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Expression of housekeeping markers in pluripotent or differentiating mouse embryonic stem cells (ESC) in response to ionising radiation (IR)*

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ESC are pluripotent cells that have the ability to self-renew indefinitely and, due to their pluripotency, can also give rise to all cell types of the three germ layers. Pluripotent cells and differentiating cells show a completely different protein expression pattern. Housekeeping markers are used for normalization when quantifying protein or RNA expression in cells. They are proteins whose expression does not vary during different stages of the cell; for example during the cell cycle, under hypoxia conditions, or in the case of pluripotent cells, during their differentiation process. Housekeeping markers are used as loading controls for normalizing the quantity of the protein or RNA.

Since exposure to IR affects the expression of genes, including housekeeping ones and the extent of this effect also depends on the cell line, it is important to assess the stability of the genes in each system [1]. Moreover, the use of the geometric mean of more than one housekeeping marker is recommended to reduce variability [2].

In the present study we analysed three housekeeping markers: 18S rRNA, GAPDH and EEF2 in differentiating embryoid bodies (EB), as described previously [3]. EBs were formed from pluripotent mouse D3 ESC which had been irradiated with 0 Gy, 3 Gy X-ray (250 kV, 16 mA) or 3 Gy C-ions (25 mm SOBP, 106-147 MeV/u) [4]. Samples were collected at different time points during the differentiation process. RNA was purified and reverse transcribed according to the manufacturer's instructions (MasterPure RNA purification kit, Epicentre; and High Capacity RNA-to-cDNA kit, Applied Biosystems). 18sRNA, GAPDH and EEF2 RNA expression was analysed using QuantiTect Primer Assays (Qiagen) with Fast SYBR Green master mix (Life Technologies) to amplify the cDNA on a StepOnePlus Real-Time PCR system (Life Technologies).

The expression pattern of the three markers is shown in **Fig. 1**. NormFinder software, a model-based aproach to estimate expression variations of normalization/ house-keeping markers was used. Favourable low variabilities are represented by low stability values (M) [5]. When the data was compared throughout the differentiation period independently of the radiation quality, 18S rRNA was the most stable marker (M=0,233). When radiation quality was analysed independently of the differentiation time, GAPDH yielded the most stable marker (M=0,478). The best stability value was obtained by the combination of 18S rRNA and GAPDH (M=0,221). Hence, the geometric mean of 18S rRNA and GAPDH will be used in subsequent studies to quantify the gene expression of mESC during differentiation after exposure to IR.

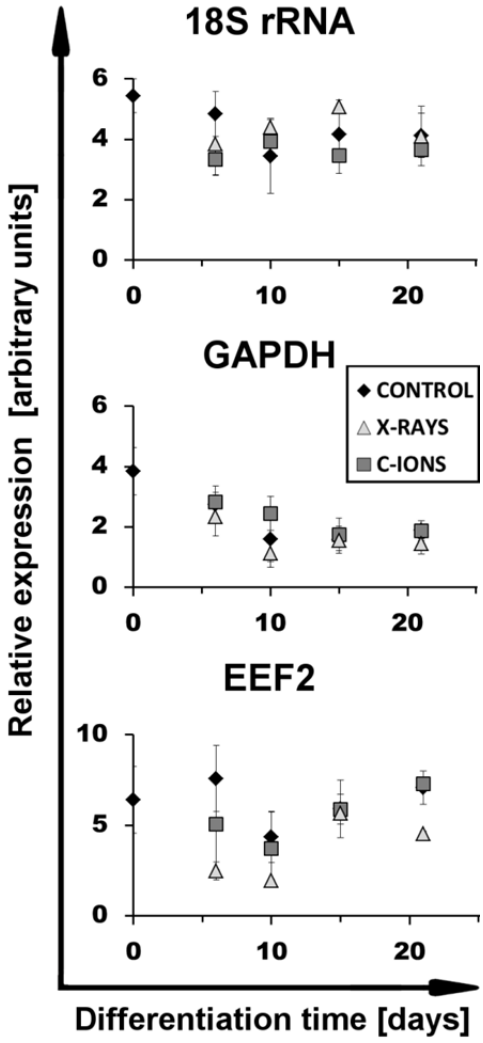

Figure 1: Expression of housekeeping markers during differentiation. 18S rRNA, GAPDH and EEF2 marker expression was quantified in differentiating EBs derived from irradiated pluripotent mouse D3-ESC. Exposure was done with X-rays (triangles) or C-ions (squares), controls were sham irradiated (diamonds).

References

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