Invited Review

The diverse role of oral fibroblasts in normal and disease

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Abstract

Fibroblasts are the major cellular component of the connective tissue. They differ both structurally and functionally based on their location. The oral fibroblasts vary from the dermal fibroblasts in their origin, properties and also functions. These cells play an important role in wound healing, tumor progression and metastasis, allergic reactions. In this review, the various functions of the oral fibroblasts are discussed in detail.

Keywords: Cancer-associated fibroblasts, fibroblasts, healing, myofibroblasts, oral fibroblasts, oral submucous fibrosis

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INTRODUCTION

Virchow (circa 1858) and later Duvall studied cells in the connective tissue using classic anatomy techniques and microscopy. The fibroblasts were then defined as cells in the connective tissue that synthesize collagen.[1] Fibroblasts are found in almost every organ and tissue of the body.[2] They are morphologically and functionally heterogeneous in nature. Even within the same tissue, they differ based on their location. For example, the dermal fibroblasts are called papillary fibroblasts (Fps), reticular fibroblasts (Frs) and dermal-subcutaneous junction fibroblasts (F-DHJs) based on their location. The Fps are tricuspid in shape and arranged close to each other. The Frs are stellate in shape and loosely arranged, whereas the F-DHJs are morphologically uneven.[3]

FIBROBLASTS ORIGIN

The head and neck fibroblasts arise from the neural crest-derived ectomesenchyme during development. The fibroblasts of other issues arise from the primary mesoderm.[4] They also arise through the epithelial-mesenchymal transition in organs like the liver, kidney; through the endothelial-mesenchymal transition in lungs, heart and also from circulating cells such as mesenchymal stromal cells or fibrocytes.[5]

STRUCTURE OF FIBROBLASTS

Within a tissue, the fibroblasts exist in either of the two states – active or quiescent. The active fibroblasts are large, ovoid with abundant basophilic cytoplasm and a pale staining nucleus. They have a prominent nucleolus, well-developed Golgi complex, abundant rough endoplasmic reticulum, secretory granules and numerous mitochondria. In adults, rarely undergo mitosis. The quiescent fibroblasts, also called fibrocyte, are small, spindle-shaped with acidophilic cytoplasm and a small, dark elongated nucleus. The nucleolus is not present.

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They contain fewer processes and less rough endoplasmic reticulum. Upon adequate stimulation, the fibrocyte can revert to the active fibroblast state and acquire synthetic functions.[6,7]

Fibroblasts usually lack intercellular junctions. However, the exceptions being, in embryonic tissue they show gap junctions, in periodontal tissue they show gap junctions and cell to cell contact of adherents type, and in the dental pulp tissue they are attached by desmosomal junctions. Fibronexus are the focal contacts formed between the fibroblasts and the extracellular matrix components.[6,7] Fibroblasts also exhibit planar polarity and cell plasticity.[1]

The fibroblasts lack epithelial, vascular and leukocyte lineage markers.[5] They express cell surface markers Cq-1, CD 40, CD 39, Thy-1 and CD 90.[2,4,8] Upon activation CD 90 regulates the proliferation and differentiation of fibroblasts. They also express pattern recognition receptors like Toll-like receptors and also possess chemokine receptors.[2,4] The cancer-associated fibroblasts (CAFs) express CD 44, CD 49b, CD 87, CD 95 and Ly-6C.[9] The pro-tumorigenesis markers are Fibroblast-activation protein, Platelet-derived growth factor (PDGF) Rα/β, podoplanin (PDPN), CD 70, vimentin, G-protein coupled receptor (GPR) 77, CD 10 and CD 74. The tumor suppressive biomarkers are CD 146, CAV1, $Saa3^{-}$.[10]

DERIVATIVES OF FIBROBLASTS

The fibroblasts are the cells of the connective tissue. They secrete collagen, proteoglycans, glycosaminoglycans, glycoproteins, prostaglandins, matrix metalloproteinases, some cytokines and growth factors. The proteoglycans are the decorin and aggrecan. The glycosaminoglycans are the hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate and keratan sulfate. The glycoproteins namely fibronectin, laminin, entactin, and tenascin.

