TREATMENT OF DIABETIC PERIPHERIAL ARTERY DISEASE WITH AUTOLOGOUS BONE MARROW OR PERIPHERIAL BLOOD DERIVED CELLS

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Diabetic foot syndrome occurs in 10,0% of cases among patients with diabetes mellitus, ulcers heal too long, are accompanied by infectious processes and occurs gradually leading to the amputation of the affected limb. Some authors suggest, that the paracrine factors secreted by mononuclear stem cells (MNCs) are these the main driving force behind its therapeutic activity. In the current study, we investigate the efficacy and safety of the therapy of ischemia using stem cells in patients with diabetes mellitus. An assessment of the paracrine functionality of the patient's cells was performed.

There were three groups: the patients from the main group were treated with intramuscular injections of mobilized autologous mononuclear cells (12) or bone marrow autologous cells (5). The patients of the control group (20) had similar conservative and endovascular treatment within the same time period without stem cells. We analyzed cell types, level of cell proliferation and viability, promising effects of MNCs conditioned medias on proliferation of lines fibroblasts, MSCs from patients without diabetes mellitus and EaHy926, migration on the wound model.

The tolerance of patients to cell therapy is high, the number and volume of surgical interventions on the foot after cell therapy are less, epithelialization of trophic ulcers and restoration of the support function of the limb are higher in the main group. There is a patient-specific correlation between the composition of the cellular product, conditioned media and their clinical effect. We suggest that MNCs conditioned medias can be employed in regenerative medicine for therapeutic angiogenesis and skin repair in difficult-to-heal conditions such as diabetic foot.

STIMULATING EFFECT OF FS-1 ON FUNCTIONAL ACTIVITY OF LYMPHOPOIESIS AND MYELOPOIESIS PROGENITOR CELLS *IN VITRO*

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Cancer is the second leading cause of death globally, and is responsible for an estimated 9,6 million deaths in 2018. Chemotherapy is a cancer treatment that uses drugs to kill rapidly growing and dividing cells, like cancer cells. Unlike radiation or surgery, which target specific areas, chemo works systemically. This affects some fast-growing healthy cells, like those of the skin, hair, intestines, and bone marrow.

Bone marrow is the spongy tissue inside the bones, which makes new blood cells. Chemotherapy affects this process, so there are side effects, such as too few blood cells. Low levels of blood cells during treatment can cause problems: more susceptible to infections; body tissues do not receive enough oxygen (hypoxia); anemia and increased blood coagulation.

Prevention and treatment of such side effects is called palliative or supportive therapy and constitutes an important part in cancer treatment.

In this article we present study results concerning new medicine FS-1 effect on bone marrow mesenchymal stem cells. FS-1 synthesized at Scientific Centre for Anti-infectious Drugs is ionic nanostructured complex formed by proteins and/or polypeptides, hydrocarbons, salts of alkali and alkaline earth metals with intercalated iodine. Patented in 2014 (RK No20129000).

In our work we used mesenchymal stem cells from mouse bone marrow (STEMCELLS Technologies, USA). Detect production of stem-cells factor (SCF), IL-7, IL-8 macrophage colony-stimulating factor (M-CSF), chemotactic stroma-derived factor-1 (SDF-1), macrophage anti-inflammatory protein (MIP)-1α and MIP-1β quantitative analysis was made using kits from R&D Systems (USA) in accordance with manufacturers' protocols.

In the process of study of FS-1 effect on production of cytokines and chemokines in mouse bone marrow mesenchymal stem cells culture we found out that FS-1 has actual stimulating effect on production of SCF ($P = 0,015$), IL-7 ($P = 0,017$), M-CSF ($P = 0,009$) and SDF-1 ($P = 0,009$).

Thus, new medicine FS-1 can influence animal bone marrow by stimulating mouse bone marrow mesenchymal stem cells to produce certain cytokines regulating myelopoiesis and lymphopoiesis, as well as certain chemokines taking part in induction of stem cells and mature cells migration from bone marrow to blood.

Obtained results can be used in development of drugs for bone marrow hematopoietic function recovery during chemotherapy of tumors.

IMPROVING CRISPR-CAS TECHNOLOGY FOR THERAPEUTIC APPLICATIONS

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CRISPR-Cas technology has rapidly become a popular and rather powerful tool of genome manipulation. It is widely used not only for scientific research but for the development of new therapeutic approaches.

Applications of CRISPR-CAS system are not limited by genome editing, they include genetic screens, modulation of gene expression either by attracting RNA polymerase or by blocking its binding to DNA, induction of histone or genome methylation and silencing, incorporation of specific sequences into the target site in the genome.

