C, Bernard G, Tremblay S, Chabaud S, Bolduc S,et al. Exosomes Induce Fibroblast Differentiation into Cancer-Associated Fibroblasts through TGFβ Signaling. Mol Cancer Res. 2018 Jul;16(7):1196-1204. doi: 10.1158/1541-7786.MCR-17-0784)
171. Hu T, Hu J. Melanoma-derived exosomes induce reprogramming fibroblasts into cancer-associated fibroblasts via Gm26809 delivery. Cell Cycle. 2019; 18(22): 3085-3094. doi: 10.1080/15384101.2019.1669380
172. Shelton M, Anene CA, Nsengimana J, Roberts W, Newton-Bishop J, et al. The role of CAF derived exosomal microRNAs in the tumour microenvironment of melanoma. Biochim Biophys Acta Rev Cancer. 2021 Jan;1875(1):188456. doi: 10.1016/j.bbcan.2020.188456.
173. Procopio MG, Laszlo C, Al Labban D, Kim DE, Bordignon P, et al. Combined CSL and p53 downregulation promotes cancer-associated fibroblast activation. Nat Cell Biol. 2015 Sep;17(9):1193-204. doi: 10.1038/ncb3228.
174. Qureshi-Baig K, Ullmann P, Haan S, Letellier E. Tumor-Initiating Cells: a criTICal review of isolation approaches and new challenges in targeting strategies. Mol Cancer. 2017 Feb 16;16(1):40. doi: 10.1186/s12943-017-0602-2
175. Sun G, Li Z, Rong D, Zhang H, Shi X, et al. Single-cell RNA sequencing in cancer: Applications, advances, and emerging challenges. Mol Ther Oncolytics. 2021 May 8;21:183-206. doi: 10.1016/j.omto.2021.04.001
176. Allinen M, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J et al. Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell. 2004 Jul;6(1):17-32. doi: 10.1016/j.ccr.2004.06.010
177. Neuzillet C, Tijeras-Raballand A, Ragulan C, Cros J, Patil Y, et al. Inter- and intra-tumoural heterogeneity in cancer-associated fibroblasts of human pancreatic ductal adenocarcinoma. J Pathol. 2019 May;248(1):51-65. doi: 10.1002/path.5224
178. Bartoschek M., Oskolkov N., Bocci M., Lövrot J., Larsson C et al. Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. *Nat. Commun.*2018;9:1–13. doi: 10.1038/s41467-018-07582-3.
179. Biffi G, Oni TE, Spielman B, Hao Y, Elyada E, Park Y, Preall J, Tuveson DA. IL1-Induced JAK/STAT Signaling Is Antagonized by TGFβ to Shape CAF Heterogeneity in Pancreatic Ductal Adenocarcinoma. Cancer Discov. 2019 Feb;9(2):282-301. doi: 10.1158/2159-8290.CD-18-07
180. Xiao Q, Zhou D, Rucki AA, Williams J, Zhou Jet al. Cancer-Associated Fibroblasts in Pancreatic Cancer Are Reprogrammed by Tumor-Induced Alterations in Genomic DNA Methylation. Cancer Res. 2016 Sep 15;76(18):5395-404. doi: 10.1158/0008-5472.CAN-15-3264
181. Bernard V, Semaan A, Huang J, San Lucas FA, Mulu FC, et al. Single-Cell Transcriptomics of Pancreatic Cancer Precursors Demonstrates Epithelial and Microenvironmental Heterogeneity as an Early Event in Neoplastic Progression. Clin Cancer Res. 2019; 25(7): 2194-2205. doi: 10.1158/1078-0432.CCR-18-1955
182. Hosein AN, Huang H, Wang Z, Parmar K, Du W, et al. Cellular heterogeneity during mouse pancreatic ductal adenocarcinoma progression at single-cell resolution. JCI Insight. 2019; 5(16): e129212. doi: 10.1172/jci.insight.129212
183. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, et al. Cross-Species Single-Cell Analysis of Pancreatic Ductal Adenocarcinoma Reveals Antigen-Presenting Cancer-Associated Fibroblasts. Cancer Discov. 2019 Aug;9(8):1102-1123. doi: 10.1158/2159-8290.CD-19-0094
184. Friedman, G., Levi-Galibov, O., David, E., Bornstein C, Giladi A *et al.* Cancer-associated fibroblast compositions change with breast cancer progression linking the ratio of S100A4+ and PDPN+ CAFs to clinical outcome. *Nat Cancer.* 2020; 1: 692–708. doi: 10.1038/s43018-020-0082-y
185. Biffi G, Tuveson DA. Diversity and Biology of Cancer-Associated Fibroblasts. Physiol Rev. 2021 Jan 1;101(1):147-176. doi: 10.1152/physrev.00048.2019.
186. El Agha E, Moiseenko A, Kheirollahi V, De Langhe S, Crnkovic S, et al. Two-Way Conversion between Lipogenic and Myogenic Fibroblastic Phenotypes Marks the Progression and Resolution of Lung Fibrosis. Cell Stem Cell. 2017; 20(2): 261-273.e3. doi: 10.1016/j.stem.2016.10.004
187. Salzer MC, Lafzi A, Berenguer-Llergo A, Youssif C, Castellanos A, et al. Identity Noise and Adipogenic Traits Characterize Dermal Fibroblast Aging. Cell. 2018; 175(6): 1575-1590.e22. doi: 10.1016/j.cell.2018.10.012.
188. Su G, Sung KE, Beebe DJ, Friedl A. Functional screen of paracrine signals in breast carcinoma fibroblasts. PLoS One. 2012; 7(10): e46685. doi: 10.1371/journal.pone.0046685
189. Sudhakar A. History of Cancer, Ancient and Modern Treatment Methods. J Cancer Sci Ther. 2009 Dec 1;1(2):1-4. doi: 10.4172/1948-5956.100000e2
190. Karpozilos A, Pavlidis N. The treatment of cancer in Greek antiquity. Eur J Cancer. 2004; 40(14): 2033-40. doi: 10.1016/j.ejca.2004.04.036
191. de Groot AE, Roy S, Brown JS, Pienta KJ, Amend SR. Revisiting Seed and Soil: Examining the Primary Tumor and Cancer Cell Foraging in Metastasis. Mol Cancer Res. 2017 Apr;15(4):361-370. doi: 10.1158/1541-7786.MCR-16-0436
192. Hawinkels LJ, Paauwe M, Verspaget HW, Wiercinska E, van der Zon JM, et al. Interaction with colon cancer cells hyperactivates TGF-β signaling in cancer-associated fibroblasts. Oncogene. 2014 Jan 2;33(1):97-107. doi: 10.1038/onc.2012.536.