The collagen fibers are inelastic, constituting approximately 25% of the total body protein. They resist tensile forces and are of approximately 27 different types. The fibroblasts are the major source of collagen. They synthesize and degrade collagen. Intracellular degradation of collagen by fibroblasts is mediated through MMPs (Matrixmetallopreoteinases) and is the important mechanism for physiologic turnover and remodeling. The fibrils are recognized by the fibroblast integrin receptor. Gelatinase A (MMP-2) partially digests the fibrils. The lysosomal enzyme cathepsin further degrades the fibrils within the phagolysosome.[6,7]

MESENCHYMAL STEM CELL-LIKE PROPERTIES OF FIBROBLASTS

Human fibroblasts possess certain phenotypic and immunologic characteristics similar to human mesenchymal stem cells. The fibroblasts from foreskin, lung and mammary tissue are known to express cell surface markers such as CD 73, CD 70, CD 105, CD 29 and CD 44 similar to the mesenchymal stem cells from bone marrow and adipose tissue. They also possess the ability to differentiate into adipocytes, chondrocytes and osteoblasts similar to mesenchymal stem cells. The immunologic characteristics such as the ability to suppress T-cell proliferation, modulate immunophenotype of macrophages and to express Human Leukocyte Antigen– DR isotype upon stimulation with Interferon-gamma are expressed similar to the mesenchymal stem cells.[11]

Upon laterally confined growth on micropatterned substrates, stem cell-like properties confer on fibroblasts. Embedding these cells into collagen-1 matrices of varying densities, mimicking different three-dimensional tissue constrains, the cells regain back their fibroblastic properties. They begin to exhibit features of reduced DNA damage, enhanced cytoskeletal gene expression, enhanced actomyosin contractility, increased matrix deposition and collagen remodeling. Therefore, the fibroblasts rejuvenation can also find its place in regenerative medicine.[12]

FIBROBLASTS IN NORMAL ORAL TISSUES

Fibroblasts play an important role in all the oral tissues. Collagen, the major secretory protein of fibroblasts, is the important component of the extracellular matrix of oral tissues such as dentin, cementum, bone, oral mucosa and salivary glands. Microscopically, the fibroblasts of all tissues appear similar. However, they vary between different connective tissue and also within the same connective tissue both in structure and function.[7]

DENTAL PULP FIBROBLASTS

The fibroblasts are the principal cell type in the dental pulp. They are numerous in the cell-rich zone of the dental pulp and have their origin from the undifferentiated ectomesenchymal cells. They synthesize and secrete collagen and ground substance. The pulp fibroblasts function as immune and inflammatory cell.[7] They are capable of producing pro-inflammatory cytokines and can express adhesion molecules. They synthesize Interleukin (IL)-8, IL-6 and express several Toll-like receptors and Nucleotide-Binding Oligomerisation Domains.[13] Human dental pulp fibroblasts have an

important role in pulp healing following pulpal injury. They express various growth factors, such as fibroblast growth factor-2 (FGF-2) and Vascular endothelial growth factor-2 (VEGF-2) and thus help in angiogenesis, revascularization, nerve-sprouting and regeneration of dentin-pulp complex.[14]

The human dental pulp fibroblasts, in the presence of Gram-positive organisms and upon stimulation by Lipoteichoic acid, produce complement components that in turn activate the complement cascade with the formation of Membrane Attack Complex and generate C5a. The C5a activate the C5aR expression by Pulp progenitor cells and also induce their gradient-dependent migration. Thus, a nonimmune, nonhepatic cell, the pulp fibroblasts, can synthesize complement components.[15,16]

