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Doing the Unthinkable: Telomerase in the Brain and in Neurodegeneration

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Abstract

Telomerase is an enzyme that in its canonical function maintains telomeres, the ends of linear chromosomes. For that function two components are required: the reverse transcriptase TERT and an inherent RNA component TERC. The latter contains the template for telomere synthesis de novo. This canonical, telomere-related function counteracts telomere shortening in dividing cells. However, in addition to this telomere-related function, the telomerase protein TERT has a number of non-canonical functions. These can contribute to tumorigenesis and also protect against oxidative stress and decrease sensitivity to apoptosis. Some of those functions are associated with a shuttling of the TERT protein from the nucleus to mitochondria.

In most human somatic tissues there are no or rather low amounts of telomerase activity due to a rapid decline during embryonic development. However, in some tissues such as the brain, a persistence of the TERT protein has been demonstrated, predominantly in neurons. These are post-mitotic cells and thus there is no need to maintain their telomeres in the nucleus. Instead, the TERT protein resides mainly outside the nucleus and protects those cells by influencing the expression of genes related to neurotrophic and other brain-related factors or by modulating protein quality mechanisms such as autophagy. All these mechanisms have also been shown to protect the brain against toxic proteins associated with neurodegeneration. This mini-review summarises the current knowledge on telomerase in the brain and its potential for novel therapeutic approaches against neurodegenerative diseases.

Keywords: *Telomerase; Brain; Neuron; Neurodegeneration; Alzheimer's disease; Parkinson's disease; Mouse models*

The Telomeric Function of Telomerase

The enzyme telomerase is a reverse transcriptase. Its main function is to counteract telomere shortening of dividing cells. Telomere shortening occurs during normal semiconservative DNA replication due to the "end replication problem" (ERP) as only the leading DNA strand can be synthesized by the DNA polymerase continuously while the lagging strand is synthesized by Okazaki-fragments with the help of a RNA-primer and afterwards linked together by DNA ligase. At the very ends of the lagging strand, the RNA primer is removed leaving a 3'overhang. Due to the telomeric hexanucleotide sequence TTAGGG in mammals including humans which are tandemly repeated up to 12 kb in humans at birth, this overhang is G-rich. It serves as the end where the telomerase enzyme, consisting of the reverse transcriptase protein *TERT* and the RNA component TERC, adds hexanucleotide *de novo* in late S-phase after DNA replication is completed. However, the enzyme is mainly active during embryonic development and then downregulated in most somatic human cells. Only germ line cells, embryonic stem cells as well as most cancer cells possess sufficiently high telomerase activity in order to avoid telomere shortening and have thus a potentially unlimited division potential. Adult stem cells are able to upregulate telomerase activity when required while T- and B-lymphocytes as well as endothelial cells have some levels of telomerase activity even in the adult human organism. The expression of *TERT* and telomerase activity are tightly regulated at various molecular levels. In contrast, cancer cells often acquire hTERT promoter mutations which are responsible for continuously high and unregulated levels of telomerase activity which is a prerequisite for cellular immortality.

Non-canonical Functions of *TERT*

In addition to those telomere-related functions, the telomerase protein *TERT* has various non-telomeric functions. Some of those functions also contribute to tumorigenesis and cancer progression. They influence processes such as epithelial-mesenchymal transition (EMT), metastasis progression, gene expression and interaction with signalling pathways such as Wnt and NFκB [1]. Some of these functions are associated with a mitochondrial localization

of *TERT* after cellular stresses [2-5]. This is a regulated biological process due to a mitochondrial localization signal [2] which seems to be a rather recent evolutionary acquisition and does not exist in lower eukaryotes such as yeast and plants. Within mitochondria, *TERT* seems to interfere with respiration [4], decreases oxidative stress, mitochondrial and nuclear DNA damage and protects from apoptosis [3-5]. Within mitochondria, *TERT* can complex with various mitochondrial RNAs and perform reverse transcription with their help [6]. However, its biological significance is not clear yet.

Interestingly, overexpressing hTERT in cancer cell models has demonstrated that this can promote protein degradation mechanisms such as proteasomal digestion [7] and autophagy [8]. These processes also play a role in the brain supporting the clearance of toxic brain proteins such as amyloid, tau and α-synuclein.

