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Canonical and non-canonical Wnt signaling in hematopoiesis and lymphocyte development

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Summary in English

The immune system of mammals is responsible for protecting our body against pathogens and foreign substances (antigens), and it consists of two discrete lines of defense. The first line called innate immunity and provide a quick and nonspecific defense. The innate immunity includes different cells types, such as mast cells, macrophages, neutrophils, eosinophils, dendritic cells and natural killer (NK) cells. The second line of defense called adaptive immunity responds in an antigen-specific manner, and comprised of B and T lymphocyte cells.

All types of immune cells mentioned above are derived from a unique cell type that resides in bone marrow (BM), called hematopoietic stem cells (HSCs). This rare cell type is characterized as multipotent cells because they can differentiate into any type of immune cells just by receiving the "right" signal, but also they have the potential to repopulate (self-renewal) in order to maintain enough pool of precursor cells.

All classes of immune cells except T lymphocytes develop and maturate in the bone marrow. For T cell development, HSCs should migrate into the thymus via the circulation and seed the thymus. Thymic seeding progenitors (formerly HSCs) will first expand in the thymus in order to sustain sufficient pool of progenitor T cells and subsequently they develop towards mature and functional T cells. It is known that thymus is the only organ in our body which could provide right signals for T cell development.

This thesis focuses on one of the signals which are known to play an important role during HSC repopulation and T cell development that is Wnt signaling pathway. Depending on the tissue/cell types (microenvironment) and specific class of Wnt proteins binding to the corresponding receptors on the developing lymphocytes, two discrete downstream pathways will be activated namely canonical or non-canonical Wnt pathway. The main aim of this thesis is to dissect the roles of these two distinct pathways during hematopoiesis and lymphocyte development in murine as a physiologically relevant animal model.

In **chapter 2** we investigated the role of canonical Wnt signaling in HSCs repopulation and differentiation in the BM. One of the most common approaches to study roles of genes is manipulation of the genes of interest e.g. knocking out or overexpressing by mutations. In this chapter, we used such a genetic tool to overexpress canonical Wnt signaling at various levels (low to high) in HSCs. The aim of this study is to solve the controversy of the previous studies observed due to the application of different gain of function and loss of function genetic models. Our results show that high levels of Wnt signaling results in the loss of stem cell repopulation. We further explored the mechanisms underlying this phenomenon using gene expression and functional analysis approaches. Our data revealed that high

levels of Wnt signaling in HSCs is in favor of differentiation thereby reduce the self-renewal of stem cells. This led to the exhaustion of HSCs pool. Therefore, an optimal dosage of Wnt signaling is crucial for the homeostasis of HSCs by maintaining sufficient pool on one hand and the induction of differentiation towards mature immune cells on the other hand.

In **chapter 3** we focused on the role of non-canonical Wnt signaling in HSCs by using another genetic model in which one of the non-canonical Wnt receptors called Ryk is knocked out. We performed functional experiments by transplanting the Ryk KO stem cells into the irradiated mice and monitored the reconstitution of the immune system over time. Our data suggest that the absence of non-canonical Wnt signaling via Ryk deficiency results in a mild decrease of stem cell repopulation. Further mechanistic studies showed that the loss of stemness is caused by an increased apoptosis (programmed cell death) and decreased proliferation in the HSCs

In **chapter 4** we performed a side by side study of the effect canonical and non-canonical Wnt signaling in lymphocyte and in particular T cell development. Here we used a gain of function approach by overexpressing canonical Wnt ligands (Wnt3a) and non-canonical Wnt ligand (Wnt5a). Our in vitro studies revealed that high levels of Wnt signaling inhibit T cell development while intermediate levels accelerate this process, confirming that the optimal dosage of Wnt signaling is also crucial during T cell development in the thymus. On the other hand, activation of Wnt5a non-canonical Wnt signaling is harmful to the T lymphopoiesis and increase apoptosis in the developing T cell progenitors. Our in vivo studies (transplantation assays) showed that overexpression of Wnt3a is in favor of lymphopoiesis while overexpression of non-canonical pathways promotes myeloid differentiation. The latter considered as an inefficient hematopoiesis which happens at the older ages and known as senescence of the stem cells. These findings (**chapters 2-4**) enhanced our understanding of the biology of HSCs and T cell development and could help us to develop more efficient protocols for HSC expansion and T cell reconstitution in future.

Aberrant Wnt signaling, e.g. genetic mutations in one of the key components of the pathway, has been reported in various types of leukemia and lymphomas (white blood cell's cancer). Mutations in TCF-1 (T cell factor) has been shown in several patients with leukemia. TCF-1 is a crucial transcription factor during T cell development and its expression is regulated by canonical Wnt signaling pathway. In **chapters 5 and 6** we studied the role Tcf-1 during normal T cell development (**chapter 5**) and during an occurrence of thymic malignancy (**chapter 6**) by using a loss of function model of TCF-1.

Our data in **chapter 5** revealed that deficiency in Tcf-1 results in partial blocks at various stages of T cell development while inducing development of non-T cells within the

thymus. Overexpression of Bcl-11b (another crucial gene for T cell commitment) rescues the T cell development even in the absence of Tcf-1 showing that Tcf-1 functions via Bcl-11b to promote T cell development. However, in order to suppress the development of non-T cells, we upregulated another gene called Gata-3. These results reveal that T cell development is controlled by a minimal transcription factor network involving Tcf1, and the subsequent division of labor between Bcl11b and Gata3, thereby ensuring a properly regulated T cell gene expression program.

At older ages, mice with deficiency of Tcf-1 develop highly metastatic thymic lymphoma. In **chapter 6** we performed mechanistic studies to understand the cause of lymphoma development. Deregulation of Wnt signaling (high expression of Wnt target genes) observed in the leukemic T cells. Further studies revealed that Tcf1 is higher expressed than Lef1 (another transcription factor downstream of Wnt pathway), with a predominance of Wnt inhibitory isoforms. Loss of Tcf1 as the repressor of Lef1 leads to high Wnt activity and is the initiating event in lymphoma development. Thus, we showed that Tcf1 acts as a molecular switch between proliferative and repressive signals during T-lymphocyte development in the thymus.

It has been a long-standing challenge to mimic the physiological signals in vitro or ex vivo, with the aim to proliferate and manipulate HSCs and/or thymocytes for clinical purposes. The possibility of stimulating these cells with Wnt ligands is a very attractive approach since this would result in a more controlled therapy, compared to approaches requiring permanent gene modification. This would ultimately result in a faster thymic reconstitution by thymic seeding cells. The finding obtained in this thesis could be used for the above-mentioned purposes in future.