ABSTRACT

PU.1 is a key hematopoietic transcription factor. Knock-out of PU.1 in mouse is embryonic lethal due to complete depletion or several disruption of differentiation of multiple blood cell lineages. Low level of PU.1 and the disruption of its regulation are associated *in vivo* with acute myeloid leukemia and other hematologic malignancies.

Myelodysplastic syndrome (MDS) is hematopoietic stem cell disorder with extremely heterogeneous features and outcome. It is characterized by improper differentiation of blood cells resulting in loss of function, dysplasia and blasts accumulation in bone marrow. About one third of MDS cases transforms into AML. MDS is also characterized by silencing of gene expression caused by aberrant DNA hypermethylation. Using DNA Methyltransferase inhibitors (DNMTi) such as 5-azacitidine (AZA) has good clinical results for the MDS patients with higher risk of disease. Indeed, AZA became standard therapy of high risk MDS in recent years. Nonetheless, our understanding of molecular mechanisms of AZA remains incomplete.

This PhD thesis reports about the role of transcription factor PU.1 in MDS. We found that significant subset of high risk MDS patients express low level of PU.1 due to DNA hypermethylation of PU.1 upstream regulatory element (URE). We also found significant relationship between levels of PU.1 expression and response of patients to AZA treatment. AZA is capable to significantly demethylate DNA of URE and may also initiate other epigenetic changes on its chromatin such as histone modifications pattern. These changes result in upregulation of PU.1 expression and triggers myeloid differentiation of transformed MDS cell lines and CD34+ progenitors isolated *ex vivo* from a patient bone marrow. Effects of AZA on PU.1 expression and myeloid differentiation can be modified – attenuated or enhanced – by pre-stimulation with the cytokines including Granulocyte-colony stimulating factor (G-CSF). AZA also inhibits cell proliferation and cause mild apoptosis in MDS cell lines.

This work collectively provides important observations, that are currently further studied to be used in the future for *in vitro* assessment of AZA efficiency.