

## CANCER CONUNDRUM



### CANCER ASSOCIATED FIBROBLASTS

Oral cancer, a major widespread public health threat does not act alone but is surrounded by microenvironment that is composed of the tumor-associated stroma, which is created by and acts for the tumor itself. Tumors are composed of reciprocally interacting cell types that include cancer-initiating cells, differentiated cancer cells, extracellular matrix (ECM) and stromal cells such as fibroblasts, endothelial cells, immune cells, pericytes, and adipocytes.<sup>[1]</sup> Various cell types in a tumor exhibit grades of plasticity when exposed to different tumor-derived factors. Based on the type of stimulus, cells in the tumor microenvironment can adopt different states of activation ranging from tumor suppression to tumor promotion.<sup>[2]</sup> Cancer-associated fibroblasts (CAFs) are the most abundant type of stromal cells in many tumors.<sup>[3]</sup> They are derived from different cell types and have distinct populations in the same stroma.<sup>[4]</sup>

There are two phenotypes of CAF: myofibroblasts and fetal-like fibroblasts.

Myofibroblasts normally are a minor fibroblast subpopulation in most organs and also the tumors exhibiting a strong desmoplasia. Origins of these cells are from embryonic progenitor stem cells and differentiation from resident tissue fibroblasts by tumor-induced fibroblast – myofibroblast differentiation under the influence of various paracrine stimuli. CAFs undergo changes in protein expression which represent an “activated” myofibroblastic phenotype, which typically

involves the upregulation of Vimentin, Actin and Myosin +ve type  $\alpha$ -smooth muscle actin. Myofibroblastic differentiation in tumors is restricted locally to the tumor-adjacent stroma.<sup>[5]</sup>

The term “fetal-like” fibroblast implies an aberrant phenotype if present exclusively in adult tissues. They have fetal-like migratory behavior compared to normal adult fibroblasts. They express and release an autocrine and paracrine factor migration-stimulating factor. These cells are not restricted to local tumor microenvironment but is also seen in distant areas.<sup>[5]</sup>

Genotype of CAF: generally, CAFs are considered phenotypically and functionally altered but genetically normal cells in tumors. However, recent evidence shows that carcinoma-adjacent stroma may be “already” genetically altered. The genetic markers identified most recently in the stroma are altered p53 suppressor gene and microsatellite instability.<sup>[5]</sup>

The heterogeneity of CAFs is due to the type of tissue in which the tumor grows, local paracrine effect and the type of cellular origin.<sup>[1]</sup> This heterogeneous population of CAFs shares some properties collectively leading to their “activation state.”<sup>[3]</sup>

There are different origins for CAFs that includes from local resident fibroblasts, pericytes within tumor microenvironment through mesenchymal - mesenchymal transition (MT).

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They can be derived from mesenchymal stem cells of bone marrow, normal or transformed epithelial cells via epithelial to mesenchymal transition (EMT), or from endothelial cells via endothelial to MT. CAFs can also arise directly from carcinoma cells through EMT<sup>[6]</sup> which allows the cancer cells to adopt a mesenchymal phenotype characterized by an enhanced migratory capacity and invasiveness.<sup>[3]</sup>

Histologically, based on their morphology CAFs are divided into two groups.

- Mature - thin, wavy, and small spindle cell morphology as normal fibroblasts
- Immature - large, plump spindle-shaped cell with prominent nucleoli.<sup>[7]</sup>

### EXPRESSION PROFILE OF CANCER-ASSOCIATED FIBROBLAST

The tumor-associated microenvironment is abnormal and complex meshwork of collagens, fibrillar glycoproteins and proteoglycans leading to aberrant tumor architecture. CAFs also express cell matrix receptors, cell adhesion molecules, growth factors and cytokines.<sup>[5]</sup>

CAFs produce different factors that are generally not expressed by the normal fibroblasts. Upon activation, these cells produce mesenchyme-specific proteins such as fibroblast-specific protein or S100A4, fibroblast-activating protein, vimentin, and  $\alpha$ -SMA. CAFs also secrete different factors such as cytokines, chemokines (interleukin-6, CXCL8, CXCL12) and growth factors like vascular endothelial-derived growth factor, transforming growth factor beta (TGF- $\beta$ ), hepatocyte growth factor, epidermal growth factor, fibroblast growth factor and express receptors such as platelet-derived growth factor receptor alpha and beta (PDGFR- $\alpha$ , PDGFR- $\beta$ ). These soluble factors either act through paracrine signaling or in autocrine loops, thus contributing to the constitution of the CAF phenotype. CAFs also have an important role in remodeling the ECM by expressing a variety of matrix components and matrix-remodeling enzymes such as fibronectin, matrix metalloproteinases-1/stromelysin-1, neuron-glia antigen, tenascin-C and Type I collagen.<sup>[1]</sup>

