NeuroGenix-Alpha: Recombinant Protein to Overcome Age Related Neurological Disorders and Aging

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Abstract

Oxidative stress is caused by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to repair the resulting damage, playing a significant role in the aging process and development of various diseases. NeuroGenix-Alpha is a theoretical recombinant protein utilizing Sirtuin-6 (SIRT6) and the catalytic domain of telomerase (TERT) specifically designed to combat the damaging effects of oxidative stress. It is designed to be activated by amyloid-beta protein, a marker of oxidative stress, to recover DNA strands and telomere length potentially offering protection against Alzheimer's disease. It can be administered as a weekly intranasal inhalation and engineered with an IgG domain allowing it to cross the blood-brain barrier. With a dual focus on telomere repair and DNA damage response, NeuroGenix-Alpha potentially represents a promising avenue for combating oxidative damage and paving the way for a breakthrough in age-delaying biologics.

Introduction

Over the past few decades, significant research has been conducted in aging, specifically endeavors aimed at averting age-associated disorders and ailments. Within these investigations, Oxidative stress Theory (OST) represents a fundamental avenue for elucidating the mechanistic underpinning of oxidative stress in aging (1). Oxidative stress ensues from a dysregulated equilibrium between free radicals and antioxidants within the organism, manifesting as a natural product of routine cellular metabolism (2). This leads to cellular damage, compromising structural integrity and functional efficacy as well as precipitates DNA damage due to its propensity to induce mutations within the genetic material, causing multiple pathological conditions (3).
The brain, characterized by its heightened metabolic activity and heightened vulnerability to ischemic insults, is a particularly susceptible target of oxidative stress. The aging process and predisposition to age-related neurological conditions is mainly due to enduring accrual DNA damage (4). Moreover, there is pathogenesis of both acute and chronic conditions including stroke, traumatic brain injury and neurodegenerative disorders (5). Therefore, the need to preserve brain health among aging individuals is of paramount significance. This research proposes a solution to slow down brain aging and reduce the risk of age-related neurological disorders by combining these two essential proteins, Telomerase and Sirtuin-6. Sirtuin-6 (SIRT6) is a NAD+ dependent protein deacetylase that plays a crucial role in brain aging and is intricately involved in regulating the oxidative stress responses. It specifically has a role in DNA repair and genomic stability by deacetylating histone H3 lysine 56 to facilitate chromatin relaxation at DNA damage sites, thereby facilitating the recruitment of DNA repair factors to damaged DNA (7). It also acts as a molecular sense of oxidative stress and orchestrates adaptive cellular responses to mitigate oxidative damage.
Specifically SIRT6 promotes the expression of antioxidant genes such as superoxide dismutase 2 and catalase, which help neutralize reactive oxygen species and alleviate oxidative stress. Moreover, SIRT6 promotes neuronal survival and neural protection in the aging brain by preventing neuronal apoptosis and preserving synaptic integrity. SIRT6 activity undergoes a decline throughout one's

lifespan (Figure 1). Its peak activity is observed during the 19th week of fetal development. Following birth and throughout one's life, the Sirtuin activity diminishes in the brain.
Telomerase is an enzyme responsible for maintaining the length of telomeres. Telomeres are protective caps at the end of chromosomes that consist of repetitive DNA sequences and associated proteins and shorten with each round of cell division. This shortening contributes to cellular aging and senescence. TERT, the catalytic domain of telomerase, acts to counteract this shortening by adding repetitive DNA sequences to the end of chromosomes, thereby preserving telomere length and promoting cellular longevity (Figure 2).
Telomerase activity is particularly relevant in the brain due to high metabolic demands and susceptibility of brain cells to oxidative stress. The brain, with its high oxygen consumption and lipid rich composition is particularly susceptible to oxidative damage caused by the imbalance between the production of reactive oxygen species and the ability of antioxidant defenses to neutralize them. This stress leads to DNA damage, protein oxidation, lipid peroxidation and mitochondrial dysfunction, all of which contribute to neuronal dysfunction and age-related cognitive decline. By maintaining telomere length, telomerase helps preserve genomic stability and protect against DNA damage. Moreover, it can directly scavenge reactive oxygen species and modulate cellular responses to oxidative stress through various signaling pathways.
Adequate telomerase activity may offer neuroprotection and delay the onset of age-related neurological disorders, neuroinflammation, synaptic dysfunction and neuronal apoptosis.
The combination of SIRT6 and Telomerase can be combined to form a new recombinant protein, named NeuroGenix-alpha, which could potentially be administered as a nasal inhalation in order to identify and recover damaged DNA thereby suppressing age-related neurodegenerative disorders, contributing to the preservation of brain health and ultimately extending the human lifespan.

