cells of the cerebellum and motor neurons in the medulla at high levels, and (ii) that the intermediate levels of TSC2 signals are expressed in most neurons of the cerebral cortex, septum, striatum, reticular formation in the medulla and substantia nigra compacta.

In the brains from individuals affected with TSC, cortical tubers, periventricular nodules and giant cell astrocytoma are the characteristic lesions (Gomez, 1988). These hamartomas in the central nervous system are considered to be due to errors in differentiation and migration of germinal cells (Nishimura et al., 1985), which result from loss of heterozygosity for the TSC2 gene observed in the abnormal tissues. Because of the heterozygosity of TSC2 DNA markers on chromosome 16p13.3 in most affected cells, a lesser amount of TSC2 products must be observed in normal tissues of the brain from TSC patients. However, neurons in the normal tissues from TSC patients do not exhibit any other degenerative features, even with the reduced amount of TSC gene products. Together with this lack of degenerative features of neurons in the brain from TSC patients, from the present finding on the high levels of TSC2 gene expression in the specific types of neurons in the normal brain, the possibility emerges that the function of TSC2 gene products in mature neurons may be quite other than proliferation, differentiation or a tumor suppressor function.

Table 1. Degree of TSC2 gene expression in various types of neurons

Brain region	Neuron type	TSC2 gene expression
Cerebral cortex	Most neurons	++
Septum	Most neurons	+
Piriform cortex	Most neurons	+++
Striatum	Medium neurons	++
	Large interneurons	+++
Hippocampus	Pyramidal cells	+++
	Dentate granule cells	+++
	Helus neurons	+++
Substantia nigra	Neurons of the pars compacta	+
Cerebellum	Purkinje cells	+++
	Granule cells	+
Medulla	Neurons of reticular formation	+
	Neurons of the facial nucleus	+++

Degree of expression: +++, high; ++, moderate; +, slight.

The most abundant expression of the TSC2 gene was exhibited specifically in large- or medium-sized neurons of the hippocampal pyramidal layer, dentate granular layer, piriform cortex, striatum, cerebellar purkinje cell layer and medullary motor nucleus, which all had the common feature of well-developed dendritic trees (McGeer et al., 1978). Especially, as novel imaging and electrophysiological techniques have revealed, these cerebellar Purkinje neurons, cortical pyramidal and hippocampal pyramidal neurons have active dendrites of which entire trees are covered with calcium channels and demonstrate calcium spikes (Wong et al, 1979; Llinas and Sugimori, 1980; Ross and Werman, 1987; Tank et al., 1988; Regehr et al 1989; Jaffe et al., 1992; Yuste et al., 1994). In contrast to the abundant expression of the TSC2 gene in these large- or medium-sized neurons, cells in the cerebellar granule layer which were characterized by their tiny cell size and poor dendritic structures (Palay and Chan-Palay, 1974) expressed a lesser amount of TSC2 signals, despite having the highest cell density in the layer. Also, small non-neural cells like oligodendrocytes or astrocytes which exist abundantly in the striatal regions rich in dendrites and fiber tracts appeared not to be stained by TSC2 mRNA signals, as seen in the radiatum, oriens and molecular layer of the hippocampus (Fig. 2).

Current studies on the molecular and cellular basis for memory and learning have accumulated evidence that the hippocampal pyramidal neurons, dentate granule cells, cortical pyramidal neurons and cerebellar purkinje cells showed a long-term potentiation (LTP) (Bliss and Lomo, 1973; Artola and Singer, 1987; Brown et al., 1988; Bashir et al., 1991; Madison et al., 1991; Bliss and Collingridge, 1993) or long-term depression (LTD) (Ito et al., 1982; Kano and Kato, 1987; Ito, 1989) thought of as activity-dependent long-term changes in synaptic efficacy. On the other hand, the morphological

basis for memory and learning was considered to lie in modifications of synaptic connectivities, especially in the activity-dependent changes of the dendritic spines as a result of intracellular molecular alterations in response to repeated stimulation (Guthrie et al., 1991; Muller and Connor, 1991; Lisman and Harris, 1993; Shepherd, 1994). It has been reported that the TSC2 gene product shares a region of homology with the GTPase-activating protein for rap1 which is predicted to interact with rap1 (Wienecke et al., 1995). This relation of the TSC2 gene product with rap1, which functions in the regulation of cytoskeletal interaction in mammalian platelets and in the budding of yeast cells (McCabe et al., 1992; White et al., 1992), strongly supports the notion that the TSC2 gene product may function in the formation of the dendritic spines, thus conferring synaptic plasticity to neurons. Since neurons showing the highest TSC2 expression were of the cell types which were deeply involved in long-term memories and were rich in synaptic plasticity, it is likely that TSC2 gene products play a role in building and maintaining the higher functions of neuron networks for memory and learning.

