Hippocampal TERT Regulates Spatial Memory Formation through Modulation of Neural Development

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SUMMARY

The molecular mechanism of memory formation remains a mystery. Here, we show that TERT, the catalytic subunit of telomerase, gene knockout (Tert−/−) causes extremely poor ability in spatial memory formation. Knockdown of TERT in the dentate gyrus of adult hippocampus impairs spatial memory processes, while overexpression facilitates it. We find that TERT plays a critical role in neural development including dendritic development and neuritogenesis of hippocampal newborn neurons. A monosynaptic pseudotyped rabies virus retrograde tracing method shows that TERT is required for neural circuit integration of hippocampal newborn neurons. Interestingly, TERT regulated neural development and spatial memory formation in a reverse transcription activity-independent manner. Using X-ray irradiation, we find that hippocampal newborn neurons mediate the modulation of spatial memory processes by TERT. These observations reveal an important function of TERT through a non-canonical pathway and encourage the development of a TERT-based strategy to treat neurological disease-associated memory impairment.

INTRODUCTION

Telomerase is found to maintain the telomere length by adding DNA bases to the end of the chromosomes for protecting genetic material. Telomerase is a ribonucleoprotein with reverse transcriptase activity that consists of telomerase RNA (TERC) and TERT, a reverse transcriptase (Blackburn, 2001; Nandakumar and Cech, 2013; Zhao et al., 2009). Telomerase is essential for the prolonged persistence of stem cell function in organs with extensive cell turnover (Rufer et al., 1999; Sarin et al., 2005; Wege and Brummendorf, 2007). Besides embryonic and cancer stem cells, proliferative neural stem cells (NSCs) also exhibit high levels of telomerase activity (Cai et al., 2002; Caporaso et al., 2003; Ferron et al., 2004, 2009). To date, the main role of TERT found in NSCs is to regulate proliferation (Zhou et al., 2011). Accordingly, in contrast to the well-studied functions of TERT in embryonic and cancer stem cells (Harley, 2008), still little is known about the function of TERT in NSCs, especially in adult hippocampal dentate gyrus (DG).

The adult brain has the ability to change its structure and function during maturation, learning, environmental challenges, or pathology (Lledo et al., 2006). The adult hippocampus, a crucial structure for the formation of certain types of memory, such as episodic memory and spatial memory (Squire, 1992b), is one of the most plastic regions in the mammalian brain. New neurons are generated continuously throughout life in the DG originating from neural progenitor cells (NPCs) (Ayala et al., 2007). The DG, which receives direct inputs from the entorhinal cortex and sends projections to the CA3 region, is traditionally considered as the information gateway to the hippocampus (Deng et al., 2010; Jaffe and Gutierrez, 2007). The development of a dendritic tree allows newborn neurons to communicate with each other by joining circuits (Silva et al., 2009). Our previous study found that hippocampal TERT is implicated in modulation of mood behaviors by regulation of proliferation of NPCs. The development of the newborn neurons contributes to the high synaptic plasticity and dynamic circuit incorporation of the DG, which plays a fundamental function in memory processing (Deng et al., 2010; Jaffe and Gutierrez, 2007; Nakashiba et al., 2012). However, the exact understanding of the molecular mechanism underlying the regulation of dendritic development and circuit formation of newborn neurons in the post-natal hippocampus is necessary (Ming and Song, 2011). Thus, in this study, we investigated the potential of hippocampal TERT in the modulation of neural development and its contribution to spatial
memory formation, as well as determining its cellular mechanism.

RESULTS

Hippocampal TERT Is Required for Spatial Memory Formation

It has been shown that TERT deficiency affects normal brain functions in mice (Yuan et al., 1999). To investigate whether TERT is implicated in hippocampus-dependent spatial memory formation, we performed the Morris water maze (MWM) test, a classic method for measuring spatial memory formation in rodents (D’Hooge and De Deyn, 2001), using the first generation of Tert−/− mice (Figure S1) and their wild-type (WT) mice. Surprisingly, Tert−/− mice displayed extremely poor ability in spatial learning and memory formation compared with WT in the MWM task (Figure 1A; Movies S1 and S2). To rule out the influence of swimming speed on platform probing, we monitored the swimming speed in each experiment. There was no difference in swimming speed between groups in each MWM task in this study (Table S1); and there was no difference in visible probe tests between groups in each MWM task (Figure S2).