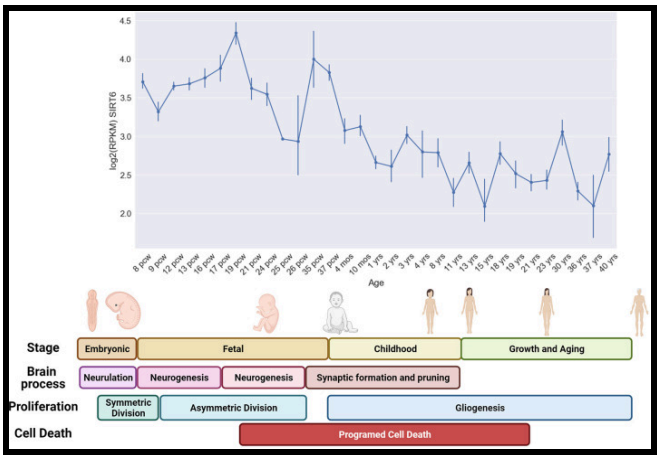

Figure 1.) Sirt6 expression during human development and life. In the upper graph, we show the mean expression level of several brain regions during specific developmental time points.

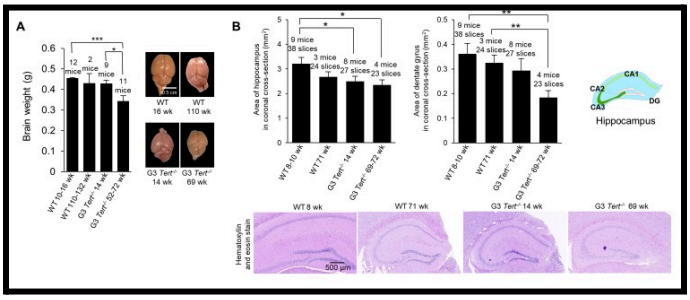

Figure 2.) Mice deficient for telomerase have smaller brains, shorter telomeres, more proliferation, more DNA damage, and less neurogenesis. (A) Brain weight and representative images of young and old wild-type and G3 Tert-/- mice. (B) Area of the hippocampus and dentate gyrus in untreated mice quantified from representative images of brain sections stained with hematoxylin and eosin.

Materials & Methods

The construction of the NeuroGenix-Alpha protein began with a literature search through the RCSB Protein Database. Using *Blender*, a 3D modeling software the development of a recombinant protein was underway. With the aid of the RCSB PDB, the program accurately found potential binding sites for both the telomerase and sirtuin-6 proteins. A

physical and real-life concept has not been created. Using the 3D modeling software, translations in the protein were created to accurately combine both proteins in place.

Results

To construct the recombinant protein involving SIRT6 and TERT, an extensive online modeling analysis of the protein was executed to anticipate potential binding interfaces and interactions. This provided valuable insights into the structural intricacies of both proteins facilitating the identification of plausible interaction sites. A comprehensive examination of the active sites within the 3D sequence annotation of SIRT6 and TERT was conducted during the online modeling process. The active sites of SIRT6 accommodating residues such as SO4, Adenosine-5-diphosphoribose, and Zinc atoms were unsuitable for interaction with the TERT subunit. We then explored alternative binding sites, such as cis-peptide bonds which play crucial roles in protein folding, conformational changes and enzymatic reactions are typically formed between the amino group of one amino acid and the carboxyl group of another. SIRT6 has a singular cis-peptide binding site and TERT exhibited two such sites, one proximal to the RNA template and another distal. To ensure structural stability and prevent interference with the functionality of the telomerase protein, cis-peptide sites positioned further from the RNA template were prioritized [Figure 3]. This decision was grounded in the understanding that the utilization of these specific binding sites would contribute to the stability of the resultant structure, aligning with the prior research on protein structure and function. The insights gained from online modeling significantly informed our experimental design, guiding the selection of appropriate binding sites for constructing the recombinant protein [Figure 4].

