（学位第３号様式）

学 位 論 文 要 旨

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題　目	Study on PyrzA with a different scaffold from known prolyl hydroxylase domain protein inhibitors: integrated analysis of hypoxic protective function and selection of higher HIF activity of PyrzA derivatives （既存プロリン水酸化酵素阻害剤と骨格を異にする PyrzA に関する研究：低酸素防御機能の統合的解析と高活性誘導体の選抜）

Hypoxia-inducible factor (HIF-α) has a pivotal function in driving anti-hypoxic responsive genes during hypoxic conditions. Under normoxic conditions, HIF-α is hydroxylated by prolyl hydroxylase domain-proteins (PHDs). Hydroxylated HIF-α of two prolyl residues is ubiquitinated and degraded via ubiquitin─proteasome system. On the other hand, under hypoxic conditions, due to a reduction in oxygen molecules, which are one of the substrates of PHDs, hydroxylated rate for HIF-α is suppressed, and HIF-α degradation current is ceased. Consequently, HIF-α stabilizes, translocalizes to the nucleus, and upregulates hypoxia-protective proteins such as erythropoietin (EPO).

HIF activators that have a capability to reversibly without exposing the low oxygen condition can be used to prevent ischemic diseases such as myocardial infarction, stroke, and chronic kidney disease (CKD), since HIF activators can protect tissues from hypoxic stress and restore functions. Several HIF activators have been approved for the treatment of renal anemia, which is one of the main complications of CKD. Almost HIF activators have a 2-oxoglutarate (2-OG) scaffold that interacts with the catalytic centers of PHDs, while previous studies have estimated that more than 60 proteins use 2-OG as a co-enzyme. Therefore, the compounds that harbor 2-OG moiety might interact with those proteins, and then 2-OG analogs may cause side effects. We have developed a novel HIF activator, 5-(1-acetyl-5-phenylpyrazolidin-3-ylidene)-1,3-dimethylbarbituric acid (PyrzA) by using the HIF-α reporter cells and revealed the synthesis processes of PyrzA.

In this study, PyrzA stabilized both HIF-α proteins in SK-N-BE(2)c, HeLa, and Hep3B cells. Furthermore, PyrzA upregulated HIF target genes such as *CA9* and *EPO* in Hep3B cells. Similarly, PyrzA also upregulated HIF target genes such as *Bnip3* and *Car9* in the kidney of six-week-old female C57B6 mice 6 h after intraperitoneal administration of PyrzA (50 mg/kg). PyrzA decreased two prolyl-hydroxylated HIF-1α proteins using immunoblot analysis. Moreover, PyrzA mimicked hydroxylation on HIF-α prolyl residues instead of 2-OG using docking simulation between PHD2. Those experiments implied that PyrzA directly inhibits the function of PHDs. Next, 22 PyrzA derivatives were selected, including 16 PyrzA derivatives with replaced phenyl and acetyl groups (Group A) and 6 PyrzA derivatives with replaced acetyl and methyl groups (Group B) to improve lipophilicities. HIF transcriptional activities and cellular viabilities of these derivatives were measured using HIF-α reporter cells. As a result, from B group, PyrzA-50 was approximately 30-fold more potent than PyrzA and had lower cytotoxicity. PyrzA-50 stabilized HIF-α proteins and upregulated *CA9* and *EPO* at lower concentrations than PyrzA. Then, PHDs recombinant proteins in SF21 insect cells or silkworms produced. As a future plan, confirmation of the physical interaction between PHD and PyrzA will be carried out using these recombinant PHD proteins.