



Title	Distinct requirements for the maintenance and establishment of mouse embryonic stem cells
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## 論文内容の要旨

氏名 ( 小西理予 )	
論文題名	Distinct requirements for the maintenance and establishment of mouse embryonic stem cells (マウスES細胞の維持と樹立における異なる要求性)
論文内容の要旨	
<p>Mouse embryonic stem cells (ESCs) are derived from the inner cell mass (ICM) of blastocysts. The culture condition with LIF and two inhibitors of glycogen synthase kinase-3-<math>\beta</math> (GSK3<math>\beta</math>) and mitogen-activated protein kinase (MEK) signaling (2iL) greatly enhances the establishment of ESC. Genomic imprinting is an epigenetic phenomenon that regulates allele-specific gene expression by DNA methylation or histone modifications, but it is lost in female ESCs under 2iL condition. This loss of imprinting causes the loss of full-term development potential of female ESCs. Therefore, a novel method to establish the ESCs maintaining normal imprinting is required. To this end, I analyzed the necessary culture condition for the maintenance and establishment of ESCs in detail. Even at low concentration of the GSK3<math>\beta</math> inhibitor and LIF (LowGiL), the expression levels of pluripotency markers and the chimera-producing ability of the cells were comparable with those of ESCs cultured in 2iL. However, blastocysts underwent spontaneous differentiation, and ESCs were not established under LowGiL condition. Time-course analysis showed that 2iL condition for three days from the initiation of culture was sufficient for the acquisition of permanent pluripotency. The female ESCs established using this LowGiL condition maintained DNA methylation at differentially methylated regions (DMRs) of imprinted genes. Gynogenetic and androgenetic ESCs established using LowGiL condition also displayed the parent-of-origin gene expression of all imprinted genes that were bi-allelic expressed in the ESCs established under 2iL condition. Taken together, the novel method proposed in this study would be the powerful tool to produce the ESCs keeping genomic imprinting.</p>	

## 論文審査の結果の要旨及び担当者

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論文審査担当者	(職)	
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## 論文審査の結果の要旨

着床前初期胚の内部細胞塊 (Inner Cell Mass: ICM) から樹立されるマウス胚性幹細胞 (Embryonic Stem Cell: ES 細胞) は、未分化状態を永続的に維持できる。しかし、ICM の未分化状態は一過的で、ES 細胞の永続的な未分化状態をもたらす分子機構は明らかにされていない。その点に着目した研究をおこない、ES 細胞の樹立過程、特に培養開始 1~3 日の間ににおいて、Mitogen-activated protein kinase kinase (MEK) シグナルの阻害することが、堅強な未分化能の獲得に必須であることを明らかにした。一方、ゲノムインプリンティングは、DNA のメチル化やヒストン修飾によって、片方のアレルからの発現を抑制することで遺伝子量を半分に調節する機構である。また、MEK 阻害剤を含む培養条件は、雌の ES 細胞で DNA の低メチル化状態を誘導し、ゲノムインプリンティングの破綻を引き起こす。この問題を克服するため、MEK 阻害剤の使用を最低限に抑えた新規の ES 細胞樹立法を考案し、ゲノムインプリンティングが維持された雌の ES 細胞の確立に成功した。以上、学位授与に相応しい研究内容であると認める。