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SUMMIT
SOFIA UNIVERSITY
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FOR INNOVATION AND
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SOFIA UNIVERSITY -
MARKING MOMENTUM
FOR INNOVATION AND TECHNOLOGICAL TRANSFER

National Recovery and Resilience Plan
OF THE REPUBLIC OF BULGARIA

RESEARCH GROUP SUMMIT 3.4
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RESEARCH AREA
STEM CELL BIOLOGY

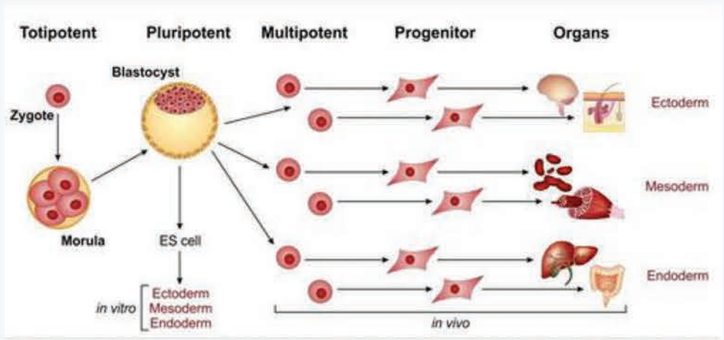
Establishment and characterization of new human embryonic stem cell line

INTRODUCTION

According to their origin - embryo, fetus, or from an adult organism, stem cells are divided into embryonic, fetal and "adult" (somatic) stem cells. A special place in this classification is occupied by induced pluripotent stem (iPS) cells. They have no analogue in embryonic development or the adult organism and are entirely a laboratory product, the result of the accumulated knowledge of stem cell biology.

iPS cells allow the creation of a patient's own (autologous, patient-specific) pluripotent cells, overcoming the biological and ethical obstacles to obtaining them by other means. The factors originally used to reprogram fibroblasts can reprogram a variety of cell types from the adult organism to iPS cells (Hanna et al., 2008; Stadtfeld et al., 2008). Many technological improvements to the method in recent years have made it possible to increase the efficiency of reprogramming from the initial 0.001% to 4.4% (Robinton and Daley, 2012). Intensive work is now underway to determine whether ES and iPS cells are functionally equivalent. Either way, both cell types are valuable pluripotent populations with the potential to make the goals of regenerative medicine a reality.

Human embryonic stem (ES) cells are most often isolated from the inner cell mass (ICM) of early embryos at the blastocyst stage. They are pluripotent self-renewing cells that, under appropriate conditions, can differentiate into all derivatives of the three embryonic germ layers (ectoderm, mesoderm, and endoderm), as well as into derivatives of the germ line. Their potential for unlimited proliferation in vitro and their ability to differentiate into all cell types that make up the human organism make these cells extremely valuable, both for the purposes of regenerative medicine and cell therapy, and as experimental model systems for studies in the field of developmental biology, cell differentiation, transdifferentiation, etc.

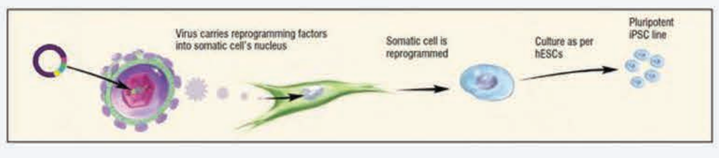


PROJECT GUIDELINES

The aim of this project is to establish Bulgarian cell lines of human embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) and to study their differentiation potential. Furthermore, we hypothesize that 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.

METHODOLOGY

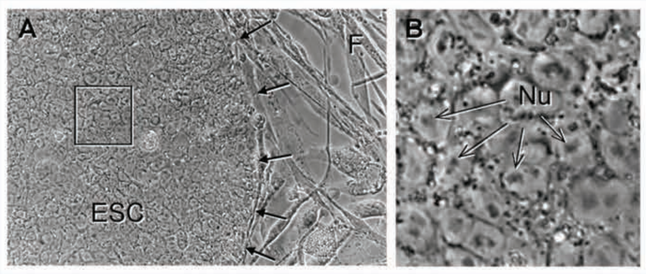
- cell culture
- reprogramming of somatic cells to iPS cells by episomal vectors
- in-vitro differentiation of stem cells
- immunofluorescence
- polymerase chain reaction (PCR)
- fluorescence-activated cell sorting (FACS)
- Immunoblotting
- cryopreservation
- karyotyping



