Introduction:
Vascular endothelial growth factor (VEGF) has been recently shown to play an important role during endochondral bone formation in hypertrophic cartilage remodelling, ossification and angiogenesis, but is not expressed in normal adult cartilage. Since genes expressed during development often re-appear in the disease state, we investigated whether VEGF and its receptors are expressed in osteoarthritic cartilage. Further more, we analyzed the regulation of the VEGF-receptor 2 (KDR) and the function of VEGF in vitro.

Methods:
VEGF production in osteoarthritic cartilage from the tibial plateau was measured by enzyme-linked immunosorbent assay (ELISA). Deposition of VEGF and its receptors (VEGFR) was determined by immunohistochemistry. Expression of mRNA for the different VEGF splice forms and for VEGFR was determined by reverse transcription-polymerase chain reaction (RT-PCR). The signaltransduction of VEGF was measured by anti-phospho-tyrosine Western-Blot (WB), pErk-WB and by Electro Mobility Shift Assay (EMSA) for AP-1. The functions of VEGF were determined by using proliferation assays, Matrix-Metalloproteinase ELISAs and Real-Time RT-PCR studies.

Results:
In addition to VEGF, also its receptor VEGFR-2 (KDR, flk-1), but not VEGFR-1 (flt-1), could be detected by RT-PCR in osteoarthritic cartilage and immunostained on osteoarthritic chondrocytes (Fig. 2).

Fig. 1:
Increased VEGF concentrations were measured in osteoarthritic cartilage from the tibial plateau whereas it could not be detected in normal cartilage. VEGF could be immunostained within the intracellular and pericellular matrix of osteoarthritic chondrocytes in the deep and superficial layers, respectively. RT-PCR and immunohistochemistry for VEGF in normal hyaline cartilage remained negative (Fig. 1 A: OA cartilage Mankin score: 10, original magnification: 200-fold; B: Detail OA cartilage chondrocytes, original magnification: 650-fold; C: negative control with inactivated antibody on OA cartilage, original magnification 650-fold; D: VEGF immunostaining remains negative in adult cartilage).

Fig. 2:
Chondrocytes incubated overnight with Epidermal Growth Factor (EGF) (100 ng/ml) or with VEGF (100 ng/ml) express significantly more VEGF-receptor 2 (KDR).

Fig. 3:
Chondrocytes incubated for 24 hours with VEGF (10 ng/ml) secreted more Matrix-Metalloproteinase-1 (MMP-1) compared to control chondrocytes (ELISA of the supernatant). The increase of MMP-1 expression is comparable to the effect of known MMP-1 inducers like interleukin-1β (IL-1β), IL-6 or Tumor Nekrosis Factor-alpha (TNF-alpha).

Conclusion:
Apart from hypertrophic chondrocytes, VEGF and VEGFR are also produced in chondrocytes of osteoarthritic cartilage. VEGF-expression is regulated by VEGF. VEGF is able to activate the signal transduction cascade which culminates in AP-1 accumulation. In addition VEGF is able to raise MMP-1 expression. In conclusion, VEGF seems to be an important factor in the pathogenesis of the OA.

Supported by a grant from the Forschungsschwerpunkt Muskell- und Skelettsystem of the Medical Faculty of the Christian-Albrechts-University of Kiel.