Abstract

Enhancers are distal cis – regulatory DNA sequences that regulate (enhance) transcription of the respective gene driven by its promoter. Enhancers are found in non-coding DNA upstream or downstream of the gene coding sequence, or in introns or coding regions that are located up to hundreds kb away from the gene.

Superenhancers are newly discovered clusters of multiple enhancers that play a vital role in activating tissue-specific genes, determining cell identity and regulating differentiation.

PU.1 is the transcription factor (TF) that is necessary for normal haematopoiesis, specifically for the development of myeloid and lymphoid blood lineages. Distinct levels of PU.1 induce differentiation of hematopoietic cells into different cell lineages whereby disruption of PU.1 levels leads to leukemogenesis. High PU.1 levels stimulate macrophage development, while intermediate levels stimulate the development of granulocytes. This diploma thesis seeks to contribute to addressing the interesting biological question of what are the regulatory mechanisms to ensure that granulocytic genes are activated only at the intermediate concentration of PU.1, whereas macrophage genes are activated only at its high levels.

The aim of this diploma thesis was to create a series of reporter vectors carrying regulatory regions of granulocyte genes MPO and MMP9, which would allow the study of mechanisms of how different levels of TF PU.1 are sensed by regulatory sequences of target genes. Based on the analysis of the regulatory regions of the MPO and MMP9 genes we identified 4 MPO and 14 MMP9 putative enhancers +/- 100 kb from the transcriptional start. Subsequently, we created 48 luciferase constructs carrying the regulatory regions of the MPO and MMP9 genes and their variants or combinations. The resulting luciferase constructs were functionally tested during the differentiation of PU.1 transgenic myeloid progenitors initiated by different levels of PU.1. Data show that 7 of 13 MMP9 and 2 out of 3 MPO of enhancer constructs that were tested had activation ability at the intermediate (granulocytic) level of PU.1. This suggests that the expression of these genes is co-regulated by multiple domains that likely constitute a superenhancer. The sequential assembly of individual MPO enhancers led to an additive activation effect. While the activation potentials of the individual enhancers were relatively low, their activity gradually increased in the presence of other enhancers and/or proximal promoter, indicating the need for the collaboration of the individual enhancers necessary for optimal tissue-specific expression of MPO and MMP9 genes.

The generated enhancer constructs were subsequently used for mutagenesis of PU.1 binding sequences with different affinities and will be utilized for the testing of PU.1 co-operative transcription factors.

Keywords: Transcription regulation, Enhancer, Superenhancer, Differentiation, PU.1