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Review Article

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New Perspectives of Dyrk1A Role in Neurogenesis and Neuropathologic Features of Down Syndrome

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Down syndrome (DS) is one of the most common genetic disorders accompanying with mental retardation, cognitive impairment, and deficits in learning and memory. The brains with DS also display many neuropathological features including alteration in neurogenesis and synaptogenesis and early onset of Alzheimer's disease (AD)-like symptoms. Triplication of all or a part of human chromosome 21, especially the 21q22.1~21q22.3 region called 'Down syndrome critical region (DSCR)', has been considered as the main cause of DS. One gene product of DSCR, dual-specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A), has been highlighted as a key contributor to the neural consequences of DS. This minireview summarizes accumulating recent reports about Dyrk1A involvement in the neuritogenesis, synaptogenesis, and AD-like neurofibrillary tangle formation, which is mainly focusing on Dyrk1A-mediated regulation of cytoskeletal proteins, such as tubulin, actin, and microtubule-associated protein tau. Understanding the molecular mechanisms of these phenomena may provide us a rational for new preventive and therapeutic treatment of DS.

Key words: down syndrome, Dyrk1A, neuritogenesis, synaptogenesis, cytoskeletal proteins

INTRODUCTION

Since Dr. John L. H. Down first described the patients with mental retardation and characteristic facial appearance [1], Down syndrome (DS) has been characterized as one of the most common genetic disorders with an incidence of 1 in every 700~800 live births. DS patients also display cognitive impairment, learning and memory deficit, a high risk of leukemia, congenital heart disease, and hypotonia [2-4]. The main cause of DS is trisomy of all or a part of human chromosome 21 [2, 5]. Several studies of the partial trisomy 21 patients characterized a region of human chromosome 21 [21q22.1~21q22.3; named as 'Down syndrome critical region (DSCR)'] as a key suspect of DS symptoms [6-8].

Among the 33 presumed genes in DSCR, dual-specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A) has been intensively studied due to its close association with various cellular and neuronal processes [9]. Dyrk1A is a proline-directed serine/threonine kinase [10] that phosphorylates more than 20 substrates involved in various cellular processes [11]. More importantly, Dyrk1A up-regulation by trisomy 21 is implicated in the neural defects observed in the patients with DS [11].

Interestingly, accumulating data for recent years have suggested that Dyrk1A is involved in the regulation of cytoskeletal proteins such as tubulin, actin, and microtubule-associated protein

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tau and the alteration in neurogenesis of DS. In this context, this minireview focuses and discusses Dyrk1A and its link to neuropathologic features of DS.

ALTERATION OF NEUROGENESIS IN DOWN SYNDROME

The brains from DS patients have shown alteration in neurogenesis and synaptogenesis. The cortices from DS infant patients (from birth to 14 years) showed 20~50 percent fewer neuronal densities compared to age-matched controls [12]. The reduction in neuron numbers was also observed in the middle-aged patients [13]. This phenomenon correlates with findings from the fetal brains [14] and cultured fibroblasts with DS [15] that showed altered cell proliferation. The arrest of neurogenesis is accompanied with early arrest of brain growth as well as higher frequency of Alzheimer's disease (AD)-like plaque and tangle formation [13, 16, 17]. Also, the brains with DS showed a significant reduction in dendritic spine number in the hippocampus [18, 19]. Alteration in synaptogenesis can be supported by a recent study that the DS model mice show significant changes in spine morphology [20].

