Angiogenesis: the fundament of osseous regeneration

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Abstract

Introduction
Bone formation, osseous regeneration and the associated physiological and pathologically altered angiogenesis are not only of vital importance in fracture repair but also play a constantly growing role in the diagnosis and treatment of chronically infected bone or osteonecrosis. The jaw and especially the mandible with its exceptional vascularization in the human body are prone to these diseases. The understanding of the molecular mechanisms of osteogenic-angiogenic coupling is the fundament of a sufficient diagnosis and therapy.

The aim of this critical review is to discuss angiogenesis from the description of the angiogenic cascade with its regulative mechanisms to the demands of regenerative medicine. We follow the process of bone formation under the influence of a sufficient angiogenesis with special regard to the key protagonist vascular endothelial growth factor, the osteogenic-angiogenic interface and their interaction.

Conclusion

The potential of angiogenesis as a pivotal factor in many areas of tissue maintenance and regeneration is obvious, and angiogenesis will play a significant role in developing therapeutically regenerative concepts.

Introduction

Angiogenesis

Angiogenesis is defined as the growth of new blood vessels on the base of existing vascular structures, whereas vasculogenesis characterizes the development of a new vessel system by sprouting, differentiating endothelial cells.

These pivotal processes mark the starting point of any form of vascular development or regeneration processes. In malignant growth, the appearance of new vessels can be a determinant of progression and poor prognosis, where a lack of vascularisation inevitably leads to loss of functional efficiency and tissue necrosis from the brain to the limbs.

The fine-tuned balance of vascular- and angiogenesis is controlled by many growth and transcription factors.

This process is often triggered by a signalling cascade that occurs upon ligand–receptor binding between vascular endothelial growth factor (VEGF) and its receptors [VEGFR1/Fit-1, VEGFR2/KDR]. These receptors are expressed by endothelial cells that line the blood vessels. The most potent trigger for angiogenesis is hypoxia.

Angiogenesis, the complex physiological sequence of vasodilatation, degradation of basement membrane, endothelial cell migration, chemotaxis, increasing vascular permeability and eventually endothelial cell proliferation and vessel formation is regulated by VEGF(R). Osteogenic and angiogenic cascade are inseparably interconnected by the functional alliance of endothelial cells, osteoblasts and growth factors.

VEGF in angiogenesis

The healthy vasculature is one prerequisite of every regenerative process. It is governed by many interacting signalling pathways, and the VEGF pathway is considered as one of the most crucial ones; it is certainly the most investigated and understood one.

The first observed function of VEGF was its ability to enhance the permeability of tumour vasculature. Later, its power as endothelial mitogen was described: VEGF attracts endothelial cells and promotes their differentiation, proliferation and survival. Today, the role of VEGF as a keeper and promoter of vascular homoeostasis in the organism has become clear.

The striking significance of VEGF becomes obvious considering the fact that the first definitive marker protein on ripening endothelial cells in the yolk sac is the VEGF receptor 2 (VEGFR2 or Flk-1). Under the influence of VEGF-A, these endothelial progenitors marked by VEGFR2 form areas of blood islands; clusters of initial erythroblasts lined by the endothelial precursors characterize these formations.

VEGF-A exists in several splice variants, with different characteristics; VEGF120 for example is thought to be an especially diffusible isoform because of the lack of a heparin-binding domain.

Three relevant receptors transmit the signal of specific VEGF binding: VEGFR1 (Flt-1), VEGFR2 (KDR/Flk-1) and VEGFR3 (Flt-4). The ligand–receptor interaction leads to cellular response on the base of receptor phosphorylation.

One regulative factor is the appearance of a soluble VEGF-Trap, VEGFR1 (sFlt-1) that acts as a so-called VEGF trap, catching VEGFA and so inhibiting the initiation of angiogenesis.

Osteogenesis

Bone tissue formation rests upon the balanced interaction between the secretory function of osteoblasts...
and the degrading activity of osteoclasts. There is intramembraneous and enchondral ossification: both processes are dependent on vascular ingrowth. Osteogenesis in general takes place in the vicinity of neovessels that mediate the delivery of osteoprogenitors, secrete mitogen for osteoblasts and transport nutrient and oxygen.

Blood vessels provide a conduit for the recruitment of cells involved in resorption and bone deposition and are therefore a crucial condition for any bone formation or regeneration. Decisive factor of the rate of bone increase is the level of vascularization of the growth plate.

Bone is the rigid form of connective tissue. It is a dynamic construct, composed of secretory cells, the osteocytes, degrading cells, the osteoclasts and the surrounding matrix, the osteoid. The interaction of its major constituents defines its mechanical characteristics: collagen type I guarantees its elasticity; calcium phosphate as structural components stand for stability and resistance; dermatan- and chondroitin sulfate represent together with collagen the main part of the extracellular matrix and determine the structural integrity of the tissue.