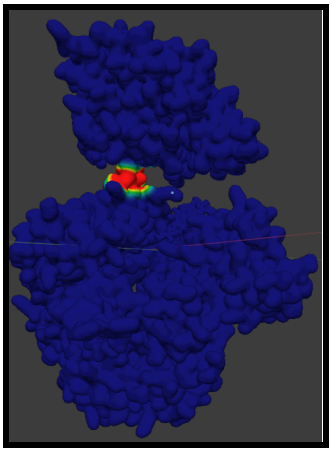

Figure 3.) Display of cis-peptide bonds in the recombinant protein structure as seen in red.

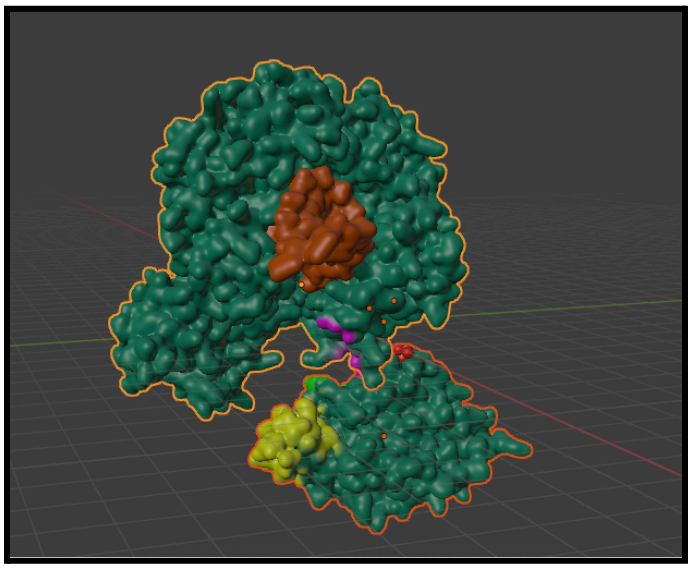

Figure 4.) 3D model of recombinant protein colored; orange represents TER, dark green represents the Sirtuin-6 and TERT recombinant, yellow/red/light green represents binding sites, and purple represents the cis-peptide bond sites.

There is inherent difficulty of many biologics to diffuse through the blood brain barrier (BBB). In order to overcome this difficulty, an IgG domain fusion is necessary. We specifically selected the human insulin receptor (HIR) monoclonal antibody (Mab) due to its abundant expression on brain endothelial cells and mediates the transport of insulin and insulin-like growth factors across the

BBB (Figure 5) (8). Intranasal administration offers several advantages due to the direct route to the CNS via the olfactory and trigeminal nerve pathways. It can also bypass a blood brain barrier and directly access the brain parenchyma delivering the protein to target sites within the brain. It also allows for rapid absorption of antibodies across the nasal mucosa into the systemic circulation and cerebrospinal fluid. There is minimization of systemic exposure to the antibody reducing the risk of side effects as well as metabolism and enzymatic degradation resulting in higher bioavailability and therapeutic efficacy. Modification of the antibody is still a challenge to be discussed. An analysis of formulation and compatibility of this solution into an intranasal approach needs to be further explored.

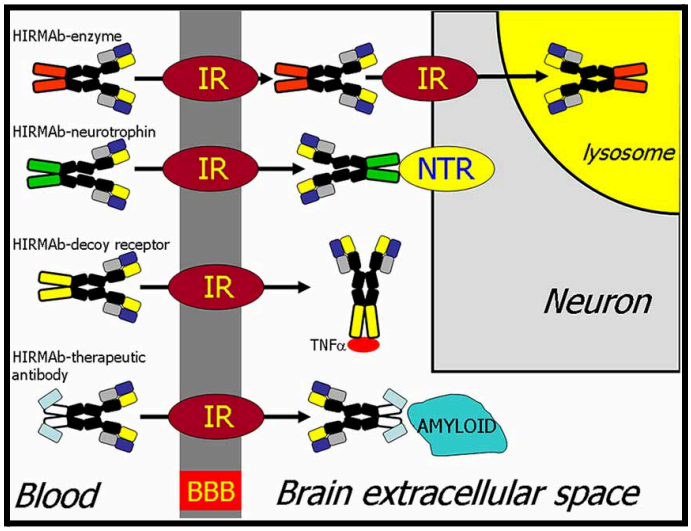

Figure 5.) Visualization of the passage of IgG domains (MAb and HIR) that can pass the Blood-Brain Barrier.

