

АКТУАЛЬНЫЕ ПРОБЛЕМЫ КЛЕТОЧНОЙ БИОЛОГИИ И КЛЕТОЧНЫХ ТЕХНОЛОГИЙ

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БИОЛОГИЯ КЛЕТКИ В КУЛЬТУРЕ

CELLS BIOLOGY IN VITRO

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CELL LINEAGE TRACING — A NEW APPROACH TO THE STUDY OF ATHEROSCLEROSIS DEVELOPMENT

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Atherosclerosis is the number one killer in Western civilization, as it is responsible for more than 40% of all deaths. Widespread use of statins and lifestyle modifications within developed nations have resulted in modest reductions in the incidence and severity of the atherosclerotic disease. However, long-term use of statins has been linked to an increased prevalence of type II diabetes, neurological side effects, and liver damage. In addition, no one has yet to procure defining evidence that statins can prevent the late-stage clinical consequences of atherosclerosis, including plaque rupture, which can lead to possible myocardial infarction or stroke. Thus, it is crucial to identify cellular and molecular mechanisms that can be potentially used for therapeutic interventions for treating and preventing atherosclerosis.

In vivo cell lineage tracing studies from our and other labs demonstrated that there is a lot of ambiguity in cell identification within atherosclerotic lesions. Herein, using a combination of the *in vivo* endothelial cells (EC) lineage tracing atherosclerotic mouse model and single-cell (sc)RNA-seq genome-wide analysis, we found that aortic EC demonstrate high phenotype diversity at the early stage of atherosclerosis development. Specifically, scRNA-seq unsupervised cluster analysis of aortic EC revealed seven unique transcriptome states, with two clusters of highly metabolically active EC. Also, using EC-specific knockout of the pluripotency factor OCT4 in combination with EC-lineage tracing, we demonstrate that OCT4 plays an athero-protective role in EC at least in part through regulating EC phenotypic plasticity. These results provide the first direct evidence showing that OCT4 plays a functional role in EC.

In summary, cell lineage tracing approach in combination with scRNA-seq analysis provides an unambiguous characterization of the heterogeneous populations of EC in atherosclerosis. Data from these bioinformatics analyses might be beneficial for exploring cellular and molecular mechanisms that contribute to the development, progression, and end-stage clinical complications of atherosclerosis.

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FUNCTIONAL IMAGING OF NANODOMAINS IN CARDIOMYOCYTES

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Cardiac myocytes represent cells with defined nanodomains on the plasma membrane that are involved in distinct signalling pathways, for example T-tubules and caveolae. I will present evidence of nanodomain function and regulation in cardiac myocytes obtained with the use of novel microscopic technique of Scanning Ion Conductance Microscopy in conjunction with other methods. Signalling events can be monitored in live cells with nanometre precision with the use of precise positioning of the scanning probe (nanopipette) and biochemical inhibitors/agonists as well as fluorescent sensors for Förster Resonance Energy Transfer and calcium. Most importantly, functional dysregulation that occur in disease can be monitored in live cells isolated from failing hearts and correlated with structural degradation of nanodomains. I will present work on rat myocytes derived from heart after myocardial infarct and from human myocytes isolated from biopsies of patients with various cardiomyopathies. Myocardial infarction leads to rearrangement of adrenergic receptors and downstream disconnection of excitation-contraction coupling, and that can be partially reversed with restoration of nanodomains on the plasmalemma. Unloading of failing heart can restore nanodomains to a degree and this leads to a partial restoration of heart function. Also, I am interested in functional heterogeneity within the heart. I will present evidence that nanodoman organisation differs in myocytes from different parts of the heart, namely apex and base of the myocardium.

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STUDY OF AMYLOIDOGENIC PROPERTIES OF HUMAN PROTEINS PHC3 AND RAD51

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Amyloids are highly ordered protein aggregates that can attach monomeric molecules of the same protein with a change of its native conformation. Amyloid fibrils are enriched with β -layers and are resistant to proteinases and detergents. The main interest in the study of amyloids

is associated with their association with a number of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease. Amyloids can also perform biologically positive functions in various organisms. The role of amyloids and amyloid-like oligomers in the formation of biofilms in bacteria, melanin synthesis, and long-term memory in animals is shown. In yeast *Saccharomyces cerevisiae* and other fungi, amyloids are transmitted in cell generations and are carriers of protein heredity.

During previous studies conducted in our laboratory, due to the novel yeast test-system [1], amyloid properties for mammalian proteins Rad51 and PHC3 (isoforms 5 and 6) has been shown.

Protein PHC3 is one of the key components of the polycomb group (PcG) — a complex necessary to maintain the repressive state of many genes, including Hox genes during the development of the body. We believe that amyloid aggregation of short isoforms of the PHC3 protein can lead to amyloidization of its full-sized isoform and regulate genes expression level.

The Rad51 protein is one of the main proteins of the DNA double-strand break repair system. We believe that Rad51 aggregation may affect genome stability in normal and cancer cells.

This work is aimed at studying the amyloid properties of human Rad51 protein and short isoforms of the PHC3 protein in vitro, in human cell cultures, human tissues, as well as studying the functional effects of amyloidization of these proteins.

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ТЕСТИРОВАНИЕ ФАРМАКОЛОГИЧЕСКИХ ИНГИБИТОРОВ АКТИВНОСТИ МИЕЛОИДНЫХ СУПРЕССОРНЫХ КЛЕТОК, ГЕНЕРИРОВАННЫХ IN VITRO В КУЛЬТУРЕ КЛЕТОК КОСТНОГО МОЗГА

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TESTING THE INHIBITORS OF MYELOID-DERIVED SUPPRESSOR CELLS IN VITRO GENERATED IN BONE MARROW CULTURE

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Миелоидные супрессорные клетки (Myeloid-derived suppressor cells, MDSC) представляют собой гетерогенную популяцию незрелых клеток миелоидного ряда, доля которых значительно возрастает при онкопатологии и хроническом воспалении. MDSC ингибируют иммунологические функции ряда эффекторных клеток. К настоящему времени разработано несколько путей фармакологического воздействия на MDSC с целью подавления их иммуносупрессорной функции, однако их применение ограничено в основном онкопатологией. Целью исследования был поиск малотоксичных или нетоксичных фармакологических препаратов, которые могут быть использованы для коррекции активности MDSC при хронических воспалительных процессах.

В ходе исследования было показано, что 4-дневная инкубация клеток мышинового костного мозга в присутствии цитокинов GM-CSF, IL-6 и TNF α приводила к генерации клеток с фенотипом MDSC (CD11b⁺Ly6G⁺LybC⁺), характеризующихся продукцией супрессорных факторов (ROS, NO, IL-10, TGF β). Добавление в культуру MDSC витамина D3 приводило к достоверному повышению доли MDSC, экспрессирующих маркер апоптоза Annexin-V, и снижению продукции активных форм кислорода (ROS). Сульфасалазин, используемый в настоящее время в терапии хронических воспалительных заболеваний, не влиял на экспрессию ни одного из исследуемых фенотипических маркеров и маркеров активности MDSC. Куркумин индуцировал увеличение в культуре MDSC доли клеток, экспрессирующих маркер дендритных клеток CD11c, а также снижение продукции ROS. Таким образом, витамин D3 и куркумин очевидно