Neurosci Bull October 1, 2010, 26(5): 411-416. http://www.neurosci.cn
DOI: 10.1007/s12264-010-0625-9

·Minireview·

The function of DNA topoisomerase IIβ in neuronal development

Xin HENG, Wei-Dong LE

Institute of Health Science, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiao Tong University School of Medicine, Ruijin Hospital, Shanghai 200025, China

Abstract: Type II DNA topoisomerases (Tops) are ATP-dependent enzymes that catalyze topological transformations of genomic DNA by the transport of one DNA double helix through another. In mammals, there are 2 isoforms of DNA Top II, termed Top IIα and Top IIβ. The IIβ isoform is abundantly expressed in cells that have undergone the final cell division and are committed to differentiation into neuronal cells. In recent years, there have been accumulating studies showing the significant role of Top IIβ in neuronal development through regulating expression of certain genes in cells committed to the neuronal fate after the final division. These genes are involved in the processes of neuronal differentiation, migration, axon guidance and so on. The present review mainly focused on the research progress on the role of Top IIβ in neuronal development over the recent decades.

Keywords: neuronal development; axon guidance; neuronal differentiation

1 Introduction

Type II DNA topoisomerase (Tops) activity is required to change DNA topology. DNA Top plays important roles in various genetic processes, such as DNA replication, transcription, recombination, chromosome condensation/decondensation and sister chromatid segregation[1]. Eukaryotic Top II is present in 2 isoforms: α and β, and they share a 72% identity in their amino acid sequences. The 2 isoforms are highly homologous in N-terminal ATPase and central core domains, but differ greatly in their C-termini[2]. Type IIα isoform is expressed in proliferating cells and is mainly required for chromosome segregation, while type IIβ isoform is enriched in post-mitotic neuronal cells in developing brain. Inhibition and/or knock-down of functional Top IIβ can inhibit the induction of expression of a subset of neuronal genes during neuronal cell differentiation[19], suggesting that the enzymatic activity of the IIβ isoform is involved in the early stage of neuronal gene expression, probably through de-condensation of the chromatin structure of the gene-containing region. The present review mainly focused on the discovery and characterization of Top IIβ, especially its function in the nervous system.

2 Structure of DNA Top IIβ

Top IIβ was initially purified from murine P388 cells by Drake *et al*.[3]. Human Top IIβ gene is located to chromosome 3p24[4,5], and its coding sequence specifies a 1621-residue protein. Based on the sequence comparisons, limited proteolysis experiments, and crystallographic studies, type II enzymes have been shown to contain several discrete domains bordered by proteolytic cleavage sites[6-9]. The N-terminal domain has an ATPase function and is the most conserved among species[6-8]. The central region is also highly conserved and possesses DNA breakage and reunion

Corresponding author: Wei-Dong LE
Tel: +86-21-64370045-611201; Fax: +86-21-54669084
E-mail: wdle@sibs.ac.cn
Article ID:1673-7067(2010)05-0411-06
Received date: 2010-06-14; Accepted date: 2010-07-15

activity, including the active site tyrosine residue[3,10]. The C-terminal domain is the least conserved region and may hold the key to any isoenzyme-specific differences. Besides, analysis of human Top IIβ sequence has revealed several putative nuclear localization signals and many potential phosphorylation sites as well as putative sites for other post-translational modifications[3]. Purification of native DNA Top IIβ allows its characterization. Top IIβ can catalyze ATP-dependent DNA strand passage activity *in vitro*, unknot P4 knotted DNA and relax super-coiled plasmid DNA[11].

3 Spatio-temporal expression pattern of DNA Top IIβ

Top IIβ has a distinct cellular localization pattern and a cell cycle expression profile. Its activity is constant throughout the cell cycle. It is localized in the nucleolus during interphase, and in the cytoplasm during mitosis[12]. In the embryonic stage, Top IIβ in the brain is a nucleoplasmic enzyme, and its expression level is higher in the differentiating neurons[13]. Top IIβ is also detected at a high level in differentiated tissues like brain.

The subcellular location of DNA Top IIβ has been extensively investigated. During mitosis, Top IIβ is predominantly cytoplasmic[12,14], although a small amount of Top IIβ is also detected in metaphase scaffolds[15]. Immunofluorescence using the monoclonal antibody has shown the exclusive nucleolar staining of Top IIβ. By using a polyclonal antibody, however, the same research group detected both nucleolar staining and punctate nuclear staining[10]. The current consensus is that during interphase, Top IIβ is located in nuclear, and binds to the fibrillar components of the nucleolus, while during mitosis, it has a predominantly cytosolic location[12,14,16].