PERIODONTAL LIGAMENT FIBROBLASTS

The periodontal ligament (PDL) fibroblasts exhibit a high turnover rate.[7] The periodontal fibroblasts have the capacity for osteogenic, adipogenic and neurogenic differentiation *in vitro* when there is ectopic expression of human Telomerase reverse transcriptase and Bone morphogenic protein-4 (BMP-4). They express osteoblasts-like characteristics and osteogenic potential when cultured *in vitro*. They also show cementum/PDL-like tissue regeneration *in vivo*. They express osteocalcin, Runxz and periostin.[17-19] These fibroblasts influence alveolar bone remodeling through Osteoprotegerin-Receptor activator of nuclear factor kappa-B ligand (RANKL) pathway and also can alter inappropriate mineralization such as during root ankylosis. They also exhibit the property of contractility that is vital during tooth eruption.[7] The human PDL fibroblasts can also be used as a source for induced pluripotent stem cells (iPSCs) under optimum conditions.[20] PDL fibroblasts acquire myofibroblasts like property during wound healing.[21] Fibroblast contractility plays an important role in posteruptive tooth movement.

Age is an important factor determining the cellular activity of gingival fibroblasts in relation to viability, total protein production and collagen synthesis. All these properties reduce with age.[22] 1 cm^3 of gingival connective tissue has about 200 million fibroblasts that is 5% in volume.[23]

ORAL MUCOSAL FIBROBLASTS

When compared to the dermal fibroblasts, the proliferating capacity of oral mucosal fibroblasts is more. Also, when exposed to Transforming growth factor-β_1 (TGF-β_1), the oral mucosal fibroblasts synthesize more collagen.[24] In regards to the healing characteristics, the oral mucosal fibroblasts exhibit fetal-tissue like phenotype.[25] The iPSCs can be generated from oral mucosal fibroblasts and the fibroblasts can be considered a potential cellular source for regenerative dentistry/medicine.[26]

BONE FIBROBLASTS

The fibroblasts situated in the perivascular and endosteal location, when appropriately stimulated, results in the formation of bone, PDL and cementum.[7]

WOUND HEALING AND FIBROBLASTS

The process of wound healing is different in the skin and oral mucosa. Oral mucosal wounds heal faster and also the formation of hypertrophic scars is rare in oral mucosa, compared to dermal wounds. The probable reason might be the difference in the keratinocyte and fibroblast properties in the two tissues.[27]

In the skin, the fibroblasts enhance the migration and proliferation of the keratinocytes in a time and density-dependent manner.[27,28] The oral keratinocytes proliferate faster than the dermal keratinocytes. However, in both the tissues, the keratinocyte proliferation is influenced by the underlying fibroblasts. Furthermore, the oral fibroblast proliferation rate is greater than the dermal fibroblasts and their doubling time is comparatively less. These cells stop proliferating as they grow to confluent in culture, while the dermal fibroblasts continue proliferation – a probable explanation for rare hypertrophic scar in oral mucosa.[29]

The oral and dermal fibroblasts express similar growth factors but in varying proportions.[29] Buccal mucosal fibroblasts show increased secretion of Keratinocyte growth factor (KGF) and Hepatocyte growth factor (HGF) with an increase in the expression of KGF mRNA and HGF mRNA than the dermal fibroblasts. KGF stimulates re-epithelialization in a paracrine manner. The inflammatory mediators IL-19, IL-20, IL-1 β, IL-6, Tumor Necrosis Factor-alpha (TNF-α) stimulate the expression of KGF in fibroblasts. KGF in turn enhances keratinocyte proliferation.[30] HGF stimulates fibroblasts to produce MMP-1 and also inhibits the expression of TGF-β. Therefore, HGF prevents fibrosis. Thus, increased expression of KGF and HGF in oral fibroblasts is responsible for faster and scarless healing of oral mucosa.[29]

Dermal wound in mice when treated with human gingival fibroblasts and human gingival fibroblasts – conditioned medium, reduce inflammation, enhance angiogenesis

and increase collagen deposition. The net result is faster wound closure. The neutrophils and macrophages at the wound site reduce and the macrophages polarize towards an anti-inflammatory phenotype. Furthermore, the anti-inflammatory cytokine IL-10 increases and the pro-inflammatory cytokine TNF-α reduce. There is also enhanced proliferation of Human Dermal Microvascular Endothelial Cells to form vessel-like structures.[31]

