Chapter 1

Introduction

1.1 Background

Blood is indispensable in human life as it is responsible for oxygen transport, immunity, and healing. Organs and tissues rely on red blood cells to receive oxygen and nutrients in order to maintain their vital function, while white blood cells play a crucial role in protecting the body against diseases and foreign threats (Cherian, 2022). Moreover, platelets are important for blood coagulation, wound healing, and maintaining homeostasis (Chen et al., 2020). As a vital life source, blood diseases and abnormalities tend to have detrimental clinical consequences and serious physiological effects. Hence, studying the underlying characteristics and molecular mechanisms of blood regulation in the human body may allow a better understanding of processes behind blood disorders and malignancies (Shah et al., 2024).

The process of making new blood cells and their components is called hematopoiesis, which begins during embryonic development and is maintained throughout adulthood in order to replenish the blood system (Dzierzak & Bigas, 2018). Hematopoiesis begins with the maintenance of hematopoietic stem cells (HSC), which will then develop into progenitor cells that can further differentiate into specialized effector cells (Höfer & Thomas, 2018). For example, the common myeloid progenitor cells will eventually differentiate into a variety of white blood cells or be specified into megakaryocyte/erythrocyte progenitor cells to produce megakaryocytes and erythrocytes, while the common lymphoid progenitor will differentiate into Natural Killer (NK) cells and B- and T-lymphocytes (Zelm et al., 2019).

The differentiation and survival of blood cells during hematopoiesis are largely controlled by a variety of signaling molecules and pathways. One of the main pathways involved in hematopoiesis includes

the phosphoinositide 3-kinase (PI3K)/Akt pathway, which is responsible for HSC maintenance, lineage commitment, cell metabolism, and cell survival. Downstream of PI3K/Akt signaling is the mechanistic target of rapamycin (mTOR) pathway, which is known to control the activation or quiescence state of HSCs by sensing signals from nutrients, growth factors, and cytokines. This specific function of the mTOR pathway is important for preserving the stem cell pool and maintaining the HSCs' self-renewal capacity during hematopoiesis (Wu et al., 2021).

The mTOR pathway is regulated by two multiprotein complexes called mTORC1 and mTORC2, which have their own specific roles. In high-energy and nutrient-rich conditions, mTORC1 is primarily activated to promote anabolic metabolism, increasing energy production, hence supporting the expansion and differentiation of progenitor cells. However, overactivation of mTORC1 would eventually exhaust the HSC pool and lead to dysregulation of cell lineages (Malik et al., 2018). While the role of mTOR and its upstream Akt signaling has been extensively studied, the role of its downstream proteins in hematopoietic development and specification remains unclear. As mTORC1 acts as a primary regulator in hematopoiesis, assessing its regulatory proteins may provide a better understanding of hematopoiesis regulation.

Among these proteins is the Akt-Associated Protein (AAP), which is thought to interact with mTORC1 by integrating growth signals in the maintenance and differentiation of HSCs. Several studies suggested that AAP may function as a conditional inhibitor for mTORC1, leading to repression of mTORC1 activity, and presumably hindering cell proliferation and cell growth. However, it was also found that the function of AAP may change and is highly dependent on cell type (Wiza et al., 2012; Zhang et al., 2019). Nonetheless, the main function of AAP in vertebrate hematopoiesis has yet to be elucidated in detail; therefore, this study aims to characterize the function of AAP in hematopoiesis by generating AAP knockout zebrafish mutants using CRISPR/Cas9 and to characterize their phenotypical hematopoietic changes by performing *in situ* hybridization of hematopoietic

lineage-specific genes and subsequent quantification using qPCR. Zebrafish will be used as the hematopoietic model in this experiment due to its genetic tractability, conserved hematopoietic program, and transparent embryos, which will provide optimal *in vivo* visualizations (Sofyantoro et al., 2024).

1.2 Objective

The aim of this study is to characterize hematopoietic defects in AAP knockout embryos through lineage-specific *in situ* hybridization, followed by qPCR to quantify their corresponding downstream gene expression.

1.3 Hypothesis

It is hypothesized that the AAP regulates hematopoietic stem cell and progenitor cell maintenance, lineage specification, and survival during zebrafish development via modulation of mTORC1 activity. The knockout of AAP is expected to show impaired hematopoietic development, which will be evident in a decrease in most hematopoietic lineage marker expression, but an increase in myeloid lineage markers.