Skeletal bone is the reservoir of calcium and phosphate that can be activated and released under endocrinological control. Its dynamic (re)modelling is a result of a permanent adaptation to changing physiological and pathological requirements. A fine-tuned balance of (physiological) stress and phases of regeneration leads to a continuous coexistence of osteogenesis and bone degradation and simultaneously to a corresponding vasculature.

Osteogenesis takes place not only during foetal development but also in the course of fracture healing or during infection. Essential osteoblastic markers are collagen type I, alpha 1 (Col1a1) and alkaline phosphatase; osteoclasts are characterized by the presence of tartrate-resistant acid phosphatase, cathepsin K and dendritic cell-specific transmembrane protein.

**Angiogenesis in osteogenesis**

The level of angiogenesis is a pivotal element of osteogenesis. The vasculature provides the transport of oxygen, nutrients, growth-regulating factors and metabolites to all tissues in the body.

Many factors act as key protagonists of bone angiogenesis: VEGF, especially its isoforms VEGF120, -164 and -188, bFGF, TGFβ and HIF are the most potential ones. VEGF in its isoforms with the corresponding receptors have emerged as the decisive coupling factors between epidermal and metaphyseal vascularization and cartilage development and therefore enchondral ossification.

The angiopoietins Ang-1 and Ang-2, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), the IGF family and the neurotrophins NGFs also have angiogenic properties.

The course of osteogenesis paves the way for the vascular anatomy: anastomosing vessel net or central artery and vein.

One well-investigated area of research in this context is the regeneration of bone, for example, in terms of skeletal development or fracture repair. The vasculatures’ role is to bring oxygen and nutrients to the metabolically active areas and to also provide the bone with precursors or inflammatory cells. As far as the cytokines are concerned, there are many factors that act as key protagonists in angiogenesis as well as in bone regeneration and remodelling: VEGFs, especially its isoforms VEGF120, -164 and -188, play a significant role. There are other relevant players too; bFGF, TGFβ and HIF are among the most potential ones.

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In fracture vascularization and repair, VEGF function is required: here the matrix-bound forms of VEGF are activated by matrix metalloproteinases, enzymes that fulfill many functions during bone and matrix degradation and remodelling.

MMP9, expressed in osteo- and chondroblasts during fracture repair, initiates cartilage resorption. This degradation process releases matrix-bound VEGF from the cartilage matrix and thus stimulates the vascularization. This callus degradation in addition provides the base for bony fracture repair in contrast to persisting cartilage non-union.

MMP13 activates VEGF release independently: whereas MMP9 depends on osteo- and enchondral function, MMP13 is expressed by hypertrophic osteo- and chondroblasts. Lack of MMP13 interferes with the proteoglycan degradation, leading to a reduced permeability of the cartilage matrix for recruited inflammatory cells and sprouting blood vessels. The result is delayed callus resorption and altered vascular invasion.

**Hypoxia: the regulative factor**

Hypoxia is one of the most potent inductors of angiogenesis. Hypoxia is defined by a deficiency of oxygen that can concern the whole organism or parts of it.

The standard is age dependent and varies from 80 to 100 mmHg; the formula is \( \text{paO}_2 = 102 - \text{age} \times 0.33 \).

A disturbance of the oxygen haemostasis can be caused on different levels: the partial pressure of the tidal air, the gaseous exchange in the lung or the peripheral tissues or the binding capacity of the erythrocytes.
The pathologies resulting from hypoxia are various: on cellular level an alteration of oxygen tension can lead to endothelial changes, among others.

Systemically persisting hypoxia results in pulmonary hypertension. The aim of the increased perfusion of the pulmonary vessels is an optimal oxygen profit during the gaseous exchange.

On a biomolecular level, hypoxia interferes with gene expression; via oxygen sensing molecules and their downstream signalling cascade the transcription of genes is promoted that induce an enhanced haematopoiesis and angiogenesis and vasculogenesis.

Cellular mechanisms of oxygen regulation concern the aerobic glycolysis, the arrest of the cell cycle end the initiation of apoptosis.

Systemic regulation includes the release of erythropoietin from the kidneys, hyperventilation and finally angiogenesis.

The interesting question is which molecule represents the sensor of a low intracellular oxygen tension.

