Hypoxia Inducible Factor 1α as Key Factor in Wound Healing Post Tooth Extraction: an Overview

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Abstract

HIF-1α play an essential role during inflammation in wound healing. They promote the recruitment and proliferation of fibroblasts, and express some of the key growth factors that stimulate angiogenesis, but its role in healing remains incompletely understood. In wounds caused by tooth extraction, there will be a repair process that includes soft tissues and hard tissues of the oral cavity. Healing tissue requires new blood vessel growth, via angiogenesis and vasculogenesis, to re-establish the vasculature necessary to deliver oxygen and nutrients to the injured tissue. For the alveolar bone formation, the role of Bone morphogenetic protein (BMP) is needed to accelerate the formation of osteoblasts. It has been shown that hypoxia stimulation regulates bone formation, maintenance, and repair. BMP plays important roles in osteoblastic differentiation and bone formation. Modulating HIF-1α expression may improve future management to accelerate wounds healing in soft and hard tissue post tooth extraction.

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Introduction

Wounds are injuries to the body that typically involves laceration or breaking of a membrane such as the skin or mucous membrane and usually damage to underlying tissues. This situation can be caused by sharp or blunt object trauma, changes in temperature, chemicals, explosions, electric shock, animal bites and tooth extraction. Some of the effects that arise when wounds occur are loss of whole or partial organ function, sympathetic stress response, bleeding and blood clotting, bacterial contamination and cell death.[1] Wounds to the body include acute and chronic wounds. Chronic wounds fail to heal, despite the use of current therapies, because they are stalled in the early inflammatory state within the wound healing stages. Acute wounds undergo a complex interactive process involving a variety of cell types that leads to a healed wound.[2-3]

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Wound healing is a complex biological process which results in the restoration of tissue integrity. Tissue repair is a simple linear process in which the growth factors cause cell proliferation, thus leading to an integration of dynamic changes that involve soluble mediators, blood cells, the production of the extracellular matrix, and the proliferation of parenchymal cells. Cell and biochemical events in wound repair can be divided into the following stages: inflammatory reaction, cell proliferation and synthesis of the elements which make up the extracellular matrix, and the posterior period, called remodeling.[4-5]

Inflammation

The first event occurring after injury is the formation of a blood clot by activated platelets. The blood plug will be composed of various cell types including platelet, red and white blood cells. The initial plug is stabilised by fibrin fibres and will be a scaffold for the various infiltrating cells. The first inflammatory cells recruited are the neutrophils. They infiltrate massively the wound during the first 24h post injury attracted by the numerous inflammatory cytokines produced by the activated platelets, endothelial cells, as well as by the degradation products from pathogens. Neutrophils enter apoptosis soon after infiltrating the wound and the release of cytokines during

this apoptotic process is an important component in macrophage recruitment. Macrophages infiltrate the wound massively 2 days post injury and exacerbate at this stage an intense phagocytic activity.[6-7]

Proliferative Phase

Epithelialization, angiogenesis, granulation tissue formation, and collagen deposition are the principal steps in this building portion of wound healing. Epithelialization occurs early in wound repair. If the basement membrane remains intact, the epithelial cells migrate upward in the normal pattern. The epithelial progenitor cells remain intact below the wound (in skin appendages), and the normal layers of epidermis are restored in 2 to 3 days. If the basement membrane has been destroyed, then epithelial cells located on the skin edge begin proliferating and sending out projections to re-establish a protective barrier. Angiogenesis, stimulated by TNF-α, is marked by endothelial cell migration and capillary formation. The migration of capillaries into the wound bed is critical for proper wound healing. The granulation phase and tissue deposition require nutrients supplied by the capillaries, and failure of this to occur results in a chronically unhealed wound. Epithelial cells located on the skin edge begin proliferating and sending out projections to reestablish a protective barrier against fluid losses and further bacterial invasion. The stimulus for epithelial proliferation and chemotaxis is epidermal growth factor (EGF) and TGF-α produced by activated platelets and macrophages. Epithelization begins shortly after wounding and is first stimulated by inflammatory cytokines. IL-1 and TNF-α upregulate keratinocyte growth factor (KGF) gene expression in fibroblasts. In turn, fibroblasts synthesize and secrete KGF-1, KGF-2, and IL-6, which simulate neighboring keratinocytes to migrate in the wound area, proliferate, and differentiate in the epidermis. It has been shown that, for humans, KGF-2 is most important for directing this process.[8-12] The final part of the proliferative phase is granulation tissue formation. Fibroblasts migrate into the wound site from the surrounding tissue, become activated, and begin synthesizing collagen and proliferate. Platelet-derived growth factor (PDGF) and EGF are the main signals to fibroblasts and are derived from platelets and macrophages. PDGF expression by fibroblasts is amplified by autocrine and paracrine signaling. Fibroblasts already located in the wound site (termed “wound fibroblasts”) will begin synthesizing collagen and transform into myofibroblasts for wound contraction, they have less proliferation compared with the fibroblasts coming in from the wound periphery. In response to PDGF, fibroblasts begin synthesizing a provisional matrix composed of collagen type III, glycosaminoglycans, and fibronectin.[8,13-16]

