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論文内容の要旨

〔 目　　的 〕

The remodeling of cell surface growth factor receptors by modification of their oligosaccharide structures is associated with certain functions and biological events. N-glycans play an important role in the folding, stability and sorting of glycoproteins. Core fucosylation is formed by reaction of alpha 1,6 fucosyltransferase, also known as Fut8, which catalyzes the transfer of a fucosyl residue from GDP- fucose to the asparagines-linked GlcNAc residue of complex N-linked glycans via alpha 1,6 linkage. N-linked oligosaccharides on EGF receptor appear to be important factors for receptor function. However, to data the roles of core fucosylation in EGF receptor-mediated functions have not been identified yet. In the present study, the effects of core fucosylation on EGF receptor-mediated signaling were investigated by using wild type cells and Fut8-null cells.

〔 方法ならびに成績 〕

We recently found that disruption of the Fut8 gene induces severe growth retardation and early postnatal death.* In order to investigate the molecular mechanism involved, we established embryonic fibroblasts of $Fut8^{+/+}$ and $Fut8^{-/-}$, derived from wild-type and Fut8-null mice, respectively. By using HPLC analysis, Fut8 activities could not be detected in $Fut8^{-/-}$ cells, Re-introduction of Fut8 gene into $Fut8^{-/-}$ cells, resulted in recovery of Fut8 activities, the levels of which were comparable with those in $Fut8^{+/+}$ cells. The reaction products of Fut8, core fucocylated N-glycans were analyzed by means of the AAL lectin, which preferentially recognizes the core fucose, in these cells. The reactive staining lost in $Fut8^{-/-}$ cells was completely rescued by restoring the Fut8 gene to $Fut8^{-/-}$ cells.

Further more, we found core fucosylation was expressed on EGFR in $Fut8^{+/+}$ cells, but abolished on EGFR from $Fut8^{-/-}$ cells. The phosphorylaition levels of EGFR stimulated with EGF were increased in a dose-dependent manner in $Fut8^{+/+}$ cells. Interestingly, the stimulation was significantly suppressed in $Fut8^{-/-}$

cells, whereas the responsiveness was rescued in the restored cells. Moreover, we found that tyrosine-phosphorylated levels of EGFR in Fut8-null embryos were lower than that in wild-type embryos.

To determine whether core fucosylation is also important for EGFR-mediated intracellular signaling, downstream signaling molecules such as JNK and ERK1/2 were examined by immunoblotting with anti-phospho-JNK and ERK antibodies. JNK and ERK phosphorylation levels, as induced by EGF, were greatly enhanced in $Fu8^{+/+}$ cells, whereas marginal activation of JNK or ERK was detected in $Fu8^{-/-}$ cells. The down-regulation of JNK and ERK activation in $Fu8^{-/-}$ cells was rescued in the restored cells.

To elucidate the mechanism by which core fucosylation affects the EGF signaling, a binding analysis of ^{125}I-EGF to EGFR in three types of cells was performed. A Scatchard analysis revealed that both low and high affinity binding of EGFR were present in $Fut8^{+/+}$ (K_d are 269 pM and 1052 pM for low and high affinity, respectively) and the restored cells (K_d are 253 pM and 1079 pM for low and high affinity, respectively), but only low affinity EGFR was detected in $Fut8^{-/-}$ cells (K_d=1233 pM). Thus these results suggest that the modulation of N-glycans by core fucosylation on EGFR may regulate the high affinity binding EGF to EGFR, which is required and sufficient for EGF-induced response, but not for the low affinity of EGFR.

〔 総　　括 〕

EGF-induced phosphorylation of EGFR was down regulated in $Fut8^{-/-}$ cells, compared with $Fut8^{+/+}$ cells. Consistent with this observation, EGFR-mediated downstream signaling such as the phosphorylation of JNK or ERK was also decreased in $Fut8^{-/-}$ cells. These inhibitory effects in $Fut8^{-/-}$ cells were completely rescued by re-introduction of the Fut8 gene to $Fut8^{-/-}$ cells. Furthermore, the levels of phosphorylation of EGFR were reduced in knockout mice, compared with wild-type mice. Our results provide clear evidence, for the first time, that core fucosylation may play an important role in EGFR-mediated biological functions.

*Wang, X., Inoue, S., Gu, J., *et al* (2005) *Proc Natl Acad Sci U S A* 102, 15791-15796

論文審査の結果の要旨

コアフコースは、糖タンパク質のN-結合型糖鎖の還元末端のGlcNAcにα1,6フコース転移酵素（Fut8）によって作られる構造である。申請者らは最近、Fut8ノックアウト（$Fut8^{-/-}$）マウスの作製に成功した。このマウスは7割が生後3日以内に死に、また生き残ったマウスも顕著な成長障害をきたすが、それらの原因は明らかでない。EGF受容体（EGFR）は細胞増殖、分化などの生理的機能に重要な役割を果たすことで広く知られている。最近の報告から、EGFRの糖鎖は、その機能に重要であることが分かってきた。そこで、本研究では野生型細胞、$Fut8^{-/-}$ マウス由来の胚線維芽細胞および $Fut8^{-/-}$ マウスの胚線維芽細胞にFut8遺伝子を再導入した細胞を用いて、EGFRにおけるコアフコースの機能を調べた。その結果、$Fut8^{-/-}$ マウス由来の胚線維芽細胞は野生型細胞に比べ、EGF刺激によるEGFRのリン酸化ならびにその下流シグナル分子JNKとERK1/2のリン酸化などが著しく抑制された。また、抑制されたEGFRのリン酸化はFut8再導入により回復された。^{125}I-EGFを用いたScatchard解析の結果、$Fut8^{-/-}$ マウス由来の胚線維芽細胞では高親和性EGFRの機能が著しく低下していることがわかった。EGFRの機能低下は、$Fut8^{-/-}$ マウスにおける成長障害に何らかの関わり持つかもしれない。本研究は、増殖因子受容体のコアフコースの生理機能を初めて明らかにしたものである。よって学位の授与に値すると考えられる。