Does telomerase protein protect our neurons?
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ABSTRACT
Telomerase is best known for its canonical function in telomere maintenance. However, a growing number of non-telomeric functions have been described. Several groups have found the telomerase protein TERT to persist in adult brain neurons. A protective role for the telomerase protein TERT had been demonstrated in cultivated mouse neurons during brain development, against excitotoxic stresses from N-methyl-D-aspartate (NMDA) and glutamate and agents known to be involved in neurodegenerative diseases such as beta amyloid peptides and hyperphosphorylated tau. In contrast, lack of telomerase and TERT protein increase oxidative stress and decrease neuronal survival. Research on telomerase and TERT protein in human neurodegenerative diseases is a relatively new field. However, there is emerging evidence of a beneficial role of telomerase in human brains and animal models of neurodegenerative diseases that suggests to explore the possibility of using telomerase activators as neuroprotective agents to combat brain ageing and to ameliorate neurodegenerative diseases such as Parkinson’s and Alzheimer’s diseases. The current mini-review summarises the knowledge about this developing novel area of brain research.
Telomerase is a reverse transcriptase best known as the enzyme that extends and maintains telomeres, the protective ends of linear chromosomes, in dividing cells. It consists of the catalytic protein TERT (Telomerase Reverse Transcriptase) and an RNA subunit which contains the template region for telomere synthesis. However, in most human somatic cells telomerase is downregulated early during development\(^1\)\(^2\) while some cell types such as endothelial cells, lymphocytes and adult stem cells express some activity throughout life. Due to its own RNA moiety, telomerase is able to extend the 3' overhang of telomeres, generated during DNA replication and thus counteract telomere shortening. Without sufficient telomerase activity present, telomeres shorten due to the inability of conventional DNA polymerases to replicate the lagging strand at the end of chromosomes, the so called “end replication problem”\(^3\)\(^4\). Importantly, also oxidative stress has been shown to be a major reason for telomere shortening\(^5\). However, telomere shortening happens mainly in dividing cells and this division is required for translating accumulated DNA damage and DNA breaks into telomere shortening\(^6\). In addition, telomeres are not well repaired\(^7\). DNA damage however, can also happen in non-dividing cells such as neurons and it was even shown that the grade of maturation might play a role with newly non-dividing cells such as neurons and it was even shown that the grade of maturation might play a role with newly non-dividing cell types such as Purkinje neurons, both DNA damage and cellular senescence as well as telomerase have been detected\(^8\)\(^9\)\(^10\)\(^12\).

The research on the involvement of telomerase in neurons and the brain was pioneered by Mark Mattson’s group more than 15 years ago. They showed that cultivated neurons from mouse embryos need telomerase activity for protection and development and that telomerase promotes their survival against trophic factor withdrawal, glutamate-induced excitotoxicity and amyloid peptides in cultured mouse primary neurons\(^13\)\(^-\)\(^16\). Analysing telomerase activity as well as the expression of its protein part TERT it became apparent that, while telomerase activity is downregulated early during brain development in humans\(^1\) and postnatally in rodents, the TERT protein seemed to persist even in adult mouse brains\(^17\)\(^18\). In addition to telomere-dependent, canonical functions of telomerase, more and more telomere-independent, non-canonical functions of the TERT protein became apparent over the last decade (for reviews see\(^19\)\(^20\)). This includes subcellular shuttling of TERT from the nucleus to mitochondria upon increased oxidative stress\(^21\)\(^24\). In addition to a nuclear localisation signal (NLS) and a nuclear exclusion signal (NES)\(^25\)\(^26\) TERT in higher organisms such as mammals, also contains a specific mitochondrial localisation signal (MLS)\(^21\). The nuclear exclusion upon oxidative stress is dependent on Src kinase and Ran GTPase\(^27\). TERT within mitochondria exerts a protective function with a decrease of mitochondrial and intracellular oxidative stress, decrease of apoptosis and protection of mitochondrial and nuclear DNA\(^22\)\(^-\)\(^24\) although the exact underlying mechanisms still remain elusive. Binding to mitochondrial DNA, improvement of mitochondrial respiration and changes in cellular antioxidants have been suggested\(^23\)\(^28\)\(^29\). Interestingly, Santos’ group demonstrated that the TERT protein can exert its reverse transcriptase function in mitochondria by using mitochondrial tRNAs as templates for reverse transcription\(^29\) although the biological significance of that novel catalytic function of telomerase within mitochondria has not been demonstrated yet. There is an ongoing debate in the telomerase field whether some non-canonical functions are dependent on the presence of the catalytic function of TERT while others are not (for review see\(^29\)). Demonstrating the catalytic function of TERT within mitochondria does not answer this question since no direct correlation between the catalytic activity of TERT in mitochondria and its protective function has been confirmed. It is also not known whether catalytic activity of TERT is essential or required for the protection of neurons by TERT. The protective role of mammalian TERT in mitochondria has recently been demonstrated even in yeast where it increased the resistance against oxidative stress without interacting with telomeres or the yeast TERT which lacks the mitochondrial localisation signal\(^30\).

