First experiments using human embryonic stem cells as a model to examine radiation effects on early embryonic development: emphasis on gene expression

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Embryonic stem cells (ESC) are derived from the inner cell mass of the blastocyst and are characterized by an unlimited self-renewal capability. They are pluripotent due to their ability to generate all tissues and cell types of an individual. Because of their key biological role, it has been proposed that ESC have a strict control of their genome integrity to prevent the transmission of the genetic damage to their progeny. Indeed, there is that the repair of DNA damage is more precise in hESC than in their differentiated counterparts (e.g. [1]). Little is known about the effects of ionizing radiation (IR) on the developing human embryo and the few data available come mostly from the atomic bomb survivors (2). This hinders an accurate estimate of the risk from a prenatal exposure. To this end, human embryonic stem cells (hESC) represent an excellent model to investigate the effects of IR on early embryonic development.

Recently we set up the protocol for hESC cultivation (line H9), characterized the cell and performed first radiation experiments. Karyotype analysis of control cultures by means of the mFISH technique (fig.1, left) revealed a low percentage of cells with numerical or structural aberrations (1.7 ± 1.1 and 3.3 ± 1.6, respectively). Apoptosis was detected by flow cytometry based on caspase-3 activity. As shown in fig.1 (right) cells exposed to 1 Gy Ni-ions (1 GeV/n) showed a significant higher number of caspase-3 positive cells (6 fold) compared to the control. Additionally, we examined by flow cytometry the presence of the pluripotency marker SSEA4 in cells exposed to 1 Gy X-rays or Ni-ions and no statistical difference was observed up to 50h post-irradiation (data not shown).

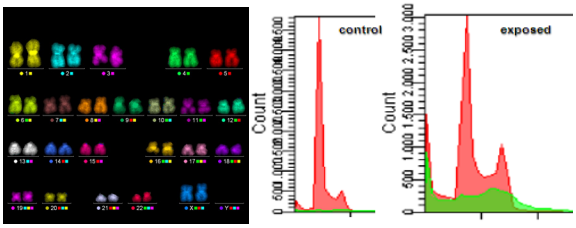

Figure 1: Normal karyotype of hESC visualized by the mFISH staining (left); Flow cytometric analysis of hESC immunolabeled against active Caspase 3 (green profile) and counterstained with DAPI (red profile) (right).

Since the signalling network of hESC is extremely intricate, the analysis of a few pluripotency markers is not conclusive. For a more detailed analysis of this complex network, gene expression profiling has to be performed. For that purpose we used the RT^2 Profiler PCR array system (Qiagen) that allows the analysis of the expression of 84 key genes involved in ESC maintenance and differentiation. Briefly, for RNA isolation sham irradiated and exposed cells were detached by 20 minutes incubation in 1% EDTA PBS, centrifuged, re-suspended in PBS and after another centrifugation stored at -80° in a tissue and cell lysis buffer (Epibio) with 0.3% proteinase K. RNA was harvested with the MasterPure™ Complete DNA and RNA Purification Kit (Epibio) following manufacturer's instructions. RNA concentration and purity were tested with a NanoDrop spectrophotometer and integrity was verified by agarose gel electrophoresis (Fig. 2, left).

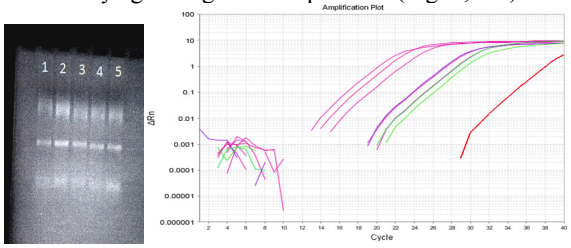

Figure 2: Agarose gel with RNA samples (left) from control (lanes 1-2, 5) and exposed samples (X-ray, lane 3 and Ti-ions, lanes 4). Amplification plot of representative genes from the Ti sample (right): The pink curves correspond to the housekeeping genes (ACTB, B2M, GAPDH, HPRT1, RPLP0), green curves represent genes for receptors (IL6ST, LIFR) and violet for transcription factor STAT3 involved in the pluripotency maintenance pathway. The red curve represents human genomic contamination.

The cDNA was obtained from 0.5 µg RNA using the RT^2 First Strand kit (Qiagen) following manufacturer's protocol. The cDNA from the exposed sample was used to test the first stem cell signalling RT^2 Profiler PCR array (Qiagen). A StepOne instrument (Applied Biosystem) was used to perform the real time PCR assay; data were collected and visualized with the Step One software.

After correction of the threshold line, the C_T (threshold cycle) values were examined and compared with the reference values reported by the manufacturer: no significant genomic DNA contamination was detected (Fig. 2, right, red line). The comparison between the C_T values for reverse transcription control and positive PCR control indicated that no inhibition of the reverse transcription occurred. The positive PCR control value is lower than the expected one reported in the manual. A high sensitivity of the Real-time cycler might be responsible for this discrepancy. Nonetheless, this deviation from the reference number is acceptable, if it is consistently observed in subsequent experiments. Further tests are needed to verify the reliability of the system used.

References

[1] Fung et al. 2011 Plos One, 6 (5): e20514.
[2] Streffer et al. 2003 Annals of the ICRP, 33: 5-206.

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