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Mechanism of MASH1 induction by ASK1 and ATRA in adult neural progenitors

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The molecular mechanisms underlying differentiation and lineage commitment in neural stem cells are just beginning to be understood, however the molecules involved in this process and their functions remain largely unknown. Here we studied the effects and downstream signals of apoptosis signal-regulating kinase 1 (ASK1) together with all-trans retinoic acid (ATRA) on neuronal differentiation in adult hippocampus-derived progenitor (AHP) cells. Following ASK1 over-expression and ATRA treatment in AHPs, a larger number of cells differentiated into neurons and the MASH1 promoter became activated. Analyzing downstream effector molecules of ASK1 or ATRA targeting the MASH1 promoter revealed that the myocyte enhancer factor 2C (MEF2C) mediated ASK1 signalling, while activation of Sp1 was involved in ATRA signalling. Chromatin immunoprecipitation assay on the promoter revealed that ASK1 induced binding of MEF2C and Ca^{2+}/calmodulin-dependent kinase II to the MASH1 promoter. Taken together, ASK1 and ATRA activate MEF2C and Sp1, respectively, and up-regulate MASH1 protein expression.

Keywords: ASK1; ATRA; MASH1; MEF2C; Chromatin

Introduction

The subgranular zone of the dentate gyrus of the hippocampus is one of the two known areas of the adult mammalian brain where neurogenesis continues to occur (Alvarez-Buylla et al., 2001; Eriksson et al., 1998; Kornack and Rakic, 1999). In adult rodent hippocampus, multipotent progenitor cells migrate and differentiate into neurons in the granule cell layer of the dentate gyrus (Kempermann et al., 2003; Kuhn et al., 1996). Adult-derived rat progenitor (AHP) cells isolated from dentate gyrus, retain the ability to differentiate to form neurons, oligodendrocytes, and astrocytes (Gage et al., 1995; Palmer et al., 1995). Identifying the signal cascades that mediate their fate determination has now become a major field of investigation. Many regulators of neurogenesis with specific activities have been identified, however the mechanism controlling the differentiation of the newly born neurons in the adult brain is still poorly characterized.

MASH1 is a basic helix–loop–helix (bHLH) transcription factor that is essential for survival and differentiation of neuronal progenitor cells, and is one of the earliest markers expressed in committed neural progenitor cells (Johnson et al., 1992; Lo et al., 1991; Parras et al., 2004; Torii et al., 1999). Over-expression and loss-of-function studies have suggested that precise temporal and spatial expression of bHLH transcription factors is critical for proper development of the nervous system (Casarosa et al., 1999; Guillemot et al., 1993). When MASH1 is expressed in differentiating neurons, they form heterodimers with a family of ubiquitously expressed bHLH factors known as E proteins, and activate gene expression by binding to the E box (Johnson et al., 1992; Kageyama et al., 1995). MASH1 has been shown to be expressed in the adult subgranular zone (SGZ) of the dentate gyrus (Elliott et al., 2001; Sharma et al., 2002).

Apoptosis-signalling regulating kinase-1 (ASK1)/MEKK5 was identified as one of the MAP kinase kinases that induces stress-mediated apoptotic signalling, through activation of c-Jun N-terminal kinase (JNK) and p38 (Ichijo et al., 1997; Matsuzawa and Ichijo, 2001; Tobiume et al., 1997). In recent studies we and others have shown that in certain cell types, ASK1 promotes cellular differentiation and survival rather than apoptosis (Faigle et al., 2004; Sayama et al., 2001; Takeda et al., 2000). We showed that ASK1 promotes neuronal differentiation by increasing transcription of the pro-neuronal *MASH1 gene* (Faigle et al., 2004), however, the exact signalling mechanism remains to be elucidated. One of the extensively studied molecules that are known to induce neuronal differentiation is all trans-retinoic acid (ATRA). ATRA

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plays an important role in developmental neurogenesis, especially for the proper generation of the spinal cord and hindbrain throughout embryonic development (Evans and Kaye, 1999; Maden, 2002; Sucov and Evans, 1995). In addition, in vitro studies have shown that exposure of embryonic stem cells to ATRA induced neuronal differentiation, which involved upregulation of MASH1 (Bain et al., 1996; Johnson et al., 1992).

In this study we have investigated the combined effect of ASK1 and ATRA on neuronal differentiation of AHP cells and found Myocyte Enhancing Factors 2C (MEF2C) and Sp1 as downstream mediators of ASK1 and ATRA signalling. MEF2C, a member of MADS (MCM1, Agamous, Deficiens, and Serum-response factor) transcription factor family, is highly expressed in neurons in the CNS (Lyons et al., 1995), and suggested to be involved in neuronal differentiation (Okamoto et al., 2000). Sp1 is essential for early embryonic development, and has been shown to play a role in activating neural-specific genes (Liu et al., 2004; Marin et al., 1997; Yan and Ziff, 1997).

A considerable number of molecules have been reported to stimulate neuronal differentiation, but the efficiency of yielding sustained neurons is still too poor for it to be applied for replacement therapy. Identifying molecules that induce neuronal differentiation and their mechanisms are, therefore, crucial for clinical use of neural stem cells. We show herein that combining ASK1 and ATRA synergistically increases the neuronal differentiation through activation of MEF2C and Sp1.

