Cancer Associated Fibroblasts (CAFs) –The Influencers of Oral Squamous cell carcinoma & its microenvironment

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Abstract: The tumor microenvironment (TME) is composed of cells from endothelial, mesenchymal, and hematopoietic origins embedded in a complex extracellular matrix (ECM), and these components of TME enter into a dynamic crosstalk with tumor cells, resulting in an environment suitable for tumor growth. Different alterations such as angiogenesis, hypoxia, ECM remodeling, interstitial pressure, metabolism changes have received recent attention as key determinants of the TME modifying cancer cell behavior and disease progression, with potential clinical applications. Among the stromal cells, activated fibroblasts(myofibroblasts) are present and they share similarities with activities of the wound healing process.
Myofibroblasts of solid tumor TME are referred to as Cancer associated fibroblasts(CAFs) and they play a critical role in the complex process of tumor cell-stroma interaction. (1,2,3)
Studies have shown that CAF-rich OSCC are associated with significantly shorter patient survival & therefore, the best independent risk factor of early OSCC death was high stromal α-SMA expression as, CAF promote many "hallmarks of malignancy".(4,5)
CAFs create "tracks" in ECM through which tumour cells invade and are led by CAF. CAF-tumour cell communication via exosomes and reciprocal paracrine interaction, neo-angiogenesis & tumor immune evasion further enhances tumor progression. CAFs help the cancer cells to travel to distant site through metastatic pathways by inhibiting anoikis (A protective mechanism against invasion and metastasis) of tumor cells.(6)
CAFs have been proposed to modulate the drug sensitivity of cancer cells.(7)
Researchers are unpicking CAF complexity and there is great optimism in the field that effective CAF-targeted therapies.
Keywords: Tumor microenvironment(TME), Epithelial-mesenchymal transition(EMT) , Cancer associated fibroblasts(CAFs), myofibroblasts, Extra-cellular matrix(ECM)

Introduction:

Tumor progression and metastatic niche formation do not only depend on cancer cell genetic and epigenetic defects but are also controlled by the tumor microenvironment (TME). The TME or tumor stroma is composed of cells from endothelial, mesenchymal, and hematopoietic origins embedded in a complex extracellular matrix (ECM), and these components of TME enter into a dynamic crosstalk with tumor cells, resulting in an environment suitable for tumor growth. Consequently, different alterations such as angiogenesis, hypoxia, ECM remodeling, interstitial pressure, metabolism changes have received recent attention as key determinants of the TME modifying cancer cell behavior and disease progression, with potential clinical applications. In addition to, the various known classical strategies used by tumor cells to escape immune surveillance (such as down regulation of antigen expression, resistance to cell-mediated lysis or expression/secretion of immunosuppressive molecules), it should be noted that tumor cell evasion from immunosurveillance is also under the control of the TME complexity. Among the stromal cells, activated fibroblasts are present and they share similarities with activities of the wound healing process subsequent to acute or chronic inflammatory signals, in which fibroblasts are activated and also observed to promote tissue fibrosis. Such fibroblasts are also known as myofibroblasts and they play a critical role in the complex process of tumor cell-stroma interaction and therefore have emerged as important regulators of the anti-tumor immune response.(1)

The tumor microenvironment (TME) is important to the development & behaviour of solid malignancies. This is attributed to the elements of the TME that evolve over time and are reprogrammed to cater for the needs of a growing tumor. A dominant cell type found in the TME of solid tumor lesions is the mesenchymal or fibroblastic cell type, also referred to as cancer associated fibroblasts (CAFs). (2, 3)

