**191** POSTER

**Recombinant endostatin prevents integrin alpha4, beta1-mediated adhesion and proliferation of vascular endothelial growth factor receptor-expressing B16 melanoma cells; antimetastatic implications**

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**Background:** Endostatin (ES) is a potent anti-angiogenic protein, but the molecular mechanism of its antitumoral action is not yet clear. Given its angiostatic effect, majority of studies have focused on effects of ES in endothelial cells. However, few studies have analyzed direct effect of ES on tumor cell behavior. Herein, we explored this possibility by analyzing ES binding to melanoma cells and its effect on tumor cell adhesion and proliferation in response to IL-18 and VEGF, prometastatic cytokines elicited in the inflammatory and angiogenic microenvironment of hepatic metastases (Carrascal et al, Cancer Res 2003; 63:491–97).
**Results:** Using alexa-conjugated ES, we detected that a subpopulation of B16 melanoma (B16M) cells binds to ES under basal culture conditions, as shown by flow cytometry. Addition of increasing concentrations of ES to B16M did not alter their basal proliferation. However, ES abrogated B16M proliferation induced in by either IL-18 or VEGF. Anti-murine VEGF antibodies abolished proliferation-stimulating activity of IL-18 on B16M. VEGF also increased B16M cell proliferation and ES, but not anti-IL-18, abrogated it. This suggests that inhibitory action of ES on B16M cell proliferation-stimulating effect of IL-18 was produced via autocrine VEGF action blockade. Five human melanoma cell lines (A375, SK23, VUP, 883 and MJM) also increased their proliferation rate in response to IL-18, and ES given to melanoma cells together with IL-18 abrogated its proliferation-stimulating effect. Anti-VEGF antibodies also abolished proliferation-stimulating activity of IL-18 on human melanoma cells. On the other hand, both IL-18 and VEGF increased adhesiveness of B16M cells to hepatic sinusoidal endothelium cells. Proadhesive effect of IL-18 on B16M cells was abrogated by anti-VEGF antibodies while anti-IL-18 did not affect VEGF-dependent adhesion. Again, both IL-18- and VEGF-dependent adhesion were neutralized in B16M cells given cytokines together with ES. VEGF increased B16M cell adhesion to an immobilized VCAM-1 substrate and addition of ES together with VEGF abrogated it. Consistent with in vitro assays, incubation of luciferase-transfected B16M with VEGF prior to be intrasplenically injected increased by 3-fold their intrasinusoidal retention on the 18th-hour postinjection, and metastasis development on the 12th-day postinjection. These prometastatic effects were abrogated by addition of ES to B16M cells 30 min prior to VEGF.
**Conclusion:** These results demonstrate the role of endostatin as inhibitor of melanoma cell proliferation and adhesion in response to proinflammatory and proangiogenic cytokines IL-18 and VEGF, and suggest that endostatin's anti-tumoral effects extend beyond an anti-angiogenic action to encompass direct effects against VEGF-stimulated melanoma cells.

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**Transplanted hair follicles give rise to blood vessels in skin visualized in nestin-driven GFP mice**

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**Background:** The neural-stem cell marker nestin is also expressed in hair follicle stem cells in transgenic mice as visualized by nestin-driven green fluorescent protein (GFP) (1). Since it is known that blood vessels can express nestin, the present study was designed to determine if hair follicles could directly generate blood vessels after transplantation.
**Materials and Methods:** Fibrissa hair follicles were transplanted from nestin-GFP C57BL6 mice to nude mice and visualized under fluorescence microscopy.
**Results:** After transplantation, nestin-GFP was visualized by fluorescence in proliferating endothelial cells and nascent vessels growing in the nude mouse skin. Nestin-GFP hair follicles were also observed to give rise to GFP expressing vessels in red fluorescent protein (RFP) expressing B16 melanoma after both were transplanted to nude mice. Transplantation of the nestin-GFP-expressing vibrissa hair follicles under the renal capsule also resulted in GFP-expressing vessels formation. I mmunohistochemical staining showed that endothelial-cell-specific markers CD31 as well as von Willebrand factor and nestin-colocalize in the nestin-GFP-expressing nascent vessels. Nestin-GFP expression was diminished or extinguished in mature blood vessels with apparent blood flow and is therefore a marker of nascent angiogenesis.
**Conclusion:** The results therefore demonstrate that hair follicles can give rise to blood vessels in the skin. This transplant model enables very early events in angiogenesis, including tumor angiogenesis to be visualized, and to used for antiangiogenesis drug screening. The results suggest potential new stem cell targets for antiangiogenesis therapy. (1) Li, L., et al. Nestin expression in hair follicle sheath progenitor cells. Proc. Natl. Acad. Sci. USA 100, 9658–9662, 2003.

