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Treatment of human embryos with the TGFβ inhibitor SB431542 increases epiblast proliferation and permits successful human embryonic stem cell derivation

Van der Jeught, M.[1]; O'Leary, T.[1]; Lierman, S.[1]; Deforce, D.[2]; Chuva de Sousa Lopes, S.[3]; Heindryckx, B.[1] and De Sutter, P.[1]

[1] Department for Reproductive Medicine, Ghent University Hospital, Ghent, Belgium
[2] Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium
[3] Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands

Introduction

Recently, it has been shown that at least two states of pluripotency exist. Mouse embryonic stem cells (mESC) are considered to be naïve, while human embryonic stem cells (hESC) have the property of being primed. It is known that the 2i condition (combined inhibition of the Mitogen-Activated Protein Kinase (MAPK) and Glycogen Synthase Kinase (GSK)3β pathways) supports the naïve state of ESC, and increases epiblast formation in mice and human blastocysts. Here, we set out to investigate if inhibition of the TGFβ pathway, an important signalling pathway to sustain pluripotency in primed ESC, increases the epiblast cell number in human blastocysts as well, and if it permits subsequent successful hESC derivation.

Materials and Methods

All patients donating research embryos for this project provided written informed consent. This study was approved by the local Ethical Committee (2009/281) and the Federal Ethical Committee for embryo research (Adv-030). For the first experiment, 169 fresh day 3 spare human embryos were randomized into three different culture groups (i): Control Cook Blastocyst medium (CB), (ii): CB + SB431542 (TGFβ inhibitor; 10µM), (iii): CB + Activin A (TGFβ activator; 50 ng/mL). The embryos were cultured at 37°C, 6% CO_2 and 5% O_2. Blastocysts were scored at day 6 and fixed using 4% paraformaldehyde. Immunocytochemistry was performed for Nanog (epiblast marker) and Gata6 (hypoblast

marker). For the derivation experiment, 103 DMSO frozen embryos were thawed and cultured in the presence or absence of SB431542 from day 3 to day 6 of development. After this initial embryo culture, day 6 blastocysts with both good and poor quality ICMs were plated on a MEF feeder layer, either in standard hESC medium or in hESC medium supplemented with SB431542. As such, we created three different conditions (i): control blastocysts that were plated in standard hESC medium, (ii): SB blastocysts that were plated in SB supplemented hESC medium, and (iii): SB blastocysts that were plated in control hESC medium.

Results

Immunostainings revealed that the number of NANOG positive ICM cells was significantly higher in the SB group compared to the control (12.0 ± 5.9 versus 6.1 ± 4.7), but not in the Activin A group (6.7 ± 3.7). There were no differences in GATA6 positive cells between the groups (8.8 ± 4.3**,** 7.2 ± 4.0 and 8.0 ± 4.6 resp.)**.**

Of the plated control blastocysts, 33.33% developed into initial outgrowths (PICMIs (Post-ICM-Intermediates)), and 16.66% formed embryonic stem cell colonies. When SB treated blastocysts were plated in SB supplemented hESC medium, no stem cells could be derived. Six days after plating the SB blastocysts in standard hESC medium, one initial outgrowth was formed (12.5%). This resulted in a new embryonic stem cell line that could be kept in culture for at least 30 passages.

Conclusions

Our findings indicate that the number of NANOG positive ICM cells significantly increased after inhibiting TGFβ with SB431542. This outcome resembles the effect of 2i treatment of human and mouse embryos. Therefore we speculate that inhibition of TGFβ signalling could be a valid approach to derive naïve hESC. Moreover, we saw that SB treatment of human embryos permits the derivation of pluripotent hESC lines.