The age-associated changes in the protein and activity levels of Top IIβ isoform in different regions of brain have been reported. The level of Top IIβ has been analyzed in the whole brain, and also in 3 specific regions, including cerebellum, cerebral cortex and midbrain. The activity of Top IIβ is the highest in "Young" rat brain, moderate in "Adult" brain and the lowest in "Old" brain. The protein level of Top IIβ correlates well with its enzymatic activity, suggesting that the enzymatic activity and protein level of Top IIβ are high in young brain, and these levels decrease with the increase of age[17].

4 Role of DNA Top IIβ in neural development

Top IIβ can affect brain development and neuronal differentiation. Mice lacking Top IIβ are reported to exhibit a perinatal death phenotype[18]. In a study of Lyu YL *et al.*, transcription profiles of the brains of wild-type and Top IIβ knockout mouse embryos were generated, and surprisingly, only a small number (1%-4%) of genes are affected in Top IIβ knockout embryos. However, the expression of nearly 30% of developmentally regulated genes is either up- or down-regulated[19]. Immunohistochemical analysis of brain sections has revealed that Top IIβ and histone deacetylase 2, a known Top IIβ-interacting protein, are preferentially expressed in neurons in the later stages of differentiation[19].

The cerebral cortex has a unique structure with laminar arrangement of cells that is formed during early postnatal development. Studies using brain-specific Top IIβ knockout mice have demonstrated an aberrant lamination pattern in the developing cerebral cortex and a similar perinatal death phenotype, suggesting an essential role of Top IIβ isoform in brain development[20]. Detailed analysis of corticogenesis has revealed that the migration of postmitotic cortical neurons is affected in Top IIβ mutant embryos. The extracellular matrix protein reelin, which is known to be important for neuronal migration during corticogenesis, is found to be down-regulated in Top IIβ-null mutants[20]. Besides, Top IIβ, which is highly expressed in differentiating cerebellar neurons, is the catalytically competent entity operating directly on chromatin DNA *in vivo*. When the cells reach terminal differentiation, this *in vivo* activity decreases with concomitant loss of the nucleoplasmic enzyme[21].

Cell cycle analysis in cultured cells shows that the level of Top IIβ is not altered significantly. Actually, it is even dispensable for cell proliferation and survival *in vitro,* since in some cell lines, the enzyme is not expressed at all[22]. These observations suggest that Top IIβ is not essential for the maintenance of general cellular activities but is rather involved in more specific processes *in vivo*.

5 Role of DNA Top IIβ in axon guidance

Axon guidance is a subfield of neural development concerning the process by which neurons send out axons to reach the correct targets. Axons often follow very precise paths in the nervous system. A previous study has shown that in the cerebral cortex of Top IIβ-null mice, neurons born at later stages of corticogenesis fail to migrate to the superficial layers. Besides, motor axon fails to contact skeletal muscles, and sensory axon fails to enter the spinal cord, suggesting that DNA Top II plays a remarkable role in neurogenesis and axon guidance[18].

Growing axons have a highly motile structure at the growing tip called the growth cone, which "sniffs out" the extracellular environment for signals that instruct the way of axon growth.

Furthermore, a recent study shows that the Top II inhibitor ICRF-193 can significantly block neurite outgrowth and growth cone formation in cultured cerebellar granule neurons (CGNs), dorsal root ganglions (DRGs) and cortical neurons (CNs). In addition, ICRF-193 also blocks neurite outgrowth and growth cone formation in PC12 cells undergoing nerve growth factor-induced differentiation. Isolated cortical neurons from Top IIβ knockout embryos develop shorter neurites than those from their wild type counterparts, confirming the role of Top IIβ in neurite outgrowth[3].

6 Role of DNA Top IIβ in the transcription of several neuronal genes

Top IIβ controls and alters the topologic states of DNA during transcription. This nuclear enzyme is involved in processes such as chromosome condensation, chromatid separation, and the relief of torsional stress that occurs during DNA transcription and replication. It catalyzes the transient breaking and rejoining of 2 strands of duplex DNA, which allows the strands to pass through one another, thus altering the topology of DNA.

A large-scale microarray analysis has revealed interesting features of gene expression profiles in the brain of Top IIβ knockout embryos. The expression of genes encoding proteins involved in neuron migration (e.g., Reln, Dab1, Sst and Robo1), cell adhesion (e.g., Catna2, Cdh4, Cdh8, Nell2 and Alcam), voltage-gated calcium channel activity (e.g., Cacna2d1 and Cacna2d3), synaptic transmission (e.g., Syt1) and cytoskeleton formation (e.g., neurofilament) is down-regulated in the mutant. The expression of some transcription factors (e.g., Myt1l, Ebf1 and Mef2c) that have been implicated in various differentiation pathways is also down-regulated[19,23-25] (Fig. 1).

