

Transcriptional and Post-transcriptional Control of Nhlh2 with Differing Energy Status

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ABSTRACT

Nescient Helix Loop Helix 2 (Nhlh2) is a member of the basic helix-loop-helix transcription factor family. Mice with a targeted deletion of Nhlh2, called N2KO mice, show adult onset obesity in both males and females. Nhlh2 regulates other genes by binding to the E-box in the promoter region of these genes. This transcription factor regulates many other transcription factors including MC4R and PC1/3 which are associated with human obesity. The Nhlh2 promoter has been analyzed for putative transcription factors binding sites. These putative binding sites have been tested to be the regulators of Nhlh2 by transactivation assays with mutant promoters, Electrophoretic Shift Assay (EMSA), and Chromatin Immunoprecipitation Assay (ChIP) as methods to investigate the DNA-protein binding.

The results of these experiments showed that the Nhlh2 promoter has five Signal Transducer and Activator of Transcription 3 (Stat3) binding site motifs at -47, -65, -80, -281, -294 and two Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NFκB) binding site motifs at -67 and -135. While NFκB acts as a negative regulator of Nhlh2, this research showed that Stat3 acts as a regulator for the Nhlh2 basal expression and leptin stimulation. The ChIP assay using chromatin from mouse hypothalamus and antibodies against Stat3 and the NFκB subunits P50, P65, and c-Rel demonstrated that all of these antibodies were able to pull down the part of the Nhlh2 promoter containing the binding sites of Stat3 and NFκB. The EMSA results not only demonstrated that NFκB and Stat3 binding site motifs are real binding sites, but also exists the possibility of a relationship between these transcription factors to regulate Nhlh2 expression with leptin stimulation.

An effort in analyzing the human NHLH2 3'UTR showed that one of the SNPs located at position 1568 in the NHLH2 mRNA (NHLH2^{A1568G}) which converts adenosine to guanine might have the potential to decrease the mRNA stability. For more investigation about this SNP, the mouse Nhlh2 tail was cloned into 2 different vectors and these vectors were subjected to site directed mutagenesis to create the 3'UTR SNP that convert A to G. One of these vectors used luciferase as a reporter gene for expression while the other one was used to measure Nhlh2 mRNA stability. These vectors were transfected into hypothalamic cell line N29/2 to test the effect of this SNP on Nhlh2 expression. This study demonstrated that this SNP down regulated luciferase expression and also decreased Nhlh2 mRNA stability.

Taken together, this study demonstrated that Nhlh2 could be regulated transcriptionally by both NFκB and Stat3 transcription factors and post-transcriptionally by the 3'UTR SNP that converts adenosine to guanine.

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