Lymphangiogenesis Induction to Accelerate Wound Healing

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As the role of lymphangiogenesis in wound healing becomes more and more evident, the question of its induction in the case of chronic wounds in order to increase healing may also arise. Despite the importance of lymphangiogenesis as a therapeutic target, there are still few experimental models to trace and study this process in vivo. An example, however, are the different lines of transgenic mice used for the fluorescent visualization of the recently reported lymphatic vessels. All these lines are based on BAC transgenic constructs targeted to the gene to express GFP, mOrange, or Tomato, fluorescent proteins under the transcriptional control of Prox-1, using VEGFR-3 as a lymphatic marker. The application of pharmacological concentrations of purified polypeptide growth factors, cytokines, and matrix molecules has resulted in the acceleration of normal repair in a wide variety of skin wound models. It would be interesting to consider the use of drugs that can modulate lymphangiogenesis. At the moment, there are both drugs capable of inhibiting lymphangiogenesis, mainly used for the treatment of neoplasms, and drugs capable of increasing this phenomenon on the market. Below is a short list of these drugs, divided into inhibitors and inducers of lymphangiogenesis. Thus far, different drugs have shown effects.

lymphangiogenesis wound healing VEGF-A

1. Simvastatin

Simvastatin is an inhibitor of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, belonging to the statin class of drugs. Simvastatin is used for lipid lowering in cardiovascular diseases. Recently, its therapeutic effects, beyond plasma cholesterol lowering, have been found in terms of fracture healing [1] and anti-inflammatory activity on skin [2]. In particular, it has been demonstrated that the topical application of simvastatin promotes wound healing in diabetic mice by augmenting angiogenesis and lymphangiogenesis [3]. Studies have evidenced that simvastatin is able to exert multiple effects on lymphatic endothelial cells, both on metabolic pathways and on stimulating factors. In fact, simvastatin stimulates the AKT/PI3K/mTOR pathway, which represents an important pathway for several biological functions of LECs. Furthermore, simvastatin has been observed to stimulate macrophages to secrete VEGF-C, which represents a fundamental growth factor in inducing lymphatic proliferation. Furthermore, simvastatin is able to induce the transformation of M1-phenotype pro-inflammatory macrophages into M2-phenotype anti-inflammatory/tissue reparative macrophages, and reduce oxidative stress-induced apoptosis [4]. In conclusion, the topical application of simvastatin may have significant therapeutic potential for improving wound healing in patients with impaired microcirculation, such as diabetics.

2. COMP-Angiopoietin 1

Angiopoietin-1 (Ang1) is a specific growth factor that has the function of generating stable and functional vascularization through the Tie2 and Tie1 receptors. Delayed skin wound healing is a serious complication accompanied by impaired cutaneous blood flow, hypoxia, accelerated inflammation, edema, and endothelial–neural dysfunction [5]. Therefore, restoring the structural and functional microvasculature via the supplementary delivery of Ang1 could be beneficial to increase the restoration of vascular regeneration and functionalization. Cho and colleagues [6] investigated the efficacy of oligomeric cartilage matrix (COMP)-Ang1, as a soluble, stable, and potent form of Ang1, on promoting the healing of skin wounds in diabetic mice. It was observed that mice treated with the COMP-Ang1 protein showed accelerated wound closure and epidermal and dermal regeneration, following the potent stimulation of angiogenesis. Furthermore, the authors have observed that angiogenesis is accompanied by also lymphangiogenesis, which characterizes strongly the region in which the wound healing takes place. Interestingly, the research indicates that COMP-Ang1 can promote wound healing in diabetes through enhanced angiogenesis and blood flow and lymphangiogenesis, which became a fundamental characteristic of wound healing [6].

