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Short communication

Dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1A) interacts with the phytanoyl-CoA α-hydroxylase associated protein 1 (PAHX-AP1), a brain specific protein

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Abstract

Down syndrome (DS) is the most common genetic defect correlated with mental retardation and delayed development. The specific genes responsible for these phenotypic alterations have not yet been defined. *Dyrk1A* (dual-specificity tyrosine-phosphorylated and regulated kinase 1A), the human ortholog of the Drosophila *minibrain* gene (*mnb*), maps to the Down syndrome critical region of human chromosome 21 and is overexpressed in Down syndrome fetal brain. In Drosophila, minibrain is involved in postembryonic neurogenesis. In human, DYRK1A encodes a serine-threonine kinase but despite its potential involvement in the neurobiological alterations associated with Down syndrome, its physiological function has not yet been defined. To gain some insight into its biological function, we used the yeast two-hybrid approach to identify binding partners of DYRK1A. We found that the C-terminal region of DYRK1A interacts with a brain specific protein, phytanoyl-CoA α-hydroxylase-associated protein 1 (PAHX-AP1, also named PHYHIP) which was previously shown to interact with phytanoyl-CoA α-hydroxylase (PAHX, also named PHYH), a Refsum disease gene product. This interaction was confirmed by co-immunoprecipitation of PC12 cells co-transfected with DYRK1A and PAHX-AP1. Furthermore, immunofluorescence analysis of PC12 cells co-transfected with both plasmids showed a re-distribution of DYRK1A from the nucleus to the cytoplasm where it co-localized with PAHX-AP1. Finally, in PC12 cells co-transfected with both plasmids, DYRK1A was no longer able to interact with the nuclear transcription factor CREB, thereby confirming that the intracellular localization of DYRK1A was changed from the nucleus to the cytoplasm in the presence of PAHX-AP1. Therefore, these data indicate that by inducing a

Abbreviations: CREB, c-AMP-response element binding protein; DS, Down syndrome; DSCR, Down syndrome critical region; DYRK1A, dual-specificity tyrosine-phosphorylated and regulated kinase 1A; eIF2Bε, eukaryotic initiation factor 2B epsilon; FKHR, forkhead in rhabdomyosarcoma; FnIII, fibronectin type III domain; mnb, minibrain; PAHX and PHYH, phytanoyl-CoA α-hydroxylase; PAHX-AP1 and PHYHIP, phytanoyl-CoA α-hydroxylase-associated protein 1; STAT3, signal transducer and activator of transcription 3

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re-localization of DYRK1A into the cytoplasm, PAHX-AP1 may contribute to new cellular functions of DYRK1A and suggest that PAHX-AP1 may be involved in the development of neurological abnormalities observed in Down syndrome patients.

Keywords: Down syndrome; DYRK1A; Minibrain; Phytanoyl-CoA α-hydroxylase-associated protein 1

1. Introduction

Trisomy 21 or Down syndrome (DS) is a major cause of mental retardation (Epstein, 2001). It is also characterized by a variety of anomalies including congenital heart defects, gastrointestinal tract malformations, a characteristic set of physical features, a decrease muscle tone at birth, a high incidence of leukemia, and an increase incidence of early onset Alzheimer-like dementia. In most cases, DS results from the presence of a third copy of chromosome 21. Studies of rare cases of partial trisomy 21 allowed to define a critical region (DSCR for Down syndrome critical region) responsible for many features of the DS phenotype to the 21q22.2 region (Delabar et al., 1993; Rahmani et al., 1989). One of the DSCR genes is *Dyrk1A*, the human homolog of the Drosophila *minibrain* gene (*mnb*) (Guimera et al., 1996; Shindoh et al., 1996; Song et al., 1996; Tejedor et al., 1995). In Drosophila, mnb appears to play an essential role during postembryonic neurogenesis in regulating the numbers of distinct type of neuronal cells (Tejedor et al., 1995). Mutant mnb flies are characterized by a marked reduction in size of the adult optic lobes and the central brain hemispheres. This is caused by an abnormal spacing of neuroblast and hence a reduction in the production of neuronal progeny (Tejedor et al., 1995). In human, *Dyrk1A* is highly expressed in several regions of the central nervous system (CNS), especially in the cerebral cortex, hippocampus, and cerebellum (Guimera et al., 1996; Marti et al., 2003; Shindoh et al., 1996; Song et al., 1996; Rahmani, Lopes, Rachidi, & Delabar, 1998a; Tejedor et al., 1995). Overexpression of *Dyrk1A* has been found in DS fetal brain (Guimera, Casas, Estivill, & Pritchard, 1999). Murine models of DS overexpressing *Dyrk1A* gene show neurodevelopmental delay, motor abnormalities and cognitive deficits (Smith & Rubin, 1997; Smith et al., 1997). These data suggest that DYRK1A may be involved in the neuropathological alterations observed in DS patients.

DYRK1A encodes a serine-threonine kinase of 763 amino acids and phosphorylates a variety of substrates in vitro such as the signal transducer and activator of transcription 3 (STAT3) (Matsuo, Ochiai, Nakashima, & Taga, 2001), the ε subunit of eukaryotic initiation factor 2B (eIF2Bε) (Woods et al., 2001a), the protein Tau (Woods et al., 2001a), the transcription factor of the forkhead family FKHR (Woods et al., 2001b), dynamin (Chen-Hwang, Chen, Elzinga, & Hwang, 2002), glycogen synthase (Skurat & Dietrich, 2004), and cyclin L2 proteins (De Graaf et al., 2004) suggesting that DYRK1A may be involved in several signaling pathways in vivo (reviewed in Galceran, De Graaf, Tejedor, & Becker, 2003). In vivo, DYRK1A has been shown to interact and activate several transcription factors (Mao et al., 2002; von Groote-Bidlingmaier et al., 2003; Yang, Ahn, & Chung, 2001) suggesting that DYRK1A may play a role in the regulation of gene expression. However, the biological significance of these interactions remains to be established. Interestingly, DYRK1A contains several striking features in its C-terminal region such as a PEST region, which is believed to initiate rapid degradation of the protein, a stretch of 13 consecutive histidine residues (amino acids 607–619), and a serine/threonine rich segment of 14 subsequent residues (Becker et al., 1998; Himpel et al., 2000; Kentrup et al., 1996). To contribute to the elucidation of the function of the C-terminal region of DYRK1A, we used the yeast two-hybrid assay to screen a human brain cDNA library. We found that PAHX-AP1, also named PHYHIP (phytanoyl-CoA α-hydroxylase associated protein 1), a brain specific protein, interacts with the C-terminal domain of DYRK1A. PAHX-AP1 was first isolated in a yeast two-hybrid screen using PAHX, also named PHYH (phytanoyl-CoA α-hydroxylase), the Refsum disease gene product, as a bait (Lee et al., 2000). DYRK1A/PAHX-AP1 interaction was confirmed in vivo by co-immunoprecipitation in PC12 cells transfected with PAHX-AP1 and DYRK1A. Immunofluorescence analysis of transfected PC12 cells