Our group also found that upon treatment with hydrogen peroxide or irradiation cancer cells exclude TERT protein in a heterogeneous manner and intriguingly, nuclear DNA damage correlated positively with TERT protein remaining within the nucleus; while cells with cytoplasmic/mitochondrial TERT localisation had lower nuclear DNA damage, lower levels of reactive oxygen species (ROS) and hardly any apoptosis. In contrast, cells with a nuclear TERT localisation had high amounts of DNA damage, oxidative stress as well as apoptosis\(^34\). This finding corresponds well with results from experiments that used a hTERT protein with a mutant NES rendering the protein unable to leave the nucleus resulting in increased nuclear and mitochondrial DNA damage as well as higher levels of oxidative stress\(^32\)\(^33\).

There are various mechanisms of neuronal cell death in the brain during neurodegeneration. While in Parkinson’s disease (PD) apoptosis might occur as the pre-dominant mechanism, in Alzheimer’s disease (AD) the exact mechanism of neuronal degeneration is still not known\(^31\). Consequently, the involvement of TERT into neuronal apoptosis has not been investigated yet.

It has not been analysed in detail whether nuclear
localisation of TERT is the default situation in all cell types. Immune cells such as lymphocytes have their TERT protein in the cytoplasm under unstimulated conditions and transport it into the nucleus upon antigen or inflammatory stimulation (see Figure 1 A) and activate telomerase activity and nuclear translocation by phosphorylating TERT on serine 227 by AKT. In contrast, it is thought that TERT and telomerase activity are localised within the nucleus under non-stressed conditions (see Figure 1 B).

Intriguingly, Iannilli et al showed that in fully differentiated neurons the majority of cytoplasmic TERT forms a complex with RNA granules and binds to the mRNA of p15INK4b, a pro-survival cell cycle inhibitor. Upon cellular stress TERT and p15 dissociated resulting in translation of p15 while TERT was free to shuttle to mitochondria. The authors also showed that the anti-apoptotic functions of TERT and p15 in cultivated rat hippocampal neurons were mutually dependent on each other. In general, Iannilli’s findings suggested that TERT protein is not necessarily localised in the nucleus of neurons corresponding to reports from Spilsbury et al. who also found TERT signals predominantly outside the nucleus in hippocampal human and cultivated mouse neurons although contradicting results exist and changes might occur with the age of the organism.

Mattson’s group also showed that presence of telomerase is able to protect against apoptosis, withdrawal of growth factors and glutamate treatment (excitotoxicity) as well as treatment with amyloid precursor protein in cultivated primary mouse neurons. Thus, a potential connection between the presence of TERT protein in adult brains and neurodegeneration was possible. Recently, Esther Priels’ group has demonstrated that boosting telomerase levels in mice by using specific compounds that activate telomerase, can ameliorate NMDA toxicity in wild type mice. This seems to correspond well to a previous study in mouse brains in vivo that showed that overexpressing TERT in neurons did not result in any telomerase activity but improved protection of neurons from NMDA excitotoxicity as well as mitochondrial functions such as mitochondrial membrane potential. Importantly, Eitan and co-workers also showed that compounds that activate telomerase can ameliorate symptoms and disease severity in a mouse model of Amyotrophic Lateral Sclerosis (ALS), again suggesting a beneficial effect of telomerase/TERT levels in the brain of adult mice of a neurodegenerative mouse model.