Hypoxia-inducible factor 1 alpha (HIF-1α) is the key regulator of cellular and systemic oxygen haemostasis. HIF-1α consists of two subunits: the alpha subunit is the eigentliche oxygen sensor. It is O2 sensitive and very unstable. In the presence of oxygen, the alpha subunit is not detectable. Under normoxic conditions, a quick ubiquination and immediate proteosomal degradation is observed. The compulsory cofactors for the degradation are oxygen and EISEN. Under hypoxic conditions, HIF-1α cannot be degraded for the lack of the cofactor oxygen; thus, it accumulates in the cell. The molecules reach the nucleus where they come in contact with the HIF-1β subunit.

The beta subunit, the so-called aryl hydrocarbon receptor nuclear translocator, ARNT, is expressed in the nucleus constantly.

Triggered by hypoxia, dimerization of alpha and beta subunits occurs that finally leads to the activation of target genes via binding to so-called hypoxia-responsive elements. The effect of HIF-1α as stimulator of bone regeneration also has been observed: in a mouse model with increased HIF activity the animals showed significantly higher bone mass. The stimulated HIF activity led to enhanced intramembraneous bone regeneration in a mouse distraction model. This critical review discusses osteogenesis and the fundament of osseous regeneration.

Discussion

Clinical implications

The functionality of HIF-1α, its capacity to transmit the need for oxygen and its ability to induce the development of vasculature has been the basic principle of innovative therapeutic concepts.

Recently, several drugs have been developed, which act as selective HIF prolyl-hydroxylase inhibitors; the inhibited degradation of HIF-1α persuades the system of a severe lack of oxygen and leads to an initiation of counter-measures.

By inhibiting HIF prolyl-hydroxylase, the activity of HIF-1α in the bloodstream is prolonged, which results in an increase in endogenous production of erythropoietin.

In addition, there have been observations that suggest that HIF pathway is not only a pivotal inductor of neoangiogenesis but also is relevant in questions of bone regeneration for example in fracture repair.

The mechanism behind this hypothesis postulates the ability of osteoblasts to instrumentalize HIF-1α as oxygen sensor and the corresponding signalling cascade to improve angiogenesis and osteogenesis concurrently. The molecular interconnection is not finally elucidated; a dynamic crosstalk between osteoblasts and endothelial progenitors is assumed.

Therefore, the application of HIF activators might improve bone healing by optimizing the angiogenic properties of the wounded bone but more important by inducing bone regeneration itself. Encouraging observations have been made in mouse fracture models where an overexpression of HIF and VEGF in long bones of mice results in pronounced vascularization. Even a separate cultivation of the special osteoblasts without the corresponding endothelial cells does not affect their proliferation and differentiation.

Angiogenesis as pivotal part of any regenerative concept

In different clinical applications, the angiogenic effect of different growth and transcription factors are used. In the context of angiogenesis, their coupling and the chance of therapeutic intervention, the administration of VEGF is the best investigated one. In fracture healing and bone regeneration, therapeutic angiogenesis finds many points of attack. Besides the acute trauma, the especially interesting indications considering bone regeneration are non-unions and distraction osteogenesis. There are several approaches to stimulate angiogenesis and consecutively bone regeneration. The administration of angiogenic factors, VEGF or FGF, is supposed to affect a direct angiogenic upregulation. Another initiator of angiogenesis is HIF; its application or the inhibition of its degradation results in angiogenic effects. Generally, these therapies aim to promote angiogenesis, to block anti-angiogenic processes and to bring endothelial progenitor cells to the wounded bone.

The effects of VEGF as a promoter not only of angiogenesis but also of bone regeneration have been reported in a femur fracture model in mice and in a rabbit radial segmental defect; improved ossification and callus maturation were observed.

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Another growth factor with angiogenic and osteogenic characteristics is PDGF that acts as mitogen for osteoblasts and upregulates VEGF expression. In animal models, the administration of PDGF came with increased mechanical stability and callus density. In human pilot projects, these positive results of PDGF application in combination with fracture stabilization could be verified.

A modern area of research dealing with VEGF as a means of vascular protection and regeneration aims at neuroprotection; VEGF has been reported to protect motor neurons from hypoxia-induced toxicity, reactive oxygen and other degrading factors. In addition, VEGF seems to be able to stimulate growth and development of neuronal stem cells as well as to recruit neuronal progenitor cells. In ALS rat models, the protective effect of VEGF in the cerebrospinal fluid has recently been reported, showing a deferred course of disease with delayed paralysis and increased survival time.

Conclusion
The potential of angiogenesis as a pivotal factor in many areas of tissue maintenance and regeneration is obvious; in future regenerative medical concepts, the manipulation of angiogenesis together with tissue regeneration itself will be an integral part and will lead to successful strategies.

Abbreviations list
HGF, hepatocyte growth factor; HIF, hypoxia-inducible factor; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth receptor.

References