Therefore, the possible function of the TSC2 gene product in the formation of dendritic spines and the use-dependent modification of synaptic connectivities may be relevant to mental retardation observed in TSC patients.

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References

1 Artola A, Singer W. Long-term potentiation and NMDA receptors in rat visual cortex. Nature 1987; 330:649–652.
2 Bashir ZI, Alford S, Davies SN, Randall AD, Collingridge GL. Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature 1991;349:156–158.
3 Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. J Physiol 1973;232:331–356.
4 Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 1993;361:31–39.
5 Brown TH, Chapman PF, Kairiss EW, Keenan CL. Long-term synaptic potentiation. Science 1988; 242:724–728.
6 European Chromosome 16 Tuberous Sclerosis Consortium, The. Identification and characterization of the tuberus sclerosis gene on chromosome 16. Cell 1993;75:1305–1315.
7 Fryer AE, Chalmers A, Connor JM, Fraser I, Povey S, Yates AD, et al. Evidence that the gene for tuberous sclerosis is on chromosome 9. Lancet 1987;1(8534):659–661.
8 Geist RT, Gutmann DH. The tuberous sclerosis 2 gene is expressed at high levels in the cerebelum and developing spinal cord. Cell Growth Differ 1995;6:1477–1483.
9 Gomez MR. Tuberous sclerosis. New York: Raven Press; 1988.
10 Green AJ, Smith M, Yates JR. Loss of heterozygosity on chromosome 16p13.3 in hamartomas from tuberous sclerosis patients. Nat Genet 1994;6:192–196.
11 Guthrie PB, Segal M, Kater SB. Independent regulation of calcium revealed by imaging dendritic spines. Nature 1991;354:76–80.
12 Hino O, Mitani H, Katsuyama H, Kubo Y. A novel cancer preposition syndrome in the Eker rat model. Cancer Lett 1994;83:117–121.
13 Ito M. Long-term depression. Annu Rev Neurosci 1989;12:85–102.
14 Ito M, Sakurai M, Tongroach P. Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. J Physiol 1982;324:113–134.
15 Jaffe DB, Johnston D, Lasser-Ross N, Lisman JE, Miyakawa H, Ross WN. The spread of Na^+ spikes determines the pattern of dendritic Ca^{2+} entry into hippocampal neurons. Nature 1992; 357:244–246.
16 Kandt RS, Haines JL, Smith M, Northrup H, Gardner RJ, Short MP, et al. Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. Nature Genet 1992;2:37–41.
17 Kano M, Kato M. Quisqualate receptors are specifically involved in cerebellar synaptic plasticity. Nature 1987;325:276–279.
18 Kobayashi T, Hirayama Y, Kobayashi E, Kubo Y, Hino O. A germline insertion in the tuberous sclerosis (*Tsc2*) gene gives rise to the Eker rat model of dominantly inherited cancer. Nat Genet 1995;9:70–74.
19 Kubo Y, Kikuchi Y, Mitani H, Kobayashi E, Kobayashi T, Hino O. Alelic loss at the tuberous sclerosis (*Tsc2*) gene locus in spontaneous uterine leiomyosarcomas and pituitary adenomas in