Maturation Phase

Also called the remodeling stage of wound healing, the maturation phase is when collagen is remodeled from type III to type I and the wound fully closes. The cells that had been used to repair the wound but which are no longer needed are removed by apoptosis, or programmed cell death. When collagen is laid down during the proliferative phase, it is disorganized and the wound is thick. During the maturation phase, collagen is aligned along tension lines and water is reabsorbed so the collagen fibers can lie closer together and cross-link. Cross-linking of collagen reduces scar thickness and also makes the skin area of the wound stronger. Generally, remodeling begins about 21 days after an injury and can continue for a year or more. Even with cross-linking, healed wound areas continue to be weaker than uninjured skin, generally only having 80% of the tensile strength of unwounded skin.[1,4,6]

The stages of wound healing are a complex and fragile process. Failure to progress in the stages of wound healing can lead to chronic wounds. Factors that lead up to chronic wounds are venous disease, infection, diabetes and metabolic deficiencies of the elderly. Careful wound care can speed up the stages of wound healing by keeping wounds moist, clean and protected from reinjury and infection.

The role of HIF-1α in wound healing post tooth extraction

In wounds caused by tooth extraction, there will be a repair process that includes soft tissues and hard tissues of the oral cavity. Soft tissue consist of gingival and hard tissue is the alveolar bone. The wound healing consists of several processes, angiogenesis and vasculogenesis to reestablish blood supply to the injured tissue; fibroplasia, the formation of fibrous tissue, which contributes to granulation tissue development; re-epithelialization driven by keratinocyte proliferation and migration; and wound contraction mediated by myofibroblasts.

Healing tissue requires new blood vessel growth, *via* angiogenesis and vasculogenesis, to re-establish the vasculature necessary to deliver oxygen and nutrients to the injured tissue. Hypoxia, through the accumulation of HIF-1α activates several angiogenic growth factor genes including VEGF.[1, 17]

HIF-1 is a member of the basic helix-loop-helix superfamily of transcription factors. Only active as a heterodimer, HIF-1 is composed of 2 subunits: HIF-1α and HIF-1β. Whereas the HIF-1β protein is readily found in all cells, HIF-1α is virtually undetectable in normal oxygen conditions. When cells are subjected to hypoxic conditions (1% oxygen), levels of the HIF-1α subunit are rapidly increased. Instead of acting on HIF-1α transcription or translation, hypoxia increases HIF-1α protein levels by inhibiting the rapid ubiquitination and degradation of HIF-1α by the proteasome. An elegant series of studies has shown that when oxygen is present, HIF-1α is modified by prolyl hydroxylation, which permits the binding of the von Hippel-Lindau protein (pVHL), a recognition component of the E3 ligase complex. This promotes the ubiquitin degradation of HIF-1.[18-21] Under normoxia, PHD hydroxylates proline residues in the oxygen dependent degradation (ODD) domain of HIF-1α, thereby promoting its binding to von Hippel Lindau protein (pVHL), leading to active ubiquitination and degradation with a half-life. Under hypoxic conditions, HIF-1a accumulates and induces expression of several angiogenic genes through binding to hypoxia responsive elements (HRE) in the promotor region. Due to the absolute importance of HIF-1a within this context, it has been the target of therapeutic strategies aiming at either inducing vessel growth.[18, 22-28]