In the adult brain, TERT is detected in various regions including the hippocampus (Caporaso et al., 2003). To determine whether TERT loss in the DG of Tert−/− mice accounts for the defect in spatial memory formation, we constructed a lentivirus (LV) expressing Flag-tagged mouse Tert full-length cDNA under control of the promoter, Ubi, and a GFP reporter gene under control of another promoter, SV40 (named LV-TERT-GFP; Figure 1B) to express the TERT protein and GFP separately. Western blot measurement showed that the TERT-Flag fusion protein was successfully detected by Flag primary antibody in cultured 293T cells 4 days after infection with LV-TERT-GFP (Figure 1B). Then, 1 μL of LV-TERT-GFP was microinjected into bilateral DGs of the hippocampi of Tert−/− mice (Figure 1C) to replenish TERT protein. As control, 1 μL of LV-GFP was microinjected into bilateral DGs of the hippocampi of Tert−/− and WT mice. Western blot measurement of Flag expression indicated that the TERT-Flag fusion protein was expressed in the DG of Tert−/− mice 14 days after injection of LV-TERT-GFP (Figure 1D). Strikingly, replenishment of TERT protein in the DG of Tert−/− mice significantly improved the ability of spatial learning and memory formation in the MWM task 30 days after infusion of LV-TERT-GFP (Figure 1E). In contrast, to specifically disrupt the expression of TERT protein in the DG, we delivered an LV vector expressing small hairpin RNA (shRNA) of TERT (LV-TERT-shRNA-GFP, Figure 1F) or LV-GFP into bilateral DGs. RT-PCR analysis showed 77.32% reduction of TERT mRNA expression level 7 days and 65.46% reduction 28 days after infusion of 1 μL of LV-TERT-shRNA-GFP into the DGs compared with 1 μL of LV-GFP infusion (Figure 1G). Importantly, an impaired ability in spatial memory formation in the MWM task of mice infused with LV-TERT-shRNA-GFP was observed 28 days after infusion, compared with mice infused with LV-GFP (Figure 1H), suggesting that specific TERT deficiency in the DG of the hippocampus impairs spatial memory formation. Altogether, these data suggest an essential role of hippocampal TERT in spatial memory formation.

High Level of Hippocampal TERT Facilitates Spatial Memory Formation

TERT overexpression in the hippocampus exerts an anti-depressant effect (Zhou et al., 2011). To test whether overexpression of TERT in adult hippocampal DG promotes spatial memory formation, we delivered a recombinant adenoviral vector carrying the gene-encoding mouse TERT and GFP reporter cDNA linked by internal ribosome entry site (named AD-TERT-GFP) into the DGs by microinjection, and measured spatial memory formation in the MWM test 30 days after virus infection. TERT mRNA content measurement in the hippocampus showed that TERT expression was enhanced at day 7, peaked at day 14, began to decline at day 21, and returned to normal levels at day 28 after infusion of AD-TERT-GFP (Figure S3). Interestingly, AD-TERT-GFP infection in the DGs significantly improved spatial memory formation of mice in the MWM test 30 days (Figure 2A), but not 7 days (Figure 2B), after hippocampal microinjection. Taken together, these results imply that the positive impact of TERT overexpression on spatial memory formation is indirect, delayed, and requires a period of about 1 month.

TERT Is Essential for Dendritic Development In Vitro and in Adult DG

Neural development, especially of hippocampal newborn neurons in adulthood, is closely associated with memory formation (Bruel-Jungerman et al., 2007; Zhao et al., 2008). It is reported that telomere shortening caused by TERC knockout disrupts neuronal differentiation and neuritogenesis (Ferron et al., 2009). Although there is extensive evidence showing that telomere length is modulated by TERT, the role of TERT in neural development remains unknown. We accordingly speculated that TERT might be involved in spatial memory process via modulation of neural development, including adjusting morphological characteristics and sculpting the dendritic arbor.

To test this hypothesis, first, in vitro NSC differentiation experiments were performed using E18 embryonic hippocampus of Tert−/− and WT mice. Four days after induction of differentiation, analysis of doublecortin (DCX, a marker
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