Target genes of Topoisomerase IIβ regulate neuronal survival and are defined by their chromatin state

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AUTHOR SUMMARY

Topoisomerases are enzymes crucial for solving topological constraints in DNA that result from DNA twisting during fundamental cellular events such as DNA replication (1). Members of the type II subfamily of topoisomerases create double-strand breaks in DNA and pass a region of duplex from the same or a different molecule through this double-stranded gap. Little is known about the cellular role of one member of this subfamily, Topoisomerase IIβ (Top2β). Here, we studied the supposed function of Top2β in neuronal development and found that it binds to gene regulatory regions and modulates the activity of genes that control neuronal differentiation and survival.

Mammals have two type II topoisomerase enzymes, Topoisomerase IIα (Top2α) and Top2β (2). Top2α is established as the main topoisomerase expressed in proliferating cells and plays important roles in DNA replication and cell-cycle processes. By contrast, Top2β is up-regulated robustly when cells reach a terminally differentiated postmitotic state, and its cellular role remains largely unclear. Genetic deletion of Top2β in mice leads to neural defects, including aberrant axonal elongation, perinatal death from lack of muscular innervation, and defective development of the brain cortex (2). Of note, very little is known about topoisomerase function in nonreplicating cells or about the relationship of its DNA-binding and enzymatic activity with its robust expression in terminally differentiated cells and the defects observed in its absence.

In this study, we investigated these questions by using an established neuronal differentiation system that progresses through defined stages in vitro, further validating the findings during neurogenesis in vivo. We show that the transition from pluripotent stem cells to terminally differentiated postmitotic neurons accompanies a switch in the expression from Top2α to Top2β. We find that stem-cell pluripotency and neuronal-progenitor specification remain unaffected by the absence of Top2β, whereas postmitotic neurons degenerate prematurely (Fig. P1A).

Using methods for global profiling of gene expression, we revealed a number of genes, predominantly associated with neurogenesis, that were down-regulated significantly in stem cell-derived neurons lacking Top2β. Moreover, similar sets of genes were down-regulated in primary cortical neurons isolated from Top2β-knockout embryos (i.e., those lacking this protein).

We next studied the consequences of inhibiting the enzymatic activity of Top2β using the established inhibitor meso-2,3-bis(2,6-dioxopiperazin-4-yl)butane, ICRF-193. We profiled the transcriptome of stem cell-derived neurons as well as of primary cortical neurons isolated from mouse embryos treated with the inhibitor. This analysis revealed transcriptional down-regulation of a set of genes similar to those of Top2β-knockout neurons, so that fewer

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Data deposition: Microarray and ChIP-chip raw data reported in this paper have been deposited in the Gene Expression Omnibus database, www.ncbi.nlm.nih.gov/geo (accession nos. GSE27245 [microarray] and GSE27246 [ChIP-chip]). Deep sequencing data reported in this paper have been deposited in the Gene Expression Omnibus database (accession no. GSE25533).

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mRNA molecules were read from these genes. This transcriptional down-regulation further accompanied reduction in chromatin accessibility at these gene promoters (i.e., the genome within the chromatin was less available to interact with regulatory factors). Moreover, such inhibitor treatment led to neuronal degeneration similar to our observations in Top2β-knockout neurons (Fig. P1B).

Among the rare genes that were commonly up-regulated in neurons lacking Top2β protein or its activity was Ngfr (nerve growth factor receptor, commonly referred to as the “neurotrophin receptor p75”). We focused further on this protein because it is a member of the TNF receptor family and previously has been implicated in neuronal death. Interestingly, we found that inhibition of Top2β enzymatic activity in p75-knockout neurons resulted in significantly reduced neuronal death compared with similarly treated normal cells. Furthermore, depletion of p75 levels in Top2β-knockout neurons led to their survival, suggesting that increased levels of p75 underlie the premature death of Top2β-knockout neurons.

We next investigated the link between the changes in gene expression observed in cells lacking either the Top2β protein or its enzymatic activity with its DNA-binding properties. Using assays to locate genomewide binding sites of the protein, we discovered that Top2β preferentially occupies promoter regions in the genome and that these regions become Top2β targets during the transition from neuronal progenitors to neurons, a time when Top2β levels are highly induced and cells cease to divide. Further analysis revealed that the histone modification defining active, open chromatin, H3K4me2, is present at almost all Top2β-enriched regions, and vice versa (Fig. P1C). However, Top2β does not correlate with the gene silencing-associated repressive mark, H3K27me3 (Fig. P1C). Moreover, the majority of Top2β-bound promoters also recruit RNA polymerase II, the enzyme responsible for producing mRNA from genes, and are actively transcribed (Fig. P1C). Importantly, we discovered that a significant number of genes showing differential expression in cells lacking either Top2β protein or its activity are bound by Top2β in normal cells. These genes are predominantly genes associated with neurogenesis.

Our results suggest that H3K4me2-enriched chromatin regions might recruit Top2β. It is known that H3K4me2 recruits proteins for a number of other activities, such as chromatin remodeling (3). It is possible that such events create topological constraints that need to be resolved by the topoisomerase activity of Top2β. Our findings regarding Top2β’s relationship to Ngfr p75 (Fig. P1D) are especially interesting given Ngfr p75’s role in neuronal degeneration and neuronal cell death during nervous system development (4). Taken together, our results provide insights into the genomic localization and cellular function of Top2β in terminally differentiated postmitotic cells.