CAFs are a 'family' or 'group' of cells that exhibit mesenchymal cell-like features and are likely mesoderm derived. They are found in the vicinity or in direct contact with neoplastic cells, and are often the dominant cell type within a solid tumor mass. Unlike CAFs, the tissue resident normal fibroblasts are a more discrete proportion of cells that resides in a given organ. The appellation 'CAFs' is often used as an umbrella term to define a complex population of

dynamically heterogeneous mesenchymal cells that have an activated phenotype, with functions that are likely distinct from those of resident tissue fibroblasts. Most researchers, describe CAFs as producers of cytokines, chemokines, metabolites, enzymes and extracellular matrix molecules that fuel the growth of cancer cells. Synchronous with the complexity of the tumor immune response, CAFs also exhibit complex tumor-associated phenotypes, suggestive of their distinct functions. They also express a number of signaling receptors that are engaged in maintaining or changing the CAF phenotypes during cancer progression. These receptors might in turn be involved in integrating signals from various cell types within the TME, thus may further influence the functioning of CAFs. (4)

They are contractile, αSMA-positive cells are generated principally through TGF-β signalling/mechano transduction, and secrete extracellular matrix (ECM) analogous to myofibroblasts found in healing wounds and fibrotic disorders. In tissue sections these are usually identified using immunochemistry for α-SMA, although this protein is also expressed by pericytes and smooth muscle cells. Thus, there is no specific single marker that accurately identifies CAF. They are also referred to as myofibroblastic CAFs (myCAFs). Most myCAF originate from local fibroblasts–pericytes, adipocytes, endothelial cells and bone marrow-derived mesenchymal stem cells,in addition to, macrophages and cancer stem cells. Thus, CAFs are heterogenous in origin and CAF subpopulations are characterised by high expression of α-SMA, however, conversely, not all α-SMA-positive CAF are myofibroblastic. Researchers have found that, CAFs can be further divided into two subclusters through differential expression of immediate early response genes, mesenchymal markers, ligands and receptors, and ECM proteins. Patel et al found on transcriptomic analysis of CAF cells of OSCC that, CAF1 was associated with increased proliferation of cancer cells but with suppressed self-renewal growth of oral stem-like cancer cells (oral-SLCCs). In contrast to this finding, CAF2 correlated positively with frequency of oral SCCs but negatively with tumour cell proliferation. BMP4 (bone morphogenetic protein 4) was differentially expressed between the two CAF populations and was therefore indicating at least partial influence of exerting the suppressive effect on cancer cells' stemness. Additionally, CAF phenotype may also vary according to molecular phenotypes of SCC. It is noteworthy, that the generation of intracellular reactive oxygen species plays a major role in CAF (and myofibroblast) differentiation, with the ROS-producing enzyme, NADPH oxidase 4 (NOX4), central to this process. However, characterisation of CAF heterogeneity still remains incomplete. (5)

Numerous studies have shown that CAF-rich OSCC are associated with significantly shorter patient survival & therefore, the best independent risk factor of early OSCC death was high stromal α-SMA expression. When clinic-pathological correlations are done, a greater clarity is obtained as to how CAFs function to promote OSCC progression. The role of CAFs is multifactorial as, CAFs have a wide range of influence on tumor behaviour as can be interpreted from tumour aggressiveness, including invasion, metastasis, absence of T-cells and therapy resistance. This interpretation is based on the results of numerous functional studies that show that CAF promote many, if not all of the "hallmarks of malignancy".(5)

Tumour invasion is enhanced through multiple mechanisms, including deposition and remodelling of ECM, by CAFs and the same is also a central myCAF function. Researchers have found that, CAFs create "tracks" in ECM through which tumour cells invade and are led by CAF. A blend of protease- and force-mediated matrix remodelling mechanisms are responsible for the creation of tracks in ECM. This occurs due to, CAF producing numerous ECM proteins and soluble factors that affect the composition of the matrix significantly & hence affect cancer invasion. A similar phenomenon is, noted in bone invasion by OSCC. Besides these phenomena, CAF-tumour cell communication via exosomes which are secreted extracellular vesicles contain proteins, lipids and nucleic acids, such as messenger RNA (mRNA), micro RNA (miRNA), long non-coding RNA (lncRNA) and others, and exosomal miR382-5p and MFAP5 have been shown to promote OSCC cell migration and invasion by many researchers. The cancer enhancing features of exosomes are a consequence of dual impact of both a transfer of pro-tumoral factors by it and also a lack of suppressive factors in the tumor mileu. There is also a reciprocal paracrine interaction(a reciprocal and convergent set of signaling activities that promote tumor growth and cancer invasion and metastasis) between tumour cells and CAF (5)

