II. Statement of Research Interests (1 page)

Cerebral cavernous malformation (CCM) is a vascular disease, which results in severe phenotypes including brain hemorrhage and stroke. The disease manifests itself principally in endothelial cells as lesions of thin, dilated, and leaky capillaries, which lack normal blood vessel-stromal interactions. CCM affects approximately .1-1% of the entire population with an increased prevalence in Hispanic populations. The disease is recessive and can be inherited or incurred through spontaneous mutation in one of three genes, CCM1/Krit1, CCM2/OSM, or CCM3/PDCD10. These genes encode for proteins that are thought to function as scaffolding proteins that work in multi-protein complexes that regulate cell-signaling networks. Disruption of this signaling in endothelial cells results in an increase in stress fiber incidence, reduced endothelial cell barrier, and reduced angiogenesis.

Current cell culture models of CCM entail the use of finite endothelial cells, which have limited usefulness in fully understanding the development of CCM lesions. Mouse models are now beginning to be utilized; however, generating an accurate animal model of spontaneous CCM, which encompasses the genetic diversity of the human population, is not possible. This basic disconnect underlines the importance of generating new human models of CCM, ideally from patients. It is thought that CCM lesions can arise in adults from endothelial progenitor cells, which are cells from an early embryological origin. Indeed, there have been reports of rapid lesion formation in patients with no familial history of CCM and currently there has been no study into the biology of CCM in terms of endothelial cell differentiation and development. For instance, homozygous genetic ablation of CCM genes results in early embryonic lethality in mouse and zebrafish models, but it is unknown whether this is due to an endothelial cell proliferation or progenitor cell differentiation defect. Thus, I hypothesize that CCM defects arise from loss of CCM1, CCM2, or CCM3 in the proper development of early endothelial cells and understanding these genes during endothelial cell development may lead to new avenues for CCM therapeutic development. Therefore, the aims for my thesis involve using induced pluripotent stem (iPS) cells to derive endothelial cells using donor cells from patients harboring CCM mutations.

This research goal will be accomplished by capitalizing on the properties of pluripotent stem cells, which are both immortal and can readily differentiate into endothelial cells in two project aims. 1) I will determine the effects of RNAi ablation of CCM1, CCM2, or CCM3 on the differentiation potential of human embryonic stem cells (hESCs) to endothelial progenitor cells and to mature endothelial cells. This aim will be realized through reporter based real-time monitoring of endothelial commitment, flow cytometry marker analysis, and in vitro assays of vasculogenesis. I will further conduct genome wide microarray analysis during important endothelial differentiation time points to discover developmentally important genes involved in CCM. 2) In the second aim, I will generate CCM pluripotent cells from CCM patients donating cells for iPS, allowing phenotypic properties and differentiation characteristics to be defined., I will generate these cells by employing recent advances in hESC biology, whereby unlimited populations of iPS cells, which mimic hESCs, are induced by ectopically expressing four transcription factors in primary somatic cells. I propose to generate these iPS cells from CCM patient blood samples with an already approved IRB protocol. I will then test iPS cells harboring CCM mutations for their ability to differentiate properly through assays defined in Aim I. Furthermore, I will test patient specific iPS cell derived endothelial cells for their response to therapeutic treatment such as inhibitors of Rho kinase (Y-27632). The completion of these aims will provide unique knowledge into developmentally important pathways in endothelial cell function, while also generating self-renewing populations of patient CCM samples, which could be beneficial for the therapeutic of screening small molecules to rescue the CCM defects.

III. State How Your Work Relates to Developmental Disabilities (1 paragraph):

My work will be particularly relevant to this fellowship because CCM can be thought of as a developmental disease of endothelial cells, which leads to debilitating conditions in adults. De-novo vasculogenesis is one of the most important early embryonic developmental processes that occurs through the recruitment and differentiation of early hemangioblasts to endothelial cells. In humans it is unknown what effect CCM protein loss has on the early differentiation of these hemangioblasts because a germ-line mutation in one of the three CCM proteins would lead to embryonic lethality. Importantly, human pluripotent cells (hESCs and iPS) proceed through the same developmental pathways as a person as they differentiate. Therefore, this work will shed light into early developmental defects from patients with CCM protein loss that is not otherwise possible. By understanding the normal development and differentiation of endothelial cells we will have a unique understanding of how CCM lesions are developed in the adult.

IV. List of Publications, Abstracts, Awards:

Publications:

Beltran AS, **Richardson BT**, Rivenbark AG, Casbas-Hernandez P, Zimmerman E, Yuan X, Graves LM, Blancafort P: Genome-wide characterization of tumor initiating cells (tics) reveals claudin-low molecular signatures. Submitted. (2010).

Beltran AS, Rivenbark AG, **Richardson BT**, Yuan X, Blancafort P: Isolation of tumor initiating cells (tics) by transduction of the oct4 transcription factor. Submitted. (2010).

Awards:

2007 – Pharmacology NIH Training Grant GM007040
2007- Graduated with honors, East Carolina University, Summa Cum Laude
2006- Mary C. Helms East Carolina Biology Scholarship
2006- East Carolina University undergraduate research stipend award
2005- Phi-Kappa-Phi