Preparatory experiments to investigate the radiosensitivity of human embryonic stem cells

S. Luft, I. Schroeder, P. Hessel, O. Arrizabalaga, P.G. Layer, M. Durante, and S. Ritter

1GSI, Darmstadt, Germany; 2Technical University, Darmstadt, Germany; 3IKERBASQUE, Foundation for Research, Bilbao, Spain

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 homogeneous 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 including daily medium changes. 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 immunocytochemistry 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 Oct4. Figure 2 shows two manually passaged ESC colonies stained with antibodies against Nanog or Oct4, 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.

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