Neuroscience Letters 594 (2015) 6–11

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Suppressed expression of mitogen-activated protein kinases in hyperthermia induced defective neural tube

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HIGHLIGHTS

- mRNA levels of JNK, p38 and ERK decreased in hyperthermia-treated neural tubes.
- Phosphorylated JNK, p38 and ERK decreased in hyperthermia-treated neural tubes.
- Phosphorylated ERK1/2 transfered into nucleus in neural tubes after hyperthermia.
- HMGB1 was downregulated in hyperthermia-treated neural tubes.

ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form 5 March 2015
Accepted 23 March 2015
Available online 25 March 2015

Keywords:
Neural tube defects
Mitogen-activated protein kinase
Hyperthermia

ABSTRACT

Neural tube defects (NTDs) are common congenital malformations. Mitogen-activated protein kinases (MAPKs) pathway is involved in many physiological processes. HMGB1 has been showed closely associated with neurulation and NTDs induced by hyperthermia and could activate MAPKs pathway. Since hyperthermia caused increased activation of MAPKs in many systems, the present study aims to investigate whether HMGB1 contributes to hyperthermia induced NTDs through MAPKs pathway. The mRNA levels of MAPKs and HMGB1 between embryonic day 8.5 and 10 (E8.5-10) in hyperthermia induced defective neural tube were detected by real-time quantitative polymerase chain reaction (qPCR). By immunofluorescence and western blotting, the expressions of HMGB1 and phosphorylated MAPKs (ERK1/2, JNK and p38) in neural tubes after hyperthermia were studied. The mRNA levels of MAPKs and HMGB1, as well as the expressions of HMGB1 along with phosphorylated JNK, p38 and ERK, were downregulated in NTDs groups induced by hyperthermia compared with control. The findings suggested that HMGB1 may contribute to hyperthermia induced NTDs formation through decreased cell proliferation due to inhibited phosphorylated ERK1/2 MAPK.

1. Introduction

NTDs are common congenital malformations. Neurulation is a fundamental embryogenesis event that culminates in the formation of the neural tube [1–2]. NTDs are influenced by both environmental and genetic factors [3]. More than 80 mutations have been identified linked to different types of rodent NTDs, with more than 100 genes implicated in neural tube formation [4]. Studies suggested the involvement of distinct molecular pathways in NTDs pathogenesis [5].

MAPKs are required for many physiological processes. All eukaryotic cells possess multiple MAPKs pathways which coordinate and regulate nearly every cell process [6]. By far, the most extensively studied groups of mammalian MAPKs are ERK1/2, JNK and p38. ERK1 and ERK2 regulate a large variety of processes, such as cell survival and proliferation [7]. p38 MAPKs have also been proved to play roles in cell apoptosis and survival [8]. Much like p38 MAPKs, JNKs are strongly activated by various cellular stresses [9].

HMGB1 is an important factor for brain development. The knockdown of HMGB1 in zebrafish resulted in severe defects in forebrain development [10]. We previously showed that HMGB1 closely associated with neurulation and NTDs induced by

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http://dx.doi.org/10.1016/j.neulet.2015.03.046

