Abstract

The process of cell differentiation is dependent on the chromatin-remodeling activity of the ISWI ATPase SMARCA5 (SNF2H) and its complexes. A series of mouse models have been used to study the functions of this ATPase, starting with a model with constitutive deletion of the *Smarca5* gene and followed by models with tissue-specific deletion of *Smarca5* gene (especially at different stages of hematopoiesis). The severity of phenotypic manifestations in mouse models varies from early embryonic lethality in the constitutive deletion model, through fetal lethality associated with erythropoiesis failure (Vav1iCre model - deletion in the definitive hematopoietic progenitor) to lymphopoiesis defects that do not limit animal survival (hCD2iCre model - deletion in T and B lymphocytes) in conditional deletion models. This work builds on the observations obtained in the study of deletion models of this *Smarca5* gene, i.e. that deletion leads to arrest of cell proliferation and their entry into apoptosis. Defects were observed already at the stem cell and hematopoietic progenitor stages, which were unable to enter the differentiation process in the absence of the SMARCA5 protein. In these models, it was not possible to study the progression of differentiation because of this reason. Therefore, we decided to create a new mouse model with a hypomorphic (expressing lower amounts of the protein) *SMARCA5* transgenic allele (*S5tg*), expressed on a *Smarca5* deletion background, which allowed us to observe hematopoietic cell differentiation at different levels of SMARCA5 protein expression. We observed that transgenic *SMARCA5* can rescue lymphocyte developmental arrest in a tissue-specific model of *Smarca5* deletion in this lineage (hCD2iCre), depending on the amount of protein expressed, as well as the lethal phenotype associated with constitutive deletion or conditional deletion in hematopoietic stem cells (Vav1iCre). The *Vav1iCre S5tg* model has shown that the expression level of SMARCA5 protein plays a crucial role in hematopoietic stem cells and progenitors; when the expression of this protein is reduced (to about 10 %), it results in the accumulation of multipotent progenitors that are unable to differentiate. The observed defect resulting from reduced SMARCA5 protein levels has the greatest impact on the differentiation of lymphocytes and their progenitors, especially in B-lymphocytes, which are the most sensitive to SMARCA5 protein levels, leading to a significant reduction in their numbers in peripheral blood. Number of erythrocytes was only minimally reduced and myelocytes were not affected by hypomorphic SMARCA5 expression. The transgenic SMARCA5 model demonstrates that the ISWI ATPase SMARCA5 is indispensable for the proper development of hematopoietic cells, especially lymphocytes. This research

contributes valuable insights to deepen the knowledge of this important epigenetic regulator, which can be used in the development of therapeutic strategies for hematopoietic disorders.

Keywords

chromatin, Snf2h, Smarca5, ATPase, mouse model, hematopoiesis, stem cells, multipotent progenitors