Editorial

Oral Submucous Fibrosis: Revised Hypotheses as to its cause

Oral submucous fibrosis (OSF), being a prototype of pathological fibrosis, remains enigmatic as regards its causation. The connective tissue production is permanent and there is no reversal of the condition even after cessation of the habit of areca-nut usage; prime suspect in its causation.[1] The bulk of the connective tissue consists of type-1 collagen[2] and its formation does not appears to be caused by excessive proliferation of fibroblasts.[3] The effect of areca nut extract on *in vitro* fibroblasts varies on a concentration gradient, predominantly suppressing rather than stimulating the growth of the cells.[4] Based on morphological characteristics, the fibroblast population in the diseased mucosa has been classified in to types F1, F2 and F3 with F3 cells producing significantly more collagen than the other two cell types. It was concluded that a change of fibroblast population has occurred in OSF and that this relative increase of F3 cells in humans, could be committed to the production of large quantities of collagen formation in OSF. It has been proposed that fibroblasts are functionally heterogeneous, the composition of any given normal or diseased connective tissue being a consequence in part of its particular mixture of fibroblast subtypes and density. Subtype deletion or amplification can result from selective cytotoxic or mitogenic responses induced by the binding environmental ligands.[5] Against this backdrop, we propose few de-novo attributes, hitherto unreported, and seem to be of relevance in the pathogenesis of OSF; namely the role of autophagy in basic cellular homeostatic process, important to cell fate decisions under conditions of stress and also ECM producing cells (fibroblasts, myofibroblasts and smooth muscle cells) derived from epithelial and endothelial cells through process termed epithelial and endothelial-mesenchymal transition.

Autophagy is a catabolic process by which components of the cytoplasm are degraded in lysosomes. The importance of autophagy to homeostasis and development has been underscored by numerous studies in yeast and mice[6,7] but its importance in human diseases and preclinical models are being elucidated.[8] Relatively few studies have addressed the role of autophagy and dysregulation of its impact on numerous human diseases including cancer and chronic obstructive lung disease.[9-11] Recent advances in our understanding of the pathogenesis of idiopathic pulmonary fibrosis (IPF) have underscored potential links with autophagy.[12] Fibroproliferative disease may affect almost all tissues and organs, including the skin, kidneys, lungs, cardiac and vascular systems, eyes, liver, pancreas and intestine, and tissue fibrosis is a leading cause of morbidity and mortality in humans.

Several lines of evidence suggest that inflammation is necessary to trigger the onset of the fibrotic process, but subsequently plays a minor role in progression of the disease.[13] Fibrogenesis is a physiological process triggered by the onset of inflammation that may lead either to tissue repair or fibrosis depending on the balance between production of ECM proteins and enzymatic degradation. These observations are in conformity with the clinical stages of OSF, where persistent inflammation and subsequent scarring are hallmarks of the disorder. A derangement in the inflammatory-reparative response of the host culminating in fibrous healing and subsequent scarification has been suggested as plausible tissue changes in OSF (Fig. 1).[14]

Several studies have demonstrated that autophagy is not induced in pathologic fibrosis (e.g. IPF), despite the upregulation of several activators of autophagy.[8] *In vitro* experiments demonstrate that the profibrotic mediator, TGF-b1 is likely responsible for decreased autophagy. Treatment of animals with an autophagy inducing agent partially protects against fibrosis. Since there are existing drugs that induce autophagy, this line of investigation offers the possibility of translation to patient care, which could be beneficial in the management of obscure and intractable fibrotic conditions like OSF.

Fig. 1: Clinically advanced OSF with limited inter-incisal clearance (<2 cm), generalized mucosal atrophy

Malignant transformation rate of OSF was found to be in the range of 7-13% and the WHO definition for an oral precancerous condition accords well with this disease.[15] But this assumption does not seems to be straight-forward and there are unsettled issues still remaining, which demands further clarification. One such issue is the development of carcinoma associated with the disease, with no cases of sarcoma reported. This sounds intriguing given the nature of

the disease primarily as a connective tissue disorder. The often cited explanation of the dense fibrosis and less vascularity of the corium in the presence of an altered cytokine activity creating a unique environment for carcinogens from both areca nut and tobacco to act on the epithelium,[16] leading to dysplasia and subsequent malignancy, seem naïve in this context and demand retrospection in the light of recent experimental evidences.

