論文の内容の要旨

論文題目

The role of a cell adhesion molecule, CADM1, in the internalization and degradation of EGFR
(細胞接着分子 CADM1 による EGF 受容体の分解制御機構の解明)

氏名　許 淑真

Lung cancer is one of the most common human malignancies in the world, and non-small cell lung cancer (NSCLC) represents a majority of lung cancers (85~90%). Although the understanding of tumorigenesis in molecular level has been improved, patients with early phase NSCLC deemed suitable for curative treatment still maintain a high rate of relapse. Clarifying the process of tumorigenesis becomes an important issue for diagnosis at early stage and for finding out new targets of treatment. Tumorigenesis is promoted due to inactivated tumor suppressors and/or activated oncoproteins. In NSCLC, numerous genetic alterations have been reported, including activation of oncogenes, such as *KRAS* mutations, *EGFR* mutations, *ALK* rearrangement, and inactivation of tumor suppressors, such as *TP53*, *RB*, *p16*, and cell adhesion molecule 1 (CADM1). Several studies reported that EGFR protein expression correlates with NSCLC tumorigenic stage, implying that EGFR might be related to tumorigenesis. Furthermore, the ratio of hypermethylation of CADM1 promoter in relatively advanced tumors was higher than early ones, which suggests that alteration of CADM1 expression could be involved in the progression of human NSCLC. In this study, we would focus on the relationship between CADM1 and EGFR.

CADM1, tumor suppressor protein, was identified by functional complementation in a NSCLC cell line, A549. In carcinoma of the lung, cervix, nasopharynx, esophagus and breast, reduced expression of CADM1 has been reported especially in their advanced stages. CADM1 is expressed at the lateral membrane in epithelial cells, and forms a cis-homodimer on plasma membrane. It connects to actin through protein 4.1 and participates in cell mobility. Furthermore, CADM1 trans-interacts with another CADM1 in adjacent cell and functions in cell-cell interaction. Since disruption of cell-cell adhesion facilitates morphological remodeling leading to cancer cell dissemination, invasion and metastasis, it is well considered that the tumor suppressive activity of CADM1 is through its adhesion function. However, the tumor suppressive activity of CADM1 was abrogated by truncating of its cytoplasmic domain, although the cell-cell adhesion activity was partially retained *in vitro*. The precise molecular mechanism of CADM1 in tumor suppress needs to be clarified.

Over-expression of EGFR or amplification of *EGFR* gene has been reported in many cancers. In NSCLC, over-expression of EGFR has been observed in more than 60% of metastatic tumors and correlated with poor prognosis. Since abnormal over-expression of EGFR and loss or decreased

expression of CADM1 have been reported in NSCLC, we speculate that there is a link between the activation of EGFR and the inactivation of CADM1. Here, we examined the internalization and following degradation of CADM1 and EGFR in NSCLC cells after stimulation with EGF.

NCI-H1838 is a NSCLC cell line which endogenously expresses CADM1 and wild type EGFR. First, I investigated whether CADM1 is involved in the degradation of EGFR. For this purpose, NCI-H1838 cells were transfected with control or full length CADM1 vector, HA-CADM1-full. With the expression of full length CADM1, the expression of EGFR was reduced to 75%. However, the mRNA expression level of EGFR did not decrease with exogenous CADM1 expression. To further confirm this phenomenon, the same experiment was performed in other NSCLC cell lines, NCI-H1993 and A549, both endogenously express wild type EGFR and with loss of CADM1 expression. The protein expression of EGFR in HA-CADM1-full transfected NCI-H1993 and A549 cells was reduced to 70% and 75%, respectively. This phenomenon was observed not only in the NSCLC cell lines, but also in the EGFR over-expressing epidermoid carcinoma cell line, A431. These results indicate that exogenous expression of CADM1 decreased protein expression of EGFR.

It has been reported that EGFR mutation occurrs in 50% in NSCLC patients. The NSCLC cell lines with mutant form of EGFR protein were also investigated in this study. The protein expression of EGFR in HA-CADM1-full transfected HCC827 and PC9 cells did not change significantly. This implies that CADM1 did not affect protein expression of mutant form of EGFR.

To identify the functional domain of CADM1 involved in EGFR regulation, the deletion mutants of CADM1 were constructed, including deletion extra-cellular domain, HA-CADM1-Δ EC, deletion cytoplasmic domain, HA-CADM1-Δ CT, and deletion the PDZ binding motif, HA-CADM1-Δ PDZ. Since CADM1 is a membrane protein, immunofluorescence staining was performed to confirm the localization of CADM1 deletion mutants. The confocal micrograph indicated that HA-CADM1-Δ CT and HA-CADM1-Δ PDZ deletion mutants were located at plasma membrane, similar to full length CADM1, but HA-CADM1-Δ EC mutant was located in cytoplasm. Since CADM1 is a membrane protein, the mutants with membrane localization (HA-CADM1-Δ CT and HA-CADM1-Δ PDZ) were used for subsequent experiments. However, expression of EGFR did not reduced in HA-CADM1-Δ CT and HA-CADM1-Δ PDZ-transfected cells. This suggests that cytoplasmic domain of CADM1 is essential for EGFR down-regulation.

To further confirm the role of CADM1 in the degradation of EGFR, chloroquine, an inhibitor for maturation of lysosome was used. Upon chloroquine treatment, CADM1 and EGFR were accumulated and co-localized in lysosome after EGF stimulation, suggesting that CADM1 and EGFR were internalized into the same endosome and degraded in lysosome by EGF stimulation. This study implies that CADM1 might participate in the internalization and degradation of EGFR during the stimulation

with EGF.

EGF stimulation induces the endocytosis and degradation of EGFR. It is well studied that EGFR was endocytosed through clathrin-dependent pathway. Depletion of clathrin expression in NCI-H1838 cells resulted in decrease of EGFR degradation by EGF treatment. This result indicates that degradation of EGFR was through clathrin-dependent pathway in NCI-H1838 cells, and also implies that EGFR go through clathrin-dependent endocytosis upon EGF stimulation. In addition, depletion of CADM1 expression in NCI-H1838 cells by siRNA reduced EGFR degradation induced by EGF stimulation. This result further confirmed that CADM1 facilitates the degradation of EGFR. Furthermore, simultaneous knockdown of CADM1 and clathrin showed no synergistic effect in EGFR expression. These data confirmed that CADM1 facilitates EGFR degradation partly through clathrin-dependent pathway and implies that CADM1 might participate in the endocytosis of EGFR.

In conclusion, our data indicate that CADM1 down-regulated EGFR expression in three NSCLC cell lines. This phenomenon was also observed in A431 cells, which has amplification of *EGFR*. Furthermore, CADM1 facilitates the degradation of EGFR partly through clathrin-dependent manner. These results imply that restoration of CADM1 expression might be a promising adjuvant approach to anti-EGFR therapy.