Consistent with the above results, studies in the cortical neurons in Top IIβ knockout mice have also demonstrated that the expression levels of certain neuronal genes (i.e. Robo1, catenin alpha 2, cadherin 8, and synaptotagmin) are down-regulated. However, the expression levels of actin and cadherin-13 are not significantly affected[26].

Chromatin immunoprecipitation analysis of the developing brain has revealed that Top IIβ binds to the 5' region of a number of Top IIβ-sensitive genes[19]. Indeed, the role of Top IIβ in hormone-induced gene expression has been demonstrated in a recent study[27]. In that study, researchers find that the signal-dependent activation of gene transcription by nuclear receptors and other classes of DNA-binding transcription factors, including activating protein 1, requires DNA Top IIβ-dependent, transient, site-specific dsDNA break formation, with subsequent activation of poly (ADP-ribose) polymerase-1 (PARP-1) enzymatic function, which causes local changes of chromatin architecture[27]. Interestingly, genomic locations of the genes identified as being controlled by the IIβ enzyme show characteristic similarities: they are relatively long and AT-rich in sequence, residing right next to long, AT-rich intergenic regions. The profile indicates that the chromatin structure of the region is quite condensed[28], suggesting that Top IIβ activity is indispensable for the induction of these genes that are most frequently required for neuronal function[29].

7 Conclusion

DNA Top II catalyzes a strand passage reaction in which one duplex is passed through a transient brake or gate in another. Completion of late stages of neuronal development depends on the presence of active Top IIβ isoform. Two research groups have reported evidence supporting this no-

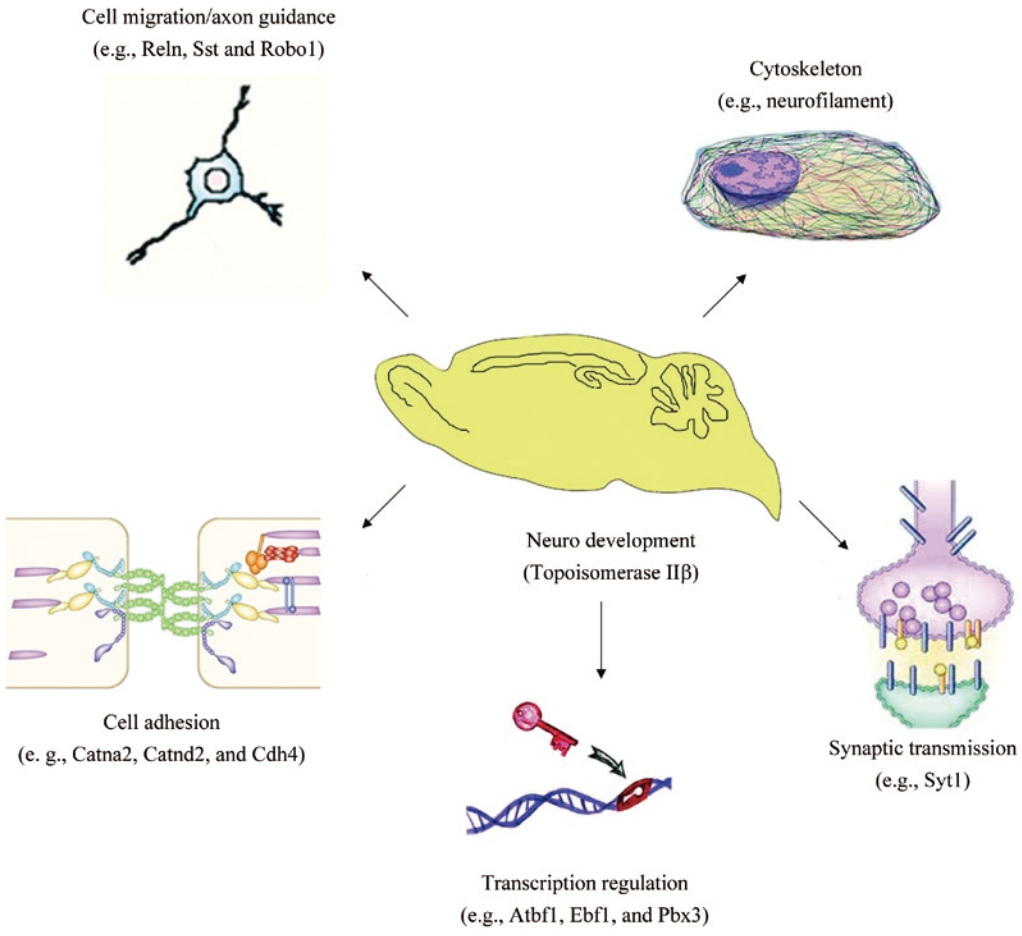

Fig. 1 Potential functions of DNA Top IIβ in neuronal development (Pictures are adopted and reproduced from internet).