DYRK1A, MICROTUBULE, AND NEURITOGENESIS

Among a number of DSCR gene products, Dyrk1A is the most attractive protein that shows close association with neuritogenesis. In nerve growth factor-induced PC12 cell neuronal differentiation (a cell line derived from a pheochromocytoma of the rat adrenal medulla), Dyrk1A overexpression prolonged mitogen-activated protein kinase cascade and promoted neurite outgrowth [21]. In contrast, stable overexpression of Dyrk1A in immortalized H19-7 hippocampal neural progenitor cells caused a failure in basic fibroblast growth factor-induced neuronal differentiation [22]. Cortical neurons from Dyrk1A transgenic adult mice also showed a reduction in the length and number of dendrites [23]. Meanwhile, knockdown of Dyrk1A by specific short hairpin RNA in cultured cortical neurons caused a reduction in neurite length and tau-1-positive axons as well as an increase in neurite branching [24]. The compromised neuritogenesis by Dyrk1A knockdown was considered as a consequence of reduced phosphorylation at serine 1392 residue of microtubule-associated protein 1B (MAP1B) and of altered microtubule dynamics [24]. In addition, a potent and specific inhibitor of Dyrk1A, harmine, showed a capacity that can reduce the number of neurites in cultured hippocampal neurons [25]. Although there are many gaps in knowledge due to different experimental systems and approaches, it is obvious that Dyrk1A protein levels may contribute to neurite formation and altered neuritogenesis seen in DS.

DYRK1A, ACTIN, AND SYNAPTOGENESIS

Dyrk1A also contributes to regulation of actin dynamics and synaptogenesis. Yeast homologue of Dyrk kinase, Pom1, interacts with Rga4 GTPase-activating protein and regulates Rga4 localization, which is involved in Cdc42 GTPase localization in yeast [26]. RNA interference screen of *Drosophila* kinome based on cell morphology identified minibrain (*Drosophila* homologue of Dyrk1A) as a regulator of actin organization [27]. Specific knockdown of minibrain caused an increase in peripheral actin and in the number of protrusions in CNS-derived cell line [27]. The repressive effect of Dyrk1A on actin dynamics is further supported by several studies with mammalian systems. Dyrk1A regulates intramolecular interaction of neural Wiskott-Aldrich syndrome protein (N-WASP) and inhibits actin-polymerizing activity of N-WASP by phosphorylating GTPase-binding domain of N-WASP [28]. While specific knockdown of Dyrk1A in COS-7 cells promoted filopodia formation, Dyrk1A overexpression caused a reduction in dendritic spine formation of cultured hippocampal neurons [28]. In strong correlation, cortical neurons from Dyrk1A transgenic mice displayed a reduction in spine density, synapse formation, and dendritic filopodia length as well as alteration in spine morphology [23]. The association of Dyrk1A with actin filaments can be further supported by co-immunoprecipitation assays with DS tissues [29].

DYRK1A, TAU, AND ALZHEIMER'S DISEASE-LIKE FEATURES IN DOWN SYNDROME

One of the major neuropathological features of DS is a sign of early onset of AD-like symptoms, characterized by the formation of amyloid senile plaques (insoluble deposits of β-amyloid) and neurofibrillary tangles (hyperphosphorylated tau aggregates) [16, 17, 30, 31]. Dyrk1A has been intensively investigated in the context of its contribution to hyperphosphorylation of tau that stabilizes microtubules. The phosphorylating capacity of Dyrk1A to threonine 212 residue of tau was first described by *in vitro* kinase assay and phospho-specific antibody [32]. In addition to *in vitro* phosphorylation, protein-protein interaction between Dyrk1A and tau was analyzed by co-immunoprecipitation [22]. Stable overexpression of Dyrk1A caused a robust increase in intracellular inclusions of phosphorylated tau in immortalized H19-7 hippocampal neural progenitor cells [22]. Consistently, Dyrk1A transgenic mouse brains displayed an increase in tau phosphorylation not only at threonine 212 residue but also at

serine 202 and 404 residues [33]. Immunoblotting with a bunch of phosphorylation site-specific antibodies revealed that Dyrk1A directly phosphorylates multiple serine and threonine residues of tau (serine 199, 202, 396, 400, 404, 422, threonine 181, 205, 212, 217, and 231) *in vitro* and the tau hyperphosphorylation occurs both in the brains from Ts65Dn DS model mice (a partially trisomic DS mouse model of mouse chromosome 16 which contains *Dyrk1A* gene) and in the temporal cortices from DS patients [34]. Dyrk1A immunoreactivity in the tau-positive neurofibrillary tangles in the DS brain strongly supports their association [35]. In the same context, specific knockdown of Dyrk1A by short hairpin RNA in cultured cortical neurons caused a significant reduction in tau phosphorylation at threonine 212 residue [24]. Recently, another possible association between Dyrk1A and tau inclusions was suggested. Dyrk1A interacts with and phosphorylates proline-rich domain of serine/arginine-rich protein 55 (SRp55) that regulates splicing of tau exon 10, an exon encoding the second microtubule-binding repeat [36]. Studies from past decade strongly suggest that one of the DSCR gene products, Dyrk1A, can contribute to the hyperphosphorylated tau and its inclusion formation, which correlates with one shown in the brains with DS.