The gingival fibroblasts also express α-actin and microfilaments, suggestive of a myofibroblastic phenotype of the gingival fibroblasts. They exhibit an efficient synthesis of Type III collagen, fibrillin, MMP-2, MMP-9 and Tissue inhibitor of metalloproteinase-2 (TIMP-2).[32]

Oral fibroblasts promote the differentiation of oral keratinocytes. The fibroblasts inhibit spontaneous cell death of the basal layer of the epithelium. The suprabasal layer expresses more CK13 and less CK 14 and CK 19. The fibroblasts also enhance terminal differentiation of the superficial layer and increase the apoptotic rate. The synthesis of the basement membrane components such as laminin and Type IV collagen at the epithelial-collagen biomatrix are also enhanced by the fibroblasts.[33]

Periodontal wound healing is a complex process since it involves the cross-talk between connective tissues, of both hard and soft tissue, as well as the epithelium. The PDL fibroblasts are involved in maintaining and remodeling of the PDL, cementum and the hard tissue, the bone. It is only the PDL fibroblasts that can create new connective tissue attachment to the root surface. Furthermore, these fibroblasts exhibit osteoblasts-like properties. They also synthesize more alkaline phosphatase than gingival fibroblasts. They can synthesize mineralized structures.[34]

After tooth extraction, there is clot formation followed by an inflammatory phase. The PDL fibroblasts then migrate to the extraction socket. The proliferation phase is marked by the proliferation of fibroblasts. The proliferation rate increases 24 h after extraction. They assume a synthetic phenotype marked by increased cellular organelles. At 3rd day, the blood clot undergoes coagulative necrosis and the coagulum is replaced by dense connective tissue later. The PDL fibroblasts differentiate into osteoblasts at around 5th day postextraction. TGF-β and FGF-2 are the important mediators of fibroblasts activation and proliferation. The endosteal and paravascular fibroblasts proliferate at a slower rate and at a later stage than the PDL fibroblasts.[35,36]

WOUND CONTRACTION AND WOUND STRENGTH

Wound contraction depends on Rho-dependent kinase (ROCK). Lysophosphatidic acid acts on fibroblasts through GPR. This in turn activates GTPase, RhoA and ROCK, resulting in assembling of actin filaments. Thus initiates wound contraction.[4]

Wound strength mainly depends on Type I collagen and the principal source of Type I collagen are the fibroblasts. After injury, platelets are activated. The activated platelets secrete PDGF. PDGF in turn activates the fibroblasts. The activated fibroblasts and macrophages secrete TGF-β, enhancing deposition of Type I collagen by fibroblasts. PDGF also acts as a potent mitogen for fibroblasts by activating the Mitogen-Activated Protein kinase and phosphatidyl inositol 3-kinase pathways via the PDGF receptor.[4,37]

FIBROBLASTS IN ORAL SUBMUCOUS FIBROSIS

Oral submucous fibrosis (OSMF) is a debilitating fibrotic disease. It involves fibrosis of the oral mucosa. The fibrosis is associated with arecanut chewing. The oral fibroblasts and their contribution in OSMF are largely explained through various studies.

The byproducts of arecanut are arecoline and arecaidine. Arecoline is cytotoxic and genotoxic to the oral fibroblasts. A high concentration of arecoline changes the fibroblasts more toward a senescent phenotype. The cells appear round with granular nucleus, scanty cytoplasm and cytoplasmic vacuoles. Also, the population doubling time increases and there is an increase in the number of postmitotic subpopulation of fibroblasts. There is repression of glutathione synthetase gene. Glutathione is a potent anti-oxidant. Arecaidine is a potent stimulator of fibroblast proliferation and collagen synthesis.[38-41]

The fibroblasts in OSMF synthesize more collagen and procollagen mRNA than normal oral fibroblasts. Also, the more resistant type I collagen trimer synthesis is increased.[42]

In OSMF, the injury from arecanut chewing leads to synthesis of various inflammatory mediators and growth factors. There is an increase in the synthesis of basic FGF, connective tissue growth factor (CTGF), TGF-β, etc., Arecanut along with TGF-β increases the expression of pro-fibrotic growth factors such as CTGF, Fibronectin1, Endothelin1, collagen stabilizing and maturation genes, Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase 2,

