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National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

Increase of iPSC reprogramming efficiency by cell membrane potential (Vm) modulation

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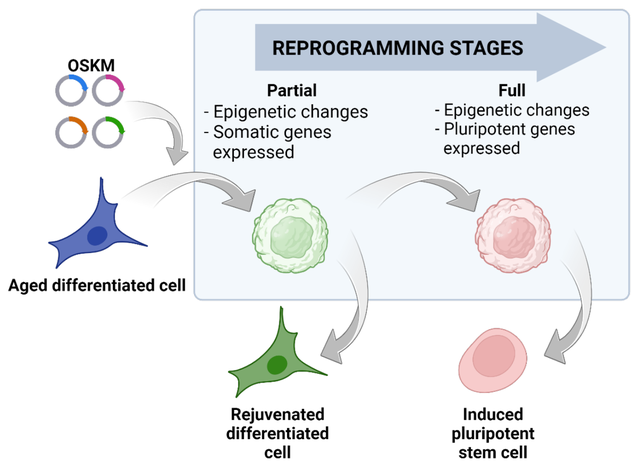
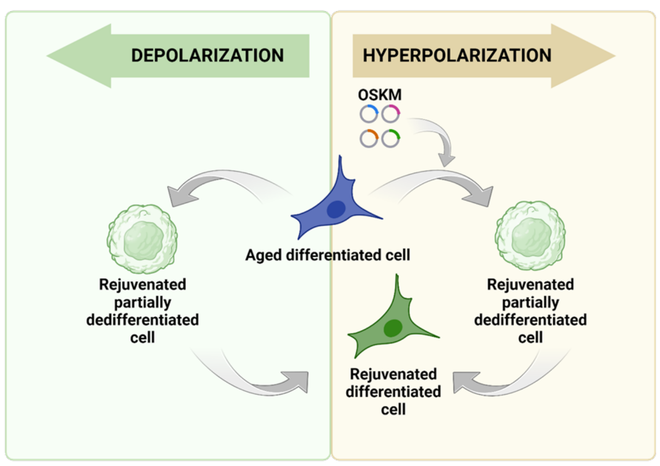
Introduction

Induced pluripotent stem (iPS) cells are obtained by reprogramming somatic cells (for example, fibroblasts) and thus acquire the potential to differentiate into all types of cells involved in the construction of the human organism. This technology, developed in 2006 by Yamanaka1, creates enormous possibilities for application in the field of regenerative medicine, in the creation of new model systems for the study of various diseases and for the development and testing of new medicinal preparations. The ability to obtain iPS cells by reprogramming a patient's own cells (patient-specific stem cells) is a huge step on the way to personalized medicine.

The cellular reprogramming process is achieved by forced expression of specific transcription factors (Yamanaka factors, Oct3/4, Sox2, Klf4, c-Myc) that activate the pluripotent gene program. Despite the efforts of scientists to improve the methods and protocols for obtaining iPS cells, the main problem remains the extremely low efficiency of the reprogramming process, which, depending on the type of cells and the methods used, varies from 0.01% for non-integrating non-viral vectors (Episomal vectors) to 1 % for integrating viral vectors (Lentiviral vectors). Since viral systems are unacceptable for regenerative medicine purposes due to the danger of negative effects caused by viral DNA integrated into the cell genome, our attention will be focused entirely on improving the efficiency of non-integrating non-viral systems such as episomal vectors. These vectors, despite their lower ability to reprogram, have a much greater perspective for application in regenerative medicine.



Reprogramming of differentiated cells (blue cell) with a specific combination of transcription factors: Oct4, Sox2, Klf4, and c-Myc (OSKM) leads to the induction of pluripotency and acquiring the characteristics of pluripotent stem cell (red cell). During the reprogramming process, the cell passes through several stages including changes in DNA methylation patterns and histone modifications. This reshapes the cell's epigenetic landscape but still leaves specific somatic genes expressed (round green cell). If the reprogramming continues, the resulting changes would lead to the activation of the pluripotent genes and, after their stabilization, the cells will become induced pluripotent cells (red cell). If the effects of Yamanaka factors are terminated before the activation of pluripotent genes, the cell can stabilize in a rejuvenated state (green cell) without losing its somatic identity. Cells in the blue box represent intermediate stages during reprogramming.



