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Short Communication

Dendritic localization of the transcriptional co-repressor Groucho/TLE1 in cortical and cerebellar neurons

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Abstract

In the present study we show that the transcription factor Groucho/TLE1 (TLE1) is expressed in virtually all major cortical subdivisions, hippocampus, amygdala, and thalamus, as well as in the cerebellum of the adult rat brain. In both neocortex and subcortical structures, TLE1 expression was mostly localized to neurons. In addition to the expected nuclear localization, TLE1 immunoreactivity was also detected in apical dendritic shafts of neocortical layer III and V pyramidal cells and in Purkinje cell dendrites. These results demonstrate that TLE1 expression occurs in the mature nervous system and suggest that this protein may perform new functions outside of the nucleus in selected cortical and cerebellar neurons.

Theme: Development and regeneration
Topic: Cerebral cortex and limbic system

Keywords: Cerebellum; Dendrite; Groucho; Hippocampus; Neocortex; Transducin-like enhancer of split 1

The *Drosophila* protein Groucho and its mammalian homologues, referred to as Transducin-like enhancer of split (TLE) 1 to 4 [16], are general transcriptional co-repressors that lack intrinsic DNA-binding ability but can be recruited to different genes through interactions with DNA-binding proteins [3,10]. Groucho/TLE proteins (TLEs) are expressed in a variety of tissues, play important roles in numerous developmental pathways, including neurogenesis [20], and are correlated with adult forms of disease [15].

To date, the majority of studies on TLE proteins have focused on their roles in cell fate determination during embryogenesis and few have examined their expression in the mature central nervous system (CNS). During mouse cortical development, it was shown that TLE1 is expressed in both neural progenitor cells and post-mitotic neurons in the outer layers of the cortical plate, whereas TLE4 is expressed by post-mitotic neurons of the inner layers [19]. Consistent with these observations, Petersen and coworkers [13] have recently shown that TLE4 is expressed in layer VI, but also in more superficial layers (II, III) of the adult mouse cortex. It has also been reported that *TLE3* transcripts are expressed in the adult rat hippocampus [6]. In contrast, less is known about the expression pattern and possible functional role of TLE1 in the adult brain.

In the present study, our primary objective was to characterize the expression of TLE1 in cortical and subcortical regions of the adult rat brain. To that end, we performed immunohistochemical studies using a panel of different antibodies previously shown to recognize TLE1 [7,10,11,19].

Adult male Sprague–Dawley rats were anesthetized with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde. The brains were removed, cryoprotected in 30% sucrose, and sectioned on a cryostat. All animal procedures followed the guidelines of the Canadian Council for Animal Care.

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Three previously characterized antibodies were used for immunohistochemical studies: a rabbit polyclonal against the SP domain of TLE1 ('anti-TLE1') [10,19,20], a rabbit polyclonal against TLE1 phosphorylated at serine239 within the CcN domain ('anti-(pS239)TLE1') [10], and a rat monoclonal against the highly conserved TLE WD40 repeat domain (this antibody recognizes all four members of the TLE family and has been termed 'panTLE') [7,11,16]. For immunohistochemistry, tissue sections were first incubated in 0.2% H_2O_2 in phosphate-buffered saline containing 0.2% Triton X-100 (PB-Tx) for 20 min, then in 10% normal goat serum (NGS) in PB-Tx for 2 h at room temperature. Sections were incubated with anti-TLE1 (1:500 in 1% NGS), anti-p(S239)TLE1 (1:500), or panTLE (1:200) antibodies overnight at 4 °C. Double labeling was performed using antibodies against MAP2 (monoclonal, 1:100; Sigma), glutamate transporter EAAC-1 (monoclonal, 1:100; Chemicon), SMI-32 (monoclonal, 1:500; Sternberger Monoclonals), NeuN (monoclonal, 1:100; Chemicon), and GFAP (monoclonal, 1:200; Sigma). After several washes in PB-Tx, sections were incubated in the appropriate fluorescein isothiocyanate (FITC)-conjugated or carboxymethylindocyanin-3 (Cy3)-conjugated secondary antibodies (1:200 in 1% NGS; Jackson ImmunoResearch). The 31 ('Standard') Series of filters (Chroma Technology Corp.) was utilized for fluorescence microscopy. For control experiments, the primary antibodies were omitted (data not shown). Immunohistochemistry was performed on coronal brain sections at the levels of (in the rostrocaudal axis) primary motor cortex (bregma +1.70 mm), septum (+0.20 mm), primary somatosensory cortex and dorsal hippocampus (−3.30 mm), primary visual cortex and optic tectum (−5.80 mm), and entorhinal cortex (−7.30 mm) according to the atlas of Paxinos and Watson [12]. The structures examined at each anatomical level are listed in Table 1. All images were captured with a DVC black and white camera mounted on a Zeiss Axioskop 2 fluorescence microscope. Grayscale images were digitally assigned to the appropriate red (Cy3) or green (FITC) channels using Northern Eclipse software (Empix).