Two interactive pathways are involved in the crosstalk between cancer and stromal cells:

- “Efferent” pathway – wherein the cancer cells trigger a reactive response in the stroma
- “Afferent” pathway - wherein the modified stromal cells in the surrounding microenvironment affect cancer cell responses.<sup>[3]</sup>

### TUMOR-PROMOTING EFFECTS OF CANCER-ASSOCIATED FIBROBLASTS

Various studies in the past decade prove that CAFs can promote tumor development and progression from the

pre-malignant stage, stimulate metastasis and support the growth of disseminated tumor cells at the metastatic site.<sup>[1]</sup>

The signals produced by CAFs directly stimulates the tumor cells and promotes proliferation, migration, invasion of cancer cells and adopts cancer stem cell phenotype by inducing EMT. <sup>[1]</sup> CAFs alter the three-dimensional ECM scaffold and support tumor cells that eventually metastasize and activate immune cells to enhance the ECM-degrading capacity.<sup>[8]</sup> CAFs also secrete a variety of proinflammatory factors for recruitment and promotion of immunosuppressive and tumor-promoting immune cells, thus establishing an immune-suppressive, tumor-permissive environment.<sup>[1]</sup>

CAFs in oral squamous cell carcinoma is associated with a diffuse pattern of invasion, preparing the microenvironment for tumor invasion and metastasis and poor prognosis.<sup>[9]</sup>

Loss of caveolin-1 is due to the autophagic destruction of mitochondria in CAFs. This leads to “reverse Warburg effect” that refers to the transfer of high-energy metabolites to neighboring tumor cells which are undergoing oxidative mitochondrial metabolism. These malignant cells thus increase the production of ATP and, thereby, exhibits increased growth and metastatic potential.<sup>[4]</sup>

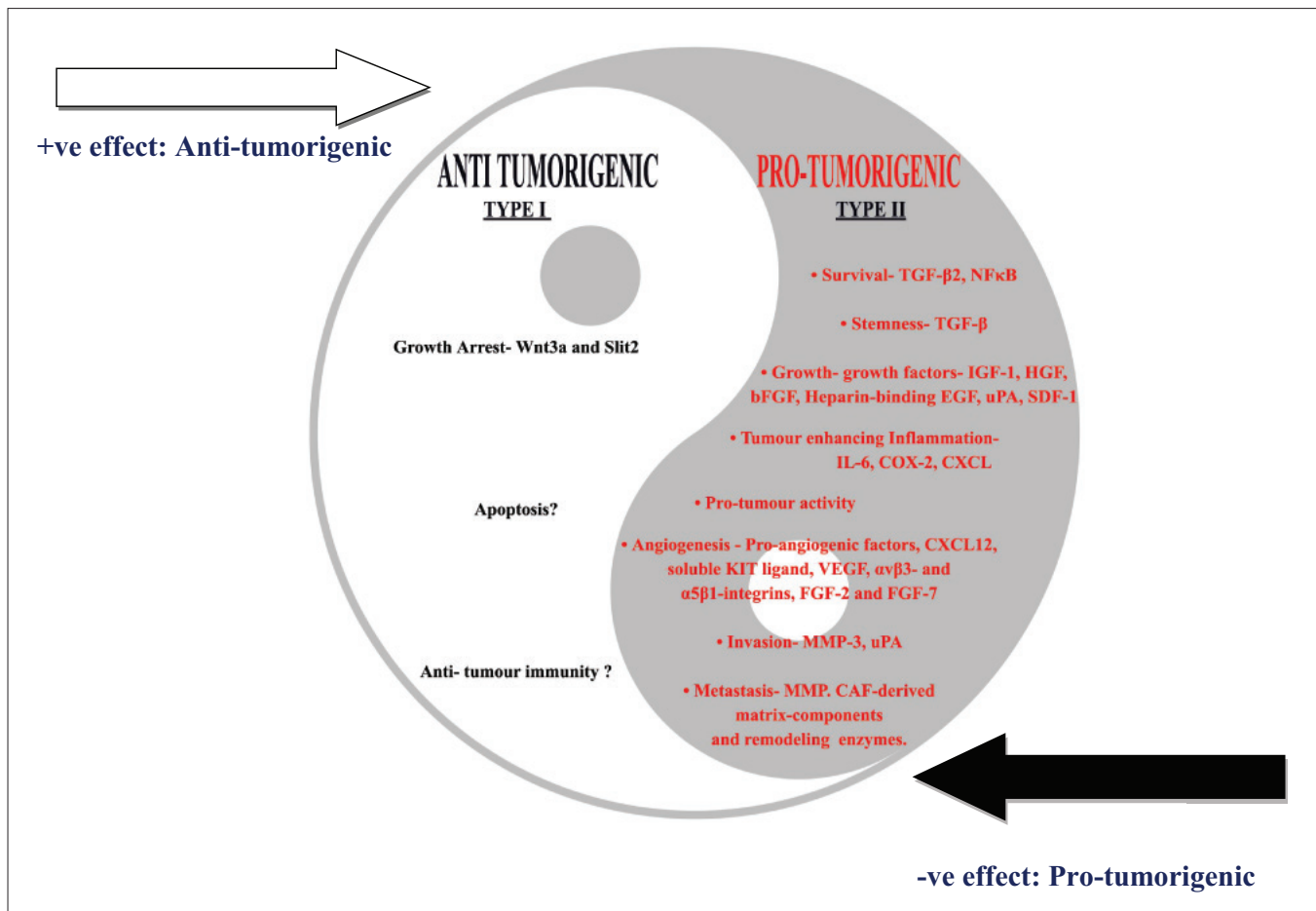
CAFs cause chemotherapy resistance by producing ECM proteins. Laminin and collagen IV provides a favorable microenvironment for tumor cell growth as they decrease the cytotoxicity of the anticancer drugs. CAFs through the action of platelet-derived growth factor control the interstitial fluid pressure which provides a barrier to uptake of chemotherapeutic compounds by cancer cells.<sup>[4]</sup>