Epithelial-mesenchymal interactions play a critical role in development and cancer progression. Tissue fibroblasts regulate the proliferation and differentiation of epithelial tissues[17,18] and transformed stroma can induce malignancy in lung and mammary epithelia.[19,20] Normal fibroblasts have been reported to convert malignant epithelia in the prostate and skin to morphologically benign lesions.[21,22] Known mediators of epithelial-mesenchymal interactions include members of the TGF-β family.[23] This family is responsible for context-dependent inhibition or stimulation of cell proliferation and neoplastic transformation.[24-26] Thus, TGF-β signaling in fibroblasts modulates the growth and oncogenic potential of adjacent epithelia in selected tissues. TGF-β positive monocytes, fibroblasts and platelets throughout the lamina propria were seen in OSF mucosa and their staining intensity was intense in these cases, compared to normal controls.[27] Furthermore, there was strong positive staining of the epithelium in OSF, whereas the normal epithelium was negative. Endothelium showed positive reaction in the lesional connective tissue and 'T' lymphocytes associated with endothelium, but the specific paracrine factors and signaling pathways involved have not been identified. The TGFBR2 mouse model illustrates that the signaling pathway known to suppress cell-cycle progression when activated in epithelial cells can also have an indirect inhibitory effect on epithelial proliferation when activated in adjacent stromal fibroblasts *in vivo*. Loss of this inhibitory effect can result in increased epithelial proliferation and may even progress to invasive carcinoma in some tissues.[28]

The main fibrogenic cells (fibroblasts, myofibroblasts) may also be derived from non-mesenchymal cells including epithelial and endothelial cells via transformation. Epithelial-to-mesenchymal transition (EMT) or endothelial-to-mesenchymal transition (EndoMT) is a key process in tissue development, carcinogenesis or organ fibrosis; and is characterized by dramatic changes in cell phenotype and function.[29] Epithelial or endothelial cells assume a spindle shape morphology, lose classical cell markers and gain typical fibroblast or myofibroblast markers such as FSP-1, alpha SMA or vimentin, and show the capacity to produce interstitial collagens and fibronectin. All these changes are due to the high plasticity of epithelial and endothelial cells. Therefore, these cell types can be considered as multipotent progenitor tissue, which can display alternative developmental pathways following injury. Recent data on the EMT and EndoMT in animal models and in human primary cells has shown strong contribution to intestinal fibrogenesis.[30,31] A change in fibroblast population in OSF and the relative increase of F3 cells in humans[32] committed to the production of large quantities of collagen, leading to this prototype of pathological fibrosis, could formulate a revised hypothesis as to its causes.

Fibroblasts present in areas of tissue injury generally have been regarded to arise by recruitment from surrounding connective tissue; however this may not be the only source of these cells. Blood - borne fibrocytes, defined as a new leukocyte sub-population, contribute to scar formation and may play an important role both in normal wound repair and in pathological fibrotic responses.[33] Cell surface analysis suggest that these cells share both leukocytic and connective tissue cell features. In addition to expressing the fibroblast components of vementin, collagen and fibronectin, fibrocytes (as it is named) also display the leukocyte common antigen (LCA) CD45 and the hematopoietic stem cell marker CD34.[34,35] Conceivably, fibrocytes that circulate in peripheral blood may comprise a population of pluripotent connective tissue cell precursors that can differentiate along either fibroblast, smooth muscle or myofibroblast lines, depending on the precise microenvironment. The deranged inflammatory-reparative response with resultant 'defective healing' (scarification) reported in OSF[14] points to the role of such contributors in the tissue reaction. Further investigations on the differentiation pathways, secretory profiles and precise tissue origin of blood-borne fibrocytes should increase significantly our understanding of the role of this cell population in tissue repair responses and in the causation of fibrotic disorders like OSF.

REFERENCES

1. Seedat HA, van Wyk CW. Submucous fibrosis (SF) in ex-betel nut chewers: a report of 14 cases. J Oral Pathol 1988;17:226-229.
2. van Wyk CW, Seedat HA, Phillips VM. Collagen in submucous fibrosis: an electron-microscopic study. J Oral Pathol Med 1990;19: 182-187.
3. Meghji S, Scutt A, Harvey W, Canniff JP. An in vitro comparison of human fibroblasts from normal and oral submucous fibrosis tissue. Arch Oral Biol 1987;32:213-215.
4. van Wyk CW, Olivier A, de Miranda CM, van der Bijl P, Grobler-Rabie AF. Observations on the effect of areca nut extracts on oral fibroblast proliferation. J Oral Pathol Med 1994;23:145-148.
5. Narayanan AS, Page RC, Kuzan F. Collagens synthesized in vitro by diploid fibroblasts obtained from chronically inflamed human connective tissue. Lab Invest 1978;39:61-65.
6. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000;290:1717-1721.
7. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, et al. The role of autophagy during the early neonatal starvation period. Nature 2004;432:1032-1036.
8. Patel AS, Lin L, Geyer A, Haspel JA, An CH, Cao J, et al. Autophagy in idiopathic pulmonary fibrosis. PLoS One 2012;7:e41394.

