Differentiation of mouse embryonic stem cells into extra-embryonic endoderm stem cells

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The embryonic response to ionizing radiations is poorly characterized. Although teratology was frequently observed after in utero exposure, little is known about the mechanisms involved. At early embryogenesis, a significant contribution to the morphogenetic program is given by the primitive endoderm (PrE) and its derivatives, visceral and parietal endoderm (VE, PE). These extra-embryonic membranes originate within the post-implantation blastocyst and were shown to directly interact with the epiblast, driving the formation of main ventral structures such as head and heart [1]. Stem cells representing the extra-embryonic endoderm (XEN) lineages can be generated *in vitro*, preserving the morphological and functional identity of the *in vivo* counterparts. As observed in recent studies, these cells retain the expression of the typical endoderm markers [2] and still maintain their morphogenetic role [3].
The *in vitro* XEN derivation would represent a simplified tool to assess the early morphogenetic events offering, therefore, the opportunity to investigate the physiologic response to ionizing radiation.

Here, extra-embryonic endoderm stem cells were generated from mouse embryonic stem cells (mESCs) in an attempt to establish a stable population. Pluripotent D3 mESCs were cultured for a prolonged time in XEN medium, and activin A and retinoic acid were employed to induce the endoderm specification according to Niakan et al. [4]. The samples were treated until the XEN morphology was detected in culture. Quantitative PCR analyses were executed to verify XEN differentiation. The expression profile of differentiated cells was evaluated after 30 days of culture and the production of pluripotency (Sox 2, Oct 4) and endoderm (Sox 7, Sox 17, Gata 6) markers was quantified and compared to the ones detected in the undifferentiated samples.
As shown in Figure 1, the pluripotent mESCs retain a higher expression of the transcription factors Sox 2 and Oct 4 and a significantly lower production of the endoderm factors can be detected at this stage.

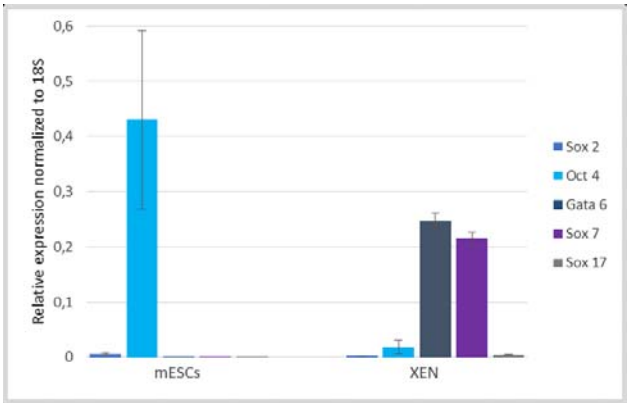

Fig.1 Gene expression profile of mESCs and in vitro derived XEN cells. An increased expression of the endodermic markers Gata 6, Sox 7 and Sox 17 can be detected in the treated cells after 30 days in XEN medium. The quantitative amount of Oct 4 and Sox 2 transcripts significantly decreases during the differentiation.

In the treated cells, however, the expression of Sox 7, Sox 17 and Gata 6 is increased, as expected for XEN lines. As already observed in previous studies, a basal production of the pluripotency markers is preserved in the XEN population, confirming their stem cells identity (fig.1, [4]). Although XEN differentiation was successful, the obtained cell population was still highly heterogeneous, which caused a delayed appearance of XEN colonies impeding the establishment of stable cell lines.
Further improvements are needed to guarantee the selection of the desired cell type and its maintenance and propagation.

References

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