Besides the role of CAFs as a promoter of tumor growth and progression, few recent reports obtained from *in vitro* studies and *in vivo* xenograft models, suggests a tumor-inhibitory role of CAFs.<sup>[1]</sup>

### TUMOR-SUPPRESSIVE EFFECTS OF TUMOR-RESIDENT FIBROBLASTS

Tumor-resident fibroblasts have the capacity to suppress growth and progression of pre-malignant lesions. Mechanisms underlying the inhibitory phenotype of CAFs are not known, but this may be due to direct inhibition of cancer cells and also modulation of immune cell behavior.<sup>[1]</sup>

Normal fibroblasts and CAFs that express Slit2 ligand inhibit the tumorigenicity of breast cancer cells by expressing the corresponding Robo1-receptor on their surface. This is because the ligand-induced Robo1 activation interfered with PI3K and  $\beta$ -catenin signaling in cancer cells and diminished their malignant potential. Slit-stimulated signaling also inhibits pro-tumorigenic stromal-derived factor 1/CXCR4-signaling pathway. It was shown that Slit expression have prognostic



**Figure 1:** Dynamism of cancer-associated fibroblasts depicted using concept of Yin and Yang

significance in predicting overall survival and occurrence of metastasis.<sup>[10]</sup>

Fibroblast-derived Wnt3a promotes as well as inhibits the growth of different, orthotopically growing patient-derived breast xenograft tumors.<sup>[11]</sup>

TGF- $\beta$ , which is mainly derived from CAFs, is known to suppress tumor initiation and early tumor growth but later promotes tumor progression and metastasis.<sup>[12]</sup>

However, the same CAFs may eventually co-express factors that by themselves suppress the action of tumor-resident cells. In prostate cancers, CAFs express several tumor-promoting factors but at the same time, molecules have been shown to suppress cancer cell growth, migration, and invasion.<sup>[1]</sup>

The pro- and anti-tumor effect of CAFs is presented in Figure 1.

### THE POLARIZATION CONCEPT TO CANCER-ASSOCIATED FIBROBLASTS

The phenotype of CAFs is determined by two components:

- Cell of origin

- Local environment in which the CAFs are embedded.

Exposure of fibroblasts to growth factors, cytokines, reactive oxygen species or a stiff matrix induces a CAF phenotype that is characterized by the ability to promote tumor growth and progression.<sup>[1]</sup>

On the contrary, CAFs can revert the protumorigenic action when exposed to some tumor-inhibitory factors, for example, TGF- $\beta$ .<sup>[13]</sup> Tumor-resident fibroblasts also can suppress the tumor growth if the microenvironment is optimal.<sup>[7]</sup>

Based on function, there are Type I and Type II CAFs that mark the two extreme poles of the spectrum. They have opposing activities in neoplasms. In the complex micromilieu of a tumor, they move on a continuum of activation states between Type I and Type II. Interestingly, the different tumor-associated cell types actively regulate the polarization status of each other.<sup>[1]</sup>

### CONCLUSION

CAFs are a heterogenous population of cells in tumor microenvironment that have contributions for tumor development, progression, and metastasis. On the contrary, the

role of these cells for tumor inhibition is emerging with few studies to support. This implies that these cells have a broader and diverse role to play with a degree of plasticity. The role of CAFs should be studied in depth for its understanding so that these cells can be targeted therapeutically.

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