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Abstract:

This dissertation examines the molecular mechanisms regulating Rac2 gene expression during cell differentiation and identification of a minimal cis-element required for the induction of Rac2 gene expression during K562 cell differentiation. The Rho family GTPase Rac2 is expressed in hematopoietic cell lineages and is further up-regulated upon terminal myeloid cell differentiation. Rac2 plays an important role in many hematopoietic cellular functions, such as neutrophil chemotaxis, superoxide production, cytoskeletal reorganization, and stem cell adhesion. Despite the crucial role of Rac2 in blood cell function, little is known about the mechanisms of Rac2 gene regulation during blood cell differentiation. Previous studies from the Skalnik lab determined that a human Rac2 gene fragment containing the 1.6 kb upstream and 8 kb downstream sequence directs lineage-specific expression of Rac2 in transgenic mice. In addition, epigenetic modifications such as DNA methylation also play important roles in the lineage-specific expression of Rac2. The current study investigated the molecular mechanisms regulating human Rac2 gene expression during cell differentiation using chemically induced megakaryocytic differentiation of the human chronic myelogenous leukemia cell line K562 as the model system. Phorbol 12-myristate 13-acetate (PMA) stimulation of K562 cells resulted in increased Rac2 mRNA expression as analyzed by real time-polymerase chain reaction (RT-PCR). Luciferase reporter gene assays revealed that increased transcriptional activity of the Rac2 gene is mediated by the Rac2 promoter region. Nested 5′ - deletions of the promoter region identified a critical regulatory region between -4223 bp and -4008 bp upstream of the transcription start site. Super shift and chromatin immunoprecipitation assays indicated binding by the transcription factor AP1 to three distinct binding sites within the 135 bp minimal regulatory region. PMA stimulation of K562 cells led to extensive changes in chromatin structure, including increased histone H3 acetylation, within the 135 bp Rac2 cis-element. These findings provide evidence for the interplay between epigenetic modifications, transcription factors and cis-acting regulatory elements within the Rac2 gene promoter region to regulate Rac2 expression during K562 cell differentiation.

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