193. Wendling O, Bornert JM, Chambon P, Metzger D. Efficient temporally-controlled targeted mutagenesis in smooth muscle cells of the adult mouse. Genesis. 2009 Jan;47(1):14-8. doi: 10.1002/dvg.20448. PMID: 18942088
194. Lazard D, Sastre X, Frid MG, Glukhova MA, Thiery JP, Koteliansky VE. Expression of smooth muscle-specific proteins in myoepithelium and stromal myofibroblasts of normal and malignant human breast tissue. Proc Natl Acad Sci U S A. 1993; 90(3): 999-1003. doi: 10.1073/pnas.90.3.999.
195. Huber MA, Kraut N, Park JE, Schubert RD, Rettig WJ, et al. Fibroblast activation protein: differential expression and serine protease activity in reactive stromal fibroblasts of melanocytic skin tumors. J Invest Dermatol. 2003; 120(2): 182-8. doi: 10.1046/j.1523-1747.2003.12035.x.
196. Roberts EW, Deonarine A, Jones JO, Denton AE, Feig C, et al. Depletion of stromal cells expressing fibroblast activation protein-α from skeletal muscle and bone marrow results in cachexia and anemia. J Exp Med. 2013; 210(6): 1137-51. doi: 10.1084/jem.20122344
197. Kahounová Z, Kurfürstová D, Bouchal J, Kharaishvili G, Navrátil J, et al. The fibroblast surface markers FAP, anti-fibroblast, and FSP are expressed by cells of epithelial origin and may be altered during epithelial-to-mesenchymal transition. Cytometry A. 2018; 93(9): 941-951. doi: 10.1002/cyto.a.23101
198. Latif N, Sarathchandra P, Chester AH, Yacoub MH. Expression of smooth muscle cell markers and co-activators in calcified aortic valves. Eur Heart J. 2015; 36(21): 1335-45. doi: 10.1093/eurheartj/eht547
199. Madsen CD, Pedersen JT, Venning FA, Singh LB, Moeendarbary E, et al. Hypoxia and loss of PHD2 inactivate stromal fibroblasts to decrease tumour stiffness and metastasis. EMBO Rep. 2015 Oct; 16(10): 1394-408. doi: 10.15252/embr.201540107
200. Yeung TL, Leung CS, Mok SC. CAF reprogramming inhibits ovarian cancer progression. Cell Cycle. 2014;13(24):3783-4. doi: 10.4161/15384101.2014.988106.
201. Vázquez-Villa F, García-Ocaña M, Galván JA, García-Martínez J, García-Pravia C, et al. COL11A1/(pro)collagen 11A1 expression is a remarkable biomarker of human invasive carcinoma-associated stromal cells and carcinoma progression. Tumour Biol. 2015 Apr;36(4):2213-22. doi: 10.1007/s13277-015-3295-4
202. Colangelo T, Polcaro G, Muccillo L, D'Agostino G, Rosato V, et al. Friend or foe? The tumour microenvironment dilemma in colorectal cancer. Biochim Biophys Acta Rev Cancer. 2017 Jan;1867(1):1-18. doi: 10.1016/j.bbcan.2016.11.001
203. Mhaidly R, Mechta-Grigoriou F. Role of cancer-associated fibroblast subpopulations in immune infiltration, as a new means of treatment in cancer. Immunol Rev. 2021 Jul;302(1):259-272. doi: 10.1111/imr.12978
204. Louault K, Li RR, DeClerck YA. Cancer-Associated Fibroblasts: Understanding Their Heterogeneity. Cancers (Basel). 2020 Oct 24;12(11):3108. doi: 10.3390/cancers12113108
205. Sandberg TP, Stuart MPME, Oosting J, Tollenaar RAEM, Sier CFM et al. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. BMC Cancer. 2019 Mar 29;19(1):284. doi: 10.1186/s12885-019-5462-2
206. Maia A, Wiemann S. Cancer-Associated Fibroblasts: Implications for Cancer Therapy. Cancers (Basel). 2021 Jul 14;13(14):3526. doi: 10.3390/cancers13143526
207. Zheng GX, Terry JM, Belgrader P, Ryvkin P, Bent ZW, et al. Massively parallel digital transcriptional profiling of single cells. Nat Commun. 2017 Jan 16;8:14049. doi: 10.1038/ncomms14049
208. Kong W, Biddy BA, Kamimoto K, Amrute JM, Butka EG, Morris SA. Cell Tagging: combinatorial indexing to simultaneously map lineage and identity at single-cell resolution. Nat Protoc. 2020; 15(3): 750-772. doi: 10.1038/s41596-019-0247-2)
209. Sebastian A, Hum NR, Martin KA, Gilmore SF, Peran I, et al. Single-Cell Transcriptomic Analysis of Tumor-Derived Fibroblasts and Normal Tissue-Resident Fibroblasts Reveals Fibroblast Heterogeneity in Breast Cancer. Cancers (Basel). 2020; 12(5): 1307. doi: 10.3390/cancers12051307
210. Das S, Shapiro B, Vucic EA, Vogt S, Bar-Sagi D. Tumor Cell-Derived IL1β Promotes Desmoplasia and Immune Suppression in Pancreatic Cancer. Cancer Res. 2020 Mar 1;80(5):1088-1101. doi: 10.1158/0008-5472.CAN-19-2080
211. Chen Z, Zhou L, Liu L, Hou Y, Xiong M, et al. Single-cell RNA sequencing highlights the role of inflammatory cancer-associated fibroblasts in bladder urothelial carcinoma. Nat Commun. 2020 Oct 8;11(1):5077. doi: 10.1038/s41467-020-18916-5
212. Dominguez CX, Müller S, Keerthivasan S, Koeppen H, Hung J, et al. Single-Cell RNA Sequencing Reveals Stromal Evolution into LRRC15+ Myofibroblasts as a Determinant of Patient Response to Cancer Immunotherapy. Cancer Discov. 2020 Feb;10(2):232-253. doi: 10.1158/2159-8290.CD-19-0644
213. Mizutani Y., Kobayashi H., Iida T., Asai N., Masamune A., et al. Meflin-Positive Cancer-Associated Fibroblasts Inhibit Pancreatic Carcinogenesis. Cancer Res. 2019;79:5367–5381. doi: 10.1158/0008-5472.CAN-19-0454
214. Miyai Y, Esaki N, Takahashi M, Enomoto A. Cancer-associated fibroblasts that restrain cancer progression: Hypotheses and perspectives. Cancer Sci. 2020 Apr;111(4):1047-1057. doi: 10.1111/cas.14346
215. Costa A, Kieffer Y, Scholer-Dahirel A, Pelon F, Bourachot B, et al. Fibroblast Heterogeneity and Immunosuppressive Environment in Human Breast Cancer. Cancer Cell. 2018 Mar 12;33(3):463-479.e10. doi: 10.1016/j.ccell.2018.01.011
216. Pelon F, Bourachot B, Kieffer Y, Magagna I, Mermet-Meillon F, ET AL. Cancer-associated fibroblast heterogeneity in axillary lymph nodes drives metastases in breast cancer through complementary mechanisms. Nat Commun. 2020 Jan 21;11(1):404. doi: 10.1038/s41467-019-14134-w