CLOSING REMARKS

As described above, Dyrk1A is closely associated with regulation of cytoskeletal protein such as tubulin, actin, and microtubule-associated protein tau through phosphorylation of various substrates. A group of substrates that are phosphorylated by Dyrk1A further contributes to regulation of neuritogenesis, synaptogenesis, and AD-like neurofibrillary tangle formation. Although many gaps in knowledge are still remaining, those extensive studies strongly suggest that the approximately 1.5-fold increase of Dyrk1A in the brains with DS may be one of the factors that lead to the neuropathologic features shown in DS patients.

Understanding molecular mechanisms of neuropathological features can offer a rationale for new preventive and therapeutic treatment of DS. One could be that inhibition of Dyrk1A activity from the excessive protein amount in DS may prevent the symptoms or make them less severe. So far, a few potent inhibitors of Dyrk1A have been identified. One of them is epigallocatechin-3-gallate (EGCG), which is the major catechin component of green tea. Treatment of EGCG promoted long-term potentiation of Ts65Dn DS model mice [37] and rescued the defects of Dyrk1A transgenic mouse brains [38]. Another candidate inhibitor of Dyrk1A is harmine although all Dyrk family proteins can be inhibited by harmine [39]. Treatment of harmine effectively reduced tau phosphorylation in neuroglioma cell line [40]. Although the current version of Dyrk1A inhibitors should be improved to get better specificity and efficacy, understanding molecular links between a strong contributor such as Dyrk1A and neuropathological features of DS and developing potent inhibitors against the identified molecular targets will bring us more closely to new preventive and therapeutic treatment of DS.

FOOTNOTES

The abbreviations used are: AD, Alzheimer's disease; CNS, central nervous system; DS, Down syndrome; DSCR, Down syndrome critical region; Dyrk1A, dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A; EGCG, epigallocatechin-3-gallate; MAP1B, microtubule-associated protein 1B; N-WASP, neural Wiskott-Aldrich syndrome protein; SRp55, serine/arginine-rich protein 55.

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REFERENCES

1. Down JL (1995) Observations on an ethnic classification of idiots. 1866. Ment Retard 33:54-56.
2. Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S (2004) Chromosome 21 and down syndrome: from genomics to pathophysiology. Nat Rev Genet 5:725-738.
3. Roizen NJ, Patterson D (2003) Down's syndrome. Lancet 361:1281-1289.
4. Hasle H, Clemmensen IH, Mikkelsen M (2000) Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet 355:165-169.
5. Lejeune J, Gautier M, Turpin R (1959) Study of somatic chromosomes from 9 mongoloid children. C R Hebd Seances Acad Sci 248:1721-1722.
6. McCormick MK, Schinzel A, Petersen MB, Stetten G,