BMP 1, cytoskeletal reorganizing genes, LIM kinase-1, Transgelin, the transcriptional factors GATA binding factor-6, Early Growth Response-2 in oral fibroblasts.[42] The growth factors are responsible for increased collagen fiber synthesis in OSMF. Also, the TGF-β enhances the transdifferentiation of fibroblasts to myofibroblasts. The myofibroblasts are α and γ-Smooth muscle actin (SMA) positive and may be the reason for persistence of fiber contraction in OSMF. Their number increases with severity of the disease.[43-48]

Arecoline augments TIMP-1 mRNA levels leading to increased accumulation of collagen fibers as TIMP is an inhibitor of MMP collagenases. The buccal mucosal fibroblasts contract in a dose and time-dependent manner, upon exposure to arecanut. The possible mechanism behind such contraction lies in the PLC/IP3/Ca^{2+}/Calmodulin and Rhokinase signaling pathways and actin filament proliferation.[49,50]

CANCER-ASSOCIATED FIBROBLASTS

The fibroblasts influence the tumor growth, invasion, progression and metastasis. They provide physical support for the tumor cells and also play a key role in promoting and retarding tumorigenesis in a context-dependent manner.[51] The tumor microenvironment (TME) and the cancer cells influence each other and thus there exists a paracrine mechanism between the tumor cells and the stromal fibroblasts. The TME consists of both immune and nonimmune stromal cells. The fibroblasts are the predominant nonimmune stromal cells.[52,53] They are called as CAFs, tumor-associated fibroblasts or activated myofibroblasts. The CAFs secrete various factors that influence the behavior and prognosis of the tumor. They are positive for α-SMA, S-100A4, nonnuclear GLI-1 (Glioma-associated oncogene homolog-1), TIMP-1.[1,54-58] The origin of these cells can be either the resident fibroblasts or the mesenchymal stem cells from the bone marrow or the oral squamous cell carcinomas (OSCC) cells that have undergone ectomesenchymal transition.[57]

In breast cancer, the genes associated with cell death regulation, stress, hypoxia and carbohydrate metabolism are upregulated. The Tricarboxylic Acid cycle genes are upregulated. There is increased glucose uptake by the tumor cells and lactate is produced by these cells even in the presence of oxygen and functional mitochondria. This process of aerobic glycolysis is called the Warburg effect. The CAFs are more towards the Warburg effect. The lactate produced by the CAFs is utilized by the cancer cells as an adaptation mechanism. Also there is downregulation of genes involved in cell mitosis and cell membrane component synthesis.[59]

There exists a bidirectional interaction between tumor cells and fibroblasts. TGF-β_1 is produced by both the tumor cells and the CAFs. They transdifferentiate the normal oral fibroblasts to CAFs. They also up-regulate the PDPN expression of tumor cells, which in turn increase the synthesis of TGF-β_1. The CAFs also activate the Protein kinase B/AKT, Epidermal growth factor receptor and Extracellular signal reductase kinase in OSCC, eventually increasing the OSCC proliferation and invasion.[55]

The CAFs serve as an important source of Hedgehog (HH) ligands and respond to the HH signaling pathway through nuclear GLI-1 (Glioma-associated oncogene homolog-1) activation. The HH cascade involved in tumor initiation and progression are mediated through paracrine activation/autocrine activation in CAFs. The extracellular vesicles released by the CAFs are shown to play an important role in tumor progression and migration.[54,56]

The CAFs produce HGF, which activates the c-Met signaling in tumor cells and also increases IL-8 expression in them.[58] They show increased expression of IL-1 βR, brain derived nuclear factor, Interferon Regulatory factor-1, IL-6 and Cox-2. All these result in tumor progression and metastasis. The CAFs express IL-1 β, MMP-1, 3 and 2 and membrane Type 1 MMPase. Amongst them, MMP-2 is associated with poor prognosis of OSCC. They enhance intratumoral microvessel density.[52,60,61]