217. Givel AM, Kieffer Y, Scholer-Dahirel A, Sirven P, Cardon M, et al. miR200-regulated CXCL12β promotes fibroblast heterogeneity and immunosuppression in ovarian cancers. Nat Commun. 2018 Mar 13;9(1):1056. doi: 10.1038/s41467-018-03348-z
218. Kieffer Y, Hocine HR, Gentric G, Pelon F, Bernard C, Bourachot B et al. Single-Cell Analysis Reveals Fibroblast Clusters Linked to Immunotherapy Resistance in Cancer. Cancer Discov. 2020; 10(9): 1330-1351. doi: 10.1158/2159-8290.CD-19-1384
219. Su S, Chen J, Yao H, Liu J, Yu Set al. CD10+GPR77+ Cancer-Associated Fibroblasts Promote Cancer Formation and Chemoresistance by Sustaining Cancer Stemness. Cell. 2018; 172(4): 841-856.e16. doi: 10.1016/j.cell.2018.01.009
220. Li H, Courtois ET, Sengupta D, Tan Y, Chen KH, et al. Reference component analysis of single-cell transcriptomes elucidates cellular heterogeneity in human colorectal tumors. Nat Genet. 2017; 49(5): 708-718. doi: 10.1038/ng.3818
221. Sugimoto H, Mundel TM, Kieran MW, Kalluri R. Identification of fibroblast heterogeneity in the tumor microenvironment. Cancer Biol Ther. 2006 Dec;5(12):1640-6. doi: 10.4161/cbt.5.12.3354
222. Zhang M, Yang H, Wan L, Wang Z, Wang H, et al. Single-cell transcriptomic architecture and intercellular crosstalk of human intrahepatic cholangiocarcinoma. J Hepatol. 2020 Nov;73(5):1118-1130. doi: 10.1016/j.jhep.2020.05.039.
223. Neri S, Ishii G, Hashimoto H, Kuwata T, Nagai K, et al. Podoplanin-expressing cancer-associated fibroblasts lead and enhance the local invasion of cancer cells in lung adenocarcinoma. Int J Cancer. 2015 Aug 15;137(4):784-96. doi: 10.1002/ijc.29464
224. Jia C, Wang G, Wang T, Fu B, Zhang Y, et al. Cancer-associated Fibroblasts induce epithelial-mesenchymal transition *via* the Transglutaminase 2-dependent IL-6/IL6R/STAT3 axis in Hepatocellular Carcinoma. Int J Biol Sci. 2020 Jul 19; 16(14): 2542-2558. doi: 10.7150/ijbs.45446
225. Chen PY, Wei WF, Wu HZ, Fan LS, Wang W. Cancer-Associated Fibroblast Heterogeneity: A Factor That Cannot Be Ignored in Immune Microenvironment Remodeling. Front Immunol. 2021 Jul 8;12:671595. doi: 10.3389/fimmu.2021.671595
226. Hornburg M, Desbois M, Lu S, Guan Y, Lo AA, et al. Single-cell dissection of cellular components and interactions shaping the tumor immune phenotypes in ovarian cancer. Cancer Cell. 2021 Jul 12;39(7):928-944.e6. doi: 10.1016/j.ccell.2021.04.004
227. Affo S, Nair A, Brundu F, Ravichandra A, Bhattacharjee S, et al. Promotion of cholangiocarcinoma growth by diverse cancer-associated fibroblast subpopulations. Cancer Cell. 2021; 39(6): 866-882.e11. doi: 10.1016/j.ccell.2021.03.012
228. Zhao X, Ding L, Lu Z, Huang X, Jing Y, et al. Diminished CD68+ Cancer-Associated Fibroblast Subset Induces Regulatory T-Cell (Treg) Infiltration and Predicts Poor Prognosis of Oral Squamous Cell Carcinoma Patients. Am J Pathol. 2020; 190(4): 886-899. doi: 10.1016/j.ajpath.2019.12.007
229. Xiang H, Ramil CP, Hai J, Zhang C, Wang H, et al. Cancer-Associated Fibroblasts Promote Immunosuppression by Inducing ROS-Generating Monocytic MDSCs in Lung Squamous Cell Carcinoma. Cancer Immunol Res. 2020 Apr;8(4):436-450. doi: 10.1158/2326-6066.CIR-19-0507
230. Cheng JT, Deng YN, Yi HM, Wang GY, Fu BS, et al. Hepatic carcinoma-associated fibroblasts induce IDO-producing regulatory dendritic cells through IL-6-mediated STAT3 activation. Oncogenesis. 2016 Feb 22;5(2):e198. doi: 10.1038/oncsis.2016.7
231. Principe DR, Timbers KE, Atia LG, Koch RM, Rana A. TGFβ Signaling in the Pancreatic Tumor Microenvironment. Cancers (Basel). 2021; 13(20):5086. doi: 10.3390/cancers13205086
232. Costea DE, Hills A, Osman AH, Thurlow J, Kalna G, Huang X, et al.. Identification of two distinct carcinoma-associated fibroblast subtypes with differential tumor-promoting abilities in oral squamous cell carcinoma. *Cancer Res.* 2013; 73: 3888–901. 10.1158/0008-5472.CAN-12-4150
233. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, et al. Phenotype molding of stromal cells in the lung tumor microenvironment. Nat Med. 2018; 24(8): 1277-1289. doi: 10.1038/s41591-018-0096-5
234. Davidson S, Efremova M, Riedel A, Mahata B, Pramanik J, et al. Single-Cell RNA Sequencing Reveals a Dynamic Stromal Niche That Supports Tumor Growth. Cell Rep. 2020 May 19; 31(7): 107628. doi: 10.1016/j.celrep.2020.107628
235. Gong L, Kwong DL, Dai W, Wu P, Li S, et al. Comprehensive single-cell sequencing reveals the stromal dynamics and tumor-specific characteristics in the microenvironment of nasopharyngeal carcinoma. Nat Commun. 2021 Mar 9; 12(1):1540. doi: 10.1038/s41467-021-21795-z
236. Alkasalias T, Flaberg E, Kashuba V, Alexeyenko A, Pavlova T, et al. Inhibition of tumor cell proliferation and motility by fibroblasts is both contact and soluble factor dependent. Proc Natl Acad Sci U S A. 2014 Dec 2;111(48):17188-93. doi: 10.1073/pnas.1419554111
237. Stoker MG, Shearer M, O'Neill C. Growth inhibition of polyoma-transformed cells by contact with static normal fibroblasts. J Cell Sci. 1966 Sep;1(3):297-310. doi: 10.1242/jcs.1.3.297
238. Trimboli AJ, Cantemir-Stone CZ, Li F, Wallace JA, Merchant A, et al. Pten in stromal fibroblasts suppresses mammary epithelial tumours. Nature. 2009 Oct 22;461(7267):1084-91. doi: 10.1038/nature08486
239. Park H, Lee Y, Lee H, Kim JW, Hwang JH, et al. The prognostic significance of cancer-associated fibroblasts in pancreatic ductal adenocarcinoma. Tumour Biol. 2017 Oct;39(10):1010428317718403. doi: 10.1177/1010428317718403.
