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Expression of MUK/DLK/ZPK, an activator of the JNK pathway, in the nervous systems of the developing mouse embryo

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Abstract

C-Jun N-terminal kinase (JNK) is implicated in regulating the various cellular events during neural development that include differentiation, apoptosis and migration. MUK/DLK/ZPK is a MAP kinase kinase kinase (MAPKKK) enzyme that activates JNK via MAP kinase kinases (MAPKK) such as MKK7. We show here that the expression of MUK/DLK/ZPK protein in the developing mouse embryo is almost totally specific for the neural tissues, including central, peripheral, and autonomic nervous systems. The only obvious exception is the liver, in which the protein is temporally expressed at around E11. The expression becomes obvious in the neurons of the brain and neural crest tissues at embryonic day 10 (E10) after neuron production is initiated. By E14, MUK/DLK/ZPK proteins are found in various neural tissues including the brain, spinal cord, sensory ganglia (such as trigeminal and dorsal root ganglia), and the sympathetic and visceral nerves. The localization of MUK/DLK/ZPK protein in neural cells almost consistently overlapped that of βIII-tubulin, a neuron specific tubulin isoform, and both proteins were more concentrated in axons than in cell bodies and dendrites. The intensely overlapping localization of βIII-tubulin and MUK/DLK/ZPK indicated that this protein kinase is tightly associated with the microtubules of neurons.

Keywords: MAP kinase; Neuron; Tubulin; Neurofilament; Microtubules; Protein; Central nervous system; Brain; Spinal cord; Sensory neuron; Sympathetic neuron; Vagus neuron; Dorsal root ganglia; Visceral nerve; Sensory ganglia; axon; Dendrite; Cervical ganglion; Adrenal gland; Pelvic ganglion; Vibrissal follicle; Eye; Olfactory nerve; Retina; Vestibulocochlear ganglion; Trigeminal ganglion; Glossofharyneal ganglion; Cerebellum; Telencephalon; Mesencephalon; Neocortex; Diencephalon; Neural tube; Midbrain; Hindbrain

1. Results and discussion

Originally identified as a subgroup of MAP kinases that are activated by cellular stress, and which regulate cell differentiation and apoptosis (Kyriakis and Avruch, 2001), JNK is now recognized as a key component of the signal transduction pathways involved in the regulation of planner cell polarity and cell migration, both of which are essential for ontogeny (Hirai et al., 2002; Weston and Davis, 2002; Xia and Karin, 2004). The multiplicity of cellular events regulated by JNK indicates that multiple upstream regulators are involved in addition to downstream targets of the JNK pathway. In fact, several MAPKKK enzymes have been identified as upstream activators of JNK. MAP kinase upstream kinase (MUK, also known as DLK and ZPK) is one of these enzymes (Hirai et al., 1996). MUK/DLK/ZPK are also grouped among the mixed lineage kinases characterized by a typical kinase domain-bearing conserved amino acid residues of both serine/threonine and tyrosine kinases (Gallo and Johnson, 2002). Northern blots have revealed the rather specific expression of MUK/DLK/ZPK in the brain, among several adult mouse or human tissues (Holzman et al., 1994; Reddy and Pleasure, 1994; Hirai et al., 1996). On the other hand, in situ hybridization studies have discovered that MUK/DLK/ZPK mRNA is expressed in the epithelial cells of several adult and embryonic mouse organs such as the gut, liver, pancreas and skin as well as in the neuronal cells in brain and dorsal root ganglia (Bouin et al., 1996; Nadeau et al., 1997). We recently showed that MUK/DLK/ZPK

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protein is expressed in young migrating neurons of the embryonic mouse telencephalon (Hirai et al., 2002). These findings provide some insight into the physiological function of this protein kinase, whereas an overview of the developmental changes in its expression remains obscure. It should also be noted that the expression of MUK/DLK/ZPK mRNA and its protein are not always correlated. In E16 mouse telencephalon, the protein expression is barely detectable level in cortical plate neurons and high in young migrating neurons, while the mRNA expression is relatively high in cortical plate neurons and low in young migrating neurons (Nadeau et al., 1997; Hirai et al., 2002). This contradiction may reflect the translational or post-translational regulation of the MUK/DLK/ZPK expression, and indicates that the clarification of the protein expression pattern is required to identify the tissue or cells wherein this protein kinase can act. Therefore, we examine the protein expression of MUK/DLK/ZPK in the mouse embryo at various gestational stages.

