УДК 577.21

DEVELOPMENT AND FUNCTIONAL EVALUATION OF A GPX3 AND SOD3 CO-EXPRESSION CONSTRUCT IN EUKARYOTIC CELLS

Bordina Y. I.[1] (student)**, Mhanna R.** [1] (student)
Scientific supervisor — Velegzhaninov I. O.[1,2]
[1]ITMO University
[2]Institute of Biology, Komi Science Centre UB RAS, Syktyvkar
b.y.e.03g@gmail.com

Abstract
Oxidative stress, driven by reactive oxygen species (ROS) imbalance, causes cellular damage and chronic inflammation via positive feedback loop. Extracellular SOD3 convert superoxide to hydrogen peroxide, while GPx3 reduces it to water, providing synergistic neutralizing potential. A bicistronic co-expression construct is developing using IRES2 and specific minding mRNA processing and protein secretion into extracellular space. The construct will be cloned into pcDNA3.4 under CMV promoter. Functionality of this construct will be assessed in HEK293T cells via assays suitable for mRNA and protein levels. This approach may enhance antioxidant defense in regenerative medicine and oxidative stress-related pathologies.

Keywords
Oxidative stress, antioxidant genes, stress resistance, overexpression

СОЗДАНИЕ И ФУНКЦИОНАЛЬНАЯ ХАРАКТЕРИСТИКА КОНСТРУКЦИИ ДЛЯ СОВМЕСТНОЙ ЭКСПРЕССИИ ГЕНОВ GPX3 И SOD3 В ЭУКАРИОТИЧЕСКИХ КЛЕТКАХ

Бордина Ю. И.[1] (магистрант), **Мханна Р.**[1] (магистрант)
Научный руководитель – кандидат биологических наук, профессор
Велегжанинов И. О.[1,2]
[1]Университет ИТМО
[2]Институт биологии Коми научного центра Уральского отделения РАН, Сыктывкар
b.y.e.03g@gmail.com

Аннотация
Окислительный стресс, вызванный накоплением активных форм кислорода (АФК), способен стать причиной возникновения клеточных повреждений и хронического воспаления за счет формирования петли положительной обратной связи. Фермент SOD3 обеспечивает преобразование супероксида кислорода в перекись водорода, пока активность GPx3 способствует утилизации перекиси в воду, что способствует синергичному действию. Была разработана бицистронная конструкция для совместной экспрессии с использованием IRES2, что обеспечит правильный процессинг мРНК и секрецию обоих белков во внеклеточное пространство. Конструкция будет клонирована в вектор pcDNA3.4 под конститутивный промотор CMV. Функциональность конструкции будет оценена в клетках HEK293T с использованием методов, позволяющих количественно определять уровни мРНК и белков. Предлагаемый подход может способствовать усилению антиоксидантной защиты в регенеративной медицине и при патологиях, ассоциированных с окислительным стрессом.

Ключевые слова
Окислительный стресс, гены антиоксидантных ферментов, стрессоустойчивость, сверхэкспрессия

Oxidative stress is an imbalance between the production of ROS and the capacity of cellular antioxidant system to neutralize them, resulting in damage to the DNA, proteins, and lipids. Accumulated ROS and oxidized biomolecules can further stimulate ROS production, creating a self-amplifying positive feedback loop that drives chronic inflammation and results

in cellular and tissue dysfunction. Co-expression of extracellular antioxidant enzymes is proposed as a strategy to interrupt this vicious cycle by efficiently neutralizing superoxide and hydrogen peroxide in the extracellular space, thereby limiting the amplification of oxidative damage and inflammation. This study aims to develop a co-expression construct for glutathione peroxidase 3 (*GPx3*) and superoxide dismutase 3 (*SOD3*) to strengthen antioxidant defense and potentially break the ROS vicious cycle [1]. The designed construct is compact and contains only about 4000 nucleotides without a marker gene. This allows it to be packaged into adeno-associated viral (AAV) vectors for direct local delivery to sites of inflammation caused by elevated ROS levels. Unlike CRISPRa-mediated activation, which has already been tested in related laboratory work and improves cell resistance, this approach creates a complete ready-to-use cassette for future in vivo validation in animal models.

SOD3 and GPx3 were selected as target enzymes due to their complementary roles in extracellular ROS neutralization and predominantly tumor suppressor effects [2]. SOD3, an extracellular Cu/Zn-superoxide dismutase, converts superoxide anions into hydrogen peroxide and oxygen, preventing superoxide-mediated damage. GPx3, a secreted selenium-dependent glutathione peroxidase, reduces H_2O_2 and lipid hydroperoxides to water using glutathione, thereby eliminating the potentially harmful product of SOD3 activity. Their synergistic action prevents H_2O_2 accumulation and interrupts the vicious cycle of oxidative stress amplification. To achieve stoichiometric and stable co-expression, the construct incorporates specific 3'UTR sequences for each gene to ensure proper mRNA processing and protein secretion into extracellular space. We utilize in our construct the internal ribosome entry site 2 (IRES2) elements to enable independent translation of both *GPx3* and *SOD3* from a single mRNA. Two modular cassette variants were designed for commercial gene synthesis: (*GPx3*-3'UTR)-IRES2-(*SOD3*-3'UTR)-IRES2-mCherry for in vitro validation by and fluorescence-based monitoring and (*GPx3*-3'UTR)-IRES2-(*SOD3*-3'UTR). These cassettes will be inserted into the pcDNA3.4 expression vector under the strong constitutive CMV promoter with a WPRE element (for enhanced mRNA stability and expression) via SacI and AgeI restriction sites, allowing enhanced expression and future recloning flexibility. Following transfection of HEK293T cells with the resulting constructs, the functionality and efficiency of our design will be assessed using qRT-PCR for mRNA level-overexpression and western blotting for protein levels.

The designed *GPx3*/*SOD3* co-expression construct using IRES2 and 3'UTR elements offers a promising platform for achieving coordinated production of secreted antioxidant enzymes in eukaryotic cells. By targeting extracellular ROS detoxification, this approach offers a safe strategy for enhancing cellular resistance to oxidative damage could find applications in regenerative medicine and therapy of oxidative stress-related pathologies.

References

1. Li Y. Oxidative Stress and Intervertebral Disc Degeneration: Pathophysiology, Signaling Pathway, and Therapy / Y. Li [et al.] // Oxidative Medicine and Cellular Longevity. – 2022. – Vol. 2022. – Art. 1984742. – DOI: 10.1155/2022/1984742.
2. Tavleeva M. M. Effects of Antioxidant Gene Overexpression on Stress Resistance and Malignization In Vitro and In Vivo: A Review / M. M. Tavleeva [et al.] // Antioxidants. – 2022. – Vol. 11, iss. 12. – Art. 2316. – DOI: 10.3390/antiox11122316.