The importance of the membrane potential (Vm) for the functioning of muscle and nerve cells. Recently, more and more studies have shown that the Vm of many and different cell types is actively involved in the regulation of basic biological processes such as cell proliferation, differentiation, wound healing, regeneration, etc. Moreover, recent studies have demonstrated the involvement of Vm in the regulation of embryonic development and morphogenesis. They prove that it is the change in the membrane potential that plays a decisive role in the exit of the cells from the pluripotent state and taking the path of differentiation in the process of gastrulation. Of particular interest are the data showing that the membrane potential can be changed not only by modulating the activity of the K+/Na+ pump, but also by purely physical effects such as an oscillating magnetic field. In addition, although without elucidating the specific mechanism, this effect has been shown to stimulate cellular reprogramming.

Hypothesis

Experimental membrane depolarization of somatic cells will significantly increase the efficiency of their reprogramming to iPS cells, thus solving one of the main problems in this field.

Objective

To investigate the effect of membrane potential modulation on the efficiency of reprogramming human fibroblasts to iPS cells.

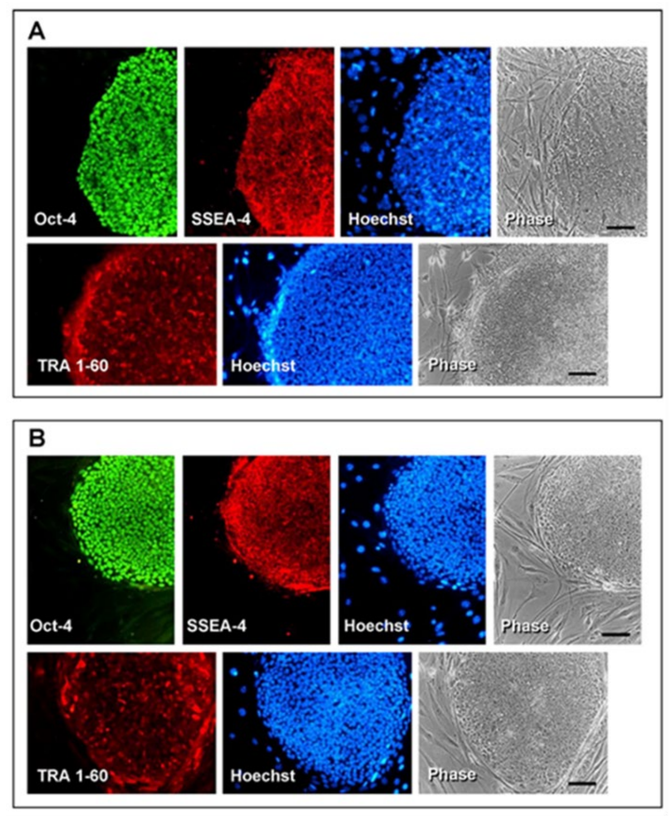
Tasks

1. Selection and approbation of methods using classic depolarizing agents (K+/Na+ pump inhibitors or increasing the concentration of K+ in the medium) that most effectively change the Vm of human fibroblasts and record the changes that occur with the highest sensitivity (selection of suitable voltage-sensitive dyes).
2. Study of changes in the efficiency of reprogramming of human fibroblasts to iPS cells after experimentally induced depolarization of the cell membrane.
3. Characterization of the pluripotency of the lines obtained from embryos and reprogrammed fibroblasts, both by specific markers and by in-vitro differentiation to ectodermal, mesodermal and endodermal derivatives.
4. Investigating the possibility of achieving an additive effect of combining membrane depolarization with already established stimulators of reprogramming to iPS cells such as hypoxia.
5. Investigating the possibility of using alternative (physical) methods, such as oscillating magnetic fields, to modulate the membrane potential of cells.