9. Kundu M, Thompson CB. Autophagy: basic principles and relevance to disease. Annu Rev Pathol 2008;3:427-455.
10. Monick MM, Powers LS, Walters K, Lovan N, Zhang M, Gerke A, et al. Identification of an autophagy defect in smokers' alveolar macrophages. J Immunol 2010;185:5425-5435.
11. Parkhitko A, Myachina F, Morrison TA, Hindi KM, Auricchio N, Karbowniczek M, et al. Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. Proc Natl Acad Sci USA 2011;108:12455-12460.
12. Korfei M, Ruppert C, Mahavadi P, Henneke I, Markart P, Koch M, et al. Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2008;178:838-846.
13. Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008;214:199-210.
14. Rajendran R, Vijayakumar T, Vasudevan DM. An alternative pathogenetic pathway for oral submucous fibrosis (OSMF). Med Hypotheses 1989;30:35-37.
15. Rajendran R. Oral submucous fibrosis: etiology, pathogenesis, and future research. Bull World Health Organ 1994;72:985-996.
16. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. Oral Oncol 2006;42:561-568.
17. Ronnov-Jessen L, Petersen OW, Bissell MJ. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. Physiol Rev 1996;76:69-125.
18. Cunha GR, Bigsby RM, Cooke PS, Sugimura Y. Stromal-epithelial interactions in adult organs. Cell Differ 1985;17:137-148.
19. Nakamura T, Matsumoto K, Kiritoshi A, Tano Y, Nakamura T. Induction of hepatocyte growth factor in fibroblasts by tumor-derived factors affects invasive growth of tumor cells: in vitro analysis of tumor-stromal interactions. Cancer Res 1997;57:3305-3313.
20. Barcellos-Hoff MH, Ravani SA. Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells. Cancer Res 2000;60:1254-1260.
21. Hayashi N, Cunha GR. Mesenchyme-induced changes in the neoplastic characteristics of the Dunning prostatic adenocarcinoma. Cancer Res 1991;51:4924-4930.
22. Cooper M, Pinkus H. Intrauterine transplantation of rat basal cell carcinoma as a model for reconversion of malignant to benign growth. Cancer Res 1977;37:2544-2552.
23. Thesleff I, Vaahtokari A, Partanen AM. Regulation of organogenesis. Common molecular mechanisms regulating the development of teeth and other organs. Int J Dev Biol 1995;39:35-50.
24. Massague J, Blain SW, Lo RS. TGF-beta signaling in growth control, cancer, and heritable disorders. Cell 2000;103:295-309.
25. Oft M, Peli J, Rudaz C, Schwarz H, Beug H, Reichmann E. TGF-beta1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells. Genes Dev 1996;10:2462-2477.
26. Yang YA, Dukhanina O, Tang B, Mamura M, Letterio JJ, MacGregor J, et al. Lifetime exposure to a soluble TGF-beta antagonist protects mice against metastasis without adverse side effects. J Clin Invest 2002;109:1607-1615.
27. Haque MF, Harris M, Meghji S, Barrett AW. Immunolocalization of cytokines and growth factors in oral submucous fibrosis. Cytokine 1998;10:713-719.
28. Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, et al. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 2004;303:848-851.
29. Speca S, Giusti I, Rieder F, Latella G. Cellular and molecular mechanisms of intestinal fibrosis. World J Gastroenterol 2012;18: 3635-3661.
30. Flier SN, Tanjore H, Kokkotou EG, Sugimoto H, Zeisberg M, Kalluri R. Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis. J Biol Chem 2010;285:20202-20212.
31. Rieder F, Kessler SP, West GA, Bhilocha S, de la Motte C, Sadler TM, et al. Inflammation-induced endothelial-to-mesenchymal transition: a novel mechanism of intestinal fibrosis. Am J Pathol 2011;179:2660-2673.
32. de Waal J, Olivier A, van Wyk CW, Maritz JS. The fibroblast population in oral submucous fibrosis. J Oral Pathol Med 1997;26:69-74.
33. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. Mol Med 1994;1:71-81.
34. Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 1984;133:157-165.
35. Katz FE, Tindle R, Sutherland DR, Greaves MF. Identification of a membrane glycoprotein associated with haemopoietic progenitor cells. Leuk Res 1985;9:191-198.

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