Talk

In vitro modelization of totipotency to study DNA damage response

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ABSTRACT

Embryonic development begins with the fusion of gametes that form a zygote, capable of differentiating into all cell types in the organism, in a state known as totipotency. This process involves intense epigenetic and metabolic reprogramming that are likely to generate high levels of stress in embryonic cells, which, paradoxically, are not only tolerable but may be necessary for the induction of totipotency (Xu & Liang, 2022). The mechanisms behind this response and their relationship to cell differentiation are still not fully understood. Furthermore, obtaining totipotent cells in the lab is a complex process, so alternative models are being developed to study these changes in vitro.

Our study aims to investigate different responses to DNA damage in an in vitro model of totipotency. To achieve this, we first generated a mouse embryonic stem cell (mESC) line that allows to detect totipotent cell by GFP expression. We transfected mESC with a plasmid that includes the GFP gene under the control of the MERVL retrotransposon promoter, whose expression is linked to the totipotent state (Ishiuchi et al., 2015). After transfection, the cells were selected and genotyped to confirm the correct insertion of the construct, thereby generating the MERVL-GFP cell line. We then performed reprogramming assays through splicing inhibition, a recently discovered method that induces a state of totipotent-like cells (Shen et al., 2021). For this, pladienolide B was used as indicated in the previous study. After a temporal study, we identified the optimal time point for reprogramming with the highest proportion of GFP-positive cells, confirmed by qPCR, sorting, and confocal microscopy.

Additionally, we performed differentiation assays and alkaline phosphatase staining, as well as embryoid body studies. The results showed that the MERVL-GFP cell line exhibits behaviour similar to non-transfected mESCs, indicating that our new cell line retains its pluripotency and differentiation capacity.

With this cell line, we aim to enrich the population of reprogrammed cells to study the effects of DNA damage in the context of the totipotent state. This model could be key to solving problems related to fertility and in vitro fertilization, opening new avenues in the study of developmental biology.

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