RESULTS

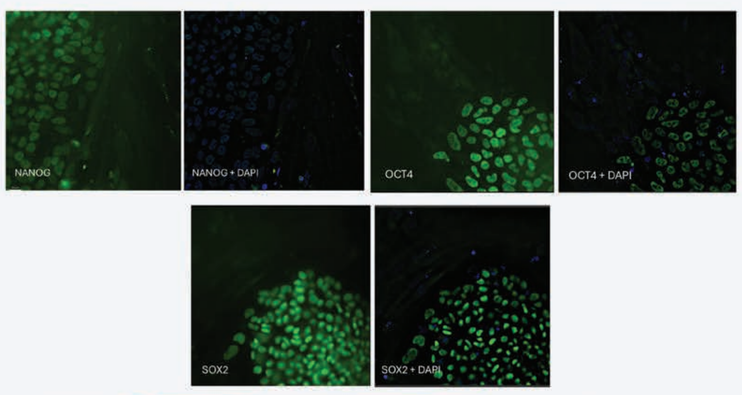
Isolation and characterization of human embryonic stem cell (hESC) line

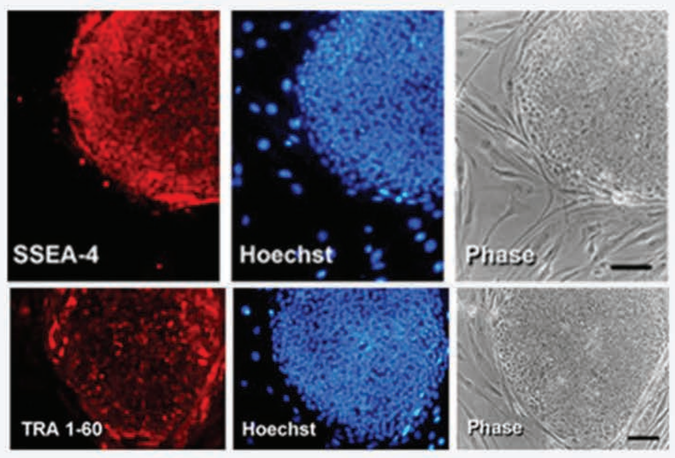
After obtaining informed consent from donors (married couples) who voluntarily provided the residual (poor quality/unsuitable) embryos obtained in the process of assisted reproduction in vitro, using immunosurgical techniques, we managed to isolate colonies of human embryonic cells grown on a feeder layer of inactivated fibroblasts.



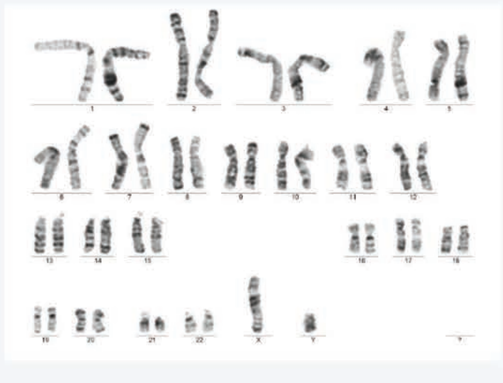
Phase-contrast images of BABE1 cells showing typical morphology of ES cells. The arrows in panel A indicate the clearly delineated borders of the ES cell (ESC) colony, (F) a feeder layer of mouse fibroblasts. A portion of the image in panel A (rectangle) is shown at higher magnification in panel B, where the well-defined nuclei (Nu) of the stem cells are clearly visible.

Using immunofluorescent techniques, we demonstrated the expression of specific pluripotency markers by our hESC line (BABE1) – Oct 4, Sox2, Nanog, SSEA-4, TRA-1-60





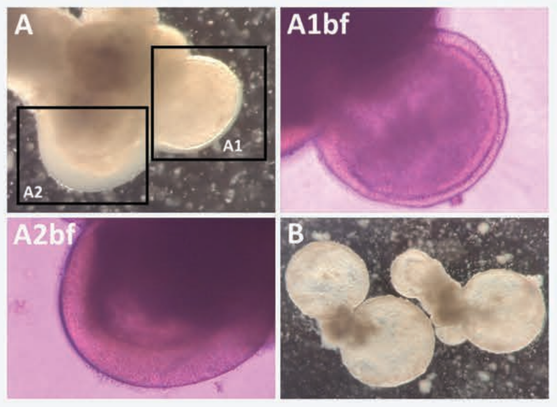
Considering that we used low-quality, residual embryos to isolate human ES cell lines, it was of particular importance for us to verify the karyotype of the isolated human ES cell lines. For this purpose, cytogenetic analysis was performed, the results of which showed that the BABE1 line has a normal male XY karyotype without numerical and structural chromosomal abnormalities.