240. D’Arcangelo, E., Wu, N.C., Cadavid, J.L. *et al.* The life cycle of cancer-associated fibroblasts within the tumour stroma and its importance in disease outcome. *Br J Cancer* 2020; **122**, 931–942 doi: 10.1038/s41416-019-0705-1
241. Monteran L, Erez N. The Dark Side of Fibroblasts: Cancer-Associated Fibroblasts as Mediators of Immunosuppression in the Tumor Microenvironment. Front Immunol. 2019; 10: 1835. doi: 10.3389/fimmu.2019.01835.
242. Barnas JL, Simpson-Abelson MR, Yokota SJ, Kelleher RJ, Bankert RB. T cells and stromal fibroblasts in human tumor microenvironments represent potential therapeutic targets. Cancer Microenviron. 2010; 3(1): 29-47. doi: 10.1007/s12307-010-0044-5
243. Fukumura D, Xavier R, Sugiura T, Chen Y, Park EC, Lu N, Selig M, Nielsen G, Taksir T, Jain RK, Seed B. Tumor induction of VEGF promoter activity in stromal cells. Cell. 1998 Sep 18;94(6):715-25. doi: 10.1016/s0092-8674(00)81731-6)
244. Guo X, Oshima H, Kitmura T, Taketo MM, Oshima M. Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer. J Biol Chem. 2008 Jul 11;283(28):19864-71. doi: 10.1074/jbc.M800798200
245. Gyotoku E, Morita E, Kameyoshi Y, Hiragun T, Yamamoto S, et al. The IL-6 family cytokines, interleukin-6, interleukin-11, oncostatin M, and leukemia inhibitory factor, enhance mast cell growth through fibroblast-dependent pathway in mice. Arch Dermatol Res. 2001 Nov;293(10):508-14. doi: 10.1007/pl00007465.
246. Jacobetz MA, Chan DS, Neesse A, Bapiro TE, Cook N, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut. 2013 Jan;62(1):112-20. doi: 10.1136/gutjnl-2012-302529
247. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science. 2009 Jun 12;324(5933):1457-61. doi: 10.1126/science.1171362
248. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012 Mar 20;21(3):418-29. doi: 10.1016/j.ccr.2012.01.007
249. Chauhan VP, Martin JD, Liu H, Lacorre DA, Jain SR, et al. Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. Nat Commun. 2013;4:2516. doi: 10.1038/ncomms3516)
250. Martin JD, Seano G, Jain RK. Normalizing Function of Tumor Vessels: Progress, Opportunities, and Challenges. Annu Rev Physiol. 2019 Feb 10;81:505-534. doi: 10.1146/annurev-physiol-020518-114700
251. Stylianopoulos T, Martin JD, Chauhan VP, Jain SR, Diop-Frimpong B, et al. Causes, consequences, and remedies for growth-induced solid stress in murine and human tumors. Proc Natl Acad Sci U S A. 2012 Sep 18;109(38):15101-8. doi: 10.1073/pnas.1213353109
252. Pickup MW, Laklai H, Acerbi I, Owens P, Gorska AE, et al. Stromally derived lysyl oxidase promotes metastasis of transforming growth factor-β-deficient mouse mammary carcinomas. Cancer Res. 2013 Sep 1; 73(17): 5336-46. doi: 10.1158/0008-5472.CAN-13-001
253. Cukierman E, Bassi DE. Physico-mechanical aspects of extracellular matrix influences on tumorigenic behaviors. Semin Cancer Biol. 2010 Jun;20(3):139-45. doi: 10.1016/j.semcancer.2010.04.004
254. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 2014 Dec;15(12):1243-53. doi: 10.15252/embr.201439246
255. Shattil SJ, Kim C, Ginsberg MH. The final steps of integrin activation: the end game. Nat Rev Mol Cell Biol. 2010 Apr;11(4):288-300. doi: 10.1038/nrm2871
256. Carracedo S, Lu N, Popova SN, Jonsson R, Eckes B, Gullberg D. The fibroblast integrin alpha11beta1 is induced in a mechanosensitive manner involving activin A and regulates myofibroblast differentiation. J Biol Chem. 2010 Apr 2;285(14):10434-45. doi: 10.1074/jbc.M109.078766
257. Zhu CQ, Popova SN, Brown ER, Barsyte-Lovejoy D, Navab R, et al. Integrin alpha 11 regulates IGF2 expression in fibroblasts to enhance tumorigenicity of human non-small-cell lung cancer cells. Proc Natl Acad Sci U S A. 2007 Jul 10;104(28):11754-9. doi: 10.1073/pnas.0703040104
258. Eble JA, Niland S. The extracellular matrix in tumor progression and metastasis. Clin Exp Metastasis. 2019 Jun;36(3):171-198. doi: 10.1007/s10585-019-09966-1
259. Kuchnio A, Moens S, Bruning U, Kuchnio K, Cruys B, et al. The Cancer Cell Oxygen Sensor PHD2 Promotes Metastasis via Activation of Cancer-Associated Fibroblasts. Cell Rep. 2015 Aug 11;12(6):992-1005. doi: 10.1016/j.celrep.2015.07.010
260. Wolf K, Te Lindert M, Krause M, Alexander S, Te Riet J et al. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. J Cell Biol. 2013 Jun 24;201(7):1069-84. doi: 10.1083/jcb.201210152
261. Acerbi I, Cassereau L, Dean I, Shi Q, Au A, Park C, Chen YY, Liphardt J, Hwang ES, Weaver VM. Human breast cancer invasion and aggression correlates with ECM stiffening and immune cell infiltration. Integr Biol (Camb). 2015 Oct;7(10):1120-34. doi: 10.1039/c5ib00040h
262. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S et al. Role of YAP/TAZ in mechanotransduction. Nature. 2011 Jun 8;474(7350):179-83. doi: 10.1038/nature10137
263. Neesse A, Frese KK, Bapiro TE, Nakagawa T, Sternlicht MD, et al. CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreas cancer. Proc Natl Acad Sci U S A. 2013 Jul 23;110(30):12325-30. doi: 10.1073/pnas.1300415110
