Chapter 1: Introduction

1.1. Background

Multiple myeloma (MM) is a hematological neoplasm characterized by growth of abnormal plasma cells (PC) that infiltrate the bone marrow (BM) (van Nieuwenhuijzen et al., 2018). MM belongs to a group of disease with elevated paraproteins, called paraproteinemia (Siegel et al., 2019). Paraproteins are monoclonal immunoglobin (Ig) or M proteins from the post-germinal B-cell lineage (Kazandjian, 2016). MM cells require survival pathways to control protein production and homeostasis due to elevated Ig production (Barwick et al., 2019; Crawford & Irvine, 2013). Misfolded or non-functional peptides produced by MM cells have to be disposed of (Barwick et al., 2019). A key survival pathway used by MM cells is the ubiquitin proteasome system (UPS). Ubiquitination, a type of post-translational modification, is mediated by three classes of enzymes, each with a specific function: activating ubiquitin, conjugating, and selecting a substrate to be ubiquitinated (Kao et al., 2018). The attachment of ubiquitin to target protein can occur as a monomer (mono-ubiquitination) or a polymer (polyubiquitination), resulting in different biological consequences (Crawford & Irvine, 2018). The most widely studied polyubiquitination are lysine linkage (K) polyubiquitination at position 48 and 63 of ubiquitin molecule (K48- and K63-linked polyubiquitination) and both types of polyubiquitination support survival pathways of MM. K48-linked polyubiquitination targets protein to be degraded by proteasome, while K63-linked polyubiquitination regulates protein for downstream signaling, protein localization, DNA damage response, and protein-protein interaction (Crawford & Irvine, 2018). K48-linked polyubiquitination is involved in the UPS. In the UPS, the proteins undergo ubiquitination, followed by proteasomal degradation. The process of ubiquitination is reversible, and ubiquitin can be removed by deubiquitinating enzymes (DUBs) (Crawford & Irvine, 2013). Ubiquitination and deubiquitination equilibrium plays a pivotal role in regulating the protein levels and their functions within the cells (Crawford & Irvine, 2018).

The administration of proteasome inhibitors (PIs) leads to build-up of misfolded or non-functional proteins, that can activate cell death through the unfolded protein response that increase

endoplasmic reticulum stress, hence activating signaling pathways for apoptosis to dispose damaged cells (Barwick et al., 2019; Hetz, 2012). The activation of unfolded protein response specific to B cells also showed that B cell Ig productions were inhibited (Hetz, 2012). Three PIs are currently clinically approved to treat MM, bortezomib, carfilzomib, and ixazomib (Gandolfi et al., 2017). However, these drugs can be limited by resistance and toxicities in some patients, such as neurotoxicity and cardiotoxicity, highlighting the need to find other target enzymes within the UPS that are more specific and less toxic (Lubbers & Mohler, 2016; Manasanch & Orlowski, 2017). Emerging targets within the UPS, such as HECT-domain containing E3 ligase (HUWE1) and ubiquitin specific protease 7 (USP7), are found to participate in oncogenesis.

HUWE1 (also known as Mule, ARF-BP1, and HECTH9) is reported to be involved in the tumorigenesis of MM through regulation of proto-oncogene, such as Myc and Mcl-1, and tumor suppressor genes, including p53 (Crawford & Irvine, 2018; Janz et al., 2019; Kao et al., 2018; Walker et al., 2018). K63-linked polyubiquitinated of the proto-oncogene c-Myc via HUWE1 can enhance transcriptional activity, activate DNA replication, regulate proliferation, and alter tumor microenvironment (Jovanović et al., 2018). HUWE1 also affects p53, a tumor suppressor gene, by targeting it for degradation via K48-linked polyubiquitination, hence inhibiting apoptosis (Hao et al., 2012). Small molecule inhibitors of HUWE1, BI8622 and BI8626, have been reported to decrease Myc protein expression via Miz1 accumulation significantly, which results in Myc target gene repression, however these inhibitors have unfavorable pharmacokinetics (Peter et al., 2014). Myeloid cell leukemia 1 (Mcl-1) has been reported to be overexpressed in MM and is related to chemotherapy resistance, cancer progression, relapse, and lower survival (Wuillème-Toumi et al., 2005). It is an anti-apoptotic protein that is important for cancer cell survival (Tron et al., 2018). HUWE1 regulates Mcl-1 during DNA damage by addition of K-48 polyubiquitin chains and thereby promoting Mcl-1 degradation (Myant et al., 2017). Expression of p53 can inhibit the expression of Mcl-1 through promoter repression (Pietrzak & Puzianowska-Kuznicka, 2008). Therefore, HUWE1 can directly or indirectly affect Mcl-1 (Myant et al., 2017; Tron et al., 2018).

USP7 is polyubiquitinated by HUWE1 at K63 to enhance deubiquitinating activity towards hypoxia inducible factor (HIF)-1α during hypoxia (Kao et al., 2018). Moreover, USP7 forms a complex with HIF-1α and other factors, hence increasing angiogenesis and metastasis (Kao et al., 2018). A study also shows that an isoform of USP7 controls the stability of HUWE1 by inhibiting its self-ubiquitylation and degradation (Khoronenkova & Dianov, 2013). The application of a USP7 inhibitor, P5091, has been reported to lower HUWE1 protein level and overcome bortezomib resistance in a pre-clinical study (Bosshard et al., 2017; Chauhan et al., 2012). A new selective allosteric USP7 inhibitor AD04, has recently been developed and is reported to stabilize p53, increase p21, and reduce mouse double minute 2 homolog (MDM2) levels in colorectal cancer and breast adenocarcinoma cell line (Gavory et al., 2018).

1.2. Objectives

In this study, we aim to elucidate the ability of USP7 inhibitor AD04 to reduce proliferation of MM cells *in vitro* and its pathway related to adhesion molecules, modulate HUWE1 protein expression and its downstream proteins, and affect cell cycle of MM cells.