Abstract

Multiple myeloma (MM) is a hematological cancer characterized by dysregulated ubiquitin proteasome system that promotes the survival of MM. Several ubiquitin enzymes are involved in tumorigenesis, including USP7, a deubiquitinating enzyme. USP7 regulates an E3 ligase, HUWE1 by preventing HUWE1 degradation, hence promoting HUWE1 protein stability. Both of the enzymes have become an attractive drug target, resulting in a recent development of USP7 inhibitor, AD04. We investigated the ability of AD04 to decrease proliferation of MM cells both in suspension and co-culture with bone marrow stromal cells (BMSCs) *in vitro*. In addition, we also elucidated the effects of AD04 towards HUWE1 and USP7 downstream protein Mcl-1, p53, c-Myc, adhesion molecule that mediates MM and BMSC interaction, and cell cycle. We observed that AD04 has greater sensitivity to certain MM cell lines both in suspension and co-culture, including OPM2 and XG-1, in a dose-dependent manner and it also promotes G1 cell cycle arrest. We also found that adhesion molecule Integrin α4 was increased, however the other types of adhesion molecule expressions have to be analyzed to further understand the implication. In addition, AD04 treatment induced accumulation of tumor suppressor p53 proteins and reduced antiapoptotic Mcl-1 proteins. Taken together, USP7 inhibitor AD04 is a potential cytostatic drug to target MM through regulating HUWE1 and its downstream proteins.