ANGIOGENESIS IN OSTEOSARCOMA IS REGULATED BY TUMOR INTERSTITIAL FLUID PRESSURE


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Introduction: We have previously shown that osteosarcomas (OS) have states of increased interstitial fluid pressure (IFP) and that these states correlate with increased proliferation and chemosensitivity.1,2 In our previous study, we noted that IFP was not related to tumor perfusion. Perfusion is a function of vessel density and blood flow within individual vessels. Reduced vascularity (ie. vessel density) as opposed to reduced blood flow within individual vessels per say could account for the reduced perfusion seen in tumors with high IFP although this possibility has not been investigated before. This study investigates the hypothesis: “Constitutively raised IFP in OS inhibits angiogenesis.”

Methods: Design: The study was based on a clinical arm, which provided proof of principle for our hypothesis, and an in vitro arm in which cells were grown under physiologically replicated states of raised IFP and assessed for markers of angiogenesis. In addition, we grew Human umbilical vein endothelial cells (HUVEC, ATCC, Manassas, VA) under pressure to exclude the possibility of a toxic effect of the raised IFP on these cells. Clinical arm: Between January 1997 and June 2000, 16 patients with the clinical diagnosis of high-grade OS underwent open biopsy with IFP and blood flow measurements at the biopsy site. Vascularity as seen in the biopsy samples was assessed in the first instance by an observational 4-grade score. Quantification of vascularity on routine hematoxylin and cosin (H&E) stained slides was done by outlining endothelial lined spaces and analyzing the area enclosed by these structures using NIHImage (NIH, Bethesda, MD). In vitro arm: We used 2 human OS cell lines, HOS and U2OS (ATCC, Manassas,VA) and compared this with a non-neoplastic osteoblast cell line HOB (Cell applications, San Diego, CA). Cells were grown at three pressures: 0 mmHg (comparable to conventional culture systems), 20 mmHg (an intermediate OS pressure observed in clinical studies) and 50 mmHg (the higher pressures observed in these studies)3. To rule out the influence of applied pressure on endothelial cell proliferation we grew human umbilical vein endothelial cells (HUVEC, ATCC, Manassas,VA) under 0 and 20 mm Hg of pressure. OS cells were evaluated using realtime PCR and immunohistochemical staining for the transcription and translation of VEGF-A, VEGF-C (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and TPA (American Diagnostica Inc.,Stamford, CT). CD 31 (DakoCytomation, Carpenteria, CA) was used to indicate vascularity on slides. Immunohistochemical staining was quantified using image analysis software (NIHimage).

Results: IFP in the OS was 33.5 ± 17.2 mmHg, which was significantly greater than that in the normal soft tissue where pressures were 2.9 ± 5.7 mmHg (p=0.00001). The 6 low vascularity tumors (Grade 1 & 2) had IFP of 49.2 ± 16 mmHg compared to 10 high vascularity tumors (Grade 3 & 4) with IFP of 24.1 ± 9.4 mmHg (p=0.0007). Tumor vascularity was inversely related to IFP (R²=0.6) as shown in Figure 1. This inhibition of vascularity was not due to a toxic effect of raised IFP on the vessels themselves – HUVEC grew well under pressure. In OS cells grown under pressure, TPA expression was upregulated compared to osteoblasts grown under pressure by quantitative PCR (Figure 2). This phenomenon was most pronounced at 20 mmHg. Growth of HOS at 20 mmHg and 50 mmHg was associated with a significant upregulation of TPA compared with growth at 0 mmHg (p=0.02 and p=0.05 respectively). Immunohistochemistry validated this observation. TPA staining on biopsy samples increased progressively with increasing tumor IFP (R²=0.75).

Figure 1. Vascularity was inversely proportional to IFP. This may account for the observation in some studies that show that blood flow is reduced in tumors with raised interstitial fluid pressure.

Discussion: Vascularity in OS is reduced by constitutively raised IFP. This was not due the inhibition of growth of endothelial cells in a pressurized environment. In OS cells grown in a physiologically replicated high-pressure environment, angiogenic factors (VEGF-A) were down-regulated and anti-angiogenic factors (TPA) and lymphangiogenic factors (VEGF-C) were up-regulated. This has important implications on intra-tumoral drug delivery which may be reduced in tumors with constitutively raised IFP due to reduced vascularity.

Bibliography: (1) Elevated physiological tumor pressure promotes proliferation and chemosensitivity in human OS (Nathan SS et al, Clin Cancer Res. 2005 Mar 15;11(6):2389-97)
(2) Simulated OS fluid pressures replicate chemotherapy-induced necrosis – novel insights into systems biology (Nathan SS et al ORS, Washington, DC 2005)
(3) Angiogenesis in OS is regulated by tumor IFP (Nathan SS et al AACR, Anaheim, CA 2005)

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Figure 2. TPA is an anti-angiogenic factor. TPA expression was increased in OS cells grown under pressure. Osteoblasts responded in a moderate but reverse manner.

Figure 3. VEGF-C expression was upregulated in OS cells grown under pressure whereas in osteoblasts, VEGF-C expression was downregulated in cells grown under raised pressure. In particular, growth of HOS and U2OS at 20 mmHg was associated with a significant upregulation (p=0.03 and p=0.01) of VEGF-C compared to growth of these cells at 0 mmHg (Figure 3). There was a strong positive correlation (R²=0.87) between VEGF-C staining intensity on immunohistochemistry and IFP in tumors.

Figure 4. VEGF-A is the archetypical angiogenic factor. VEGF-A expression was decreased in OS cells grown under pressure. Osteoblasts responded in a moderate but reverse manner.