The TGFβ pathway plays a pivotal role in early embryo lineage segregation

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Introduction

The exact molecular mechanism of second lineage segregation of the inner cell mass (ICM) into epiblast and hypoblast remains largely unknown. Recently, it has been shown that inhibition of the Mitogen-Activated Protein Kinase (MAPK) and Glycogen Synthase Kinase (GSK)3β during mouse embryonic development prevents hypoblast formation**.** A possible alternative pathway of interest is the Transforming Growth Factor (TGF)β pathway, which has been shown to regulate Nanog expression in embryonic stem cells. It remains to be investigated whether TGFβ is also involved in the second lineage segregation in both mouse and human. Hence we verified the effect of TGFβ signalling activation and inhibition on the allocation of the ICM towards epiblast and hypoblast during mouse and human embryonic development.

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 this experiment, 134 donated fresh day 3 spare human embryos were used. These fresh embryos did not meet the IVF laboratory criteria for transfer or cryopreservation and 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). In parallel, 107 B6D2F1

reconstructed parthenogenetic mouse 2-cell embryos were randomized into these groups as well, using sequential KSOM/CB medium. Both human and mouse embryos were cultured at 37°C, 6% CO_2 and 5% O_2. Blastocysts were scored at day 5 and 6, for mouse and human respectively, and fixed using 4% paraformaldehyde. Immunocytochemistry was performed for Nanog (epiblast marker) and Gata (Gata6 in human, Gata4 in mice; hypoblast markers). Blastocyst formation rates were analysed by contingency table analysis followed by Chi-square or Fisher's exact tests. P-values of ≤ 0.05 were considered to be significant. Parameters of ICM allocation were compared using one-way analysis of variance (ANOVA) followed by Tukey post-test when the significance level reached $P<0.05$.

Results

In mice, embryos developed into blastocysts at similar rates in the different treatment groups (92, 90 and 90% resp.). Immunostaining revealed significantly higher numbers of Nanog (12.6 ± 4.8 versus 8.3 ± 3.0; $P<0.001$) and lower numbers of $Gata^+$ cells (7.5 ± 3.8 versus 10.1 ± 2.7 resp.; $P<0.05$) in the SB431542 group compared to the control. Activin A treated blastocysts showed significantly lower numbers of $Gata^+$ cells in their ICMs compared to the control (5.5 ± 2.3 versus 10.1 ± 2.7 resp.; $P<0.01$), while the number of $Nanog^+$ cells remained similar to the control (6.4 ± 1.9 versus 8.3 ± 3.0). In human, blastocyst development was also comparable between the groups (29, 31 and 27% resp.), while the number of $Nanog^+$ cells tended to be higher in the SB431542 group compared to the control (11.0 ± 7.2 versus 5.7 ± 4.4), but not in the Activin A group (6.7 ± 3.7). There were no differences in $Gata^+$ cells between the groups (8.6 ± 3.2**,** 8.0 ± 4.6 and 9.2 ± 5.1 resp.)**.**

Conclusions

In mice, inhibition of TGFβ signalling by SB431542 resulted in ICMs with more $Nanog^+$ and less $Gata^+$ cells. Activation of the pathway reduced the number of $Gata^+$ cells as well, but the number of $Nanog^+$ cells remained the same. In human, the number of $Nanog^+$ ICM cells seemed to increase after TGFβ inhibition as well. Therefore, we can conclude that the TGFβ pathway plays an active role during early lineage segregation, which could affect stem cell derivation efficiency.