Athanasios Athanasopoulos

Dr. sc. hum.

Regulation of neovascularization during wound repair: the role of the *Staphylococcus aureus*-derived Extracellular Adhesion Protein (Eap) and of the endogenous CYR61 (CCN1) protein in integrin-dependent endothelial cell functions

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Promotionsfach: Innere Medizin

Doktorvater: Prof. Dr. Med. P.P. Nawroth

Inflammation and angiogenesis are important in wound healing and repair mechanisms. Both processes are governed by integrin-dependent adhesive interactions of circulatory, vascular and tissue-adherent cells with each other as well as with the extracellular matrix (ECM). Two mechanisms interfering with such repair mechanisms were identified in the present study. An exogenous factor, Eap, a protein derived from the infectious agent, *Staphylococcus aureus* was found to affect wound healing in a negative way by interfering with both inflammatory and angiogenic pathways of the host (Athanasopoulos *et al*, 2006). In contrast, an endogenous pro-adhesive factor, CYR61, was identified to mediate an autocrine loop between osteoblasts and endothelial cells with potential implications in bone angiogenesis and fracture repair (Athanasopoulos *et al*., publication in revision).

In the first part of the studies, we explored the anti-inflammatory role of Eap and studied whether *S.aureus*-derived Eap may interfere with wound healing process. *S.aureus* continues to be a major human pathogen being responsible for infections of skin and other

organs and interfering with host cell functions. Impaired wound healing is often observed in *S.aureus* infected wounds, and here, the extracellular adherence protein of *S.aureus* is shown to inhibit wound healing by exerting both anti-inflammatory and potent anti-angiogenic properties. Local administration of Eap delayed wound healing *in vivo*, whereby Eap prevented recruitment of inflammatory cells and pro-inflammatory factors, as well as significantly reduced neovascularization and impaired perfusion. *In vitro*, Eap was found to block transendothelial migration of leukocytes and reduced the activation of the pro-inflammatory transcription factor NFκB in these cells and the expression of NFκB-related genes, such as tissue factor. On endothelial cells, Eap reduced both the rapid vascular endothelial growth factor (VEGF)-driven as well as the delayed cytokine-induced increase in paracellular permeability. In addition, Eap blocked the αv-integrin-mediated endothelial cell migration towards different ECM proteins. In a three-dimensional spheroid culture endothelial capillary tube formation *in vitro*, as well as neovascularization in matrigels *in vivo* was markedly blocked by Eap. Collectively, together with its potent anti-inflammatory functions, these newly identified anti-angiogenic properties of Eap provide a potential underlying mechanism for the impaired wound healing frequently seen in *S.aureus* infected wounds. Finally, Eap may serve as a lead compound for new anti-inflammatory and anti-angiogenic therapies in several pathologies.

In the second part we investigated the role of osteblast-derived CYR61, another matrix protein with capacity to interact with cell integrins, in endothelial cell functions related to angiogenesis. It is well known that angiogenesis is indispensable during bone growth and fracture repair and VEGF has been implicated in these processes. The immediate early gene CYR61 (CCN1) is an ECM signalling molecule that is essential for successful vascular development through its interactions with several endothelial integrin receptors. CYR61 has been previously shown to localize in newly formed osteoid and to be upregulated in the reparative phase of fracture healing; however, the role of CYR61 in this newly formed osteoid

remains unclear. Here, the regulations of CYR61 expression in osteoblasts, as well as the consequences thereafter were studied. Stimulation of osteoblasts with VEGF resulted in a significant upregulation of both CYR61 mRNA and protein. VEGF-mediated upregulation of CYR61 was dose- and time-dependent. In addition, we observed an upregulation of both cell-surface associated CYR61 as well as ECM-associated CYR61 in osteoblasts. The supernatant of VEGF-prestimulated osteoblasts was chemotactic for vascular endothelial cells, thereby increasing their migration and stimulated capillary sprout formation. These effects could be attributed to the presence of CYR61 in the supernatant, as they were prevented in the presence of an antibody against CYR61. Moreover, the supernatant of VEGF-prestimulated osteoblasts stimulated angiogenesis in the matrigel plug *in vivo* in a CYR61-dependent manner. Taken together, these data demonstrate a potential paracrine loop consisting of the VEGF-mediated upregulation of CYR61 in osteoblasts that attracts vascular endothelial cells and promotes angiogenesis. Such a loop may be operative in angiogenesis during bone growth and fracture healing.