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論文題目	AMPK activation reverts mouse epiblast stem cells to naive state (AMPK 経路の活性化はマウスエピブラスト幹細胞をナイーブ状態に戻す)		

（論文内容の要旨）

There are two pluripotent states: naive and primed. Naive mouse embryonic stem cells (mESCs) which derived from inner cell mass at E4.5 can be maintained in leukemia inhibitory factor (LIF) and two inhibitors (2i) of the MEK and GSK3 pathways. Primed mouse epiblast stem cells (mEpiSCs) which derived from post-implantation epiblast at E6.5 can be maintained in fibroblast growth factor-2 (FGF2) and Activin A. They show quite distinct characteristics in colony morphology, gene expression, specific protein distribution, and the most evidently that naive mESCs can contribute to chimera formation and germline transmission after the blastocyst injection but primed mEpiSCs cannot. Two ways in general were known to revert primed mEpiSCs to naive state. One is induction with specific naive genes, such as Klf4, Klf2, Nanog, Prdm14 and Gbx2. The other is small molecule treatment, such as parnate, inhibitors of ALK4/5/7, MEK, FGFR, GSK3, and/or casein kinase 1 alpha. However, which signal is the most critical for revert primed to naive pluripotency is still unclear. Although adenosine monophosphate (AMP)-activated protein kinase activators have been reported to maintain naive mESCs, whether AMPK activation can revert primed mEpiSCs to naive state remains unknown.

In this study, three AMPK activators, AICAR (5-aminoimidazole-4-carboxamideribo-nucleotide), A769662, and metformin were shown to revert mEpiSCs to naive state. When primed mEpiSCs were cultured with serum (not for naïve nor primed cell culture), addition of AMPK activator alone induced appearance of naive-like cells. Those cells were maintained and grown in naïve cell culture condition (2iL) and expressed naive pluripotent makers with naïve-specific protein distribution. Global gene expression analysis with RNA-seq using 34,489 genes revealed that reverted cells (after expansion in the naïve cell culture condition) were clustered in cell populations close to naïve mESCs. Reverted cells contributed to chimera formation and germline transmission. All these results suffice most of the naïve criteria. Combination with LIF enhanced the AMPK activator-elicited reversion efficiency, reaching 100% success rate in AICAR with LIF condition.

p38 has been previously demonstrated to be functional downstream of the AMPK pathway in maintaining naive mESCs. In this study, inhibition of p38 blocked the reversion process induced by AMPK activation. A p38 gain-of-function experiment with an inducible expression of a constitutively active form of p38 showed that activation of p38 successfully reverted mEpiSCs to the naive state though the reversion efficiency was lower than that of AMPK activators, suggesting that p38 was critical downstream of the AMPK pathway in the naive reversion process.

Single cell RNA-seq was performed to track the reversion process. After the AICAR with LIF treatment, primed mEpiSCs once lost Oct4 expression (day 8), but a new Oct4-positive cluster appeared on day 16. After being maintained and expanded in 2iL, a distinct Oct4-positive cluster appeared closer to naive mESCs. Dppa5 and Dazl were found to be highly expressed in the Oct4-positive cluster on day 8. The addition of AICAR and/or LIF to mEpiSCs increased Dppa5a and Dazl mRNA expressions and which were blocked by p38 inhibitors, suggesting that Dppa5 and Dazl should be potent candidates for downstream of AMPK and p38 in the reversion process.

Collectively, this study provides valuable insights into the molecular machinery regulating naive and primed pluripotency and thus contributes broadly to developmental biology and regenerative medicine including rejuvenation.

（論文審査の結果の要旨）

多能性幹細胞には、ナイーブ型とプライム型の２つの多能性状態が知られている。ナイーブ型は着床前胚盤胞の内細胞塊に、プライム型は着床後のエピブラストに相当するが、それらの多能性制御機構については未だ十分に解明されていない。本研究では、アデノシンーリン酸活性化プロテインキナーゼ（AMPK)の活性化がプライム型マウスエピブラスト細胞(mEpiSCs)をナイーブ状態に戻すことを示した。すなわち、AMPK 活性化剤 AICAR、A769662、metformin の単独投与または Leukemia inhibitory factor (LIF)との共投与により mEpiSCs にナイーブ細胞マーカー発現が誘導され、ナイーブ細胞培養条件において継代維持が可能であり、コロニー形状、キメラ形成能や生殖細胞への寄与などナイーブ型細胞の条件を満たした。AMPK 活性化剤の効果は p38 阻害剤により減弱し、活性型 p38 発現によりナイーブ化効果が認められたことから、p38 が AMPK によるナイーブ化誘導の重要な下流分子と考えられた。単一細胞 RNA-seq 解析によりプライム型からナイーブ型に至る過程が示唆された。AMPK-p38経路はナイーブ型多能性を誘導する新たなシグナル経路と考えられた。

以上の研究は幹細胞の多能性制御機構の解明に貢献し、幹細胞研究に寄与するところが多い。

したがって、本論文は博士（　医学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和　5　年　7　月　12　日実施の論文内容とそれに関連した研究分野並びに学識確認のための試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降