The CAFs also play an important role in lymphangiogenesis and angiogenesis. OSCCs stimulate NOTCH 3 expression in the stromal fibroblasts. The NOTCH positive fibroblasts induce angiogenesis by Human umbilical vein endothelial cell. CAFs regulate lymphangiogenesis through C-Met/PI3K/AKT signaling pathway. They secrete increased levels of HGF than normal oral fibroblasts. The HGFs produced promote proliferation, migration, invasion and tube formation of Human lymphatic endothelial cells, thereby enhancing lymphangiogenesis in OSCCs.[62,63]

The CAFs influence bone penetration of OSCCs through RANKL pathway. They induce the immunosuppressive and protumoral phenotype of tumor-associated macrophages.[64,65]

NICKEL TOLERANCE OF ORAL TISSUES

Oral tissues exhibit increased tolerance to nickel and show less hypersensitivity towards it than the skin. The

possible reason could be the difference in the fibroblasts of both the tissues. Human gingival fibroblasts upon exposure to nickel ions show reduced expression pro-inflammatory NF-κB levels, IL-1 β mRNA and chemokine ligand-2; increase in anti-inflammatory IL-10 than human dermal fibroblasts. The dermal fibroblasts express very high VEGF m RNA levels, therefore increasing vascularization and endothelial permeability and immune cell invasion. Supernatants of dermal fibroblasts inhibit dendritic cell migration, while gingival fibroblasts increase dendritic cell migration. Therefore, the dermal fibroblasts promote proallergenic microenvironment and gingival fibroblasts favor protolerogenic anti-inflammatory microenvironment.[66]

MYOFIBROBLASTS

Giulio Gabbiani and Hartroft, in 1971, first observed fibroblasts like cells, with abundant cytoplasmic filamentous structures and named as myofibroblasts. Myofibroblasts are spindle or stellate-shaped cells that possess bundles of microfilaments in their cytoplasm. They exhibit fibronexus junction between cells and extracellular matrix; focal adhesions and also gap junctions.

Myofibroblasts are present in both normal and disease. In the oral cavity, they are seen in the gingival, palatal mucosa and PDL. They are positive for α-SMA, vimentin, non-muscle myosin, desmin, smooth muscle myosin and extradomain A cellular fibronectin. The contraction of myofibroblasts is calcium mediated. The precursors for the myofibroblasts include fibroblasts, smooth muscle cells, pericytes, resident mesenchymal progenitor cells, adipose tissue cells, bone marrow derived circulating fibrocytes and mesenchymal stem cells. The malignant epithelial and endothelial cells also serve as a source for myofibroblasts through their epithelial to mesenchymal transition.

In normal wound healing, the myofibroblasts undergo apoptosis after the tissue integrity is restored. However, in case of hypertrophic scar, keloid, fibromatoses, TME, they escape the process of apoptosis and persist for a longer period. The survival of the myofibroblasts is influenced by TGF-β_1 and endothelin-1 via the AKT pathway. In wound healing myofibroblasts present in the granulation tissue, synthesize and secrete the extracellular matrix that replaces the provisional matrix. Before evolving into a fully differentiated α-SMA positive myofibroblasts, they acquire the protomyofibroblasts phenotype expressing only β and γ cytoplasmic actins.

Increased number of myofibroblasts in salivary gland neoplasms such as adenoid cystic carcinoma and mucoepidermoid carcinoma indicates poor prognosis. These cells are present more in aggressive odontogenic cysts like odontogenic keratocyst than the less aggressive dentigerous cyst. In OSMF, increased myofibroblasts indicate a higher rate toward malignant transformation. In OSCC, the myofibroblasts play an important role in tumor angiogenesis, thereby contributing towards the spread of the tumor mass.[67-69]

SUMMARY

The fibroblasts are a diverse population of cells with various unique characteristics. They further exhibit unique features in the oral tissues that show differences in various processes than the skin. Their function in different oral tissue, wound healing, OSMF, OSCC and nickel allergy are summarized. Due to their uniqueness, oral fibroblasts can be used extensively in the future studies.

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