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Midbrain Dopaminergic Transcription Factor Nurr1 Represses the Induction of Dopaminergic Phenotypes in Human Neural Stem Cells

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Expression of tyrosine hydroxylase (TH) gene, the rate-limiting enzyme of cathecolamine biosynthesis, is regulated at transcriptional level during neuronal development. Nurr1, a member of the orphan nuclear receptor superfamily, plays a pivotal role in terminal differentiation of midbrain precursor cells into a complete DA phenotype and is expressed in midbrain DA cells in SN and ventral tegmental area during development. To determine if Nurr1 may be responsible for transcriptional regulation of human TH gene during dopaminergic neurogenesis, we constructed and fused a deletion series of TH promoter gene into a luciferase reporter gene and assessed the activity of promoter in neuronal and non-neuronal cell lines. In 5'-flanking sequence of human TH gene, 3 consensus elements for Nurr1 (NBREs) are located at position -2413 to -2406 (NBRE A), -1440 to -1433 (NBRE B) and -833 to -824 (NBRE C), respectively. Analysis of a deletion series of human TH promoter demonstrated that TH promoter activity occurred in a neuron cell type-dependent manner and NBRE-A and -B regions induced Nurr1-mediated transcriptional activation in human dopaminergic neuroblastoma SH-SY5Y cells. Among these putative motifs, EMSA and competition assay show that NBRE-A, but not NBRE-B or NBRE-C, has high binding affinity to Nurr1. Consistent with these results, site-directed mutational analysis confirmed that that NBRE-A was most critical for mediating the transcription by Nurr1. In addition, supershift experiments showed that Nurr1 dimerize with other cofactors such as RXR-alpha or RAR in NBRE-A site. These data suggests that Nurr1 directly mediated transcriptional activation of TH gene through NBRE-A site and a key regulating complex of TH expression may be formed in NBRE-A site during dopaminergic neurogenesis. We will further examine the possible cooperative action of Nurr1 and other transcription factors in regulation of human TH gene expression.