the Eker rat model. Jpn J Cancer Res 1995;86: 828–832.
20 Kumar A, Kandt RS, Wolpert C, Roses AD, Pericak-Vance MA, Gilbert JR. Mutation analysis of the TSC2 gene in an African-American family. Hum Mol Genet 1995;4:2295–2298.
21 Kumar A, Wolpert C, Kandt RS, Segal J, Pufky J, Roses AD, et al. A de novo frame-shift mutation in the tuberin gene. Hum Mol Genet 1995;4: 1477–1480.
22 Lisman JE, Harris KM. Quantal analysis and synaptic anatomy: integrating two views of hippocampal plasticity. Trends Neurosci 1993; 16:141–147.
23 Llinas R, Sugimori M. Electrophysiological properties of in vitro Purkinje cell dendrites in mammalian cerebellar slices. J Physiol 1980;305: 197–213.
24 Madison DV, Malenka RC, Nicoll RA. Mechanisms underlying long-term potentiation of synaptic transmission. Annu Rev Neurosci 1991; 14:379–397.
25 McCabe PC, Haubruck H, Polakis P, McCormick F, Innis MA. Functional interaction between p21rap1A and components of the budding pathway in *Saccharomyces cerevisiae*. Mol Cell Biol 1992;12: 4084-4092.
26 McGeer PL, Eccles JC, McGeer EG. Molecular neurobiology of the mammalian brain. New York: Plenum Press; 1978. p.501–535.
27 Muller W, Connor JA. Dendritic spines as individual neuronal compartments for synaptic Ca^{2+} responses. Nature 1991;354:73–76.
28 Nice EC, Fabri L, Hammacher A, Holden J, Simpson RJ, Burgess AW. The purification of a Rap1 GTPase-activating protein from bovine brain cytosol. J Biol Chem 1992;267:1546–1553.
29 Nishimura M, Takashima S, Takeshita K, Tanaka J. Immunocytochemical studies on a fetal brain of tuberus sclerosis. Pediatr Neurol 1985;1:245–248.
30 Ohno K, Takeshita K, Arima M. Frequency of tuberous sclerosis in San-in district (Japan) and birth weight of patients with tuberous sclerosis. Brain Dev 1981;3:57–64.
31 Palay SL, Chan-Palay V. Cerebellar cortex: cytology and organization. Berlin: Springer-Verlag; 1974.
32 Povey S, Burley MW, Attwood J, Benham F, Hunt D, Jeremiah SJ, et al. Two loci for tuberus sclerosis: one on 9q34 and one on 16p13. Ann Hum Genet 1994;58 (Pt 2):107–127.
33 Regehr WG, Connor JA, Tank DW. Optical imaging of calcium accumulation in hippocampal pyramidal cells during synaptic activation. Nature 1989; 341:533–536.
34 Ross WN, Werman R. Mapping calcium transients in the dendrites of Purkinje cells from guinea-pig cerebellum in vitro. J Physiol 1987;389:319–336.
35 Rubinfeld B, Crosier WJ, Albert I, Conroy L, Clark R, McCormick F, et al. Localization of the rap1GAP catalytic domain and sites of phosphorylation by mutational analysis. Mol Cell Biol 1992;12:4634–4642.
36 Rubinfeld B, Munemitsu S, Clark R, Conroy L, Watt K, Crosier WJ, et al. Molecular cloning of a GTPase activating protein specific for the Krev-1 protein p21rap1. Cell 1991;65:1033–1042.
37 Saji M, Kimura M, Ishida G, Ohno K. Deafferentation-induced c-*fos* gene expression in subthalamic nucleus and substantia nigra reticulata is reduced by non-NMDA receptor antagonist. Brain Res 1995; 703:165–174.
38 Shepherd GM. Neurobiology. New York: Oxford University Press; 1994. p.644–647.
39 Tank DW, Sugimori M, Connor JA, Llinas RR. Spatially resolved calcium dynamics of mammalian Purkinje cells in cerebellar slice. Science 1988; 242:773–777.
40 White GC, Crawford N, Fisher TH. Cytoskeletal interactions of Rap1B in platlets. In: Authi KS, ed. Mechanisms of Platelet Activation. New York: Plenum Press; 1992. p.187–194.
41 Wienecke R, Konig A, DeClue JE. Identification of tuberin, the tuberous sclerosis-2 product. Tuberin possesses specific Rap1GAP activity. J Biol Chem 1995;270:16409–16414.
42 Wong RKS, Prince DA, Basbaum AI. Intradendritic recordings from hippocampal neurons. Proc Natl Acad Sci USA 1979;76:986–990.
43 Yuste R, Gutnick MJ, Saar D, Delaney KD, Tank DW. Ca^{2+} accumulation in dendrites of neocortical pyramidal neurons: an apical band and evidence for two functional compartments. Neuron 1994;13:23–43.

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