Using a previously characterized anti-TLE1 antibody [10,19,20], nuclear TLE1 expression was detected within most of the adult brain regions examined and was primarily co-localized with the neuronal marker NeuN, indicating that the majority of TLE1+ cells were neurons (Table 1; Fig. 1B, panel vi; and data not shown). TLE1 expression appeared to be mostly confined to forebrain structures, such as the hippocampus, cortex, thalamus, and amygdala (Figs. 1A and D, Table 1). TLE1 expression was observed in caudal regions of neocortex (i.e., visual and retrosplenial cortices) but was absent in subcortical structures caudal to the thalamus (i.e., substantia nigra, tectum, pons). Nuclear TLE1 expression was also detected in the cerebellum where it was primarily restricted to the Purkinje cell layer (Fig. 1C, panel i) with few labeled cells in the granule cell layer.

Table 1
Distribution of TLE1 expression in the adult rat brain

Bregma (mm)	Area	TLE1-ir
+1.70	Primary motor cortex (M1)	+
	Piriform cortex	+
	Cingulate cortex	+
	Caudate nucleus	−
	Claustrum	−
	Nucleus accumbens	+
+0.20	Medial septum	−
	Lateral septum	−
	Ventral pallidum	−
−3.30	Primary somatosensory cortex (S1)	+
	Primary auditory cortex	+
	CA1	+
	CA2	+
	CA3	+
	CA4 (hilus)	+
	Dentate gyrus	+
	Ventral posterior thalamus	+
	Lateral dorsal thalamus	+
	Mediodorsal thalamus	+
	Basolateral amygdala	+
−5.80	Primary visual cortex (V1)	+
	Retrosplenial cortex	+
	Periaqueductal grey	−
	Substantia nigra reticulata	−
	Superior colliculus (deep grey layer)	−
	Subiculum	−
	Medial geniculate	−
−7.30	Medial entorhinal cortex	+
	Lateral entorhinal cortex	+
	Pedunculopontine tegmentum	−
	Inferior colliculus	−

Abbreviation: ir, immunoreactivity. +/− indicates presence (+) or absence (−) of nuclear TLE1 expression.

Unexpectedly, anti-TLE1 immunoreactivity was detected in the apical dendrites of a large number of layer V, and to a lesser extent layer III, pyramidal cells in nearly all major subdivisions of the neocortex (Figs. 1A and B, panel i). TLE1 expression appeared to be prominent among type 1 pyramidal neurons, as indicated by co-expression with both glutamate transporter EAAC-1, a general pyramidal cell marker protein, and non-phosphorylated neurofilament proteins recognized by the SMI-32 antibody [18] (Fig. 1B, panels iv and v). To confirm the specificity of this dendritic immunoreactivity, similar studies were performed with a previously characterized anti-(pS239)TLE1 antibody that recognizes TLE1 phosphorylated at serine239 [10]. In layer V of visual cortex, anti-TLE1 and anti-(pS239)TLE1 immunoreactivities were both present within groups of apical dendrites resembling dendritic bundles [17] (Fig. 1B, panels i and ii). These dendritic bundles extended dorsally to superficial layers forming "tufts" near the cortical surface. Importantly, we observed an overlapping dendritic staining with a panTLE monoclonal antibody that recognizes all four mammalian TLE family members [7,10,11,16] (Fig. 1B, panel iii), further indicating the specificity of the observed immunoreactivity. At the cortical level shown in Fig. 1B (panel iii), nuclear and dendritic TLE1 expression was absent in superficial cortical