264. Lenzi P., Bocci G., Natale G. John Hunter and the origin of the term ‘‘angiogenesis” *Angiogenesis.*2016;19:255–256. doi: 10.1007/s10456-016-9496-7
265. Elwakeel E, Weigert A. Breast Cancer CAFs: Spectrum of Phenotypes and Promising Targeting Avenues. Int J Mol Sci. 2021 Oct 27;22(21):11636. doi: 10.3390/ijms222111636)
266. Folkman J. Anti-angiogenesis: New concept for therapy of solid tumors. *Ann. Surg.*1972;175:409–416. doi: 10.1097/00000658-197203000-00014
267. San Martin R, Barron DA, Tuxhorn JA, Ressler SJ, Hayward SW, et al. Recruitment of CD34(+) fibroblasts in tumor-associated reactive stroma: the reactive microvasculature hypothesis. Am J Pathol. 2014 Jun;184(6):1860-70. doi: 10.1016/j.ajpath.2014.02.021
268. Inoue KI, Kishimoto S, Akimoto K, Sakuma M, Toyoda S, et al. Cancer-associated fibroblasts show heterogeneous gene expression and induce vascular endothelial growth factor A (*VEGFA*) in response to environmental stimuli. Ann Gastroenterol Surg. 2019 Apr 9;3(4):416-425. doi: 10.1002/ags3.12249
269. Wang FT, Sun W, Zhang JT, Fan YZ. Cancer-associated fibroblast regulation of tumor neo-angiogenesis as a therapeutic target in cancer. Oncol Lett. 2019; 17(3): 3055-3065. doi: 10.3892/ol.2019.9973
270. Herrera, A., Herrera, M., Guerra-Perez, N. *et al.* Endothelial cell activation on 3D-matrices derived from PDGF-BB-stimulated fibroblasts is mediated by Snail1. *Oncogenesis* 7, 76 (2018). doi: 10.1038/s41389-018-0085-z
271. Unterleuthner D, Neuhold P, Schwarz K, Janker L, Neuditschko B, et al. Cancer-associated fibroblast-derived WNT2 increases tumor angiogenesis in colon cancer. Angiogenesis. 2020; 23(2): 159-177. doi: 10.1007/s10456-019-09688-8
272. Huang B, Huang M, Li Q. Cancer-Associated Fibroblasts Promote Angiogenesis of Hepatocellular Carcinoma by *VEGF*-Mediated *EZH2/VASH1* Pathway. Technol Cancer Res Treat. 2019 Jan-Dec;18:1533033819879905. doi: 10.1177/1533033819879905
273. Wu Y., Deng J., Rychahou P.G., Qiu S., Evers B.M., Zhou B.P. Stabilization of Snail by NF-KappaB Is Required for Inflammation-Induced Cell Migration and Invasion. *Cancer Cell.*2009; 15: 416–428. doi: 10.1016/j.ccr.2009.03.016
274. Christenson JL, Butterfield KT, Spoelstra NS, Norris JD, Josan JS, et al. MMTV-PyMT and Derived Met-1 Mouse Mammary Tumor Cells as Models for Studying the Role of the Androgen Receptor in Triple-Negative Breast Cancer Progression. Horm Cancer. 2017 Apr; 8(2): 69-77. doi: 10.1007/s12672-017-0285-6
275. Sewell-Loftin, M.K., Bayer, S.V.H., Crist, E. *et al.* Cancer-associated fibroblasts support vascular growth through mechanical force. *Sci Rep* 2017; 7: 12574. doi: 10.1038/s41598-017-13006-x
276. Albertini S, Martuscelli L, Borgogna C, Boldorini R, Gariglio M et al. Cancer-associated fibroblasts exert a pro-angiogenic activity in Merkel cell carcinoma. Journal of investigative dermatology. 2022; 142(10): P2837. doi: 10.1016/j.jid.2022.08.003
277. Poltavets V, Kochetkova M, Pitson SM, Samuel MS. The Role of the Extracellular Matrix and Its Molecular and Cellular Regulators in Cancer Cell Plasticity. Front Oncol. 2018 Oct 9;8:431. doi: 10.3389/fonc.2018.00431
278. Olkhanud P.B., Baatar D., Bodogai M., Hakim F., Gress R., et al. Breast Cancer Lung Metastasis Requires Expression of Chemokine Receptor CCR4 and Regulatory T Cells. *Cancer Res.*2009;69:5996–6004. doi: 10.1158/0008-5472.CAN-08-4619
279. Yang Y., Shaffer A.L., Emre N.C.T., Ceribelli M., Zhang M., et al. Exploiting Synthetic Lethality for the Therapy of ABC Diffuse Large B Cell Lymphoma. *Cancer Cell.*2012; 21: 723–737. doi: 10.1016/j.ccr.2012.05.024
280. Tan M.C.B., Goedegebuure P.S., Belt B.A., Flaherty B., Sankpal N., et al. Disruption of CCR5-Dependent Homing of Regulatory T Cells Inhibits Tumor Growth in a Murine Model of Pancreatic Cancer. *J. Immunol.*2009; 182: 1746–1755. doi: 10.4049/jimmunol.182.3.1746
281. Vickman RE, Broman MM, Lanman NA, Franco OE, Sudyanti PAG, et al. Heterogeneity of human prostate carcinoma-associated fibroblasts implicates a role for subpopulations in myeloid cell recruitment. Prostate. 2020 Feb;80(2):173-185. doi: 10.1002/pros.23929)
282. Bordignon P, Bottoni G, Xu X, Popescu AS, Truan Z, et al. Dualism of FGF and TGF-β Signaling in Heterogeneous Cancer-Associated Fibroblast Activation with ETV1 as a Critical Determinant. Cell Rep. 2019 Aug 27;28(9):2358-2372.e6. doi: 10.1016/j.celrep.2019.07.092)
283. Okeke EB and Uzonna JE. The Pivotal Role of Regulatory T Cells in the Regulation of Innate Immune Cells. Front. Immunol. 2019; 10: 680 doi: 10:680. doi: 10.3389/fimmu.2019.00680
284. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. Nat Rev Immunol. 2019 Jun;19(6):369-382. doi: 10.1038/s41577-019-0127-6
285. Yan S, Wan G. Tumor-associated macrophages in immunotherapy. FEBS J. 2021 Nov;288(21):6174-6186. doi: 10.1111/febs.15726
286. Freeman P, Mielgo A. Cancer-Associated Fibroblast Mediated Inhibition of CD8+ Cytotoxic T Cell Accumulation in Tumours: Mechanisms and Therapeutic Opportunities. Cancers (Basel). 2020 Sep 21;12(9):2687. doi: 10.3390/cancers12092687.
287. Özdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell. 2014 Jun 16;25(6):719-34. doi: 10.1016/j.ccr.2014.04.005. Erratum in: Cancer Cell. 2015 Dec 14;28(6):831-3
288. Gorchs L, Fernández Moro C, Bankhead P, Kern KP, Sadeak I, Meng Q, Rangelova E, Kaipe H. Human Pancreatic Carcinoma-Associated Fibroblasts Promote Expression of Co-inhibitory Markers on CD4+ and CD8+ T-Cells. Front Immunol. 2019 Apr; 10: 847. doi: 10.3389/fimmu.2019.00847
289. Yu Y, Xiao CH, Tan LD, Wang QS, Li XQ, Feng YM. Cancer-associated fibroblasts induce epithelial-mesenchymal transition of breast cancer cells through paracrine TGF-beta signalling. *Br J Cancer* (2014) 110:724–32. 10.1038/bjc.2013.768
290. Fiaschi T, Giannoni E, Taddei ML, Cirri P, Marini A, et al.. Carbonic anhydrase IX from cancer-associated fibroblasts drives epithelial-mesenchymal transition in prostate carcinoma cells. *Cell Cycle* (2013) 12:1791–801. 10.4161/cc.24902
291. Goulet, C.R., Champagne, A., Bernard, G. *et al.* Cancer-associated fibroblasts induce epithelial–mesenchymal transition of bladder cancer cells through paracrine IL-6 signalling. BMC Cancer. 2019; **19**: 137. doi:10.1186/s12885-019-5353-6
292. You J, Li M, Cao LM, Gu QH, Deng PB, Tan Y, Hu CP. Snail1-dependent cancer-associated fibroblasts induce epithelial-mesenchymal transition in lung cancer cells via exosomes. QJM. 2019; 112(8): 581-590. doi: 10.1093/qjmed/hcz093
293. Dang TT, Prechtl AM, Pearson GW. Breast cancer subtype-specific interactions with the microenvironment dictate mechanisms of invasion. Cancer Res. 2011; 71(21): 6857-66. doi: 10.1158/0008-5472.CAN-11-1818
294. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med. 2006; 4(1): 38. doi: 10.1186/1741-7015-4-38.
295. Conklin MW, Gangnon RE, Sprague BL, Van Gemert L, Hampton JM, et al. Collagen Alignment as a Predictor of Recurrence after Ductal Carcinoma *In Situ*. Cancer Epidemiol Biomarkers Prev. 2018 Feb;27(2):138-145. doi: 10.1158/1055-9965.EPI-17-0720
296. Osuala KO, Sameni M, Shah S, Aggarwal N, Simonait ML, et al. Il-6 signaling between ductal carcinoma in situ cells and carcinoma-associated fibroblasts mediates tumor cell growth and migration. BMC Cancer. 2015 Aug 13;15:584. doi: 10.1186/s12885-015-1576-3
297. Bernard S, Myers M, Fang WB, Zinda B, Smart C, et al. CXCL1 Derived from Mammary Fibroblasts Promotes Progression of Mammary Lesions to Invasive Carcinoma through CXCR2 Dependent Mechanisms. J Mammary Gland Biol Neoplasia. 2018 Dec;23(4):249-267. doi: 10.1007/s10911-018-9407-1
298. Cirri P. and Chiarugi P. (2012). Cancer-associated-fibroblasts and tumour cells: a diabolic liaison driving cancer progression. 31, 195-208. doi:10.1007/s10555-011-9340-x)
299. De Wever O, Van Bockstal M, Mareel M, Hendrix A, Bracke M. Carcinoma-associated fibroblasts provide operational flexibility in metastasis. Semin Cancer Biol. 2014 Apr;25:33-46. doi: 10.1016/j.semcancer.2013.12.009.
300. Ji, Q., Zhou, L., Sui, H. *et al.* Primary tumors release ITGBL1-rich extracellular vesicles to promote distal metastatic tumor growth through fibroblast-niche formation. *Nat Commun* 2020; **11**, 1211. doi: 10.1038/s41467-020-14869-x
301. O'Connell JT, Sugimoto H, Cooke VG, MacDonald BA, Mehta AI, et al. VEGF-A and Tenascin-C produced by S100A4+ stromal cells are important for metastatic colonization. Proc Natl Acad Sci U S A. 2011 Sep 20;108(38):16002-7. doi: 10.1073/pnas.1109493108.
