ABSTRACT

Neutrophils are essential cells of the immune system. They engage in pathogen clearance, inflammatory response, and wound healing. Proper production and activation of neutrophils is critical for the health of an individual, since several disorders are related to neutrophilic alterations. In this thesis, we explore three previously uncharacterized mechanisms that might be involved in the regulation of neutrophilic differentiation. First, we addressed the role of the canonical Wnt signaling pathway. This signaling is executed by interaction of β-catenin with TCF/LEF transcription factors. We employed a murine model that specifically inactivates β-catenin-TCF/LEF-mediated transcription by expressing a dominant negative form of TCF4 (dnTCF4). Using this model in combination with several *in vitro* and *in vivo* assays we demonstrated that β-catenin-TCF/LEF signaling directly upregulates expression of G-CSF receptor in hematopoietic progenitors, imposing myeloid commitment and favoring neutrophilic differentiation. This appeared to be especially important during the response to systemic infection, termed emergency granulopoiesis, as dnTCF4-expressing mice showed high susceptibility to *Candida albicans* infection. Remarkably, the critical role of β-catenin-TCF/LEF signaling for neutrophil differentiation was demonstrated also in human primary cells. Second, we investigated the function of the transcription factor C/EBPγ, whose function in granulopoiesis was, unlike the function of other members of the C/EBP family, uncharacterized. To this aim, we generated a hematopoietic-specific *Cebpg* knock-out mouse. Surprisingly, our results demonstrated that C/EBPγ is dispensable for both steady-state and emergency granulopoiesis. Third, we focused on a completely unknown gene, *EVI2B*, which was found to be directly upregulated by the transcription factor C/EBPα, a master regulator of neutrophilic differentiation. With the use of *EVI2B* knock-down approaches in human and murine cell lines, primary cells, and *Evi2b* deficient mice we showed that the transmembrane protein EVI2B is necessary for proper neutrophil differentiation. Altogether, our work deepens our understanding of the processes regulating the production of neutrophils, and presents novel mechanisms that could be clinically modulated to interfere with granulopoiesis.