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Research report

# The effect of brain-derived neurotrophic factor on radiation-induced neuron architecture impairment is associated with the NFATc4/3 pathway

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ABSTRACT

Irradiation to developing brains results in progressive cognitive dysfunction. Changes in the morphology of mature neurons are thought to be related to impairments of cognitive function. However, little is known about the effects of radiation on neurite outgrowth of immature neurons. Therefore, we sought to evaluate the structural alterations of immature neurons following X-ray irradiation and determine potential strategies to reverse it. Our data revealed damage to the neurite outgrowths of cultured neurons after 2 Gy and 8 Gy irradiation at 1 d and 3 d, respectively. De-phosphorylation of nuclear factor of activated T-cells c4/3 (NFATc4/3) was inhibited post-irradiation. Extraneous brain-derived neurotrophic factor (BDNF) ameliorated impairment of neurite growth and activated the NFATc4/3 signaling pathway. These data indicate that BDNF confers neuroprotective effects against irradiation by modulating the NFATc4/3 pathway.



## 1. Introduction

Pediatric patients treated for central nervous system malignancies with cranial irradiation often endure neurological deficits (Padovani et al., 2012). As the brains of children are developing, tremendous numbers of immature neurons are gradually integrating into the hippocampal circuitry. Irradiation at this stage could lead to future progressive cognitive deficits (Duffner, 2004; Padovani et al., 2012; Tang et al., 2017). Impairments to the neuronal architecture in the dentate gyrus are thought to be associated with deficits in hippocampus function. Abnormal neural structure has been implicated in the pathogenesis of various conditions, including Alzheimer's disease (Siskova et al., 2014) and Rett syndrome (Kulkarni and Firestein, 2012). Even 1 Gy of irradiation could lead to obvious damage to the architecture of mature neurons (Parihar and Limoli, 2013). While a wealth of evidence has shown irradiation-induced impairment to neurogenesis (Son et al., 2015; Zou et al., 2013), little is known about the effects of radiation on neurite outgrowth of immature neurons. Therefore, we sought to evaluate the alterations of immature neurons after exposure to radiation.

According to available knowledge, numerous transcription factors have been described in the regulation of neurite outgrowth, among which the nuclear factor of activated T-cells family has been proven to be indispensable (Serrano-Perez et al., 2015). There are five different NFAT family members, namely, NFATc1/2/c, NFATc2/1/p, NFATc3/4/x, NFATc4/3 and NFAT5/TonEBP (Macian, 2005). NFATc4/3 promotes neural survival, synaptic plasticity, and neurite outgrowth. It does not directly affect neurite outgrowth but regulates the transcription of target genes, the expression of which affects synaptic plasticity, neurogenesis and neurite outgrowth (Bradley et al., 2005; Quadrato et al., 2012; Schwartz et al., 2009; Zagrebelsky and Korte, 2014). The NFATc4/3 signaling pathway could be activated by neurotrophins, such as BDNF.

*Abbreviations:* NFAT, nuclear factor of activated T-cells; BDNF, brain-derived neurotrophic factor; NS, normal saline; SD, Sprague–Dawley; WBI, Whole brain irradiation; GFP, green fluorescent protein; DAPI, 4,6-diamidino-2-phenylindole; ROIs, regions of interest.

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BDNF plays pivotal roles in the regulation of neural circuit development and neuroplasticity (Mizui et al., 2016; Murray and Holmes, 2011; Park and Poo, 2013). The upregulation of BDNF actuates morphological plasticity, including altered dendritic fields and increased axonal branching (Danzer et al., 2002). The increased intracellular calcium levels induced by BDNF could promote NFATc4/3 de-phosphorylation. De-phosphorylated NFATc4/3 transfers into the nucleus and initiates the transcription of target genes. Our previous studies demonstrated that forced running exercise ameliorates the impairments of hippocampal neurogenesis and cognitive function through the BDNF-pCREB signaling pathway in irradiated rats (Ji et al., 2014). Here, we attempted to determine whether activated de-phosphorylation of NFATc4/3 by BDNF could attenuate the effects of radiation on neurite outgrowth.

## 2. Results

### 2.1. Radiation inhibits NFATc4/3 de-phosphorylation

At day 1 and day 3 post-irradiation, 2 Gy and 8 Gy irradiation significantly decreased the levels of de-phosphorylated NFATc4/3 (day 1: $P < .05$; day 3: $P < .05$) (Fig. 2A) in cultured neurons. Phosphorylated NFATc4/3 (P—NFATc4/3) in the 2 Gy and 8 Gy group was increased at both time points (day 1: $P < .05$; day 3: $P < .05$) (Fig. 2B). Additionally, changes in the levels of de-phosphorylated NFATc4/3 and phosphorylated-NFATc4/3 were both dose-dependent (Fig. 2).

### 2.2. Exogenous BDNF pretreatment promotes NFATc4/3 de-phosphorylation

Exogenous BDNF pretreatment upregulated dephosphorylated NFATc4/3 in all groups at day 1 (0 Gy: $P < .05$; 2 Gy: $P < .05$; 8 Gy: $P < .05$) and day 3 post-irradiation (0 Gy: $P < .01$; 2 Gy: $P < .05$; 8 Gy: $< 0.05$) (Fig. 3A). Decreased phosphorylated NFATc4/3 in all groups at day 1 (0 Gy: $P < .05$; 2 Gy: $P < .05$; 8 Gy: $P < .01$) and day 3 (0 Gy: $P < .05$; 2 Gy: $P < .05$; 8 Gy: $P < .01$) (Fig. 3B) following exposure was also observed.