HIF-1α is the critical transcription factor that regulates adaptive responses to hypoxia. HIF-1α stability and function is regulated by oxygen-dependent soluble hydroxylases targeting critical proline and asparaginyl residues. HIF-1α plays a pivotal role in wound healing, and its expression in the multistage process of normal wound healing has been well characterized. In essence, HIF-1α is necessary for expression of multiple angiogenic growth factors, cell motility, and recruitment of endothelial progenitor cells.[29-32] In addition to hydroxylation by PHDs, HIF-1a is also regulated by factor inhibiting HIF-1 (FIH) in an oxygen-dependent manner. HIF-1α has two transactivation domains, TAD-N and TAD-C, which bind to coactivators, such as CREP-binding protein (CBP) and p300. In the presence of oxygen, FIH hydroxylates an asparagine residue within TAD-C, preventing interaction between HIF-1α and the transcriptional coactivators. This results in decreased transcriptional ability of HIF-1. Regulation by FIH is believed to be a secondary regulatory mechanism for HIF-1a proteins that evade the primary PHD-mediated regulation. Levels of HIF-1a protein increase exponentially as oxygen concentration declines. Under hypoxic conditions, HIF-1a degradation is limited by reduced presence of oxygen cofactor and by the depletion of PHDs and FIH. Stabilized HIF-1 is then activated by phosphorylation, allowing dimerization of HIF-1awith HIF-1b. The active transcription factor translocates to the nucleus where it binds to the cis-acting hypoxia regulatory element in the promoter region of HIF-inducible genes along with transcriptional coactivators p300 and CBP.[33-37]

Healing tissue requires new blood vessel growth, *via* angiogenesis, vasculogenesis, and arteriogenesis to re-establish the vasculature necessary to deliver oxygen and nutrients to the injured tissue. Mitochondria are the largest consumers of cellular O2 and are likely candidates for the location of a cellular oxygen sensor. Mitochondria are the source of ATP and maintain oxygen homeostasis at both the systemic and cellular levels. Initial evidence supporting mitochondria as an oxygen sensor came with the discovery that mitochondrial-depleted Hep3B cells fail to respire and activate mRNAs of erythropoietin, glycolytic enzymes or VEGF during hypoxia.[38-40] The three processes involved in the formation of new blood vessels are referred to angiogenesis, vasculogenesis, and arteriogenesis. Early studies postulated that as the cell mass expands, angiogenic factors would be released, but the triggers for the so-called angiogenic switch, a phenomenon in which a tumor progresses from a non-angiogenic to an angiogenic phenotype, remained obscure. The hypoxic microenvironment caused by the increased oxygen consumption of hyperplasia and/or hypertrophy and the decreased oxygen delivery due to the increase in diffusion distance was assumed to contribute to the angiogenic switch. An important link between hypoxia and angiogenesis was the discovery that the expression of the potent vascular endothelial