Results

ASK1 and ATRA synergistically increase neuronal differentiation of AHP cells

We have previously shown that ASK1 induces neuronal differentiation in a p38-dependent manner. In order to evaluate the combined effect of ASK1 and ATRA on differentiation of AHPs, cultured AHP cells were infected with a constitutively active ASK1 (ASK1-ΔN) on DIV 2 at moi 5, treated with ATRA 24 h later, and incubated for another 5 days. Cells infected with an moi higher than 5 and subsequently treated with 0.5 μM ATRA, detached from plates and were thus not able to be quantified. The treated cells were fixed and immunostained for MAP2ab, a marker for mature neurons. Treatment with both ASK1-ΔN and ATRA resulted in a more than 3-fold increase in the number of MAP2ab positive cells compared with AHP cells treated only with ATRA. The same combination treatment resulted to more than 2-fold increase in MAP2ab positive cells compared to cultures infected with ASK1-ΔN, and to more than 5-fold increase when compared to no treatment controls (Fig. 1).

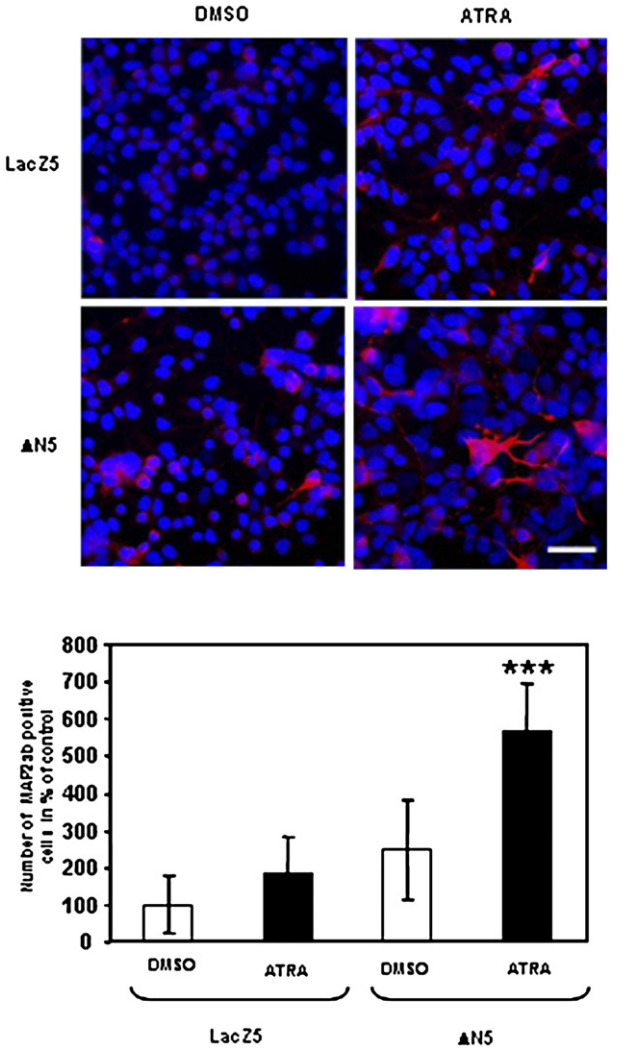

Fig. 1. ASK1 and ATRA synergistically increase neuronal differentiation of AHP cells. AHPs were infected with either Ad-LacZ or Ad-ASK1-ΔN on DIV 2, treated with ATRA or DMSO 24 h later and after additional 5 days processed for immunofluorescent staining for MAP2ab. The figure depicts the number of MAP2ab positive cells in percent of control. Scale bar, 50 μM.

ASK1 and ATRA induce MASH1 in AHP cells

To further study the effect of ASK1 and ATRA we next wanted to determine if they both targeted MASH1, known to be expressed in the adult SGZ of the dentate gyrus (Elliott et al., 2001; Sharma et al., 2002). AHPs were infected with either ASK1-ΔN or control virus on DIV 2 at moi 20, and subsequently processed for either MASH1 immunostaining or Western blot. Overexpression of ASK1-ΔN resulted in a significant increase of MASH1-expressing AHPs compared to control infected cells (Figs. 2A, B).

Next we examined whether ATRA induces MASH1 at the transcriptional level. We performed a luciferase assay where AHP cells were transfected with the MASH1 promoter-luciferase (Luc) reporter at DIV 2, followed by treatment with different concentrations of ATRA and subsequently, cells were harvested for luciferase measurement. Fig. 2C shows that ATRA significantly induces MASH1 transcription activity at an optimal concentration of 0.5 μM. Thus, in the following experiments all treatments with ATRA were given at this concentration.

On the protein level, the exposure of ATRA to the AHP cells lead to an increase in MASH1 expression already within 30 min, declining within 3 h, and was almost absent after 8 h (Fig. 2D). The control treated AHPs on the other hand had a stable low expression of MASH1 protein during these 8 h. Thus, ATRA induced a rapid transient expression of MASH1 protein. Furthermore, in order to study the combined effect of ASK1 and ATRA on differentiation of AHPs, cells were transfected with either a kinase mutant form of ASK1 (ASK1-KM) or ASK1-ΔN together with the MASH1 promoter-Luc, and subsequently treated with or without ATRA. As shown in Fig. 2E, ASK1-ΔN and ATRA were both separately capable of inducing MASH1 promoter transcription, and in combination they yielded a synergistic effect.