RETINOIC ACID–DEPENDENT ACTIVATION OF GUANYLYL CYCLASE/NATRIURETIC PEPTIDE RECEPTOR-A GENE TRANSCRIPTION AND EXPRESSION BY ENHANCED RECRUITMENT OF SP1, ETS-1, AND ACETYLATED HISTONES TO THE CORE PROMOTER.

Prerna Kumar, Gevoni Bolden, and Kailash N. Pandey.

Department of Physiology, Tulane University Health Sciences Center and School of Medicine, New Orleans, LA.

Activation of guanylyl cyclase/natriuretic peptide receptor-A (GC-A/NPRA) by cardiac hormones atrial and brain natriuretic peptides produces the second messenger cGMP, which activates downstream signaling and biological effects of NPRA including vasorelaxation, anti-mitogenic, and anti-hypertrophic effects. The objective of the present study was to gain insight into the signaling mechanism of all-trans retinoic acid (ATRA) in the regulation of Npr1 (coding for GC-A/NPRA) gene transcription and expression. Mouse mesangial cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and ITS (insulin, transferrin, and sodium selenite) and were transiently transfected using Lipofectamine-2000. The role of ATRA-mediated transcriptional regulation of Npr1 gene was studied by luciferase assay, real time RT-PCR, and sequential chromatin immunoprecipitation assay (ChIP). The results showed that ATRA significantly increased mRNA, protein expression, and guanylyl cyclase activity of NPRA. Furthermore, the Npr1 promoter from the region -365 to +55 base pairs showed a 10-fold increase in luciferase activity after treatment with ATRA. As confirmed by ChIP assays, ATRA enhanced in vivo binding of Ets-1 and Sp1 to Npr1 promoter. Moreover, retinoic acid receptor α (RARα) was recruited by both Ets-1 and Sp1 to form a transcriptional regulation complex with their binding sites in Npr1 promoter. Furthermore, ATRA increased acetylation levels of both histones H3 and H4 and enhanced their binding in the Ets-1/Sp1 binding sites region of the Npr1 promoter. Collectively, our results show that retinoic acid induces Npr1 gene transcription and expression via RARα, Ets-1, and Sp1 transcription factors in target cells. The identification of retinoic acid signaling as a regulator of Npr1 gene will have important implications in hypertension and cardiovascular regulation. Work supported by NIH grants HL57531 and HL62147.