TERT and the Brain

Initial experiments suggesting a role of telomerase in the brain were already conducted more than 20 years ago and pioneered by Mark Mattson. The group used overexpression of telomerase in primary hippocampal neurons as well as inhibition of activity using antisense technique in pheochromocytoma cells. They found that telomerase is important during neuronal development and prevents from apoptosis and protects against neurotoxic agents such as amyloid-β in cultured neurons [9]. Although still without discrimination between telomerase activity and non-canonical functions of *TERT* as the latter ones were not known yet twenty years ago, these studies demonstrated for the first time a link between telomerase/*TERT* and brain cells such as neurons. Klapper, et al. (2001) also demonstrated that telomerase activity in mouse brain persists until a few weeks postnatally [10]. In contrast to mice, in humans telomerase activity is downregulated very early during development and decreases substantially already after early postconception states [11] due to a decrease in the RNA subunit TERC. In adult brain there are only very few areas with telomerase activity which are mainly restricted to neural stem cells although telomerase can also be transiently induced after different types of brain injury. The Saretzki group also described that *TERT* expression decreases during ageing in mouse brain [12] while they did not find a decrease in *TERT* protein due to neurodegeneration [13]. Interestingly, Miwa, et al. (2016) found that *TERT* is also excluded from the nucleus by rapamycin which does not trigger any oxidative stress and was used as a mimetic for dietary restriction. While brains from wild type mice decreased ROS (reactive oxygen species) after rapamycin treatment this effect was not detected in *TERT* knock-out mice suggesting that the effect of ROS reduction was dependent on the presence of *TERT*. A similar lack of a ROS effect was found in cells when nuclear *TERT* exclusion was inhibited using the Src inhibitor bosutinib or in the absence of *TERT* [12]. This study was the first to demonstrate that not only oxidative stress but also other triggers are able to induce a nuclear *TERT* exclusion although the exclusion effect was much weaker with rapamycin than with oxidative stress. Importantly, Spilsbury, et al. (2015) detected a persistent presence of the *TERT* protein in adult hippocampal neurons as well as in microglia cells while it was absent in astrocytes [13].

The Role of *TERT* in Neurodegenerative Diseases and the Benefit of Boosting its Levels

The first group to analyse a role of telomerase in neurodegenerative diseases was that of Esther Priel on ALS (amyotrophic lateral sclerosis). They used a synthetic telomerase activator (the aryl compound AGS499) which seemed able to ameliorate the disease in a mouse model [14]. The same group also demonstrated the shuttling of *TERT* to mitochondria in cultured brain cells caused by glutaminergic stress [15]. In a different study, they analyzed the influence of *TERT* on a cellular model of Alzheimer's disease (AD). By treating a mixed brain cell culture with amyloid-β they found several gene expression changes of plasticity-related genes and neurotrophic factors [16]. Using the same aryl compound telomerase activator as previously they were able to reverse some of these changes confirming the importance of the telomerase component *TERT* in counteracting adverse processes due to neurodegeneration-associated toxic proteins.

Spilsbury, et al. (2015) demonstrated an accumulation of *TERT* protein within mitochondria in hippocampal neurons (CA1-3) from Braak stage 6 AD brains compared to age-matched healthy control brains [13]. However, it is difficult to discriminate whether mitochondrial *TERT* has a protective function here or is rather the result of increased oxidative stress which is known to be involved in neurodegeneration. Intriguingly, the authors also found that in Braak stage 3-6 neurofibrillary tau tangles as well as neuropil threads and the *TERT* protein are mutually exclusive. However, it is not clear whether pathological tau displaced *TERT* protein or whether those neurons with high levels of *TERT* were protected from tau pathology. In order to conduct more mechanistic experiments the authors employed primary embryonic mouse neurons from wild type and *TERT* knock-out mice and transduced them with pathological tau (p301L). They found that *TERT* k.o. neurons had higher ROS levels in dendrites while the soma displayed higher levels of lipid peroxides (analysed using an antibody against 4-hydroxynonenal) [13]. Together, these results demonstrate the involvement and protective effect of the *TERT* protein in neurodegenerative diseases such as AD.