tion by showing that Top IIβ is required in the late stage of neural differentiation probably through transcriptional induction of neuronal genes[19,29]. More recently, other studies have demonstrated the regulatory role of Top IIβ in the transcriptional activation of some inducible genes. Indeed, a higher-order chromatin structure of a genomic region containing a cluster of genes which require Top II for their expression becomes accessible to DNase I during cell differentiation. The opening or decondensation is completely suppressed when Top IIβ is inhibited or knocked out. It is quite possible that Top IIβ induces a regional opening of chromatin structure, leading to the transcriptional activation of genes nearby.

In the Top IIβ-deficient mouse embryo, cell differentiation and organ development appear to be normal, except for brain development. Therefore, the transcriptional induction that is dependent on Top IIβ may be rather specific to neuronal genes.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (No. 39070925), Shanghai International Science and Technology Cooperation Program (No. 075407066), and National High Technology Research and Development Program of China (No. 2006AA02A408)

References:

[1] Wang JC. Cellular roles of DNA topoisomerases: A molecular

perspective. Nat Rev Mol Cell Biol 2002, 3: 430-440.

[2] Austin CA, Sng JH, Patel S, Fisher LM. Novel hela topoisomerase II is the II beta isoform: Complete coding sequence and homology with other type II topoisomerases. Biochim Biophys Acta 1993, 1172: 283-291.

[3] Drake FH, Zimmerman JP, McCabe FL, Bartus HF, Per SR, Sullivan DM, *et al.* Purification of topoisomerase II from amsacrine-resistant p388 leukemia cells. Evidence for two forms of the enzyme. J Biol Chem 1987, 262: 16739-16747.

[4] Jenkins JR, Ayton P, Jones T, Davies SL, Simmons DL, Harris AL, *et al.* Hickson ID: Isolation of cdna clones encoding the beta isozyme of human DNA topoisomerase II and localisation of the gene to chromosome 3p24. Nucleic Acids Res 1992, 20: 5587-5592.

[5] Tan KB, Dorman TE, Falls KM, Chung TD, Mirabelli CK, Crooke ST, *et al.* Topoisomerase II alpha and topoisomerase II beta genes: Characterization and mapping to human chromosomes 17 and 3, respectively. Cancer Res 1992, 52: 231-234.

[6] Wang JC. DNA topoisomerases. Annu Rev Biochem 1996, 65: 635-692.

[7] Watt PM, Hickson ID. Structure and function of type II DNA topoisomerases. Biochem J 1994, 303 (Pt 3): 681-695.

[8] Caron PR, Wang JC. Appendix. II: Alignment of primary sequences of DNA topoisomerases. Adv Pharmacol 1994, 29B: 271-297.

[9] Austin CA, Marsh KL, Wasserman RA, Willmore E, Sayer PJ, Wang JC, *et al.* Expression, domain structure, and enzymatic properties of an active recombinant human DNA topoisomerase II beta. J Biol Chem 1995, 270: 15739-15746.

[10] Zini N, Martelli AM, Sabatelli P, Santi S, Negri C, Astaldi Ricotti GC, *et al.* The 180-kda isoform of topoisomerase II is localized in the nucleolus and belongs to the structural elements of the nucleolar remnant. Exp Cell Res 1992, 200: 460-466.

[11] Drake FH, Hofmann GA, Bartus HF, Mattern MR, Crooke ST, Mirabelli CK. Biochemical and pharmacological properties of p170 and p180 forms of topoisomerase II. Biochemistry 1989, 28: 8154-8160.

[12] Chaly N, Chen X, Dentry J, Brown DL. Organization of DNA topoisomerase II isotypes during the cell cycle of human lymphocytes and hela cells. Chromosome Res 1996, 4: 457-466.

[13] Tsutsui K, Hosoya O, Sano K, Tokunaga A. Immunohistochemical analyses of DNA topoisomerase II isoforms in developing rat cerebellum. J Comp Neurol 2001, 431: 228-239.