**193** POSTER

**Histone deacetylase inhibitors LAQ824 and LBH589 inhibit proliferation and modulate angiogenesis-related genes in human endothelial cells**

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Histone deacetylase (HDAC) inhibitors exert their antitumor effect mainly through inhibition of growth and survival in transformed cells. Recently, we have shown that proliferating endothelial cells and angiogenesis are also potential targets for this novel class of agents (Qian DZ et al Proc AACR 2004). In this study, both vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) stimulated human endothelial cell (HEC) proliferation and survival were inhibited in a dose-dependent fashion by hydroxamic-acid analogs LAQ824 and LBH589. Interestingly, proliferating HEC were more sensitive to the antiproliferative effects of both drugs than PC3 human prostate carcinoma cells. High nanomolar concentrations of LAQ824, which are achievable plasma concentrations in mice, caused cell cycle arrest in PC3 cells and apoptosis in HEC. In addition, expression of angiogenesis-related genes regulated by VEGF/VEGFR2 signaling, such as angiopoietin-2 (Ang-2), survivin and chemokine receptor CXCR4, was inhibited by both LAQ824 and LBH589 in HEC. However, in contrast to the VEGF receptor tyrosine kinase inhibitor PTK787, LAQ824 and LBH589 inhibited both VEGF-dependent and VEGF-independent endothelial cell expression of Ang-2, survivin, and CXCR4. To investigate the potential mechanisms underlying the regulation of these genes by HDAC inhibitors experiments in HEC with transient transfection of a CXCR4-promoter-luciferase reporter gene are ongoing and the results will be presented at the meeting. Taken together, our findings indicate that hydroxamic acid derivatives target critical pathways for endothelial cell proliferation and survival, and may have a promising activity in combination with molecular targeted angiogenesis inhibitors.

# Apoptosis

**194** POSTER

**BARD1 catalyzes of p53 phosphorylation by DNA-damage response kinase and reverses malignant phenotype of ovarian cancer cells**

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The abrogation of apoptotic pathways is a hallmark of carcinogenesis often associated with inactivating mutations of p53. The tumor suppressor and BRCA1 binding partner, BARD1, plays an important role as mediator of apoptosis, by binding to and stabilizing p53, and BARD1-repressed cells are resistant to apoptosis (Irminger-Finger et al., Mol Cell 2001). Here we report that rat ovarian cancer cells, NuTu-19, are defective in apoptosis induction and upregulation of p53 protein levels in response to doxorubicin-induced genotoxic stress in spite of the fact that these cells express wild type p53. However, NuTu-19 cells express aberrant forms of BARD1, derived from differential splicing and mutations and deficient of the regions required for apoptosis induction, emphasizing the importance of BARD1 in the apoptosis pathway. Stabilization and phosphorylation of p53, as well as apoptosis in response to genotoxic stress in NuTu-19 cells can be restored by the exogenous expression of wild type BARD1. Stable expression of BARD1-EGFP fusion protein under the control of EF1-alpha in NuTu-19 cells abolishes their malignant phenotype and no tumors are formed by Nutu-19 cells transduced with virally delivered BARD1, when injected intraperitoneally. The pathway of tumor suppression implies BARD1-p53 interaction. Co-immunoprecipitation and colocalization studies show that BARD1 binds to both unphosphorylated and serine-15 phosphorylated forms of p53 in several cell types and the regions required for binding are identical with the regions sufficient for apoptosis induction. In addition, BARD1 binds to Ku-70, the regulatory subunit of DNA-PK, suggesting that the mechanism of p53-induced apoptosis requires BARD1 to augment the phosphorylation of p53 by DNA-PK. Since overexpression of BARD1 can induce p53 phosphorylation in different cell types it is suggested that BARD1 catalyzes p53 phophorylation by binding to p53 and DNA-PK.