Prompted by this association the Saretzki group also used telomerase activators: TA-65, a highly enriched plant extract from Mongolian milkvetch (Astragalus membranaceus) as well as a synthetic derivative of its main ingredient-cycloastragenol called GRN510. Since, as outlined previously, there are only very few areas in the brain with telomerase activity (mainly neural stem

cells), these compounds would not primarily increase telomerase activity, but instead boost the level of *TERT* expression promoting its non-canonical functions.

Initially the authors conducted a pilot study on two-year-old mice treating them orally with each compound on a daily basis for 3-4 months. They found a significant increase of *TERT* expression as well as an improvement of motor function and balance by using a static rod [17]. While young mice stayed on the rod easily, old control mice were not able to perform as well. In contrast, mice treated with the activators could stay much longer on the rod. In order to confirm that the telomerase activators mainly targeted neurons, the authors cultured embryonic mouse neurons and treated them with both activators for 2 days and again found a significant increase in *TERT* gene expression confirming their hypothesis. In the main part of the study they employed a mouse model of Parkinson's disease (PD) which had been developed by Masliah, et al. (2000) previously [18]. These transgenic mice express human wild type α-synuclein under the control of the PDGF promoter predominantly in the hippocampus, neocortex and olfactory bulb [19]. Interestingly, the transgenic α-synuclein only increases substantially after around 12 months of mouse age and coincides with a decrease in dopamine, the main agent compromised by PD [19]. As expected, after applying TA-65 and GRN510 orally daily for 14 months starting at 4 month there was a significant increase in *TERT* expression in the brain of treated mice compared to control mice who had received the solvent DMSO. The authors performed various behavioural tests in order to characterise some PD-related motor symptoms such as motor behaviour, balance and gait. Intriguingly, some of those tests, as for example the rota-rod test showed a strong sex-dependent result. While TA-65 improved performance of female mice significantly, in males it was GRN510. The underlying mechanisms of those differences have not been analysed, but one can speculate about differences of male and female brains which have been described previously, for example for distinctive gene expression patterns. In contrast, other tests such as the stride length test which is very similar to gait tests used for human PD patients, did not show any sex difference. Both telomerase activators increased stride length of both hind legs of the treated mice and decreased variability substantially in male as well as in females with both telomerase activators. For an illustration, please see (figure 1).

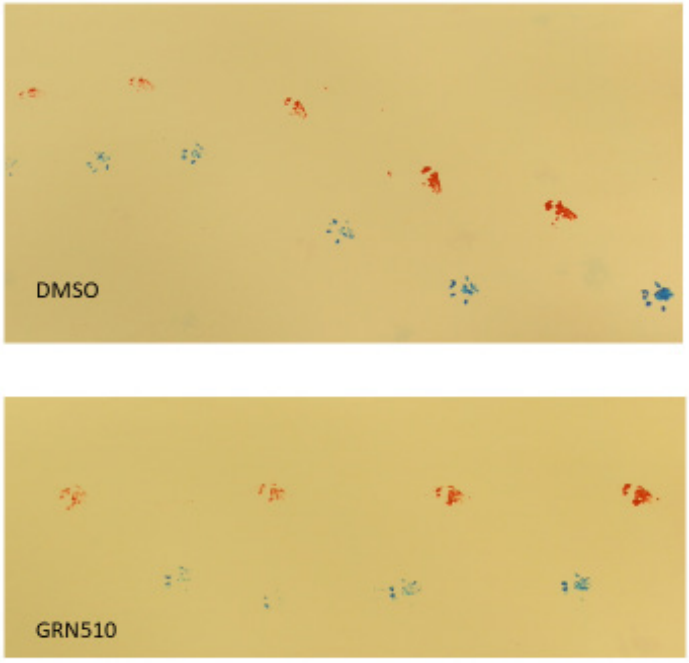

Figure 1: Representative images from a stride length test in a control mouse (upper) treated with DMSO and a GRN510 treated mouse (lower). For the test hind legs were painted blue (right leg) and red (left leg) and mice walked over a white sheet of paper. Length of stride as well as variation of stride length were analyzed subsequently.

The authors also characterized the capability of isolated brain mitochondria to release hydrogen peroxide which is a read-out for oxidative stress. Interestingly, without discriminating for sexes, in this test only TA-65, but not GRN510 decreased the forward and reverse electron flow by using different substrates in an Amplex Red test [17]. This result suggests that any potential mitochondrial localization of TERT would most likely not be the main underlying cause for any beneficial effects of telomerase activator treatment as GRN510 in various tests also showed a significant improvement of PD-related parameters.