302. Kong J, Tian H, Zhang F, Zhang Z, Li J, et al. Extracellular vesicles of carcinoma-associated fibroblasts creates a pre-metastatic niche in the lung through activating fibroblasts. Mol Cancer. 2019 Dec 3;18(1):175. doi: 10.1186/s12943-019-1101-4
303. Fang T, Lv H, Lv G, Li T, Wang C, et al. Tumor-derived exosomal miR-1247-3p induces cancer-associated fibroblast activation to foster lung metastasis of liver cancer. Nat Commun. 2018 Jan 15;9(1):191. doi: 10.1038/s41467-017-02583-0
304. Li Y, Wang R, Xiong S, Wang X, Zhao Z, et al. Cancer-associated fibroblasts promote the stemness of CD24+ liver cells via paracrine signaling. J Mol Med (Berl). 2019 Feb;97(2):243-255. doi: 10.1007/s00109-018-1731-9,
305. Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim Biophys Acta*. 2014: 1840: 2506–19. doi: 10.1016/j.bbagen.2014.01.010
306. Meran L, Baulies A, Li VSW. Intestinal stem cell Niche: the extracellular matrix and cellular components. *Stem Cells Int* 2017; 2017:7970385. 10.1155/2017/7970385
307. Malanchi I, Santamari a-Martínez A, Susanto E, Peng H, Lehr HA, Delaloye JF, Huelsken J. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature. 2011; 481(7379): 85-89. doi: 10.1038/nature10694
308. Vaziri N, Shariati L, Zarrabi A, Farazmand A, Haghjooy Javanmard S. Cancer-Associated Fibroblasts Regulate the Plasticity of Breast Cancer Stemness through the Production of Leukemia Inhibitory Factor. Life (Basel). 2021; 11(12): 1298. doi: 10.3390/life11121298
309. Kasper M, Jaks V, Fiaschi M, Toftgård R. Hedgehog signalling in breast cancer. Carcinogenesis. 2009; 30(6): 903-11. doi: 10.1093/carcin/bgp048
310. Cazet AS, Hui MN, Elsworth BL, Wu SZ, Roden D, et al.. Targeting stromal remodeling and cancer stem cell plasticity overcomes chemoresistance in triple negative breast cancer. *Nat Commun.* 2018; 9: 2897. 10.1038/s41467-018-05220-6
311. Cameron K, De Jong JH, Borovski T, et al.. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol.* (2010) 12:468–U121. doi: 10.1038/ncb2048
312. Chen WJ, Ho CC, Chang YL, Chen HY, Lin CA, Ling TY, et al.. Cancer-associated fibroblasts regulate the plasticity of lung cancer stemness via paracrine signalling. *Nat Commun.* 2014; 5: 3472. doi: 10.1038/ncomms4472
313. Baulida J. Epithelial-to-mesenchymal transition transcription factors in cancer-associated fibroblasts. Mol Oncol. 2017 Jul; 11(7): 847-859. doi: 10.1002/1878-0261.12080
314. Li Z, Sun C, Qin Z. Metabolic reprogramming of cancer-associated fibroblasts and its effect on cancer cell reprogramming. Theranostics. 2021 Jul 13;11(17):8322-8336. doi: 10.7150/thno.62378.
315. Lyssiotis CA, Kimmelman AC. Metabolic Interactions in the Tumor Microenvironment. Trends Cell Biol. 2017 Nov; 27(11): 863-875. doi: 10.1016/j.tcb.2017.06.003
316. Zhang D, Wang Y, Shi Z, Liu J, Sun P, Hou X. et al. Metabolic reprogramming of cancer-associated fibroblasts by IDH3α downregulation. *Cell Rep.*2015;10:1335–48
317. Shan T, Chen S, Chen X, Lin WR, Li W, Ma J. et al. Cancer-associated fibroblasts enhance pancreatic cancer cell invasion by remodeling the metabolic conversion mechanism. *Oncol Rep.*2017;37:1971–9.
318. Warburg O., Wind F., Negelein E. The metabolism of tumors in the body. *J. Gen. Physiol.*1927;8:519–530. doi: 10.1085/jgp.8.6.519
319. Fu Y, Liu S, Yin S, Niu W, Xiong W et al. The reverse Warburg effect is likely to be an Achilles’ heel of cancer that can be exploited for cancer therapy. *Oncotarget* **2017**, *8*, 57813–57825
320. Reyes M.E., de La Fuente M., Hermoso M., Ili C.G., Brebi P. Role of CC Chemokines Subfamily in the Platinum Drugs Resistance Promotion in Cancer. *Front. Immunol.*2020;11:901. doi: 10.3389/fimmu.2020.00901
321. Jones V.S., Huang R.Y., Chen L.P., Chen Z.S., Fu L., Huang R.P. Cytokines in cancer drug resistance: Cues to new therapeutic strategies. *Biochim. Biophys. Acta.*2016;1865:255–265. doi: 10.1016/j.bbcan.2016.03.005
322. Sun Y, Fan X, Zhang Q, Shi X, Xu G, et al. Cancer-associated fibroblasts secrete FGF-1 to promote ovarian proliferation, migration, and invasion through the activation of FGF-1/FGFR4 signaling. Tumour Biol. 2017 Jul;39(7):1010428317712592. doi: 10.1177/1010428317712592
323. Ratajczak-Wielgomas K, Grzegrzolka J, Piotrowska A, Gomulkiewicz A, Witkiewicz W, et al. Periostin expression in cancer-associated fibroblasts of invasive ductal breast carcinoma. Oncol Rep. 2016 Nov;36(5):2745-2754. doi: 10.3892/or.2016.5095
324. Calon A, Tauriello DV, Batlle E. TGF-beta in CAF-mediated tumor growth and metastasis. Semin Cancer Biol. 2014 Apr;25:15-22. doi: 10.1016/j.semcancer.2013.12.008
325. Qiao Y., Zhang C., Li A., Wang D., Luo Z., et al. IL6 derived from cancer-associated fibroblasts promotes chemoresistance via CXCR7 in esophageal squamous cell carcinoma. *Oncogene.*2018;37:873–883. doi: 10.1038/onc.2017.387
326. Huynh P.T., Beswick E.J., Coronado Y.A., Johnson P., O’Connell M.R., et al. CD90(+) stromal cells are the major source of IL-6, which supports cancer stem-like cells and inflammation in colorectal cancer. *Int. J. Cancer.*2016;138:1971–1981. doi: 10.1002/ijc.29939.
327. Ebbing E.A., van der Zalm A.P., Steins A., Creemers A., Hermsen S., et al. Stromal-derived interleukin 6 drives epithelial-to-mesenchymal transition and therapy resistance in esophageal adenocarcinoma. *Proc. Natl. Acad. Sci. USA.*2019;116:2237–2242. doi: 10.1073/pnas.1820459116.
