THE ROLE OF NEUROFIBROMIN IN REGULATION OF ENDOTHELIAL CELL AND VASCULAR SMOOTH MUSCLE CELL FUNCTIONS IN RESPONSE TO NEUROFIBROMA DERIVED GROWTH FACTORS

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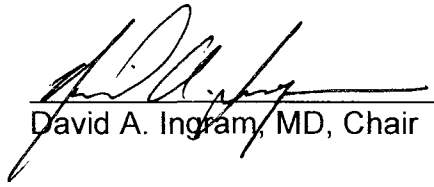

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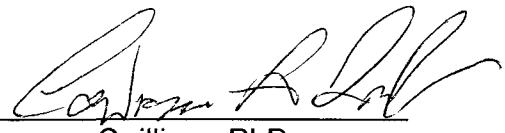

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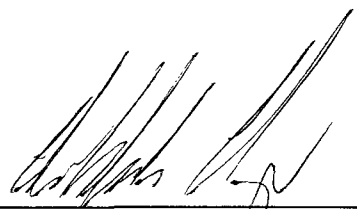

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ABSTRACT

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THE ROLE OF NEUROFIBROMIN IN REGULATION OF ENDOTHELIAL CELL AND VASCULAR SMOOTH MUSCLE CELL FUNCTIONS IN RESPONSE TO NEUROFIBROMA DERIVED GROWTH FACTORS

Neurofibromatosis Type I (NF1) is a common autosomal dominant genetic disorder caused by mutations in the *NF1* tumor suppressor gene that encodes for neurofibromin, a GTPase activating protein (GAP) that negatively regulates Ras activity. Ras signaling pathways regulate many different cellular processes and Ras is activated in many cancers. NF1 patients develop multiple cutaneous benign neurofibromas that are highly vascular, plexiform neurofibromas, cardiovascular disease, vascular occlusive disease, and malignant myeloid disease in childhood. Endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) are essential components of vessels. Recent studies suggest that *Nf1+/-* mice have increased angiogenesis *in vivo* and demonstrate Ras signaling in VSMCs is important in the pathogenesis of arterial occlusive disease. However, the function of neurofibromin in human ECs and VSMCs and the biochemical mechanism by which neurofibromin regulates neoangiogenesis are not known. In addition how Ras signals are terminated in these cells is essentially unknown. In our work here utilizing *Nf1+/-* mice, endothelial progenitor cells derived from NF1 patients, VSMCs harvested from *Nf1+/-* mice, and shRNA targeted to *NF1* to silence neurofibromin deficient ECs and VSMCs have increased proliferation and migration *in vitro* in response to neurofibroma derived growth factors via a discrete Ras effector pathway, the Ras-Mek-Erk pathway. In addition, we demonstrate *Nf1+/-* mice have increased angiogenesis

in vivo in response to neurofibroma derived growth factors via the Ras-Mek-Erk pathway. Furthermore, utilizing shRNA to silence expression of p12-GAP, the other mammalian RasGAP, we show p120GAP deficient primary human ECs have decreased migration in response to neurofibroma derived growth factors. These studies suggest p120GAP and neurofibromin have distinct functions in ECs. Collectively these studies demonstrate neurofibromin functions as a GAP for Ras in vascular cells and identify a unique pathway in *Nf1+/-* ECs as a potential therapeutic target in the neurofibroma microenvironment. Also, they identify neurofibromin as a novel regulator of Ras activity in VSMCs which provides a framework for understanding cardiovascular disease in NF1 patients and a mechanism by which Ras signals are attenuated for maintaining VSMC homeostasis in blood vessel walls.

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