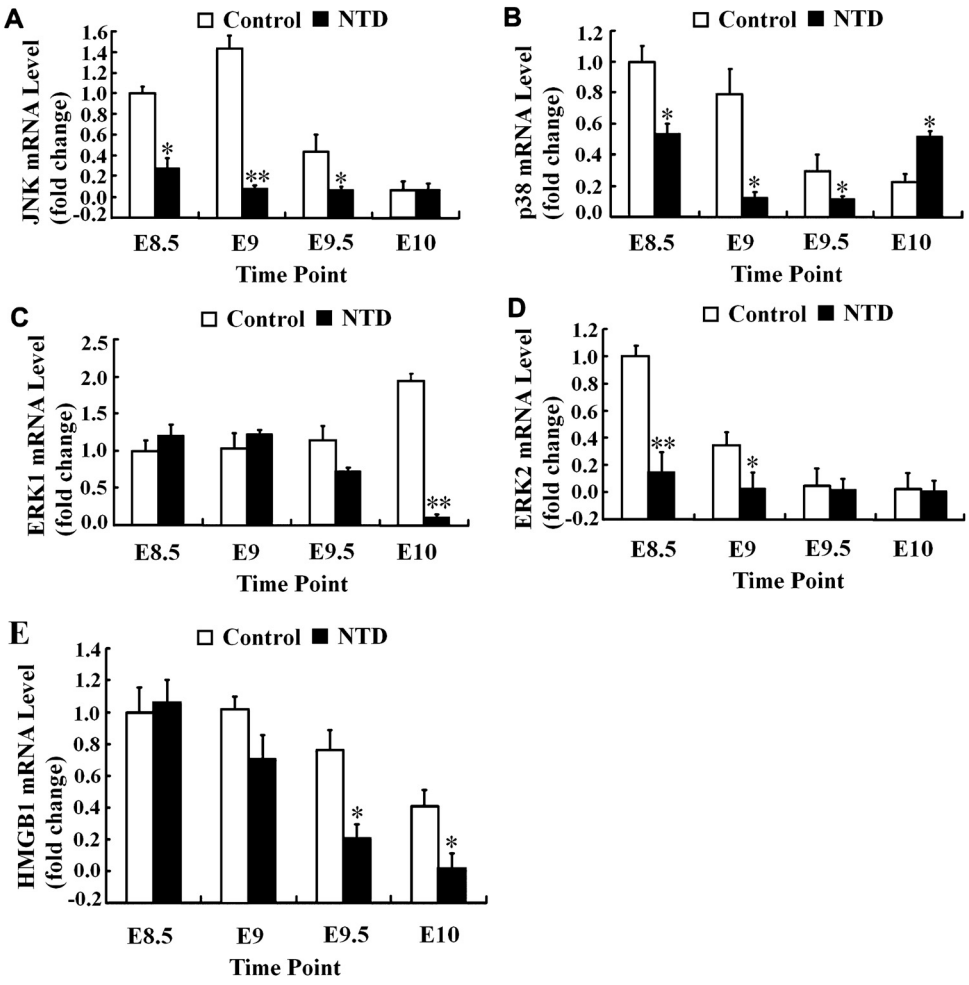

Fig. 1. The relative mRNA levels of JNK, p38, ERK and HMGB1 in the neural tube of NTDs and control groups analyzed by real-time PCR. β-actin was used as an internal control. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control littermates.

hyperthermia [11]. MEKK4 phosphorylates and activates MKK4/MKK7 and MKK3/MKK6, leading to the activation of JNK and p38, respectively [12]. Chi et al. [13] found that Mekk4-null embryos demonstrated highly penetrant neural tube defects and a loss of JNK and p38 activation, implying the important roles of JNK and p38 in neural tube formation. It has been reported that extracellular HMGB1 could activate MAPKs and transduce the signal inside neural and gliocyte cells [14]. Yu et al. found that heat stress could activate MAPKs signaling pathways [15]. The present study aims to investigate whether HMGB1 contributes to hyperthermia-induced NTDs through MAPKs pathway.

2. Materials and methods

2.1. Experimental animals and tissue preparation

Mature golden hamsters (Vital River Laboratory, Beijing, China) were crossbred, and the day when vaginal plug appeared was considered as day one of embryonic development (E1). Pregnant hamsters were randomly divided into NTDs and control groups. In golden hamster, neural tube development occurs from E8 to E10. For the NTDs groups, the hamsters were heated in a water bath at 42 °C for 20 min at 3:00 PM on E8 to establish hyperthermia induced NTDs animal model. For control, the hamsters were placed in a water bath at 37 °C for 20 min at 3:00 PM on E8 [16].

Pregnant hamsters in NTDs and control groups were divided into four subgroups, respectively. At E8.5, E9, E9.5 and E10, pregnant hamsters in each subgroup were deeply anesthetized and the neural tube tissues of the embryos were rapidly removed under a dissection microscope and frozen immediately in liquid nitrogen. The neural tubes from the litters of the same mother were regarded as one sample for subsequent qPCR and western blot analyzes. For immunofluorescence analysis, embryos were fixed in 4% paraformaldehyde overnight at 4 °C and embedded in paraffin mounting medium before coronal sectioning into 5 μm slices. All experiments were in accordance with Institutional Animal Care and Use Committee guidelines and the study was approved by Institutional Research Ethics Committee of Weifang Medical University.

2.2. RNA extraction and qPCR

Total RNA was extracted from the neural tube samples using TRIzol reagent according to the supplier's instructions (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from 2 mg RNA with M-MuLV reverse transcriptase and random 18 oligo dT according to manufacturer's instructions (Takara, Otsu, Japan). The cDNA products were stored at −70 °C until use. Next, triplicate cDNA samples, approximately 50 ng of cDNA in a total volume 20 μL with 10 μM of each primer, were assessed for target mRNA levels by qPCR with SsoFast EvaGreen Supermix (Bio-Rad Hercules, CA, USA) on a Bio-Rad IQ5 real-time PCR system. The primers used in the experiment were designed to span one or more intron(s) to prevent genomic DNA contamination and were synthesized by Takara. All assay efficiencies were monitored