Preparatory experiments to investigate the radiosensitivity of human embryonic stem cells*

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Introduction

In humans the effect of ionizing radiation on the early embryonic development is not yet understood in detail. *In vivo* studies on mice have shown that lethality is the most frequent outcome after exposure in the pre-implantation stage [1].To estimate the risk of an *in utero* exposure, e.g. in case of accidental exposure or a medical treatment of an expectant mother [2] we chose H9 human embryonic stem cells (ESCs) as a model system. Differentiating ESCs *in vitro* resembles the early developmental stages of the embryo *in vivo*.

Cell culture system

Since human ESCs are very sensitive to spontaneous differentiation *in vitro*, an indispensable requirement for our studies is a cell culture that maintains a pluripotent and homogenous cell population. First experiments, where cells were enzymatically passaged resulted in a heterogeneous culture, containing pluripotent as well as a big proportion differentiated cells. Therefore, we set up the manual passaging of cells. This method is highly labour-intensive. However, it allows to select those colonies that most likely consist of cells being pluripotent (i.e. appear compact and homogenous) and reduce the fraction of those colonies that exhibit differentiated cells (heterogeneous colonies). In this weekly passaging, cells are cut in squares manually under the microscope under sterile conditions, transferred into a new petri dish on mouse embryonic feeder cells, and cultured for one week inlcuding daily mediumchanges. Seven days after seeding, colonies with compact and homogeneous morphology are passaged again.

Quality control of ESCs via immunocytochemistry

To verify the quality of the ESC culture, we established several immunocytochemisty methods to detect the presence of pluripotency markers. Since the network of proteins maintaining the pluripotency state in a cell is highly complex and interactive, we focused on several key proteins such as Nanog and Oct3/4. Figure 2 shows two manually passaged ESC colonies stained with antibodies against Nanog or Oct3/4, respectively. The fluorescence image reveals a clearly stained colony with a typical nuclear localisation of the pluripotency factors. In the lower panel, the colony growth is clearly visible, resulting in a more intense staining at the border of the colony due to higher cell density in this area. This method will also be used in future experiments to investigate differences between irradiated and non-irradiated ESCs.

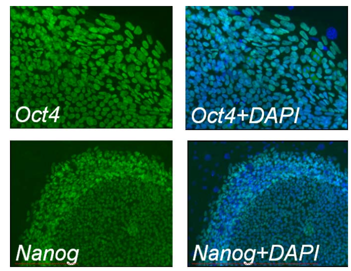

Figure 1: Immunocytochemical staining of pluripotency markers Oct4 (upper panel) and Nanog (lower panel) in human ESCs.

Differentiation capability into definitive endoderm

Another specific feature of ESCs is their differentiation capability. In general, pluripotent cells are able to differentiate into all three germ layers, namely endoderm, ectoderm and mesoderm that later give rise to a functional organism. Recently, we started investigating the differentiation capability of ESCs into definitive endoderm. We established a protocol based on studies of Baetge and colleagues [3, 4], in which ESCs are grown on matrigel for five days. Additionally, by adding specific supplements (like Activin A and Wnt3A) to the cell culture medium the differentiation into the endodermal lineage is triggered, while those cells that are not committed to endoderm undergo apoptosis. Following the differentiation protocol, the expression of genes specific for definitive endoderm like SOX17 and AFP will be measured by quantitative polymerase chain reaction.
In conclusion, the basis for an ESC-based *in vitro* assessment of irradiation effects on early human development has been established.

References

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