Rapidly growing tumours create a hypoxic microenvironment and CAF play a major role in neo-angiogenesis, producing angiogenic factors such as VEGFA (vascular endothelial growth factor), and also attracting other cells, such as macrophages, which also contribute to the angiogenic process. CAFs are the main source of interleukin-6 in OSCC. Through an autocrine signalling loop the secreted IL-6 further induces the secretion of VEGFA in CAF and also in OSCC cells. (5)

Both innate and adaptive immunity are affected owing to the tumor immune invasion, which is a result of several mechanisms that are altered by CAF influence . The CAF influences these mechanisms by secretion of cytokines, chemokines, or other soluble factors, and thereby shapes the TME and favors the recruitment of innate immune cells, such as monocytes or neutrophils. Alongwith the acquisition of an immunosuppressive phenotype of monocytes or neutrophils, CAFs also affect cytotoxic function and

cytokine production of NK cells. Based on the immunomodulatory secretome of innate immunity, CAFs might also interfere with the adaptive anti-tumor immune response at different levels, leading to a disruption of T cell function in the TME. he CAF secretome can shape the T cell-dependent antitumor immune response by affecting several populations such as DCs, MDSCs, by switching CD4+ TH lymphocytes from a TH1 to a TH2 phenotype, by affecting Tregs and TH17 cells, by affecting CD8+ T cell functions or eventually by expressing some ligands of immune checkpoint receptors.(1,5)

CAFs help the cancer cells to travel to distant site through metastatic pathways by inhibiting anoikis (A protective mechanism against invasion and metastasis) of tumor cells. CAFs play a significant role in anoikis resistance (AR) to cancer cells which is a dominant beneficial quality that is governed by cancer cells and it imparts intrinsic characteristics for metastasis.

CAFs play the role in AR through various mechanisms for the cancer cells as follows:

1. CAFs facilitate invasion via EMT. EMT is induced by expression of TGF-β by CAFs. TGF- β of CAFs has an effect on the MAPK/ERK and PI3K/AKT signaling pathways and these pathways are responsible for the increase in resistance to anoikis in cancer cells.
2. CAFs exhibit two vital processes to overcome anoikis for EMT to occur. Firstly, they secrete Matrix metalloproteinase (MMP) 2 and 9 which tend to cleave the E cadherins. Additionally, CAFs assist the release of reactive oxygen species (ROS) via Rac1b/cyclooxygenase-2 pathways and thereby help the cancer cells to overcome anoikis for EMT.
3. Researchers also found that, CAFs have stabilizing effect on antiapoptotic proteins which are responsible for anoikis through Insulin like Growth factor receptor (IGFR).
4. EMT in cancer cells is the consequence of its promotion by CAFs and that results in increase in their metastatic potential via regulation of stem-cell traits.

   Ultimately, "anoikis resistant CAFs" inhibits anoikis of cancer cells by paracrine signaling and carry them to distant site for metastasis.(3)

The metabolic abnormalities occurring in tumor cells are therefore, not simple alterations of a single metabolic pathway but are rather subversive alterations in the entire cellular network of metabolism. As a consequence of metabolic reprogramming, the limited nutrients and/or intermediate metabolites are prioritized to be utilized in various biosynthetic pathways that are necessary to support the energy-demanding anabolic processes for tumor cell proliferation. A similar reprogramming also occurs in the TME.

