RESEARCH ARTICLE

The wonderful new world of telomerase in the brain and its possible implications for neurodegenerative diseases

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Abstract

While telomerase is best known as the enzyme that maintains telomeres in dividing cells a number of TERT (Telomerase Reverse Transcriptase)-related non-canonical functions have been described. These functions are implicated in tumour development and stress response and recently have also been demonstrated in the brain. In contrast to other cells and tissues, in the brain the telomerase RNA component TERC is down regulated early during development resulting in the loss of telomerase activity in most cells except for neural stem cells. In contrast, the presence of the telomerase protein TERT persists during lifetime in neurons of the human brain. Although we are far from understanding the role of telomerase in the brain, the review aims to summarise our current knowledge. In addition to physiological functions in healthy neurons, there might be implications for neurodegenerative diseases.

Introduction

For a long time telomerase was best known for its canonical function in maintaining telomeres in cells and tissues where the enzyme is active. For this function two essential components- the catalytic subunit TERT (Telomerase Reverse Transcriptase) and its integrated telomerase RNA (TR or TERC: telomerase RNA component) are required. The RNA component contains a template whereby telomeric hexanucleotide repeats can be added *de novo* onto the G-rich telomeric overhang. This forms the basis for telomerase enzymatic activity. However, this activity is differentially expressed in different species and cell types. In protozoans is serves to maintain and cap telomeres of small chromosomes, it is active in many animal species, germline tissue of plants, and has a characteristic expression pattern between metazoan species as well as different tissue and cell types within the same species. Some insects such as diptera (for example Drosophila melanogaster) don't have telomerase; here transposons fulfil the function of telomere maintenance[1].

Telomerase activity in rodents and humans

In rodents the enzyme is active lifelong in most tissues, but often strongly downregulated during development in other mammals including humans. In humans telomerase is active during early stages of embryonic development, but in most tissues downregulated soon after[2]. Only a few tissues such as T-and B-lymphocytes, endothelial cells and adult stem cells still express the enzyme or are at least able to upregulate it upon stimulation. The downregulation of telomerase activity is in most tissues achieved via downregulation of the catalytic TERT subunit while the TERC component is still constitutively expressed[3]. Thus, initially scientists suggested that TERT expression is the limiting component[4]. This is also the basis for reconstituting telomerase activity in somatic cells by just overexpressing the TERT subunit[5]. However, others described that TERC can be the limiting component in heterozygous mice and diseases such as dyskeratosis congenita [6, 7].

Non-canonical functions of TERT-the mitochondria connection

In addition to telomerase activity, various non-canonical functions of the protein part TERT on its own have been described. There is now a large amount of such non-canonical functions known which are detailed in various reviews [8-10]. One of these non-telomeric functions is the shuttling of the TERT protein into mitochondria upon external or internal oxidative stress [5,11-13]. This is a regulated process in higher eukaryotes that depends on a specific signalling sequence[11] driving the import of TERT into the mitochondrial matrix in addition to nuclear export signals[14]. In the organelles TERT fulfils different functions such as protecting mitochondrial as well as nuclear DNA, decreasing cellular oxidative stress and sensitivity for apoptosis [5, 11, 12, 15].

Telomerase, TERT and TERC in brain

Interestingly, in brain and specifically in neurons, the TERT component was found persisting throughout human life without any measurable telomerase activity[16]. But what about TERC? If TERT is present and TERC

constitutively active-then there should also be activity? Our group demonstrated on brains from early human embryos that in this tissue type telomerase is downregulated at very early gestational stages of post-conception weeks 10-14[17]. This downregulation was associated with a decrease in TERC RNA while TERT persisted in its expression. With other words-brain is apparently different in its biological mechanism of regulating telomerase activity from most other human tissues. Another group has even described a specific mutated/deleted TERC component in mouse brain which interacts with mTERT and might be involved in the regulation of TERC levels in brain[18]. Intriguingly, this alternative telomerase RNA is suggested to protect cells from oxidative stress[18].

In addition, we and others also found that other stimuli than oxidative stress are able to drive TERT protein into mitochondria. For example, rapamycin which decreases the mTOR pathway and promotes autophagy is able to direct TERT into mitochondria specifically in brain tissue, but not in other tissues such as for example liver [19]. Others have described brain specific neurotransmitter substances such as glutamate involved in excitotoxic stress to be responsible for mitochondrial localisation of TERT in neurons [20].