3. Retinoic Acids

Retinoic acids (RAs) are composed of biologically active metabolites of vitamin A and are involved in a broad range of biological processes in vertebrate development by regulating genes important for cell proliferation, differentiation, apoptosis, and metabolism [7][8]. RAs have been shown to arrest cell cycle progression and cell proliferation in several cell types. However, the proangiogenic effect of RAs has also been documented, and RAs have been observed to stimulate the transcription and translation of vascular endothelial growth factor (VEGF) in nonendothelial cells. Recently, all-trans RA has been shown to be important in the early steps of lymphatic development during embryogenesis [9]. Furthermore, experiments have indicated that RAs are able also to potently induce post-developmental lymphatic regeneration. In particular, a specific RA metabolite, -cis retinoic acid (9-cisRA), is able to induce lymphangiogenesis, but not angiogenesis. The 9-cisRA seems to represent a specific inductor of the proliferation and differentiation of LECs through both genomic and nongenomic actions. The transcription-dependent genomic action of 9-cisRA consists of the regulation of PROX1 gene expression. The nongenomic action consists of the downregulation and phosphorylation of $p27^{Kip1}$. These findings make RAs a valid tool to assist in wound healing, whereas, in particular, 9-cisRA becomes a promising therapeutic agent for the specific treatment of patients with lymphedema [10].

4. CCBE1

Vascular endothelial growth factor C (VEGF-C) is a key factor in promoting lymphatic endothelial cells' proliferation and migration by its receptor VEGFR-3. VEGF-C is secreted as a monomeric 58 kDa precursor; it is first proteolytically processed to a 43 kDa polypeptide, and then C-terminally processed to the 29/31 kDa pro-VEGF-C form and finally fully processed to the 21 kDa mature form. The several forms produced have different degrees of

receptor-binding capacity for VEGFR-3 and VEGFR-2, with the last proteolytic form having the highest activity towards VEGFR-3 [11][12].

Recent studies have indicated collagen and calcium-binding EGF domain-1 (CCBE1) as the key factors involved in promoting VEGF-C proteolysis by the A disintegrin and metalloproteinase with thrombospondin motifs 3 (ADAMTS3) [12][13]. In particular, Song and colleagues [14] have evidenced, in a colon cancer experimental model, that CCBE1 enhances VEGF-C and facilitates tube formation and the migration of LECs, promoting tumor lymphangiogenesis and consequently the lymphatic metastasis of colon cancer cells. Furthermore, the same authors have evidenced that transforming growth factor beta (TGF-β) downregulates the transcription and lymphangiogenic function of CCBE1 through the direct binding of SMAD proteins (they modulate the activity of transforming growth factor beta ligands) at the gene locus by CCBE1. Even if the study of Song and colleagues is focused on colon cancer tumor, and the role of lymphangiogenesis in promoting tumor metastasis via lymphatic vessels, nevertheless, more generally, these findings demonstrate the role of CCBE1 in promoting lymphangiogenesis and the role of TGF-β signaling as a regulatory factor [15].

5. circEHBP1

VEGF-C and VEGF-D play a key role in embryonal lymphangiogenesis. VEGF-C accompanies the first step in the migration and formation of tubular elements that characterizes the lymphatic plexus, whereas VEGF-D intervenes in the last step of lymphatic plexus maturation, perhaps performing and assisting the functional aspect of lymph flow in vessels. Zhu and colleagues [14] have revealed that the formation of new lymphatic vessels in bladder cancer (BCa) is exclusively VEGF-D-dependent. The authors have observed that circEHBP1, a circular RNA, is upregulated in bladder cancer and positively correlated with lymphatic metastasis and poor prognosis. Circular RNAs (circRNAs) consist of a class of single-stranded molecules with development-specific expression patterns; they are characterized by a covalently closed structure and generated from the back-splicing of pre-mRNA transcripts. In bladder cancer, circEHBP1 sponges miR-130-3p, which has a suppressive effect on lymphangiogenesis in BCa. miR-130-3p regulates the expression of the TGF-β receptor (TGFβR1), inhibiting its expression. TGFβR1 is a phosphorylase of the TGF-β/SMAD3 signaling pathway; this pathway is essential in inducing specifically the expression of VEGF-D and promoting lymphangiogenesis [14].

In summary, circEHBP1 induces in BCa lymphangiogenesis, promoting the expression of VEGF-D, by sponging miR-130-3p, giving free expression to TGFβR1.

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