http://dx.doi.org/10.5607/en.2013.22.4.244

Driscoll DJ, Cantu ES, Tranebjaerg L, Mikkelsen M, Watkins PC, Antonarakis SE (1989) Molecular genetic approach to the characterization of the "Down syndrome region" of chromosome 21. Genomics 5:325-331.
7. Korenberg JR, Kawashima H, Pulst SM, Ikeuchi T, Ogasawara N, Yamamoto K, Schonberg SA, West R, Allen L, Magenis E, Ikawa K, Taniguchi N, Epstein CJ (1990) Molecular definition of a region of chromosome 21 that causes features of the Down syndrome phenotype. Am J Hum Genet 47:236-246.
8. Delabar JM, Theophile D, Rahmani Z, Chettouh Z, Blouin JL, Prieur M, Noel B, Sinet PM (1993) Molecular mapping of twenty-four features of Down syndrome on chromosome 21. Eur J Hum Genet 1:114-124.
9. Park J, Oh Y, Chung KC (2009) Two key genes closely implicated with the neuropathological characteristics in Down syndrome: DYRK1A and RCAN1. BMB Rep 42:6-15.
10. Himpel S, Tegge W, Frank R, Leder S, Joost HG, Becker W (2000) Specificity determinants of substrate recognition by the protein kinase DYRK1A. J Biol Chem 275:2431-2438.
11. Park J, Song WJ, Chung KC (2009) Function and regulation of Dyrk1A: towards understanding Down syndrome. Cell Mol Life Sci 66:3235-3240.
12. Wisniewski KE, Laure-Kamionowska M, Wisniewski HM (1984) Evidence of arrest of neurogenesis and synaptogenesis in brains of patients with Down's syndrome. N Engl J Med 311:1187-1188.
13. Mann DM, Yates PO, Marcyniuk B, Ravindra CR (1987) Loss of neurones from cortical and subcortical areas in Down's syndrome patients at middle age. Quantitative comparisons with younger Down's patients and patients with Alzheimer's disease. J Neurol Sci 80:79-89.
14. Contestabile A, Fila T, Ceccarelli C, Bonasoni P, Bonapace L, Santini D, Bartesaghi R, Ciani E (2007) Cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinal matrix of fetuses with Down syndrome and in Ts65Dn mice. Hippocampus 17:665-678.
15. Kimura M, Cao X, Skurnick J, Cody M, Soteropoulos P, Aviv A (2005) Proliferation dynamics in cultured skin fibroblasts from Down syndrome subjects. Free Radic Biol Med 39:374-380.
16. Wisniewski KE, Wisniewski HM, Wen GY (1985) Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome. Ann Neurol 17:278-282.
17. Wisniewski KE, Dalton AJ, McLachlan C, Wen GY, Wisniewski HM (1985) Alzheimer's disease in Down's syndrome: clinicopathologic studies. Neurology 35:957-961.
18. Ferrer I, Gullotta F (1990) Down's syndrome and Alzheimer's disease: dendritic spine counts in the hippocampus. Acta Neuropathol 79:680-685.
19. Suetsugu M, Mehraein P (1980) Spine distribution along the apical dendrites of the pyramidal neurons in Down's syndrome. A quantitative Golgi study. Acta Neuropathol 50:207-210.
20. Haas MA, Bell D, Slender A, Lana-Elola E, Watson-Scales S, Fisher EM, Tybulewicz VL, Guillemot F (2013) Alterations to dendritic spine morphology, but not dendrite patterning, of cortical projection neurons in Tc1 and Ts1Rhr mouse models of Down syndrome. PLoS One 8:e78561.
21. Kelly PA, Rahmani Z (2005) DYRK1A enhances the mitogen-activated protein kinase cascade in PC12 cells by forming a complex with Ras, B-Raf, and MEK1. Mol Biol Cell 16:3562-3573.
22. Park J, Yang EJ, Yoon JH, Chung KC (2007) Dyrk1A overexpression in immortalized hippocampal cells produces the neuropathological features of Down syndrome. Mol Cell Neurosci 36:270-279.
23. Martinez de Lagran M, Benavides-Piccione R, Ballesteros-Yañez I, Calvo M, Morales M, Fillat C, Defelipe J, Ramakers GJ, Dierssen M (2012) Dyrk1A influences neuronal morphogenesis through regulation of cytoskeletal dynamics in mammalian cortical neurons. Cereb Cortex 22:2867-2877.
24. Scales TM, Lin S, Kraus M, Goold RG, Gordon-Weeks PR (2009) Nonprimed and DYRK1A-primed GSK3 beta-phosphorylation sites on MAP1B regulate microtubule dynamics in growing axons. J Cell Sci 122:2424-2435.
25. Göckler N, Jofre G, Papadopoulos C, Soppa U, Tejedor FJ, Becker W (2009) Harmine specifically inhibits protein kinase DYRK1A and interferes with neurite formation. FEBS J 276:6324-6337.
26. Tatebe H, Nakano K, Maximo R, Shiozaki K (2008) Pom1 DYRK regulates localization of the Rga4 GAP to ensure bipolar activation of Cdc42 in fission yeast. Curr Biol 18:322-330.
27. Liu T, Sims D, Baum B (2009) Parallel RNAi screens across different cell lines identify generic and cell type-specific regulators of actin organization and cell morphology. Genome Biol 10:R26.
28. Park J, Sung JY, Park J, Song WJ, Chang S, Chung KC (2012) Dyrk1A negatively regulates the actin cytoskeleton through threonine phosphorylation of N-WASP. J Cell Sci 125:67-80.
29. Dowjat K, Adayev T, Kaczmarski W, Wegiel J, Hwang YW (2012) Gene dosage-dependent association of DYRK1A with the cytoskeleton in the brain and lymphocytes of Down

