Hematopoiesis is a highly orchestrated process, in which a single hematopoietic stem cell (HSC) gives a rise to all blood cellular components. For myeloid and lymphoid development precise controlled expression of the PU.1 transcription factor is needed. Deletion of PU.1 gene in mouse is lethal and its dysregulation during hematopoietic differentiation is associated with blood malignancies including acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS). MDS and AML are serious blood disorders characterized by expansion of immature blood cells and lack of differentiated functional cells. Not only genetic but also epigenetic aberrations represent a very important field for studying pathophysiology of leukemia genesis and dysregulation of the PU.1 gene represents intensively studied candidate mechanism. Modern therapy of selected MDS and subset of AML patients is based on treatment with DNA hypomethylating agent Azacytidine (AZA) interfering in PU.1 gene regulatory mechanism. However, poor response or resistance to this therapy often occurs.

In this thesis we present data obtained from AZA-resistant clones of MDS/AML cell line OCI-M2. We analysed DNA methylation and DNA hydroxymethylation at the key regulatory element of the PU.1 gene (URE). We found that these epigenetic modifications at URE strongly influence the PU.1 gene expression. We found that in subset of AZA-resistant clones, AZA was not sufficient to demethylate DNA within URE, leading to low PU.1. Furthermore we identified an enzyme from TET protein family, TET3, responsible for DNA methylation to DNA hydroxymethylation conversion in OCI-M2. Collectively, data presented in my thesis bring a new insight into epigenetic regulatory mechanism of the PU.1 gene targeted by AZA therapy in AML and MDS.