Importantly, analysing brain pathology in the hippocampal areas CA1 and CA3 as well as the neocortex regarding the levels of total, phosphorylated as well as aggregated α-synuclein they found a dramatic decrease of all those forms due to the treatment with

both activators (for illustration please see figure 2). After initially using fluorescently labelled antibodies as shown in figure 2, they confirmed the results again using a different primary antibody against total α-synuclein together with common immuno-histochemistry [17].

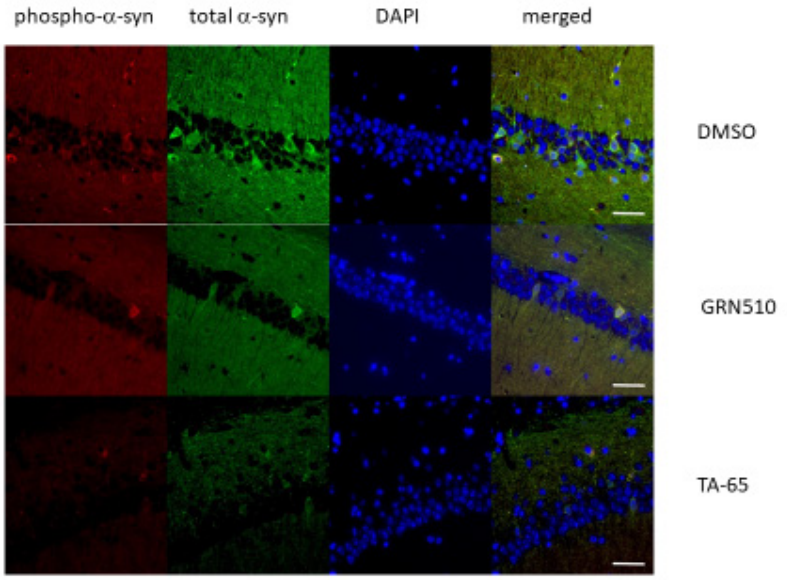

Figure 2: Representative immuno-fluorescence images of total (green) and phosphorylated (red) α -synuclein of DMSO (control), GRN510 and TA-65 treated hippocampal region CA1 using identical exposure times. Numbers and intensity of positively stained pyramidal neurons and their processes is decreased in activator treated brains.

In order to characterise a possible underlying molecular mechanism the authors analysed two components of macro-autophagy: the adaptor protein p62 as well as the protein LC3B on the surface of auto-phagosomes which both decreased upon activator treatment suggesting a possible involvement of this protein degradation mechanism in the degradation of α-synuclein upon activator treatment. Autophagy is known to clear and digest primarily oligomeric and aggregated toxic proteins such as α-synuclein and others while other mechanisms such as proteasomal degradation primarily degrade monomeric proteins. Both of those protein degradation mechanisms have been demonstrated to be activated by TERT over-expression previously [7,8]. It is also well established that protein degradation mechanisms decrease in their activity during the ageing process. Considering the rather late increase in α-synuclein levels in the transgenic mouse model (Amschl, et al. 2013) it seems possible that indeed there is a causal association between diminishing activity of the protein quality mechanisms and the increase of toxic neurodegeneration-related proteins at higher ages [19]. Moreover, as already mentioned, the activation of autophagy by hTERT overexpression has been demonstrated in a cellular model previously [8]. However, more studies are required to confirm this hypothesis.

Recently, a new study from the de Pinho laboratory demonstrated that activation of TERT in brain and neurons in different models was able to counteract various AD parameters such as amyloid pathology and synaptic dysfunction [20]. The authors found expression changes of various AD-related genes in TERT-haploinsufficient mice including upregulation of the *APP* (amyloid precursor protein) gene and the gene for the APP-processing enzyme Bace2. The reduction of TERT gene dosage resulted in the suppression of various neuronal pathways including neuronal differentiation, axon extension, and transmission of neuronal pulses and regulation of action potential. Similar to the results from Baruch-Eliyahu, et al. (2019), the authors identified a decrease of brain-derived neurotrophic factor (BDNF) in their mouse model [16]. Moreover, in a triple transgenic (tg) mouse model of AD as well as in induced pluripotency (iPSC)-derived neurons which possessed a duplication of the *APP* gene from AD patients with this genetic defect, the authors found decreased *TERT* expression [20]. These findings are in contrast with the results of Spilsbury and co-authors (2015) who did not find any changes in the level TERT protein in the hippocampus of spontaneous AD patients compared to healthy control brains [13]. It is possible that the presence of various mutations in the models of Shim, et al. (2021) exacerbated the TERT phenotype which could not be identified in spontaneous AD [20]. In summary, the results from Shim, et al. (2021) demonstrate that decreased *TERT* expression leading to changes in crucial AD- and brain-related signalling pathways are able to contribute to AD pathogenesis. To