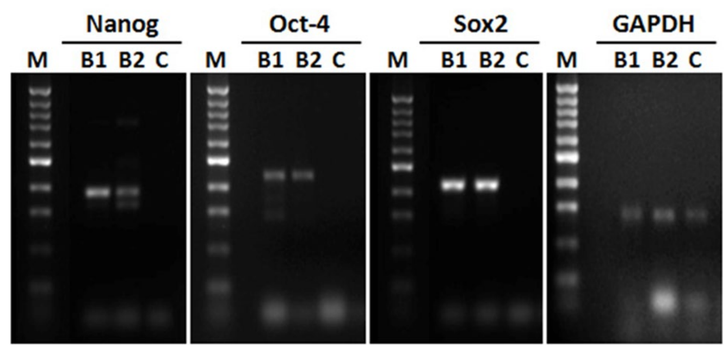
Methodology

- cell culture
- reprogramming of somatic cells to iPS cells by episomal vectors
- in-vitro differentiation of stem cells
- immunofluorescence
- polymerase chain reaction (PCR)
- fluorescence-activated cell sorting (FACS)
- immunoblotting

Results



Immunofluorescence expression analysis of pluripotency markers in cells of the BAM1 (A) and BABE1 (B) cell lines established by B. Arabadzhiev. Immunofluorescence images obtained after treatment with antibodies against Oct-4, SSEA-4 and TRA 1-60.

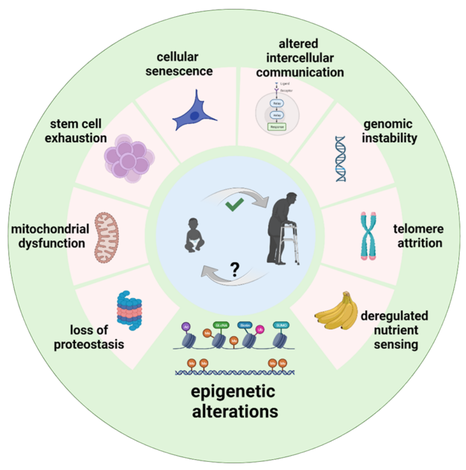


PCR expression of the pluripotency markers Nanog, Oct-4, and Sox 2 and the control, constitutively expressed glyceraldehyde-3 phosphate dehydrogenase (GAPDH) gene demonstrated by RT-PCR in BABE1 (B1), BABE2 (B2), and control cells from human MRC-5 fibroblasts (C).

Ongoing work and perspectives

As a result of the implementation of the present project, we expect:

- To experimentally prove our hypothesis that membrane depolarization of somatic cells will significantly increase the efficiency of their reprogramming to iPS cells.
- To propose optimized protocols for more efficient iPS cell generation based on membrane depolarization and conventional stimulators.
- To establish the possibility of using the oscillating magnetic fields as a modulator of the membrane potential and their applicability in the process of reprogramming to iPS cells.
- To create a team and raise the qualifications of young scientists and students with interests in the field of stem cell biology.



Alternative potential application of modulating the Vmem to enhance rejuvenation by partial reprogramming using Yamanaka factors is to use artificial hyperpolarization of the cell membrane as a safeguard against cell identity loss during the process. As discussed above, low Vmem (depolarized) prevents embryonic stem cells from differentiation, and high Vmem (hyperpolarized) promotes cell differentiation. It is only logical to assume that the opposite is also true, and hyperpolarizing the cells will prevent them from dedifferentiating. If this is the case, artificially hyperpolarizing the cell membrane can be used to enhance partial reprogramming by Yamanaka factors in two ways. Firstly, it will add an additional level of safety by preventing unwarranted dedifferentiation of the cells, which is one of the main hurdles of this emerging technology. Secondly, it will potentially expand the window for partial reprogramming, improving the efficiency of epigenetic rejuvenation.

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Research field: Stem Cell Biology

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