# 論　文　内　容　の　要　旨

〔 題　　名 〕　Regulatory mechanisms of neural stem cell proliferation by hypoxia-inducible factor
（低酸素誘導因子による神経幹細胞の増殖制御機構）

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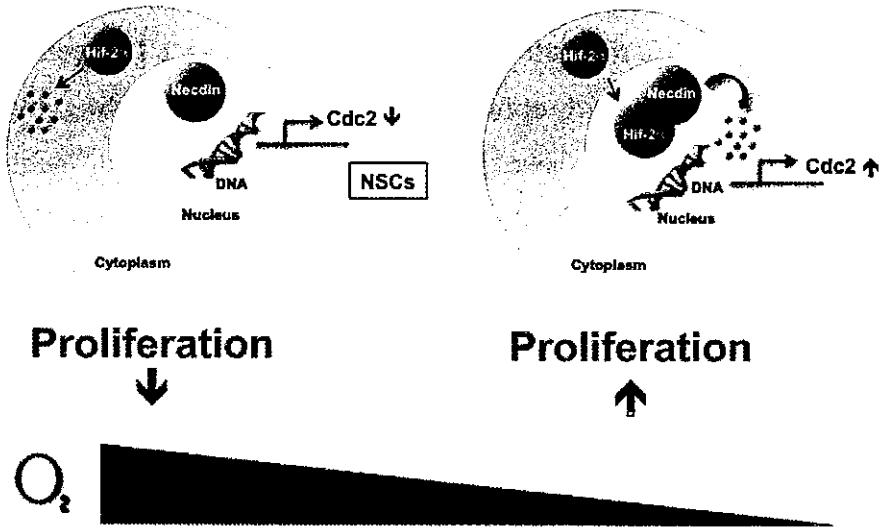
Oxygen levels are crucial for almost all organisms, but in the organs and tissues of animals, especially in the brain the oxygen levels are low under physiological conditions. Stem cells reside in complex microenvironments, termed niches, and many factors such as extracellular matrix proteins, temperature, and oxygen levels can influence the fate of stem cells.

Neural stem cells (NSCs) reside in vivo in hypoxic environments, and NSC proliferation is enhanced in vitro under hypoxic conditions. Various adaptive responses to hypoxia are mediated by hypoxia-inducible factors (HIFs), a family of basic helix-loop-helix Per-Arnt-Sim (PAS) transcription factors. HIFs regulate the expression of at least 180 genes involved in energy metabolism, cell survival, erythropoiesis, and vascular remodeling by binding to hypoxia response elements (HREs) in these genes. Under hypoxic conditions, nonhydroxylated HIF-α protein escapes proteasomal degradation, accumulates within the nucleus, and dimerizes with the HIF-β subunit forming the active HIF complex, and further cause HIF protein accumulates. Necdin, a MAGE (melanoma antigen) family protein, is expressed abundantly in postmitotic neurons and possesses potent anti-mitotic and anti-apoptotic activities. Ectopic expression of necdin strongly suppresses the proliferation of several cell lines. Several lines of evidence indicate that necdin is expressed in multipotent stem cells or committed progenitors of mesodermal/mesenchymal origin.

We here report that hypoxia induces degradation of the necdin protein in primary NSCs by HIF-mediated ubiquitin-proteasome system. Necdin was expressed in the ganglionic eminence (GE) where most of the neural stem cells reside. Necdin was also expressed in primary NSCs and cultured neurospheres prepared from the GE of mouse embryos. According to the neurosphere assay, hypoxia enhanced neurosphere formation of NSCs, in which the necdin protein level was significantly reduced. To ascertain whether endogenous necdin modulates proliferation of NSCs, wild type and necdin-deficient NSC were prepared. Primary NSCs prepared from necdin-deficient mice exhibited higher rates of proliferation and apoptosis than those from wild-type mice in normoxia, whereas there were no significant

differences in the proliferation and apoptosis rates between necdin-deficient and wild-type NSCs in hypoxia. Compare to the HIF-1α, the protein and mRNA levels of HIF-2α was significantly higher, suggesting that HIF-2α was predominantly expressed in hypoxic NSCs. Evidences also showed that the expression of HIF-responsive genes was upregulated, such as vascular endothelial growth factor (Vegf), glucose transporter 1 (Glut1), Cyclin D1 and erythropoietin. The expression of Cdc2, a gene downregulated by necdin, also increased in hypoxic NSCs. According to the co-immuno- precipitation assay, HIF-2α interacted with necdin via its PAS domain and necdin interacted with HIF-2α via its MAGE (melanoma antigen) homology domain. By using the MG132, a proteasome inhibitor, we showed that necdin is targeted for proteasomal degradation in primary NSCs, and that necdin undergoes proteasomal degradation even in normoxia. The interaction between HIF-2α PAS domain and necdin can also enhance necdin ubiquitination. Lentivirus-mediated expression of the PAS domain in primary NSCs promoted necdin degradation and enhanced NSC proliferation in normoxia, whereas the expression of Cdc2 also increased. A small-molecule inhibitor of HIF-2α translation was used to examine the effects of HIF-2α downregulation on the necdin protein level and proliferation rate of NSCs. The inhibitor can reduced the HIF-2α level and stabilize the necdin protein in hypoxic NSCs, which also reduce the NSC proliferation. These results suggest that oxygen tension regulates the necdin protein level in NSCs through HIF-2α-mediated proteasomal degradation to modulate their proliferation (the figure is the schematic diagram).

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# 論文審査の結果の要旨及び担当者

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## 論文審査の結果の要旨

哺乳類の脳において高次神経機能を担う神経細胞は、発生初期に存在する神経幹細胞から分化する。成体脳における神経細胞の数は、初期の神経管で産生される神経幹細胞の数と相関するため、神経幹細胞の増殖制御機構は、哺乳類における脳発達の分子的基盤を明らかにする上で重要である。哺乳類における胎仔期の神経幹細胞の増殖は、低酸素濃度下で著しく亢進することが知られている。また、低酸素環境の細胞内では、転写因子である低酸素誘導因子(hypoxia-inducible factor, HIF)によって、種々の低酸素反応に関連する遺伝子の発現が調節される機構に関しても、既に多くの研究がある。

本論文において、神経幹細胞では低酸素濃度下では細胞増殖抑制能をもつ Necdin の発現量が著しく低下すること、および、Necdin 遺伝子変異マウスの胎仔から調製した神経幹細胞の増殖は、正常酸素濃度下では顕著に亢進するのに対し、低酸素濃度下では増殖亢進が認められないことが明らかになった。このことは、神経幹細胞内に存在する Necdin の蛋白質量が酸素濃度によって増減することにより、神経幹細胞の増殖が制御されていることを示唆する。また、神経幹細胞では HIF の中でも HIF-2α が優位に発現しており、低酸素条件下で安定化されると Necdin と直接結合することにより、Necdin をユビキチン化してプロテアソームでの分解を促進することも明らかになった。さらに、レンチウイルスベクターによる遺伝子導入系を用いて、神経幹細胞で HIF-2α の Necdin 結合領域のみを発現させると、正常酸素濃度下でも Necdin の分解と同時に神経幹細胞の増殖が誘導されることを証明した。

以上のように、哺乳類の神経幹細胞の増殖制御には、酸素濃度に依存した蛋白質分解系が関与することを明らかにした本論文は、幹細胞の増殖制御機構の解明に大きく貢献するものと考えられる。よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。