328. Öhlund D., Elyada E., Tuveson D. Fibroblast heterogeneity in the cancer wound. *J. Exp. Med.*2014;211:1503–1523. doi: 10.1084/jem.20140692
329. Hazlehurst L.A., Argilagos R.F., Dalton W.S. Beta1 integrin mediated adhesion increases Bim protein degradation and contributes to drug resistance in leukaemia cells. *Br. J. Haematol.*2007;136:269–275. doi: 10.1111/j.1365-2141.2006.06435.x
330. Lwin T., Hazlehurst L.A., Dessureault S., Lai R., Bai W., et al. Cell adhesion induces p27Kip1-associated cell-cycle arrest through down-regulation of the SCFSkp2 ubiquitin ligase pathway in mantle-cell and other non-Hodgkin B-cell lymphomas. *Blood.*2007; 110: 1631–1638. doi: 10.1182/blood-2006-11-060350
331. Yu T, Yang G, Hou Y, Tang X, Wu C, et al. Cytoplasmic GPER translocation in cancer-associated fibroblasts mediates cAMP/PKA/CREB/glycolytic axis to confer tumor cells with multidrug resistance. Oncogene. 2017 Apr; 36(15): 2131-2145. doi: 10.1038/onc.2016.370
332. Tavares-Valente D, Baltazar F, Moreira R, Queirós O. Cancer cell bioenergetics and pH regulation influence breast cancer cell resistance to paclitaxel and doxorubicin. J Bioenerg Biomembr. 2013 Oct; 45(5): 467-75. doi: 10.1007/s10863-013-9519-7
333. Rong G, Kang H, Wang Y, Hai T, Sun H. Candidate markers that associate with chemotherapy resistance in breast cancer through the study on Taxotere-induced damage to tumor microenvironment and gene expression profiling of carcinoma-associated fibroblasts (CAFs). PLoS One. 2013 Aug 8;8(8):e70960. doi: 10.1371/journal.pone.0070960
334. Domogauer JD, de Toledo SM, Howell RW *et al.* Acquired radioresistance in cancer associated fibroblasts is concomitant with enhanced antioxidant potential and DNA repair capacity. *Cell Commun Signal* 19, 30 (2021). doi: 10.1186/s12964-021-00711-4
335. Ohuchida K, Mizumoto K, Murakami M, Qian LW, Sato N, Nagai E, Matsumoto K, Nakamura T, Tanaka M. Radiation to stromal fibroblasts increases invasiveness of pancreatic cancer cells through tumor-stromal interactions. Cancer Res. 2004 May 1;64(9):3215-22. doi: 10.1158/0008-5472.can-03-2464
336. Li D, Qu C, Ning Z, et al. Radiation promotes epithelial-to-mesenchymal transition and invasion of pancreatic cancer cell by activating carcinoma-associated fibroblasts. *Am J Cancer Res.*2016;6(10):2192–206.
337. Mantoni TS, Lunardi S, Al-Assar O, et al. Pancreatic stellate cells radioprotect pancreatic cancer cells through β1-integrin signaling. *Cancer Res.*2011;**71**(10):3453–8.
338. Horsman MR, Overgaard J. The impact of hypoxia and its modification of the outcome of radiotherapy. *J Radiat Res.*2016;**57**(Suppl 1):i90–8.
339. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell. 2014 Jun 16;25(6):735-47. doi: 10.1016/j.ccr.2014.04.021
340. Chen K, Wang Q, Li M, Guo H, Liu W, et al. Single-cell RNA-seq reveals dynamic change in tumor microenvironment during pancreatic ductal adenocarcinoma malignant progression. EBioMedicine. 2021 Apr;66:103315. doi: 10.1016/j.ebiom.2021.103315
341. Brechbuhl HM, Finlay-Schultz J, Yamamoto TM, Gillen AE, Cittelly DM, et al. Fibroblast Subtypes Regulate Responsiveness of Luminal Breast Cancer to Estrogen. Clin Cancer Res. 2017 Apr 1;23(7):1710-1721. doi: 10.1158/1078-0432.CCR-15-2851
342. (Alexeyenko A, Alkasalias T, Pavlova T, Szekely L, Kashuba V, et al Confrontation of fibroblasts with cancer cells in vitro: gene network analysis of transcriptome changes and differential capacity to inhibit tumor growth. J Exp Clin Cancer Res. 2015 Jun 18;34(1):62. doi: 10.1186/s13046-015-0178-x)
343. Degeorges A, Tatoud R, Fauvel-Lafeve F, Podgorniak MP, Millot G, et al. Stromal cells from human benign prostate hyperplasia produce a growth-inhibitory factor for LNCaP prostate cancer cells, identified as interleukin-6. Int J Cancer. 1996; 68(2): 207-214. doi: 10.1002/(SICI)1097-0215(19961009)68:2<207::AID-IJC12>3.0.CO;2-7
344. Paland N, Kamer I, Kogan-Sakin I, Madar S, Goldfinger N, et al. Differential influence of normal and cancer-associated fibroblasts on the growth of human epithelial cells in an in vitro cocultivation model of prostate cancer. Mol Cancer Res. 2009; 7(8): 1212-23. doi: 10.1158/1541-7786.MCR-09-0073
345. Narra K, Mullins SR, Lee HO, Strzemkowski-Brun B, Magalong K, Christiansen VJ, et al. Phase II trial of single agent Val-boroPro (talabostat) inhibiting fibroblast activation protein in patients with metastatic colorectal cancer. Cancer Biol Ther 2007; 6: 1691–9

Legends to Figures:

Figure 1: Potential cellular sources of Cancer associated fibroblast and their activarion factors. Cancer-associated fibroblasts originate from various sources, which are in quiescent state and convert into CAFs after communicating with malignant cells and express different markers differentiating from its progenitor state

Figure 2: Diverse signals involved in recruitment and activation of cancer associated fibroblasts

Figure 3: Common signal pathway for activation of fibroblast to cancer associated fibroblast under the influence of signals from cancer cell and the viscous cycle it generates.

Figure 4: Diverse intracellular, surface as well as extra-cellular markers associated with CAF. The heterogeneity of such markers in distinct solid tumor types and even expression of some of these markers in normal tissue pose a difficult situation when studying the crucial role of CAFs and their molecular and functional properties in cancer.

Figure 5: Multiple cancer associated functional expression of CAF that support cancer cell population and also other members of tumor microenvironment.