1.1. Induction of MUK/DLK/ZPK expression at the early stage of neurogenesis

The expression of MUK/DLK/ZPK was below detectable levels in most of the E10 embryo (Fig. 1A–C), obvious in the junctional region between the midbrain and hindbrain (Fig. 1B,G) and weak in the primordium of dorsal root ganglia (Fig. 1C,H). At this stage, neurogenesis becomes evident and differentiated neurons labeled with antibody against the βIII isoform of tubulin (Menezes and Luskin, 1994) were detected in the brain, neural tube and primordium of dorsal root ganglia (Fig. 1D–F). MUK/DLK/ZPK was expressed in some of these newly differentiated neurons.

The expression of MUK/DLK/ZPK became more evident at E11 and was obvious in divergent neural tissues including the brain, spinal cord, and ganglia of peripheral and autonomic nerves (Fig. 2A–C). In the central nervous system, the expression was restricted to the marginal zone of the spinal cord, diencephalon, mesencephalon, and hindbrain (arrows in Fig. 2C) that corresponded to

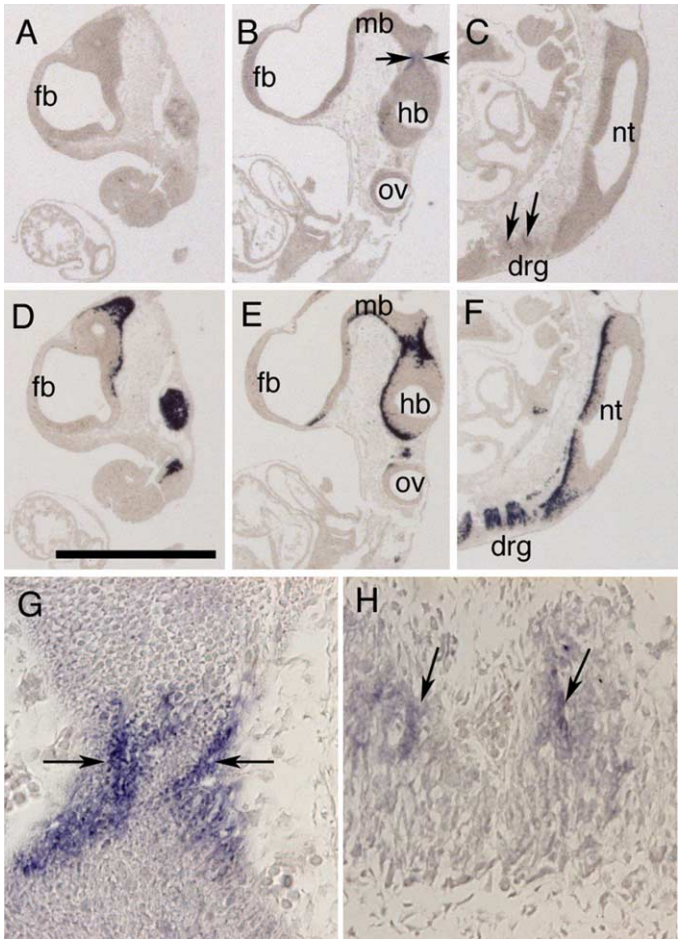

Fig. 1. Expression of MUK/DLK/ZPK in differentiated neurons of E10 mouse embryo. (A)–(C) Sagittal sections immunostained with MUK/DLK/ZPK antibody. (D)–(F) Adjacent sections immunostained with βIII-tubulin antibody. (G) Higher magnification view of junctional region of midbrain and hindbrain shown in (B). (H) Higher magnification view of spinal primordium derived from neural crest shown in (C). Arrows indicate areas of MUK/DLK/ZPK protein expression. Abbreviations: fb, forebrain; mb, midbrain; hb, hindbrain; ov, otic vesicle; nt, neural tube; drg, primordium of dorsal root ganglia. Scale bar, 1 mm.