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Tonang Dwi Ardyanto 学位論文審査要旨

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主論文

$CoCl_2$-induced HIF-1α expression correlates with proliferation and apoptosis in MKN-1 cells: A possible role for the PI3K/Akt pathway

（$CoCl_2$誘導によるHIF-1α発現は、MKN-1細胞における増殖とアポトーシスに関与する：PI3K/Akt経路への役割について）

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Hypoxia-inducible factor-1α (HIF-1α) is a heterodimer that is critical to cell survival under hypoxic conditions. Its expression is maintained at low level by ubiquitin-proteosome pathway under normoxia. In hypoxia, the expression is stabilized, translocated to nucleus, and initiates the transcription of target genes. $CoCl_2$ is one of the hypoxic mimicking agents that work by replacing the iron in iron-binding center in prolyl hydroxylases so that the HIF-1α is stabilized. HIF-1α is detected immunohistochemically in some human cancers and is suggested to play a role in cancer cell growth and survival, tumor development, tumor angiogenesis and poor clinical prognosis. A few studies on gastric cancer have been reported, however, the mechanisms of its role have not been elucidated in details. Many components of the PI3K/Akt pathway have been reported to be involved in regulation of HIF-1α. However, the exact role is still a matter of debate. We examined the expression of HIF-1α by $CoCl_2$ induction that is dependent on the PI3K/Akt pathway, and elucidated its correlation with cell proliferation and apoptosis in a human gastric cancer cell line, MKN-1.

方 法

A human gastric caner cell line, MKN-1, was treated with or without 500 μM $CoCl_2$ for 0, 2, 4, 6, 8, 10, 12, 24 and 36 h, and with 25, 50 and 100 μM LY294002, an Akt inhibitor, in DMEM containing 10% fetal bovine serum. The concentrations and duration of treatment were obtained from preliminary experiments. Immunocytochemistry was performed to detect the expression of HIF-1α and its translocation to the nucleus during the treatment. Western blot was performed to detect the expression and correlation among the HIF-1α, Akt, phosphorylated Akt (Ser473), Cyclin-B1, P27,

SKP-2, Bcl-2, Bcl-XL, Bax, Caspase-9, cleaved-Caspase-9 and P53. Cell viability index was measured to examine the correlation between HIF-1α expression and cell proliferation. The flow cytometry was performed to examine the treatment effect to the cell cycle after 36 h of treatment. Apoptotic Index was measured to examine and calculate the apoptotic cells after 0, 2, 4, 6, 8, 10, 12, 24 and 36 h of treatment as well as the correlation with HIF-1α expression. Another western blot was performed to examine the effect of LY294002, a PI3K/Akt inhibitor, on the HIF-1α expression as well as the cell viability under co-treatment.

結　果

In MKN-1, HIF-1α expression showed a pattern of an increase followed by a decrease in a time dependent manner until 36 h after the $CoCl_2$ treatment. The negative feedback mechanism was suggested to induce the dual phase of expression. Immunocytochemistry showed nuclear translocation of HIF-1α in numbers on 4 h and scarcely on 36 h of treatment, while the control cells showed little cytoplasmic immunoreactivity especially after 36 h of treatment. The cell viability index showed a correlation with the two phases pattern of HIF-1α expression, while apoptotic index showed an increase in the decrease phase of HIF-1α expression. Flow cytometry showed an increase in apoptotic area and marked G_2/M arrest after 36 h of treatment. Expression of the cell cycle- related proteins of P27, Skp2 and Cyclin-B1 was correlated with that of HIF-1α. Expression of the apoptosis-related proteins including Bcl-2, Bcl-xL, Bax and cleaved-Caspase-9 components was associated with that of HIF-1α in two phases. Addition of LY294002 partially inhibited the expression of HIF- 1α in a dose-dependent manner. The addition also inhibited the effect of HIF-1α to increase the cell viability.

考　察

This study reported that the two phases of expression of HIF-1α induced by $CoCl_2$ occur in a time dependent manner as shown by western blot and immunocytochemistry. The expression was correlated with the cell viability that peaked at 4 h of treatment as the HIF-1α expression also peaked. Regarding cell cycle, flow cytometry showed an increase of pre-G_1 area and G_2/M arrest after 36 h of treatment. It might be a

temporary state before being escaped and enters the long-term G_0/G_1 arrest, as also reported by another study on colon cancer cell line. HIF-1α expression showed a correlation with apoptotic index, as it was correlated with the apoptosis-related protein expression. The $CoCl_2$ treatment inhibited the Akt phosphorylation after 36 h. Addition of PI3K/Akt inhibitor partially inhibited the HIF-1α expression, supporting the previous report on the PI3K/Akt-GSK3β-HIF pathway, although there is still possibility of cell-type specific pattern. The partial inhibition of PI3K/Akt inhibitor showed that there are some other mechanisms regulating HIF-1α.

結　論

$CoCl_2$-induced HIF-1α expression was correlated with the cell proliferation and apoptosis in a human gastric cancer cell line, MKN-1, possibly through PI3K/Akt pathway.