further confirm this conclusion, the authors then overexpressed inducible TERT specifically in mouse neurons *in vivo* and crossed it with an AD mouse model [20]. As expected, TERT induction resulted in a decrease of pathological amyloid as well as neuro -inflammation in non-neuronal cells such as astrocytes and microglia and also improved learning and cognition capabilities. Importantly, these phenotypes were again associated with a modification of gene expression for synaptic signalling, plasticity, transmission and neuronal projection in an opposite direction to that found in TERT-haploinsufficient mice [20]. Importantly, the authors obtained similar changes in gene expression in the iPSC-derived neurons when a catalytically inactive TERT without enzymatic activity was employed. This experiment confirmed and emphasized the non-canonical function of TERT in their setting.

Conclusion

A number of recent studies confirmed that the telomerase protein TERT is able to counteract different pathological proteins known to be associated with neurodegenerative diseases. AD-related toxic proteins such as tau and amyloid-β were able to change the expression of brain- and disease-relevant genes and to decrease oxidative stress in neurons. Most of these protective TERT functions are thought to work in a non-canonical manner while others also demonstrated some telomere-related improvements in brain pathology and functions in late generations of telomerase knock-out mice.

Importantly, the successful use of various available telomerase activators on *in vitro* and *in vivo* models of neurodegenerative diseases such as AD and PD that were able to boost TERT expression and to decrease disease-related symptoms and parameters [13,14 and 16] is encouraging for a potential translation into novel therapeutic options to delay and ameliorate those devastating neurodegenerative diseases in the future.

References

1. Saretzki G. Extra-telomeric functions of human telomerase: cancer, mitochondria and oxidative stress. Current pharmaceutical design. 2014 Dec 1;20(41):6386-403. https://doi.org/10.2174/1381612820666140630095606
2. Santos JH, Meyer JN, Skorvaga M, Annab LA, Van Houten B. Mitochondrial hTERT exacerbates free radical mediated mtDNA damage. Aging cell. 2004 Dec;3(6):399-411. https://doi.org/10.1111/j.1474-9728.2004.00124.x
3. Ahmed S, Passos JF, Birket MJ, Beckmann T, Brings S, Peters H, Birch-Machin MA, von Zglinicki T, Saretzki G. Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. Journal of cell science. 2008 Apr 1;121(7):1046-53. https://doi.org/10.1242/jcs.019372
4. Haendeler J, Dröse S, Büchner N, Jakob S, Altschmied J, Goy C, Spyridopoulos I, Zeiher AM, Brandt U, Dimmeler S. Mitochondrial telomerase reverse transcriptase binds to and protects mitochondrial DNA and function from damage. Arteriosclerosis, thrombosis, and vascular biology. 2009 Jun 1;29(6):929-35. https://doi.org/10.1161/ATVBAHA.109.185546
5. Singhapol C, Pal D, Czapiewski R, Porika M, Nelson G, Saretzki GC. Mitochondrial telomerase protects cancer cells from nuclear DNA damage and apoptosis. PloS one. 2013 Jan 9;8(1):e52989. https://doi.org/10.1371/journal.pone.0052989
6. Sharma NK, Reyes A, Green P, Caron MJ, Bonini MG, Gordon DM, Holt IJ, Santos JH. Human telomerase acts as a hTR-independent reverse transcriptase in mitochondria. Nucleic acids research. 2012 Jan 1;40(2):712-25. https://doi.org/10.1093/nar/gkr758
7. Im E, Yoon JB, Lee HW, Chung KC. Human telomerase reverse transcriptase (hTERT) positively regulates 26S proteasome activity. Journal of Cellular Physiology. 2017 Aug;232(8):2083-93. https://doi.org/10.1002/jcp.25607
8. Ali M, Devkota S, Roh JI, Lee J, Lee HW. Telomerase reverse transcriptase induces basal and amino acid starvation-induced autophagy through mTORC1. Biochemical and biophysical research communications. 2016 Sep 23;478(3):1198-204. https://doi.org/10.1016/j.bbrc.2016.08.094
9. Fu W, Lu C, Mattson MP. Telomerase mediates the cell survival-promoting actions of brain-derived neurotrophic factor and secreted amyloid precursor protein in developing hippocampal neurons. Journal of Neuroscience. 2002 Dec 15;22(24):10710-9. https://doi.org/10.1523/JNEUROSCI.22-24-10710.2002
10. Klapper W, Shin T, Mattson MP. Differential regulation of telomerase activity and TERT expression during brain development in mice. Journal of neuroscience research. 2001 May 1;64(3):252-60. https://doi.org/10.1002/jnr.1073
11. Ishaq A, Hanson PS, Morris CM, Saretzki G. Telomerase activity is downregulated early during human brain development. Genes. 2016 Jun 16;7(6):27. https://doi.org/10.3390/genes7060027
12. Miwa S, Czapiewski R, Wan T, Bell A, Hill KN, von Zglinicki T, Saretzki G. Decreased mTOR signalling reduces mitochondrial ROS in brain via accumulation of the telomerase protein TERT within mitochondria. Aging (Albany NY). 2016 Oct;8(10):2551. https://doi.org/10.18632/aging.101089
13. Spilsbury A, Miwa S, Attems J, Saretzki G. The role of