syndrome patients. J Neuropathol Exp Neurol 71:1100-1112.
30. Teller JK, Russo C, DeBusk LM, Angelini G, Zaccheo D, Dagna-Bricarelli F, Scartezzini P, Bertolini S, Mann DM, Tabaton M, Gambetti P (1996) Presence of soluble amyloid beta-peptide precedes amyloid plaque formation in Down's syndrome. Nat Med 2:93-95.
31. Mann DM (1988) Alzheimer's disease and Down's syndrome. Histopathology 13:125-137.
32. Woods YL, Cohen P, Becker W, Jakes R, Goedert M, Wang X, Proud CG (2001) The kinase DYRK phosphorylates protein-synthesis initiation factor eIF2Bepsilon at Ser539 and the microtubule-associated protein tau at Thr212: potential role for DYRK as a glycogen synthase kinase 3-priming kinase. Biochem J 355:609-615.
33. Ryoo SR, Jeong HK, Radnaabazar C, Yoo JJ, Cho HJ, Lee HW, Kim IS, Cheon YH, Ahn YS, Chung SH, Song WJ (2007) DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease. J Biol Chem 282:34850-34857.
34. Liu F, Liang Z, Wegiel J, Hwang YW, Iqbal K, Grundke-Iqbal I, Ramakrishna N, Gong CX (2008) Overexpression of Dyrk1A contributes to neurofibrillary degeneration in Down syndrome. FASEB J 22:3224-3233.
35. Wegiel J, Dowjat K, Kaczmarski W, Kuchna I, Nowicki K, Frackowiak J, Mazur Kolecka B, Wegiel J, Silverman WP, Reisberg B, Deleon M, Wisniewski T, Gong CX, Liu F, Adayev T, Chen-Hwang MC, Hwang YW (2008) The role of overexpressed DYRK1A protein in the early onset of neurofibrillary degeneration in Down syndrome. Acta Neuropathol 116:391-407.
36. Yin X, Jin N, Gu J, Shi J, Zhou J, Gong CX, Iqbal K, Grundke-Iqbal I, Liu F (2012) Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1A) modulates serine/arginine-rich protein 55 (SRp55)-promoted Tau exon 10 inclusion. J Biol Chem 287:30497-30506.
37. Xie W, Ramakrishna N, Wieraszko A, Hwang YW (2008) Promotion of neuronal plasticity by (-)-epigallocatechin-3-gallate. Neurochem Res 33:776-783.
38. Guedj F, Sébrié C, Rivals I, Ledru A, Paly E, Bizot JC, Smith D, Rubin E, Gillet B, Arbones M, Delabar JM (2009) Green tea polyphenols rescue of brain defects induced by overexpression of DYRK1A. PLoS One 4:e4606.
39. Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur JS, Alessi DR, Cohen P (2007) The selectivity of protein kinase inhibitors: a further update. Biochem J 408:297-315.
40. Frost D, Meechoovet B, Wang T, Gately S, Giorgetti M, Shcherbakova I, Dunckley T (2011) β-carboline compounds, including harmine, inhibit DYRK1A and tau phosphorylation at multiple Alzheimer's disease-related sites. PLoS One 6:e19264.

 http://dx.doi.org/10.5607/en.2013.22.4.244