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Research Report

# Role of DNA topoisomerase IIβ in neurite outgrowth

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ABSTRACT

Failure to establish neuromuscular junctions is a major phenotype of *top2*β knockout mice. However, the precise mechanism for this defect is not known. In the current study, we have investigated the role of TopIIβ in cultured neurons. We showed that the TopII inhibitor ICRF-193 significantly blocked 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 blocked neurite outgrowth and growth cone formation of PC12 cells undergoing NGF-induced differentiation. Isolated cortical neurons from *top2*β knockout embryos elaborated shorter neurites than did those from their wild type counterparts, confirming the role of TopIIβ in neurite outgrowth. Together, these results demonstrate a critical role of TopIIβ in neurite outgrowth in cultured neurons. Furthermore, we demonstrated that neurons derived from *top2*β knockout mice failed to form contacts with muscle cells in co-cultures. These results suggest that the defect in establishing neuromuscular junctions in *top2*β knockout mice could be due to the lack of TopIIβ-mediated neurite outgrowth.

## 1. Introduction

The two mammalian TopII isozymes, TopIIα and TopIIβ, share about 72% identity in their primary sequences and possess the same enzymatic activity *in vitro* (Austin et al., 1993; Drake et al., 1989; Jenkins et al., 1992; Tsai-Pflugfelder et al., 1988). However, the two enzymes are regulated very differently. For example, immunohistochemical studies have shown that TopIIα is only present in proliferating tissues, including tumors, while TopIIβ is present in all tissues, including terminally differentiated tissues (Bauman et al., 1997; Hsiang et al., 1988; Tsutsui et al., 2001a). Furthermore, the level of TopIIα rises during S phase, reaching its peak at the G2/M phase of the cell cycle (Heck and Earnshaw, 1986; Niimi et al., 2001; Woessner et al., 1991). TopIIα, but not TopIIβ, has also been shown to be located in replication foci in S phase, possibly through its interaction with PCNA (Earnshaw et al., 1985). In addition, TopIIα, identified as the major chromosome scaffold protein Sc1, forms the structural scaffold, together with condensin, in mitotic chromosomes (Maeshima and Laemmli, 2003; Sumner, 1996; Swedlow and Hirano, 2003; Taagepera et al., 1993). Thus, accumulating evidence has

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*Abbreviations:* CHX, cycloheximide; DRB, 5,6-dichloro-1-beta-ribofuranosyl-benzimidazole; ICRF-193, Imperial Cancer Research Fund-193; NGF, nerve growth factor

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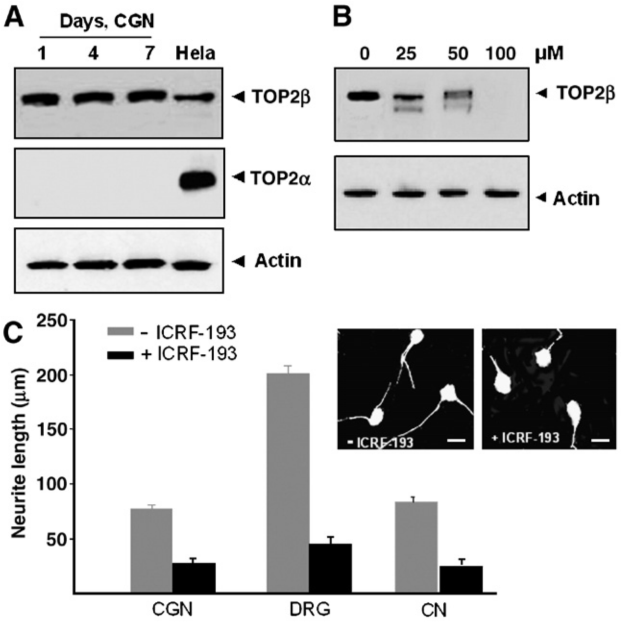