It had been demonstrated that telomerase activity is downregulated very early during human brain development. However, not much research had been performed on adult human brains. Our group used human brain tissue from the Newcastle Brain Tissue Resource (NBTR) and analysed the hippocampus of donors with different Braak stages of Alzheimer’s disease (AD) and compared them to age-matched healthy controls. We found that only neurons, but not astrocytes expressed any TERT protein while there was some expression in human microglia, presumably due to their nature being macrophages. While our initial hypothesis was that TERT protein might decrease due to disease progression, we could not find any evidence of that using different techniques such as Western blotting.

Figure 1: Subcellular localisation of TERT protein as well as the presence or absence of telomerase activity (TA) in different cell types. A: Lymphocytes: TERT enters nucleus upon stimulation, the role of ROS for changes in TERT localisation and TA are not known. B: Cancer cells: TERT localises in the nucleus as default, but can shuttle to mitochondria upon oxidative stress (ROS). C: Adult neurons: TERT is localised in the cytoplasm and might form complex with other molecules that is interrupted upon oxidative stress and localises within mitochondria.
and immuno-fluorescence staining for TERT protein\textsuperscript{39}. However, we found that there was a significant increase in the amount of mitochondrial TERT localisation in hippocampal areas CA1-3 of advanced AD cases (Braak stage 6) compared to healthy controls (Braak stage 0). Oxidative stress and mitochondrial dysfunction increase during brain ageing and are suggested to be involved in the pathogenesis of Alzheimer’s disease (AD) and other neurodegenerative diseases. In both AD and animal models of neurodegenerative diseases, chronically increased oxidative stress levels have been found to represent one of the earliest changes before specific pathologies such as amyloid-\(\beta\) (A\(\beta\)) plaques and neurofibrillary tangles occur\textsuperscript{41,42}. Our findings together with that of Iannilli et al.\textsuperscript{37} suggest that TERT protein enters neuronal mitochondria from the cytoplasm due to increased oxidative stress where it might exert a protective function (see Figure 1C). This suggestion is also supported by our results of a lack of co-localisation of hyperphosphorylated tau protein (either as neuropil threads or neurofibrillary tangles) and TERT protein in human hippocampi of advanced Braak stages in AD brains where we found a mutual exclusion of both proteins: neurons expressing TERT did not show any tau pathology while cells with neuropil threads or neurofibrillary tangles did not show any TERT expression\textsuperscript{38}. Whether high TERT expression was really protective against hyperphosphorylated tau or tau expressing neurons did displace or downregulate TERT can only be speculated at that point. However, in order to model tau pathology in an experimental system we used cultivated mouse embryonic neurons expressing TERT protein (P301L mutation known from human tauopathies\textsuperscript{45}), while the TERT protein persisted) with mutated human tau protein at higher age might help to prevent or ameliorate brain ageing as well as the onset and progression of various neurodegenerative diseases. Thus, telomerase might be viewed as a novel target for future strategies to combat neurodegenerative diseases. First results on mouse models of neurodegeneration are encouraging but have to be scrutinised carefully in a human setting.

Taken together, there is growing evidence for a cytoplasmic localisation of TERT protein in adult neurons that seems to correlate to a protective function in the brain that is quite distinct from telomerase enzymatic activity on telomeres. Consequently, these results suggest that an increase of TERT protein at higher age might help to prevent or ameliorate brain ageing as well as the onset and progression of various neurodegenerative diseases. Thus, telomerase might be viewed as a novel target for future strategies to combat neurodegenerative diseases. First results on mouse models of neurodegeneration are encouraging but have to be scrutinised carefully in a human setting.

**References**


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