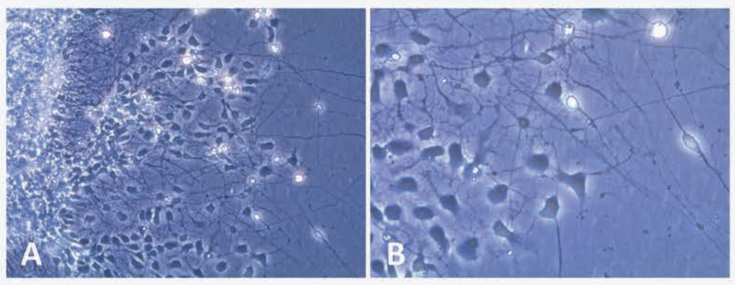
Furthermore, we managed to demonstrate the expression of stem cell markers also on a protein level (using immunoblotting techniques) and via genetic analyses (qRT-PCR) which confirmed the pluripotency markers possession of BABE1 cell line (data not shown).

Study of the pluripotency of hESC line BABE1

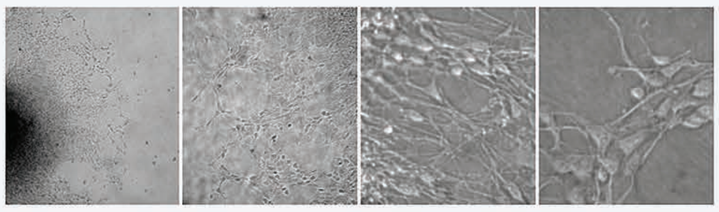
To investigate the ability of BABE1 cells to form embryoid bodies and differentiate *in vitro* into derivatives of the three embryonic germ layers (ectoderm, mesoderm, and endoderm), colonies measuring 300-500 µm were mechanically detached from the feeder layer and left as a suspension culture in culture medium in the absence of FGF2 and cultured in non-adhesive plates.



Embryoid body (EB) differentiation of human embryonic stem cells after suspension culture for 3 weeks. (A) 10X dark-field microscopy of a 10-day-old EB. (A1bf) 10X bright-field microscopy of A1. (A2bf) 10X bright-field microscopy of A2. (B) 4X dark-field microscopy of a cystic EB on day 21.



Spontaneous differentiation of neuron-like cells from human embryonic stem cells after subculture of neural rosettes (figure above). (A) 20X magnification phase-contrast microscopy of neuron-like cells. (B) 40X magnification phase-contrast microscopy of neuron-like cells.



Using a directed differentiation protocol, we obtained cultures in which over 90% of the cells had a typical neuronal morphology (figure above), demonstrating the ability of human ES cells from the BABE1 line to be directed towards neuroectoderm and, in particular, neurons.

Furthermore. we managed to observe spontaneous and directed differentiation of BABE1 human embryonic stem cells into mesoderm and cardiomyocytes, as well as into germ cells (data not shown).

CONCLUSION

The human embryonic stem cell line BABE1 expresses all major markers of pluripotency, has a normal XY karyotype, can form embryoid bodies and differentiate into ecto-, endo- and mesoderm, as well as germ cells. This allows us to define BABE1 as a full-fledged human pluripotent embryonic stem cell line.

At least to our knowledge, this is the first successful application of technology for generation of human embryonic stem cell line in Bulgaria. BABE1 is the first characterized Bulgarian human embryonic stem cell line which opens a broad range of opportunities for scientific and exploratory work in the field of stem cell biology and regenerative medicine.

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NATIONAL RECOVERY AND RESILIENCE PLAN PART OF THE PROGRAM TO ACCELERATE ECONOMIC RECOVERY AND TRANSFORMATION THROUGH SCIENCE AND INNOVATION