Whatever modification of the CRISPR-Cas technology is used, it relies deeply on the target recognition by CRISPR-Cas complex, based on the complementarity of a rather short oligonucleotide sequence. The efficiency and

specificity of this binding of spacer part of the guide RNA to DNA are especially important for therapeutic applications of CRISPR-Cas system. However, its selectivity is still insufficient for routine *in vivo* use. Uncontrolled insertion or removal of genes and their fragments in the "hot spots" of the genome can lead to oncotransformation of cells after genome editing ex vivo or *in vivo*. Another important problem for therapy is targeted delivery of the preformed RNA-protein complex or insufficient level of nuclear localization of synthetic guide RNAs if they are not part of the protein.

Here we use combinations of known and novel chemical RNA modifications to improve the characteristics of precise genome editing for its potential applications in biomedicine. These novel modifications stabilize RNA *in vivo* and are less toxic then phosphorothioates which are widely used to prevent RNA degradation of synthetic RNA molecules *in vivo*. We identify patterns of RNA modifications which can further improve therapeutic efficacy of CRISPR-Cas system without decrease of genome editing efficiency and compare them to the known gRNA modifications patterns. We also analyze the effects of these RNA modifications on the protein-RNA complex activity and discuss possible alterations in the structure of Cas9 effector complex caused by these modifications.

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TRANSLATIONAL 3D CELL CULTURE SYSTEMS FOR CLINICALLY COMPLIANT EXPANSION OF ADULT STEM CELLS AND ISOLATION OF STEM CELL-DERIVED EXTRACELLULAR VESICLES

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Since its early days, ex vivo mammalian cell culture has been conducted on flat two-dimensional (2D) glass or polystyrene surfaces. Although 2D cell culture is still widely used, it is known to result in unnatural cell polarity and morphology in addition to lacking the three-dimensional extracellular matrix. These drawbacks are especially evident if the cultivated cells are clinically relevant cell types including but not limited to stem cells. In this context, 2D cultivation is known to interfere with stem cell proliferation and to alter the cell phenotype.

In this talk, different approaches to stem cell cultivation, differentiation, and assessment of migration in 3D will be discussed. In particular, biocompatible natural and synthetic scaffolds for the cultivation of adult human neural crest-derived stem cells and human mesenchymal stem cells of different origin will be presented. Moreover, the impact of extracellular vesicles derived from adult human stem cells will be discussed in the context of their regenerative potential.

АНАЛИЗ ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ МЕЗЕНХИМНЫХ СТВОЛОВЫХ КЛЕТОК СОБАКИ, ЭКСПРЕССИРУЮЩИХ ГЕНЫ-СУПРЕССОРЫ ОПУХОЛИ И ГЕНЫ-ИММУНОМОДУЛЯТОРЫ *IN VITRO*

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Схожая патофизиология онкологических заболеваний собак и человека делает собак перспективной моделью для исследования эффективности противоопухолевой терапии. Мезенхимные стволовые клетки (МСК) — негемопоэтические клетки-предшественники, которые способны избирательно мигрировать в опухолевые ниши. Поэтому МСК могут быть использованы для целевой доставки противоопухолевых агентов. Одними из таких агентов могут быть цитокины — белки иммунной системы. Многочисленные исследования на клеточных культурах и животных моделях опухолей показали, что цитокины обладают широкой противоопухолевой активностью.

МСК были выделены из жировой ткани собаки. Выделенные клетки были в значительной степени положительны по поверхностным маркерам мезенхимных стволовых клеток собаки CD105, CD10, Stro-1 и Thy-1. Мультипотентность МСК была подтверждена путем дифференцировки в хондроциты, остеобласты и адипоциты. МСК были генетически модифицированы рекомбинантным лентивирусом, кодирующим родственный фактору некроза опухоли апоптоз-индуцирующий лиганд собаки (TRAIL), интерферон β-1 собаки (IFNβ1) и фосфатазу с двойной субстратной специфичностью собаки (PTEN). Полученную клеточную линию отбирали путем культивирования в среде с бластицидином S (5 мкг/мл) в течение 10 дней. Экспрессия генов была подтверждена ПЦР в режиме реального времени. Для того, чтобы оценить противоопухолевую активность нативных и генетически модифицированных МСК, 2×10^5 МСК культивировали в течение 24 часов, затем собирали кондиционированную среду (КС). Клетки рака толстой кишки человека HCT-116 культивировали в свежей среде RPMI-1640, либо в КС от нативных или генетически модифицированных МСК. Через 48 часов пролиферативная активность клеток HCT-116, культивировавшихся в КС генетически модифицированных МСК, была значительно ниже (78,97±9,23%) по сравнению с клетками, культивировавшимися в КС нативных МСК (99,71±4,38%) и в свежей среде (100,00±4,36%).

В дальнейших экспериментах будет проанализирована противоопухолевая активность полученных МСК на модели спонтанного возникновения опухолей у собак крупных пород in vivo.

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