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NUMB mediates the interaction between Wnt and Notch to modulate primitive erythropoietic specification from the hemangioblast

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In the developing mouse embryo, the hematovascular and cardiovascular progenitors arise from distinct mesodermal subpopulations that develop in sequential waves from epiblast cells. Studies using ES cell differentiation have led to the identification of a common precursor of the hematopoietic and vascular lineages, the blast colony forming cell (BL-CFC) representing the *in vitro* equivalent of the hemangioblast, as well as the multipotent common progenitors for cardiomyocyte and vascular lineages (cardiovascular colony forming cell, CV-CFC). Using an ES cell line with the green fluorescent protein cDNA targeted to the *brachyury* locus, we previously demonstrated that both of these progenitors co-express BRACHYURY-GFP (GFP-Bry) and FLK1 but develop in a distinct temporal order. The molecular mechanisms that control the sequential establishment of the hemangioblast and the cardiovascular progenitor and the subsequent lineage specification from these two precursors remain poorly understood. Studies using different model systems have shown that Wnt and Notch signaling are involved in many aspects of hematopoietic and cardiac differentiation. Here we show that Wnt and Notch signaling interact through NUMB (an inhibitor of Notch signaling) to regulate primitive erythropoiesis and cardiogenesis. We found that various components of Wnt and Notch signaling pathways are expressed in the developing blast colonies, and that activated NOTCH1 and membrane-associated NUMB proteins are localized in early blast colonies and E7.5 embryos. Taking advantage of soluble inhibitors of Wnt signaling and doxycyclin-induced overexpression of stabilized beta-catenin, we demonstrated that canonical Wnt signaling is critical for primitive erythroid but not other hematopoietic specification. Further studies revealed that the overexpression of constitutively activated NOTCH1 (N1C) in GFP-Bry+/Flk1+ cells specifically inhibits primitive erythropoietic specification and promotes ectopic cardiogenesis, leaving other hematopoietic lineages largely unaffected, while the overexpression of numb specifically promotes primitive erythropoiesis and dramatically potentiates canonical Wnt signaling in inducing primitive erythropoiesis. The interaction between Wnt and Notch signaling mediated by NUMB was confirmed by TOP/FOP reporter assay for β-catenin activity, and quantitative PCR revealed upregulation or downregulation of a series of Wnt inhibitors upon the overexpression N1C or NUMB respectively. These data suggest a mechanism that regulates primitive erythropoiesis versus cardiogenesis through the interplay of the two signaling pathways. Our studies have revealed an essential role of canonical Wnt signaling and a novel role of NUMB in mediating the interaction between Notch and Wnt signaling to induce primitive erythropoiesis from the hemangioblast.

***Keywords*:** primitive erythropoiesis, cardiogenesis, Wnt, Notch1, Numb

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