growth factor (VEGF) was induced by hypoxia. Angiogenesis is essential for development, wound healing, tissue or organ regeneration, but it is also part of pathological processes, such as cancer and certain retinopathies. It is an intricate multistep and temporally ordered process that involves a great number of genes, modifiers and pathways. Many of these genes are directly induced by HIF-1a, such as nitric oxide synthases, angiogenic and vascular growth factors (VEGF) and genes regulating matrix metabolism (urokinasetype plasminogen activator receptor; uPAR). Others are independently regulated by hypoxia and might be influenced by secondary mechanisms, but a central role of HIF-1α is well established: it is required for proper vascularization of the mouse embryo[41-44] and for coordinating the complex cooperation of angiogenic growth factors. This was demonstrated in transgenic animals where the overexpression of VEGF alone led to hypervascularity with hyperpermeability in the skin, whereas in contrast the vessels induced by a stable HIF-1α transgene driven by the same skin specific promoter were not leaky. The individual steps of angiogenesis require distinct changes to a variety of cells. Endothelial cells have to be transformed from a stable growth-arrested state to a plastic proliferative phenotype. The basement membrane has to be digested and the extracellular matrix remodeled so that the endothelial cells are able to migrate. HIF-1α signaling pathways have been demonstrated to influence factors such as uPAR, collagen prolyl-4-hydroxylases, matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases.[45-47] The hypoxic signaling is not restricted to mere target gene upregulation. A well-studied example of how multifaceted the hypoxic response can be is VEGF. VEGF is directly induced by HIF-1α and biological activity is further increased by the hypoxic upregulation of VEGF receptor-1 (VEGFR-1/Flt-1). VEGF mRNA stability is also increased under hypoxic conditions. Deletion of HIF-1α in endothelial cells disrupts an autocrine signaling loop for hypoxic induction of VEGFR-2 by VEGF signaling through VEGFR-1, which ultimately leads to impaired vascularization of xenografts. Despite the multitude of insights into individual molecular pathways involved in angiogenesis, such as increased migration and tube formation, which may be predicted to induce angiogenesis in vitro, these analyses in isolated systems clearly have their limitations, especially when the large scale of interconnections and complexity involved in the process of angiogenesis in vivo are considered.[48-54]

Wound healing after tooth extraction also involves hard tissues, which is alveolar bone. For the alveolar bone formation, the role of Bone morphogenetic protein (BMP) is needed to accelerate the formation of osteoblasts. It has been shown that hypoxia stimulation regulates bone formation, maintenance, and repair. BMP plays important roles in osteoblastic differentiation and bone formation. Hypoxia stimulation increased mRNA and protein levels of BMP-2 by qPCR, Western blot and ELISA assay in osteoblastic cells MG-63, hFOB and bone marrow stromal cells M2-10B4. Integrin-linked kinase (ILK) inhibitor (KP-392), Akt inhibitor (1L-6-hydroxymethyl-chiro-inositol-2-[(R)-2-O-methyl-3-O-octadecylcarbonate]) or mammalian target of rapamycin (mTOR) inhibitor inhibited the potentiating action of hypoxia. Exposure to hypoxia increased the kinase activity of ILK and phosphorylation of Akt and mTOR. Hypoxia enhances BMP-2 expression in osteoblasts by an HIF-1alpha-dependent mechanism involving the activation of ILK/Akt and mTOR pathways.[55]

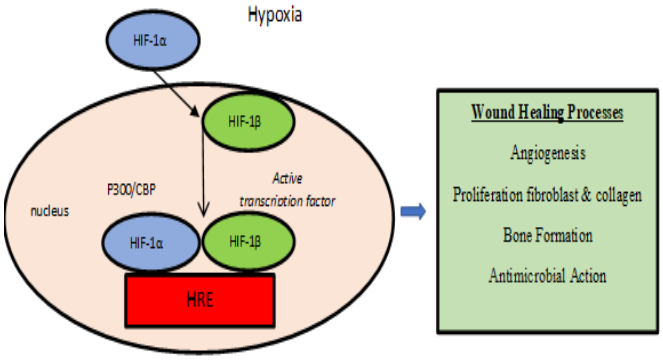

Figure 1. Under hypoxia, HIF-1α is stabilized and binds to the HIF-1b subunit to form the active transcription factor HIF-1, HIF-1 directly regulates genes involved in wound healing process.

Recruitment of HIF-1 at sites of injury

Oxygen tension in healthy tissues is 17.5–63 mm Hg or 2.5-9% oxygen, much lower levels (<1% oxygen) are present in wounds. Many bacterial pathogens survive well and proliferate under anaerobic conditions, thus phagocytes must be adapted to function effectively in microbial eradication in the same