**Fig. 1 – ICRF-193 inhibits neurite outgrowth in cultured neurons. (A) Expression of TopII isozymes in rat cerebellar granule neurons (CGNs). Cerebellar granule neurons isolated from 6-day-old rat pups were seeded onto PLL-coated plates. Neurons were collected at different times after *in vitro* culturing and lysed in SDS–sample buffer. Proteins were separated by SDS–PAGE (8%) and transferred onto a nylon membrane. Western blotting was performed using anti-TopIIβ as described in Experimental procedures. The membrane filter was re-probed sequentially with anti-TopIIα and anti-actin antibodies. Arrows indicate the positions of TopIIβ, TopIIα and actin. (B) ICRF-193 induced down-regulation of TopIIβ in cerebellar granule neurons. Cerebellar granule neurons were seeded onto PLL-coated plates and incubated for 2 h. ICRF-193 was then added to a final concentration of 25, 50 or 100 μM and incubated for 16 h. Neurons were collected and lysed with SDS–sample buffer, and Western blotting was performed using anti-TopIIβ and anti-actin antibodies. (C) ICRF-193 inhibits the growth of neurites in cultured CGNs, CNs and DRGs. Neurons (CGNs, CNs and DRGs) isolated from 6-day-old rat pups were seeded onto PLL-coated glass coverslips and allowed to attach for 2 h. ICRF-193 was then added to a final concentration of 50 μM and the incubation was continued for 24 h. Neurons were fixed and immunostained with anti-neurofilament antibodies. Images were captured (insert) using a fluorescent microscope (Carl Zeiss, Axioplan 2). The total neurite length/neuron was determined as described in Experimental procedures. Bars represent the mean ± SEM ($n=3$). Neurite outgrowth in the presence and absence of ICRF-193 showed significant difference ($p<0.05$).**

suggested that TopIIα plays an important role in cell cycle events such as DNA replication and chromosome condensation/segregation (Cuvier and Hirano, 2003; Grue et al., 1998; Lyu and Wang, 2003; Wang, 2002).

By contrast, TopIIβ is present in all cells, and its level is not significantly changed during the cell cycle (Feister et al., 1997; Liu and Wang, 1987; Lyu and Wang, 2003; Tsao et al., 1989; Tsutsui et al., 2001b; Watanabe et al., 1994; Yang et al., 2000). The abundance of TopIIβ in terminally differentiated cells suggests that it may play a role in DNA metabolism other than, or in addition to, DNA replication and chromosome condensation/segregation. However, the precise function of TopIIβ remains elusive. A number of recent studies have suggested that TopIIβ plays a role in neuronal differentiation. First, studies of whole-body *top2*β knockout mice have demonstrated a perinatal death phenotype. It has been demonstrated that motor neuron axons fail to innervate the diaphragm muscles and sensory neuron axons fail to enter the spinal cord, suggesting a role of TopIIβ in axon growth and/or guidance (Yang et al., 2000). Studies of brain-specific *top2*β knockout mice have also revealed a major defect in corticogenesis during brain development (Lyu and Wang, 2003). Second, studies of developing rat cerebellum have demonstrated that post-mitotic granule cells in the external germinal layer show an abrupt transition of expressed TopII isoforms from TopIIα to TopIIβ, again suggesting an association between TopIIβ and neuronal differentiation (Kondapi et al., 2004; Lyu and Wang, 2003; Tsutsui et al., 2001a,b).

In the current study, we show that TopIIβ is required for neurite outgrowth and growth cone formation in cultured cerebral cortical neurons, cerebellar granule neurons, and dorsal root ganglion as well as PC12 cells. In addition, the expression of a number of neuronal genes requires TopIIβ. These results support the notion that TopIIβ is required for neurite outgrowth during neuronal differentiation, possibly at the level of gene expression.

## 2. Results

### 2.1. *The topoisomerase II inhibitor ICRF-193 inhibits neurite outgrowth of cultured neurons*

Cerebellar granule neurons (CGNs) isolated from 6-day-old rat pups were cultured *in vitro* on PLL-coated plates or coverslips to induce neurite outgrowth. As shown in Fig. 1A, CGNs, unlike the control HeLa cells, did not express any detectable amount of TopIIα after 1, 4, and 7 days in culture. By contrast, the expression level of TopIIβ was significantly higher in cultured CGNs than in HeLa cells (Fig. 1A), consistent with the notion that TopIIα and TopIIβ are preferentially expressed in proliferating and differentiated cells, respectively (Kondapi et al., 2004; Tsutsui et al., 2001a).

The catalytic inhibitor of TopII, ICRF-193, is known to down-regulate TopIIβ by activating a 26S proteasome pathway (Mao et al., 2001; Xiao et al., 2003). To demonstrate that ICRF-193 indeed inhibited TopIIβ in neurons, the level of TopIIβ in ICRF-193-treated CGNs was monitored. As shown in Fig. 1B, ICRF-193 effectively down-regulated TopIIβ in CGNs in a dose-dependent manner, suggesting that ICRF-193 is effective in inhibiting TopIIβ in CGNs. We studied the cytotoxic activity of ICRF-193 in neurons. We did not find any significant cytotoxic activity of ICRF-193 at or below 100 μM in neurons (data not shown). In order to evaluate the role of TopIIβ in neuronal differentiation, the TopII inhibitor ICRF-193 was employed in neurite outgrowth assays (Tsutsui et al., 2001b). As shown in