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Effects of Activated Ras Protein on Endothelial Gene Expression

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The gene coding for the Ras protein can be altered and activated by numerous environmental mutagens and therapeutics such as vinyl chloride, chemotherapy agents, and radiation. Ras activation is known to promote tumorigenesis by activating a molecular switch, controlling cellular growth and differentiation; however, the effect of Ras on other genes is not well understood. Our hope is to lay the groundwork for determining expression patterns of genes regulated or affected by Ras activation. We are working to determine the expression patterns of Ras-induced genes in human endothelial cells, a cell type where Ras mutations have been linked with angiosarcomas. Changes in expression are currently being measured by microarray for wide-spectrum gene analysis, as well as quantitative RT-PCR for 84 selected cancer pathway genes. Human Umbilical Vein Endothelial Cells (HUVECs) were cultured in the presence of Green Fluorescent Protein (GFP) or an activated Ras (V12) mutant co-expressed with GFP in serum-free conditions for approximately 12 hours. RNA was isolated from cultured cells and sent for Affymetrix microarray analysis or processed into cDNA using reverse transcriptase followed by qPCR using SuperArray® technology. Data is currently being collected and compiled for both methods of gene expression analysis. Hopefully, this knowledge will lead to a more in-depth understanding of the molecular changes that surround Ras activation and the process leading to the formation of angiosarcomas and other more common tumors.