

Abstract

Chromatin remodeling protein Smarca5 participates on many cellular processes, which are important for tissue development and tumorigenesis. Among these processes utilizing ATPase activity of Smarca5 belong also transcription, replication and DNA repair. We hypothesized that Smarca5 represents essential molecule for chromatin modulation primarily at early developmental stages at the level of fast-dividing progenitors of many origins, in whose the ATPase is highly expressed. To such tissues may belong also hematopoiesis, in which the Smarca5 has highest expression. The subject of my doctoral thesis is therefore analysis of the effect Smarca5 depletion on proliferation and differentiation of hematopoietic progenitors *in vivo* and a search for mechanisms behind the resulted developmental defects. We utilized conditionally knockout allele of Smarca5 in blood precursors to study in a mouse model how depletion of the ISWI ATPase causes accumulation of earliest progenitors inhibited from further maturation to erythroid and other myeloid lines. The proerythroblasts became dysplastic and the majority of basophilic erythroblasts ceased cycling around the G2/M stage. An expected mechanism for observed changes appeared the activation of stress pathway of protein p53 that is often associated with unrepaired DNA damage. Analysis of Smarca5 deletion in progenitors of lymphocytes also revealed that this lineage is very sensitive to loss of chromatin remodeling activities of Smarca5. We also observed that a block of development in very early T and B cell stages, similarly to erythroblasts, also resulted in an activation of p53. By mating a null allele of *Trp53* into our mouse model of *Smarca5* conditional deletion we observed a very mild improvement of the phenotype. We therefore concluded that activation of p53 signaling pathway is not a major determinant of cell cycle arrest of Smarca5-null lymphocytes. Most prominent were changes in the expression profile of immature T lymphocytes that were markedly developmentally delayed as exemplified by the expression of earliest transcripts that were not silenced while transcripts belonging to more mature stages were not induced. These data thus strongly suggest that Smarca5 plays significant roles in setting the developmentally-specific gene expression pattern. This possibility we next explored further in detail to study genes regulated by a major epigenetic transcription factor called CTCF. We found that SMARCA5 regulates recruitment of CTCF on DNA near *SPI/PU.1* gene to temporally repress its expression during myelopoiesis. We think that this mechanism might be hijacked by acute myeloid leukemia cells in blocking the myeloid differentiation pattern of gene expression. In conclusion our data revealed that the ISWI ATPase Smarca5 has substantial yet not known

indispensable roles in early hematopoietic and lymphoid development to guide gene expression of differentiation control.