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Inhibition of Angiogenesis and Tumor Growth by Interleukin-12 in a Canine Hemangiosarcoma Xenograft Model

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The study of angiogenesis relies on in vivo models. Improved treatment of malignancy that has progressed to the angiogenic phenotype requires relevant models to test the safety and efficacy of innovative antiangiogenic therapies. Systems to study angiogenesis in the laboratory are limited by the transient nature of angiogenic events and limited accessibility to angiogenic tissue. We developed an in vivo angiogenesis model in NOD-SCID mice with malignant canine endothelial cells derived from a spontaneous subcutaneous hemangiosarcoma of a dog. An in vitro cell line, SB-HSA, was established from a fresh tumor biopsy implanted into a bg/nu/XID mouse. Flow cytometry of cultured SB-HSA cells from the tumor explant showed uniform expression of alphav beta3 integrin, an adhesion molecule present on angiogenic endothelial cells, and bright staining with P1H12 (CD146), an endothelial marker. Cells were further sorted for highest expression of these markers. RT-PCR indicated the cells expressed message for VEGF, VEGF receptors-1 and -2, bFGF, IL-8, and MMP-2 and -13, suggesting the cells might stimulate their own growth and migration. Subcutaneous injection of SB-HSA cells into NOD-SCID mice reliably induced growth of a tumor within two weeks comprised of endothelial cells that form vascular channels histologically indistinguishable from the original canine tumor. Immunohistochemical staining of fresh frozen and fixed tumor from NOD-SCID mice confirmed expression of alphav beta3 integrin, CD31, and von Willebrand’s factor, all validating an endothelial lineage. In addition, these cells stimulated robust neovascularization in the corneal angiogenesis assay and in our subcutaneous matrigel/sponge assay. Therefore, these cells can be used to investigate the angiogenic response they stimulate in mice. More importantly, it may now be possible to use this model in experiments intended to evaluate antiangiogenic agents, using inhibition of SB-HSA tumor formation as an indicator of the antiangiogenic effect.

To this end, we evaluated in vivo inhibition of SB-HSA-induced neovascularization by interleukin-12 (IL-12). IL-12 has potent antiangiogenic and antitumor effects in some models. We found that IL-12 targeted to angiogenic endothelial cells by a peptide (CDCRGDCFC) completely suppressed angiogenesis triggered by SB-HSA cells in BALB/c mice using our matrigel/sponge assay. Due to the highly vascularized nature of the SB-HSA tumors formed in NOD-SCID mice, and the rapid formation of neovessels induced by SB-HSA cells in the matrigel/sponge assay, we hypothesized that IL-12 would inhibit growth of SB-HSA tumors in immunocompromised mice. In NOD-SCID mice, continuous infusion of IL-12 delivered by osmotic pump suppressed SB-HSA tumor growth by at least 3-fold compared with tumor growth in control-treated mice. This result suggests that IL-12 may be developed as a viable treatment for canine hemangiosarcoma. Thus, our canine SB-HSA hemangiosarcoma model is providing new opportunities to evaluate novel strategies to control angiogenesis in cancer and for canine hemangiosarcoma in particular.

Biographical Profile

Dr. Stuart Helfand is Associate Professor of Oncology at the School of Veterinary Medicine, University of Wisconsin, in Madison, Wisconsin. He is a graduate of Colorado State University College of Veterinary Medicine and did his residency in
medical oncology at the University of California, Davis. Dr. Helfand is a clinical oncologist in the Veterinary Medical Teaching Hospital at the University of Wisconsin and his laboratory has studied tumor immunology and cancer immunotherapy for nearly 15 years. Recent work has focused on approaches to target immunostimulatory and antiangiogenic compounds to the tumor microenvironment.

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