microenvironments. Genetic experiments in which mouse phagocyte HIF-1α levels were manipulated revealed the pivotal role of the transcriptional control pathway in phagocyte bactericidal activity. Macrophages from mice deficient in HIF-1α show diminished capacity to kill Gram-negative and Grampositive bacteria when compared to macrophages from wild-type littermates and HIF-1α deficient animals were more susceptible to invasive skin infection produced by Streptococcus pyogenes. While HIF-1α deletion (or overexpression through vHL elimination) did not alter phagocyte production of reactive oxygen species through the respiratory burst, the expression of a number of other molecular effectors of host innate defense was significantly correlated to HIF-1α levels. These included cathelicidin antimicrobial peptides, the granule proteases cathepsin G and elastase, tumor necrosis factor-α (TNFα), and nitric oxide (NO) produced by the inducible NO synthetase (iNOS). In addition to its microbicidal properties, NO is known to stabilize HIF-1α by redistributing intracellular oxygen and inhibit prolyl hydroxylase activity.[56-59] In response to inflammatory stimuli, local vascular permeability increases, resulting in the increased delivery of effector cells and nutrients to the inflamed tissue. The combination of reduced circulation at the site of inflammation and increased metabolic demand from infiltrating immune cells and pathogens eventually leads to the local depletion of O2, resulting in hypoxia. Hypoxia and associated HIF activation have been observed in tissue specimens from patients with inflammatory conditions.[60]

HIF Regulation in Inflammatory Cells

HIF promotes NF-kB activity in macrophages, neutrophils, and nonimmune cells. Hypoxia inhibits PHD1 activity and thereby IKK hydroxylation, resulting in IKK activation and phosphorylation of IkB. Subsequent IkB degradation liberates NF-kB from the cytoplasm, resulting in the transcription of downstream target genes, including inflammatory cytokines. NF-kB signaling in activated macrophages directly regulates Hif-1α transcription. Macrophages lacking IKKb cannot stabilize HIF1-α after hypoxic or microbial challenge and exhibit decreased HIF target gene expression. However, activation of NF-kB alone is insufficient for HIF-1α stabilization, indicating that maximal HIF-1α accumulation depends on both transcriptional regulation by NF-kB and posttranslational regulation by hypoxia. This coordinated response suggests a mechanism for graded HIF-1α expression in immune cells, in which maximal HIF1α activity is induced in cells located at the site of most severe inflammation, where hypoxic and inflammatory stresses are simultaneously present.[60-63]

Research in progress

Periapical lesion is an inflammatory and immune response caused by anaerobic polymicrobial infection of the dental pulp and root canals. This inflammatory condition damages tissues and characteristically results in the formation of granulation tissue and loss of the alveolar bone surrounding the dental root apex. Recent study using dimethyloxalylglycine (DMOG) and adenovirusinduced constitutively active HIF-1α (CA-HIF1A), attenuated periapical bone loss via downregulation of NF-κB and osteoclastogenesis (Acp5, Ctsk). However, the effect of both DMOG and CAHIF1A on the expression of osteogenic genes, including Runx2, Sp7 and Atf4 was marginal. Collectively, HIF mediated attenuation of periapical bone loss seems to be dependent on inhibition of osteoclasts rather than promotion of osteoblasts and bone formation. The effect of activated HIF-1 on its downstream molecules is not clear. The explanation for this unresponsiveness is unknown and needs to be determined in further well-designed studies. Additional studies are required to further elucidate the role of HIF in this chronic inflammation. In particular, the HIF-1/HIF-2 switch in immunomodulation, HIF altered cell metabolism, and the molecular adaptive responses, including regulation of mitochondrial reactive oxygen species (ROS) in periapical lesions are of interest.[63]

HIF-1 signalling contributes to hypoxia-induced activation of EpSCs at the wound edge, which accelerates wound healing. Injection of exogenous FG-4592 (a prolyl hydroxylase inhibitor that stabilizes HIF-1α in normoxia), simulated the positive effect of hypoxia on the switching of Epidermal stem cells (EpSCs) from a quiescent state to an activated state. Understanding this mechanism may enable the development of a novel and precise wound care strategy.[64]

Conclusions

In present review we have discussed that wound healing involves various processes including inflammation, angiogenesis, vasculogenesis and arteriogenesis, which are all regulated by HIF-1α target genes, the stabilization of HIF-1α by PHD inhibition can be used to effectively treat tissue injuries and wounds. HIF-1α are also upregulated under inflammatory conditions, suggesting their role in maintaining homeostatic conditions and protecting against cellular inflammation in soft and hard tissue post tooth extraction.

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Declaration of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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