Journal of Dental Sciences (2015) 10, 323–333

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

ORIGINAL ARTICLE

Expression of hypoxia inducible factor-1α and vascular endothelial growth factor-C in human chronic periodontitis

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Received 30 May 2014; Final revision received 26 August 2014
Available online 3 July 2015

KEYWORDS
hypoxia;
hypoxia-inducible factor-1α;
periodontitis;
vascular endothelial growth factor-C

Abstract *Background/purpose:* Evidence shows that there is a relationship between hypoxia and inflammatory response in periodontitis. Hypoxia-inducible factor (HIF)-1α is a major regulator of energy homeostasis and cellular adaptation to low oxygen stress. Although experimental results demonstrate an association between HIF-1α and vascular endothelial growth factor (VEGF)-C in tumor angiogenesis, the role of HIF-1α and VEGF-C in the pathogenesis of periodontitis is still ambiguous. So far, limited attention has been given to the role of hypoxia and VEGF-C in periodontitis. The present study aimed to investigate the expression and distribution of HIF-1α and VEGF-C in gingival tissue samples from patients with different stages of chronic periodontitis and healthy individuals.
Materials and methods: A total of 56 samples were involved in this study, including moderate chronic periodontitis ($n = 20$), advanced chronic periodontitis ($n = 20$), and healthy control tissues ($n = 16$). The gingival specimens were stained with hematoxylin and eosin for histopathology. The expression of HIF-1α and VEGF-C in gingival tissues was detected by immunohistochemical staining.
Results: HIF-1α and VEGF-C were found in gingival tissues from patients with different stages of chronic periodontitis as well as healthy control tissues. HIF-1α protein was expressed mainly in the epithelial layer of gingival tissues, and VEGF-C protein was mostly located in the connective tissue papilla of gingival tissues. Compared with healthy controls, the expression of HIF-1α and VEGF-C in chronic periodontitis groups was significantly higher ($P < 0.01$), and the density of HIF-1α and VEGF-C in advanced chronic periodontitis group was even significantly higher than that in the moderate chronic periodontitis group ($P < 0.01$).

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http://dx.doi.org/10.1016/j.jds.2014.09.004

Conclusion: Our results suggest that the expression of HIF-1α and VEGF-C increased with severity of periodontitis. So, we conclude that HIF-1α may play an important role in the pathophysiology of human periodontitis and may be related to the function of VEGF-C during periodontitis.

Introduction

Periodontitis is a common chronic infectious disease characterized by the destruction of tooth-supporting tissue, finally leading to tooth loss.[1] In periodontitis, both oxygen supply and demand in the periodontium may be significantly shifted, leading to inflammation-associated tissue hypoxia and metabolic acidosis, disturbing microcirculation and increasing leukocyte infiltration, particularly myeloid cells such as polymorphonuclear leukocytes (PMNs) and monocytes.[2] Low-oxygen tolerance is supported by an adaptive response that includes a coordinate shift in metabolism and the activation of a transcriptional program that is driven by the hypoxia-inducible factor (HIF) pathway.[3] A few affected pathways generally characterize HIF-mediated adaptation responses, including upregulation of angiogenic, erythropoietic, and glycolytic transcripts.[4] Experiments have shown that hypoxia appears to stimulate both innate and adaptive immune responses.[5] Results by Motohira et al[6] showed that hypoxia could stimulate the periodontal ligament cells to produce vascular endothelial growth factor (VEGF), interleukin (IL)-6, and prostaglandin (PG)E_2, which could result in the resorption of alveolar bone in periodontitis.

HIF-1α is a transcription regulatory factor that is encoded by activated gene when organization is under hypoxic.[7] HIF-1α is a major regulator of energy homeostasis and cellular adaptation to low-oxygen stress.[8] HIF-1 functions as a master regulator of cellular and systemic homeostatic response to hypoxia by activating the transcription of many genes, including those involved in energy metabolism, angiogenesis, and apoptosis, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia.[9] HIF-1 thus plays an essential role in embryonic vascularization, tumor angiogenesis, and pathophysiology of ischemic disease.[10] At the molecular level, HIF-1α binds to hypoxia response elements (HREs) in the promoters of target genes, such as VEGF and erythropoietin, and promotes their expression.[11] Hypoxia through activation of HIF-dependent transcription of proangiogenic factors such as VEGF is a major mechanism behind tumor angiogenesis. Experiments showed that hypoxia-induced VEGF expression in both neuroblastoma and breast cancer cell lines is primarily driven by HIF-2α at prolonged hypoxia, whereas HIF-1α is the major VEGF inducer during acute hypoxic conditions.[12] So far, knowledge is limited in the role of expression of HIF-1α and VEGF in the pathogenesis of periodontitis.

VEGF produced by many normal and tumor cells plays a key role in regulating normal and abnormal angiogenesis. VEGF is an important growth factor proven to be specific and critical for a mitogen for vascular endothelial cells derived from arteries, veins, and lymphatics, but it is devoid of consistent and appreciable mitogenic activity for other cell types.[13] It binds to endothelial cell surface receptors and activates various functions of the cell including angiogenesis.[14] The periodontal vasculature is affected profoundly during the progression of periodontitis.[15] VEGF primarily stimulates endothelial cell proliferation, chemotaxis, migration, and survival, as well as increasing microvascular permeability and the secretion of proteolytic enzymes.[16,17] VEGF-C increases vascular permeability, which can contribute to the formation of inflammation in the early stages of periodontal disease. VEGF-C seems to be crucial for lymphangiogenesis during periodontal disease development, and upregulation of VEGF-C in recruited immune cells is likely important for the growth of lymphatic vessels.[18] The role of VEGF-C in the pathogenesis of periodontitis is still ambiguous. Although Mkonyi et al[19] have already observed that increased numbers of immune cells expressed VEGF-C in the gingiva after infection, along with upregulation of IL-1β and tumor necrosis factor (TNF)-α at protein levels. On the contrary, Ozcelik et al[20] demonstrated that the expression of VEGF-A and VEGF-C was significantly lower in patients with scleroderma, when compared with the controls. Although higher levels of inflammatory infiltrate and microvessel density were found in the gingival biopsy samples.[20]

Oxygen metabolism has a critical role in maintaining the normal physiological functions of periodontal tissues.[21] During the progression of periodontitis, the status of ischemia and hypoxia in tissue induced the expression of HIF-1α, which can accelerate the expression of VEGF-C. Moreover, VEGF-C is a receptor activator of nuclear factor κB ligand (RANKL) target gene in osteoclasts, and functions as an autocrine factor regulating osteoclast activity. Thus, VEGF-C plays a prominent role in the process of enhancing the resorptive activity of osteoclasts.[22] Therefore, the present study aimed to investigate the expression and distribution of HIF-1α and VEGF-C in human gingival tissues at different stages of chronic periodontitis.

Materials and methods

Gingival tissue collection

The gingival biopsies were obtained from the patients attending the Department of Periodontology, Liwan Stomatological Teaching Hospital of Jinan University,