TERT and neurodegeneration

While in mice *TERT* expression level decreases with age [19, 21] we could not find such a decrease in human brains with age[17] although a larger sample number has to be analysed for a final evaluation. Likewise, we could not find any differences in the amount of TERT protein from hippocampi of Alzheimer's disease (AD) brains compared to healthy age-matched cases[16]. Instead, we found a stronger mitochondrial localisation of TERT protein in hippocampal neurons of Braak stage 6 AD cases[16]. Modelling the influence of pathological proteins involved in AD such as tau (p301L mutant) in primary embryonic mouse neurons we demonstrated that its overexpression is associated with increased oxidative stress and more lipid peroxidation products[16]. This result shows that toxic proteins involved in neurodegeneration such as tau, but most likely also other proteins such as alpha-synuclein are able to increase mitochondrial ROS generation while TERT protein localised to the organelles might be an attempt of the neuron to counteract mitochondrial dysfunction. Our results suggest that the absence or low levels of TERT might render neurons more susceptible to the action of toxic proteins.

Telomerase and autophagy in brain

In addition to the induction of mitochondrial ROS toxic proteins associated with various neurodegenerative disease are known to exist in different protein forms such as monomers, oligomers and aggregates. The latter form cellular inclusions such as neurofibrillar tangles (NFT) and Lewy bodies (LB). Other cellular mechanisms such as the proteasome and different forms of autophagy can degrade and thereby partly detoxify such proteins[22]. Interestingly, it was recently demonstrated that increased TERT/telomerase levels in non-neuronal cellular models promote the activity of both the proteasomal [23] as well as the activity of autophagy [24]. Although there is

so far no clear evidence that TERT has similar properties in neurons and brain *in vivo*, one could speculate about this novel non-canonical function of TERT in brain.

It was demonstrated that also excitotoxic stress using glutamate can promote mitochondrial localisation of TERT [20]. Our group had previously shown that upon rapamycin treatment TERT protein can potentially interact with mTOR signalling in mouse brain on a functional level[19]. This was associated with a partial mitochondrial localisation of TERT and a decrease in mitochondrial oxidative stress. Although others have shown a direct complex formation between TERT, mTOR and other molecules in immune and cancer cells [25, 26] we don't know yet whether a similar interaction occurs in mammalian brain. Importantly, rapamycin seems to be the only trigger that promotes TERT shuttling into mitochondria not by increased oxidative stress. It is so far unknown what other physiological stimuli or specific cellular signalling pathways are able to induce TERT shuttling into mitochondria in addition to oxidative stress.

Telomerase activators and the brain

Based on the knowledge of a beneficial effect of TERT in the brain research had been initiated by the Priel group to use telomerase activators in an attempt to combat the severity of neurodegenerative diseases. The group had demonstrated in a mouse model of Amyotrophic Lateral Sclerosis (ALS) that treatment with a synthetic telomerase activator (aryl compound) was able to delay the severity of disease symptoms [27]. The same group has recently used telomerase activators in an AD related *in vitro* neuronal model and achieved an increase of different neurotrophic and plasticity proteins involved in the disease [28]. In the same study they have also used a short term treatment of mice with the same activator and demonstrated a similar expression change of those proteins in the mouse brains.

Our group has recently performed a preclinical trial on a mouse model of PD using different telomerase activators which showed a beneficial effect in improving PD-related symptoms and a decrease in alpha-synuclein levels in brain pathology (manuscript under review).

In addition, Maria Blasco's group explored the significance of short telomeres for brain neurogenesis and neurodegeneration and used telomerase gene therapy for extending short telomeres [29]. They used old wild type mice as well as late generation TERT-/- mice to demonstrate that these mice have a compromised cognition in an NOR (Novel object recognition) test and an increased amount of phosphorylated endogenous tau protein. Using telomerase gene therapy by introducing AAV (Adeno associated virus)-mediated TERT into brain neurons of those above mentioned mice with short telomeres in different brain regions. Treatment resulted in less DNA damage, inflammation, increased neurogenesis and spatial memory[29]. The authors suggest that increased telomerase can be used to treat neurodegenerative diseases such as AD.

Conclusion

Although there are still a lot of white patches in our knowledge of mechanisms how the

telomerase protein TERT protects neurons during ageing and neurodegeneration, either via it's canonical role on protecting and preserving telomeres as well as non-canonical functions of TERT which could be due to interaction with other molecular mechanisms such as protein degrading pathways. Telomerase in the brain is a rapidly developing new field of research which receives increasing interest.

It is intriguing to find that brain is different in the regulation of telomerase with a persistent presence of the TERT protein in neurons but without the presence of a functional TERC RNA component that is rather ubiquitously expressed in most other human tissues. Thus, the biology of telomerase in brain and in particular neurons seems to differ greatly from that of other tissues and cell types. This should warrant novel findings with a therapeutic relevance for brain ageing and various neurodegenerative disease in the future.

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