In order to trigger intracranial activation of NeuroGenix-Alpha, its interaction with amyloid-beta protein will be investigated. Amyloid beta protein interacts with cell adhesion molecules and synaptic proteins to regulate signaling pathways. It modulates synaptic transmission, regulation of neurogenesis in adults and can activate microglia which can contribute to neuronal dysfunction and neurodegeneration. It has specific roles in oxidative stress with high levels of oxidative stress leading to elevated concentrations of amyloid beta protein in the brain (Figure 6).

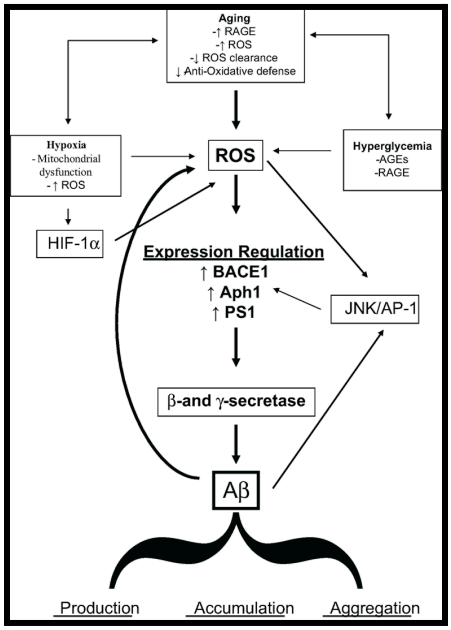

Figure 6.) Flow chart displaying the progress of oxidation promoting the concentration of amyloid-β in the brain.

Discussion

The present study proposes that a combination of Telomerase Reverse Transcriptase with an integrated Telomere RNA component connected to the Sirtuin-6 enzyme could enhance the brain's anti-aging mechanisms. The added activation of both telomeric growth and DNA damage response provides an effective function. NeuroGenix-Alpha is activated by Aβ proteins. This provides added protection against Alzheimer's, one of the most progressive neurological disorders to date. Studies support the fact that telomere shortening increases oxidative stress on DNA strands (6). A mouse model displays the relation between these two factors. This mouse model, featuring telomerase-deficient mice with shortened telomeres, illustrates premature aging and telomere dysfunction, marked by DNA damage response proteins. In another study, DNA telomere growth in

telomere-diminished mice was conducted using the TERT and TER proteins' functionality (6). Increasing the telomerase activity levels in the mouse brain had a significant effect on the decrease of cognitive impairment which is an important hallmark of neurodegenerative diseases. The authors found more neurogenesis but less inflammation and DNA damage in the brains with increased telomerase (6). However, the basic need for telomere growth cannot be the only factor in the process of aging. Further studies indicate that overexpression of telomeric entities could be a factor leading to Alzheimer's Disease AD (7). Hence, emphasizing control and DNA regeneration is vital. Sirtuin-6 emerges as a pivotal participant, particularly effective against highly perilous DNA damage like Double-Strand Breaks (DSB) (8). Adding on, SIRT6 can proactively activate downstream signaling for DNA remodeling, repair, and protection. DNA Damage Response (DDR) is crucial to the slow-down of brain aging. A rapid and efficient approach to identifying damage is necessary to prevent damage from oxidative stress. This recombinant protein, therefore, serves dual functions: (1) Telomere Repair and (2) DNA Damage Response, both in response to oxidative damage of the DNA.

Limitations

Even though our study is based on the (OST) we cannot leave out the various factors that play into the factors of aging. Aging is caused by the inevitable accumulation of various damage forms resulting from errors, misbalance, and unintended activities of biomolecules and biological processes. This accumulation of damage is considered to be inevitable in non-dividing cells, compromising cellular function and ultimately leading to senescence and death (9). A protein focused on only the protection of Oxidative Stress is not a definite and absolute pill for reverse aging.

Future Studies

The innovation of an intranasal recombinant protein can allow for the daily supplementation of age-delaying biologics. Further research and testing of this model, could in turn develop new technologies and perhaps permanent reverse anti-aging biologics. Our biological recombinant protein has the potential to delay the progression of age-related neurological diseases and reduce the chances of acquiring these diseases. The biological age of one’s brain will slow down from our protein's function of rejuvenating and fixing DNA. This is a breakthrough for the reverse aging journey. People will be able to live longer and healthier lives through the usage of NeuroGenix-Alpha.

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