CDB SEMINAR

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16:00~17:00 C1F Auditorium

LIF/STAT3 signaling in human and rabbit pluripotent stem cells

Summary

Human and rabbit embryonic stem cells (hESCs and rbESCs, respectively) depend on FGF2 and Activin/Smad signaling for self-renewal in the primed pluripotent state. In contrast, mouse ESCs (mESCs) utilize LIF/STAT3 signaling to sustain naive pluripotency. However, it remains unclear whether LIF/STAT3 can also sustain self-renewal in non-rodent species. To examine this hypothesis, we engineered hESCs with STAT3-ER^{T2}, a mutant estrogen receptor fused to mouse STAT3. The resulting F-OS3 cells could be specifically stimulated by tamoxifen. Treating these F-OS3 cells with hLIF and tamoxifen activated their STAT3 target genes. After propagation in the presence of both factors, F-OS3 cells progressively lost their bFGF dependency and continued to self-renew in the undifferentiated state. Evidence for this was the formation of tight colonies that expressed alkaline phosphatase and pluripotency-associated transcription factors Oct4, Nanog, Sox2, and SSEA4. These cells were passaged up to 60 times while maintaining a normal karyotype (46, XY). They also retained the capacity to differentiate into derivatives of the three embryonic germ layers both *in vitro* by forming embryoid bodies and *in vivo* by forming teratomas. Thus, these new cells expressed the cardinal markers and properties of pluripotency. These cells were designated TL-OS3 (TL for tamoxifen + LIF-dependency). TL-OS3 cells were strictly dependent on both LIF and tamoxifen for differentiation blockade. Whole transcriptome analysis using microarrays showed that TL-OS3 cells had a distinct gene expression profile characterized by the activation of STAT3 target genes. TL-OS3 cells also became permissive to dissociation into single cells by trypsinization and formed mouse ESC-like colonies when cultured in 2i medium. Furthermore, numerous genes associated with naive pluripotency in rodents have been activated. Collectively, these results showed that LIF and hormone-dependent STAT3 in conjunction with GSK3 and MEK inhibitors could push hESC towards naive pluripotency.

The paucity of pre-implantation embryos in humans is a major limitation for deriving ESCs. Rabbits are a good alternative since one superovulated female produce up to 30 embryos, and all rbESC lines generated to date exhibited characteristics of primed pluripotency similar to hESCs. Our newest results indicate that LIF facilitates the derivation of ESCs, but does not support naïve pluripotency in rabbits. Moreover, when rbESCs were propagated in the presence of LIF, they often acquired chromosomal abnormalities. Thus, LIF-dependent rbESCs cannot be propagated under stringent conditions without frequent chromosomal rearrangement-based adaptations.

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