Table-1 Various influences of CAFs in tumor mileu (6)

a major producer of ECM components, such as collagen and laminins, and remodeling enzymes
secrete a variety of pro-inflammatory mediators (e.g., interferon-γ, C-X-C motif chemokine ligand 12 (CXCL12), tumor necrosis factor (TNF)-α and several interleukins (ILs)), and growth factors (e.g., transforming growth factor β (TGF-β), fibroblast growth factor (FGF)), as well as, pro-angiogenic factors like vascular endothelial factor (VEGF) and endothelial growth factor (EGF)- leading to PROTUMOROGENIC EFFECT
Glucose metabolism influence- a) Regulators & active participants of important metabolic processes.eg.- tumor cells show aerobic glycolysis "Warburg effect"& under their influence CAFs show "reverse Warburg effect" b) Activation of TGF-β signaling in CAFs increase oxidative stress, autophagy/mitophagy, and aerobic glycolysis, which may be associated with downregulation of caveolin-1 (Cav-1) c) CAFs derived from oral squamous carcinoma (OSCC) exhibited significantly higher ITGB2 expression, it was correlated with poor clinical characteristics and outcomes in OSCC patients, indicating that ITGB2 hi CAFs might promote OSCC cell proliferation
Aminoacid metabolism influence- The amino acids required for tumor cell growth are manufactured by CAFs through the TCA cycle. Among them,Glutamine (Gln), an important source of carbon and nitrogen, is supposed to play a key role in tumor anabolism, and therefore, if glutamine levels in the TME are decreased in some manner, it would affect the viability of tumor cells. Glutamine dependence thus, governs the CAF migration from the glutamine-depleted core of tumors to more glutamine-rich areas. Glutamine deprivation promoted CAF migration and invasion, is thereby enabling and facilitating the movement of tumor cells toward nutrient-rich territories. CAFs are also capable of producing other amino acids as nitrogen sources. Crosstalk between SCC cells & CAFs occurs via the aspartate/glutamate transporter, & leads either glutamate is generated through the TCA cycle to produce aspartate. Aspartate is further utilized by CAFs to biosynthesize and maintain cell proliferation. This glutamate also forms glutathione (GSH) in CAFs to balance the redox status of cells and promote ECM remodeling

Lipid metabolism influence- Fatty acids are components of the membrane matrix, & are also important secondary messengers, and can also serve as fuel sources for energy production CAFs undergo a reprogramming of liposome metabolism in some carcinomas
The role of pro-glycolytic CAFs in the metabolic reprogramming of tumor cells(local effect)- CAFs control the metabolic remodeling of tumor cells by the following modes: (I) directly/indirectly exporting nutrients, (II) providing mitochondria, (III) regulating the activity and oxidative properties of metabolic enzymes, and (IV) participating in ECM formation
Effect of CAFs on Systemic Metabolism in cancer a) Cancer cachexia is characterized by skeletal muscle protein loss and reduction of body lipid stores during metabolic processes & it is linked to inflammatory response in cancer b) Inflammatory host response, may in turn may lead to systemic elevation of glucocorticoids and immunosuppression.

Now an oral carcinoma is recognized as, a complex tumor tissue where many different cell types and extracellular matrix components, interact in a multipart ecosystem thereby, creating a distinct microenvironment within the tumor. One of the most crucial elements of the tumor microenvironment is, the cancer-associated fibroblasts (CAFs). These cells modulate the tumor's fate by increasing tumor growth, epithelial-to-mesenchymal transition (EMT), the invasive potential, and metastasis, by secretion of soluble factors or by modification of extracellular matrix components. High density of CAF within the tumor microenvironment is associated with tumor progression and vascular and perineural invasion that correlates with high rate of local recurrence. CAFs have been proposed to modulate the drug sensitivity of cancer cells also their major role in EMT. (7)

Therefore,in addition to their tumour-promoting functions, CAF have been shown to confer resistance to different types of cancer therapy, including cetuximab and anti-PD-1/PD-L1 checkpoint immunotherapy, as well as radiotherapy and cisplatin chemotherapy, suggesting that therapeutic CAF targeting could increase response rates for a diverse range of treatments. With the advent of new technologies, particularly single-cell RNA-sequencing, researchers are unpicking CAF complexity and there is great optimism in the field that effective CAF-targeted therapies. (5)

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