### 2.3. Exogenous BDNF altered the subcellular distribution of NFATc4/3 affected by radiation

In incubated neurons of the 0 Gy group, the distribution of NFATc4/3 was predominantly cytoplasmic, with a nuclear-to-cytoplasmic ratio of 0.93 on day 1 and 0.86 on day 3 post-irradiation. After radiation exposure, more phosphorylated NFATc4/3 was retained in the cytoplasm, with a nuclear-to-cytoplasmic ratio of to 0.56 in the 2 Gy group ($P < .05$) and .42 in the 8 Gy group ($P < .01$) on day 1 post-irradiation (Fig. 4B) and 0.52 in the 2 Gy group ($P < .05$) and .38 in the 8 Gy group ($P < .05$) on day 3 post-irradiation (Fig. 4C). In irradiated neurons pretreated with BDNF, more de-phosphorylated NFATc4/3 was translocated into the nucleus, presenting no significant difference with the 0 Gy group at both time points (Fig. 4B, C).

### 2.4. Radiation inhibited neurite growth of cultured neurons

Neurite length increased from day 1 to day 3 post-irradiation in all groups. Neurons without irradiation showed longer neurite length than irradiated neurons. Compared with the results for the 0 Gy group, the total neurite length decreased by 28% ($P < .001$) and 36% ($P < .001$) after a single dose of 2 Gy or 8 Gy at day 1 (Fig. 5B). However, the mean values of branching points were not markedly lower post irradiation. Three days after irradiation, reduced total neurite length (2 Gy: 24%, $P < .001$; 8 Gy: 32%, $P < .001$) and branching points (2 Gy: 29%, $P < .001$; 8 Gy: 36%, $P < .001$) were observed compared with the control group (Fig. 5B, C).

### 2.5. Exogenous BDNF ameliorated the inhibitory effects of radiation on neurite outgrowth in vitro

Pretreatment with exogenous BDNF led to a significant increase in total neurite length (0 Gy: 22%, $P < .001$; 2 Gy: 34%, $P < .001$; 8 Gy: 41%, $P < .001$) and branching points (0 Gy: 31%, $P < .001$; 2 Gy: 27%, $P < .01$; 8 Gy: 43%, $P < .001$) (Fig. 6A) 1 d post irradiation compared with the NS group. By day 3 following exposure, there was an increase in the total neurite length (0 Gy: 21%, $P < .01$; 2 Gy: 28%, $P < .05$; 8 Gy: 33%, $P < .001$) and branching points (0 Gy: 30%, $P < .001$; 2 Gy: 42%, $P < .001$; 8 Gy: 44%, $P < .001$) (Fig. 6B) after BDNF treatment.

### 2.6. BDNF attenuated dendritic growth impairment of newborn granule neurons induced by whole-brain irradiation

Compared with the 0 Gy group, the total dendritic length of newborn neurons decreased significantly at 14 dpi (day post retrovirus injection) (31%, $P < .01$) and 28 dpi (27%, $P < .01$) in the irradiated group (Fig. 6B). No significant difference between the 0 Gy

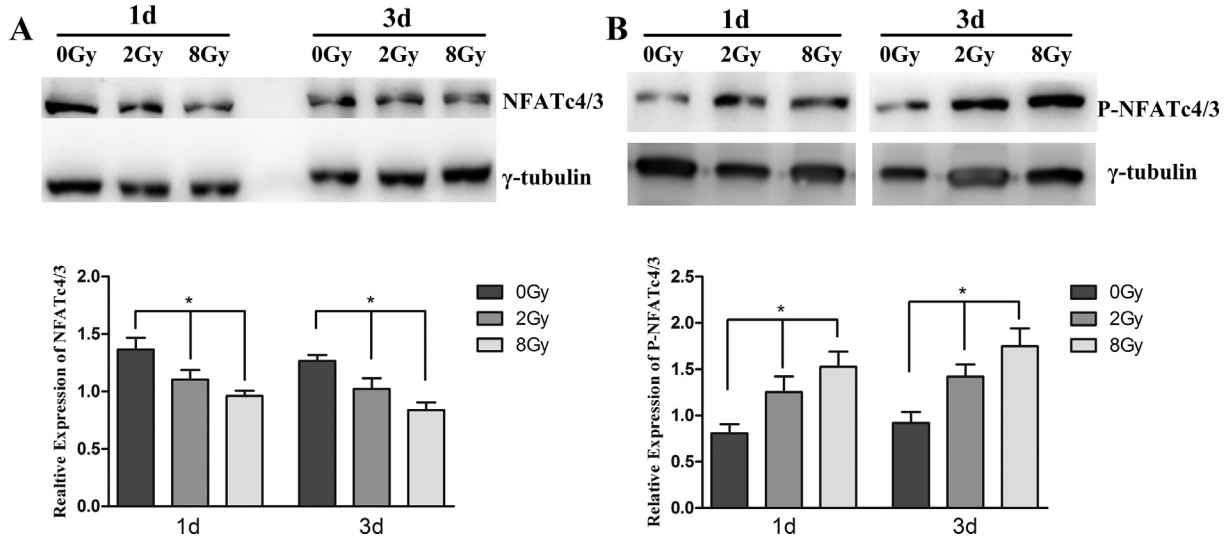


**Fig. 1.** Experimental design and timeline of procedures for the *in vivo* study. BDNF was stereotaxically injected into the hippocampus four days after cranial irradiation.