[14] Meyer KN, Kjeldsen E, Straub T, Knudsen BR, Hickson ID, Kikuchi A, *et al.* Cell cycle-coupled relocation of types I and II topoisomerases and modulation of catalytic enzyme activities. J Cell Biol 1997, 136: 775-788.

[15] Taagepera S, Rao PN, Drake FH, Gorbsky GJ. DNA topoisomerase II alpha is the major chromosome protein recognized by the mitotic phosphoprotein antibody mpm-2. Proc Natl Acad Sci U S A 1993, 90: 8407-8411.

[16] Chaly N, Brown DL. Is DNA topoisomerase II beta a nucleolar protein? J Cell Biochem 1996, 63: 162-173.

[17] Kondapi AK, Mulpuri N, Mandraju RK, Sasikaran B, Subba Rao K. Analysis of age dependent changes of topoisomerase II alpha and beta in rat brain. Int J Dev Neurosci 2004, 22: 19-30.

[18] Yang X, Li W, Prescott ED, Burden SJ, Wang JC. DNA topoisomerase IIbeta and neural development. Science 2000, 287: 131-134.

[19] Lyu YL, Lin CP, Azarova AM, Cai L, Wang JC, Liu LF. Role of topoisomerase IIbeta in the expression of developmentally regulated genes. Mol Cell Biol 2006, 26: 7929-7941.

[20] Lyu YL, Wang JC. Aberrant lamination in the cerebral cortex of mouse embryos lacking DNA topoisomerase IIbeta. Proc Natl Acad Sci U S A 2003, 100: 7123-7128.

[21] Tsutsui K, Tsutsui K, Sano K, Kikuchi A, Tokunaga A. Involvement of DNA topoisomerase IIbeta in neuronal differentiation. J Biol Chem 2001, 276: 5769-5778.

[22] Chen M, Beck WT. Teniposide-resistant cem cells, which express mutant DNA topoisomerase II alpha, when treated with non-complex-stabilizing inhibitors of the enzyme, display no cross-resistance and reveal aberrant functions of the mutant enzyme. Cancer Res 1993, 53: 5946-5953.

[23] Allen MP, Xu M, Zeng C, Tobet SA, Wierman ME. Myocyte enhancer factors-2b and -2c are required for adhesion related kinase repression of neuronal gonadotropin releasing hormone gene expression. J Biol Chem 2000, 275: 39662-39670.

[24] Garel S, Marin F, Grosschedl R, Charnay P. Ebf1 controls early cell differentiation in the embryonic striatum. Development 1999, 126: 5285-5294.

[25] Romm E, Nielsen JA, Kim JG, Hudson LD. Myt1 family recruits histone deacetylase to regulate neural transcription. J Neurochem 2005, 93: 1444-1453.

[26] Nur EKA, Meiners S, Ahmed I, Azarova A, Lin CP, Lyu YL, *et al.* Role of DNA topoisomerase IIbeta in neurite outgrowth. Brain Res 2007, 1154: 50-60.

[27] Ju BG, Lunyak VV, Perissi V, Garcia-Bassets I, Rose DW, Glass CK, *et al.* A topoisomerase IIbeta-mediated dsdna break required for regulated transcription. Science 2006, 312: 1798-1802.

[28] Gilbert N, Boyle S, Fiegler H, Woodfine K, Carter NP, Bickmore WA. Chromatin architecture of the human genome: Gene-rich domains are enriched in open chromatin fibers. Cell 2004, 118: 555-566.

[29] Sano K, Miyaji-Yamaguchi M, Tsutsui KM, Tsutsui K. Topoisomerase IIbeta activates a subset of neuronal genes that are repressed in AT-rich genomic environment. PLoS One 2008, 3: e4103.

拓扑异构酶 IIβ 在神经发育中的作用

衡鑫，乐卫东

中国科学院上海生命科学研究院健康科学研究所，上海交通大学医学院，瑞金医院，上海 200025

摘要：II型DNA拓扑异构酶是一类ATP依赖性酶，能引导双链DNA绕过另一个双链上的缺口来催化基因组DNA拓扑结构的改变。在哺乳动物中，II型DNA拓扑异构酶又分为α和β两个亚型。β亚型高表达于已完成终末分裂并能分化为神经细胞的一类细胞中。近年来，越来越多的研究表明拓扑异构酶IIβ在神经发育中有至关重要的作用。细胞进行终末分裂以后，拓扑异构酶IIβ能够调控决定神经命运的一些特定基因的表达。这些基因主要与神经分化、迁移以及轴突导向等过程相关。本文就近几十年内关于拓扑异构酶IIβ在神经发育中作用的研究进展进行综述。

关键词：神经发育；轴突导向；神经分化