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学位論文名	Function of Akt Signaling in Pluripotent Stem Cell Systems （多能性幹細胞システムにおける Akt シグナルの機能）
論文審査委員	（主査） 教　授　仲野　　徹 （副査） 教　授　濱田　博司　　教　授　近藤　寿人

論　文　内　容　の　要　旨

Embryonic stem (ES) cells are pluripotent stem cells derived from the inner cell mass of blastocysts. They can self-renew indefinitely without losing their differentiation ability to all three germ layers and germ lineage. Pluripotency of mouse ES cells can be maintained by leukemia inhibitory factor (LIF), which activates the Janus kinase-signal transducer and activator of transcription-3 (JAK/STAT3) signaling pathway. Similarly Wnt/β-catenin signaling also participates in the control of pluripotency of ES cells. In addition to these signaling pathways, ES cell-specific transcription factors such as Nanog and Oct3/4 are also required for the maintenance of ES cell pluripotency.

Phosphoinositide 3-kinase (PI3K) pathway is one of the crucial intra-cellular signaling systems and generates the second messenger molecule, phosphatidylinositol (3,4,5)-triphosphate PIP_3, from PIP_2. PIP_3 transmits the signals through downstream effectors including Akt, a serine/threonine kinase, and regulates several physiological and pathological processes. On the other hand, the tumor suppressor PTEN is a lipid phosphatase which antagonizes PI3K/Akt signaling. From the studies of conditional Pten-deficient mice, it has been proposed that PI3K/Akt signaling plays a pivotal role in various stem cell systems, including the formation of embryonic germ (EG) cells from primordial germ cells and self-renewal of neural stem cells.

In this study, we analyzed the effect of the PI3K/Akt signaling on the maintenance of ES cell pluripotency. Introduction of myristoylated, active form of Akt (myr-Akt) into mouse ES cells maintained the undifferentiated phenotypes even after the withdrawal of LIF ; expression of all the stem cell markers, i.e. *Nanog* and *Oct3/4*, was retained and differentiation markers were not induced in the absence of LIF. Although STAT3 and β-catenin signaling pathways control pluripotency in ES cells, Akt signaling maintained the undifferentiated state independent of these signaling pathways. The effects of myr-Akt were reversible, because LIF dependence and pluripotent differentiation activity were restored by the subsequent deletion of myr-Akt. The ES cells from which the floxed myr-Akt was excised by Cre recombinase differentiated upon the withdrawal of LIF. In the *in vitro* differentiation systems, these cells produced a variety of hematopoietic cells and neurons. When

transplanted into nude mice, these cells produced teratomas composed of various differentiated cells. Furthermore these ES cells were also incorporated and contributed to the development of chimeric mice when injected into blastocysts. These results clearly demonstrated that Akt signaling sufficiently maintains pluripotency in mouse ES cells. Taken together with the previous results, PI3K/Akt signaling axis regulates 'stemness' in various stem cell systems.

論文審査の結果の要旨

胚性幹細胞（ES 細胞）は、様々な細胞へと分化する能力を持つ未分化な細胞である。ES 細胞の分化多能性を発揮する為には、細胞の未分化性の維持が重要である。PI3 キナーゼ（PI3K）は、細胞増殖を促進するシグナルとして発見されたが、最近では、幹細胞の未分化性維持に関与することが明らかになりつつある。申請者は、PI3K の下流分子セリン・スレオニンキナーゼ Akt の活性化型を発現するマウス ES 細胞を用いた実験をおこない、PI3K/Akt シグナルの活性化により LIF シグナル非依存的にマウス ES 細胞の分化多能性を維持できることを明らかにした。幹細胞システム制御における PI3K/Akt シグナルの機能を明らかにすることは、個体の恒常性を維持する幹細胞システムの理解にもつながり、多能性幹細胞や他の組織幹細胞を用いた再生医療への応用が期待できる点を評価し、学位に値するものと認める。