telomerase protein TERT in Alzheimer's disease and in tau-related pathology in vitro. Journal of Neuroscience. 2015 Jan 28;35(4):1659-74. https://doi.org/10.1523/JNEUROSCI.2925-14.2015

14. Eitan E, Tichon A, Gazit A, Gitler D, Slavin S, Priel E. Novel telomerase increasing compound in mouse brain delays the onset of amyotrophic lateral sclerosis. EMBO molecular medicine. 2012 Apr;4(4):313-29. https://doi.org/10.1002/emmm.201200212

15. Eitan E, Braverman C, Tichon A, Gitler D, Hutchison ER, Mattson MP, Priel E. Excitotoxic and radiation stress increase TERT levels in the mitochondria and cytosol of cerebellar Purkinje neurons. The Cerebellum. 2016 Aug;15(4):509-17. https://doi.org/10.1007/s12311-015-0720-6

16. Baruch-Eliyahu N, Rud V, Braiman A, Priel E. Telomerase increasing compound protects hippocampal neurons from amyloid beta toxicity by enhancing the expression of neurotrophins and plasticity related genes. Scientific reports. 2019 Dec 2;9(1):1-4. https://doi.org/10.1038/s41598-019-54741-7

17. Wan T, Weir EJ, Johnson M, Korolchuk VI, Saretzki GC. Increased telomerase improves motor function and alpha-synuclein pathology in a transgenic mouse model of Parkinson's disease associated with enhanced autophagy. Progress in neurobiology. 2021 Apr 1;199:101953. https://doi.org/10.1016/j.pneurobio.2020.101953

18. Masliah E, Rockenstein E, Veinbergs I, Mallory M, Hashimoto M, Takeda A, Sagara Y, Sisk A, Mucke L. Dopaminergic loss and inclusion body formation in α-synuclein mice: implications for neurodegenerative disorders. Science. 2000 Feb 18;287(5456):1265-9. https://doi.org/10.1126/science.287.5456.1265

19. Amschl D, Neddens J, Havas D, Flunkert S, Rabl R, Römer H, Rockenstein E, Masliah E, Windisch M, Hutter-Paier B. Time course and progression of wild type α-Synuclein accumulation in a transgenic mouse model. BMC neuroscience. 2013 Dec;14(1):1-4. https://doi.org/10.1186/1471-2202-14-6

20. Shim HS, Horner JW, Wu CJ, Li J, Lan ZD, Jiang S, Xu X, Hsu WH, Zal T, Flores II, Deng P. Telomerase reverse transcriptase preserves neuron survival and cognition in Alzheimer's disease models. Nature Aging. 2021 Dec;1(12):1162-74. https://doi.org/10.1038/s43587-021-00146-z