EXPRESSION AND REGULATION OF THE ANGIOGENIC PEPTIDE VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN FETAL AND DEGENERATIVE TENDONS

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Introduction
The most frequent localization for chronic tendinosis of the Achilles tendon is located in a hypovascular region 3-6cm above the calcaneal insertion (3). This might be inconsistent to histopathological findings which describe an increased vascularity in biopsies of patients with tendinosis (3) and with results from Dopplerflowmetry of patients with chronic tendinosis (1). Astrom and Westlin (1) concluded that their study gives evidence that “a deficient blood supply does not initiate the lesion in chronic Achilles tendinopathy”. Angiogenesis is controlled by a variety of mitogenic, chemotactic or inhibitory peptides that act on invading endothelial cells. One of the most important angiogenic factors is the vascular endothelial cell growth factor (VEGF) which binds to the signalling tyrosine kinase receptors VEGFR-1 and VEGFR-2.

As the most probable candidate for angiogenic peptides we chose VEGF, and determined its presence in degenerative, normal adult and fetal human Achilles tendons. Furthermore, in a rat cell culture model we elucidated some of the factors responsible for the regulation of the VEGF expression in tenocytes.

Materials and Methods
Biopsies of human Achilles tendons from patients with chronic tendinosis were obtained from orthopaedic surgery. Fetal tendons and controls of healthy adult tendons were dissected at routine autopsies.

Immunohistochemistry: 20 tissue samples were immunostained with anti-VEGF, anti-VEGFR-1 and anti-VEGFR-2 (1:80; Santa Cruz).

ELISA: Frozen tissue samples were crushed and homogenized in 150 mM NaCl 20 mM Tris/HCl-buffer, pH 7.4, a soluble fraction obtained by centrifugation (48 000 xg, 60 min), and aliquots (100 µl) analyzed by a sandwich ELISA (R&D Systems, MN, USA) that detects VEGF.

Western Blot was carried out according to standard techniques.

RT-PCR for VEGF splice variants: Frozen samples were analyzed using the following primers: (1) Nonselective for all VEGF splice variants 5'-ATG-GCA-GAA-GGA-GGG-CAG-CAT-3' (sense) and 5'-TTG-GTG-AGG-TTT-GAT-GCG-CAT-CAT-3' (antisense) yielding a 255 bp fragment (40 cycles, annealing temperature 55°C); (2) selective for VEGF splice variants 5'-CCA-TGA-CTT-CTT-GGT-GCT-GTT-CAT-CAT-3' (sense) and 5'-ATC-CTT-TCT-TCC-TGT-TCT-3' (antisense) yielding different bp fragments [40 cycles, annealing temperature 55°C].

Culture and stimulation of rat tenocytes: Cells from Achilles tendons from postnatal (2-5 days old) rats were cultured in monolayer cultures under normoxic conditions. EGF(ng/ml) was carried out according to standard techniques.

Results
By immunocytochemistry, VEGF can be visualized in tenocytes of fetal and tendinotic human Achilles tendons (Fig. 1), but not in healthy adult ones. Endothelial cells in microvessels of degenerative and fetal but not of normal tendons were intensely stained for VEGFR-1 and VEGF-2.

The highest VEGF concentrations were found in tendons with chronic tendinosis (in areas of the lesion) and lower ones in fetal tendons whereas the VEGF content in healthy adult tendons was negligible (Fig. 2). These results could also be verified in Western blotting experiments which further prove the specificity of the immunoreaction.

From all samples of the tendons investigated, two PCR products were obtained: One with 526 bp corresponding to VEGF121 and one with 658 bp corresponding to VEGF165. Both splice products were also obtained with other VEGF-positive human tissues (rheumatoid synovium, gliomas) whereas a human cartilage sample yielded different splice forms (VEGF121 and VEGF165 not shown) illustrating that the method has the resolution necessary to detect other forms.

Discussion
The essential role of VEGF and its receptors, VEGFR-1 and -2, for embryonic vasculogenesis and angiogenesis have been clearly demonstrated by gene knockout studies in mice (2). Peptides or cytokines which are upregulated during development often reappear in the disease state. We could show that VEGF is expressed in chronic tendinosis and fetal tendon tissue whereas this angiogenic peptide was undetectable in normal tendons. VEGF re-expression in tenocytes of degenerative tendons should likely be induced by degenerative process. EGF as well as hypoxia strongly enhanced VEGF secretion from tenocytes, especially the combination of both showed a synergistic effect.

EGF induces VEGF expression in many cell types and this molecule is produced during inflammation by invading monocyes, thrombocytes, lymphocytes or fibroblasts. Chronic tendinosis has been associated with hypoxia (4). Therefore, hypoxia and inflammatory reactions in tendons may be the cause for the observed VEGF production and subsequent angiogenesis. Angiogenesis might contributes to the repair and remodeling of the injured tendon, but may also weaken its mechanically stability by proteolysis of the extracellular matrix by the invading endothelial cells.

References
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