論文の内容の要旨

論文題目　肺癌における新規治療標的分子 TMEM209 の同定及び機能解析

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Lung cancer is the leading cause of cancer deaths in Japan and nearly 70,000 people die of lung cancer each year. In spite of some advances in the early detection and recent improvements in its treatment, the prognosis of the lung cancer patients is not much improved. Recently many genetic alterations associated with development and progression of lung cancers have been reported and contributed to the better understanding of the molecular mechanisms of pulmonary carcinogenesis. Several molecular targeting agents like Irresa have been developed and are used in clinical practice.　However, each of the new regimens can provide survival benefits to a small subset of the patients and may cause serious adverse effects.　Hence, the development of novel molecular targeting drugs providing better clinical benefits without severe adverse reactions are eagerly required. To screen druggable targets, I analyzed genome wide gene expression profile of 120 lung cancer samples by means of a cDNA microarray. Tumor cells were purified by laser capture microdissection in this analysis. Through the screening of data obtained by cDNA microarray analysis, I selected genes which is over-expressed in lung cancers and showed no or low expression in normal tissues. As a result, nearly 600 genes were selected for further analysis. To confirm data from cDNA microarray analysis, I performed semi-quantitative RT-PCR and northern blotting using RNA from lung cancer tissues, cell lines, and normal tissues. Finally, 5 genes was validated. Then I performed RNA interference assay using lung cancer cell lines to investigate whether these genes are essential for cancer cell growth. Through this screening, I identified *TMEM209* (Transmembrane protein 209) as an candidate molecular target for lung

cancer.

Semi-quantitative RT-PCR analysis revealed that *TMEM209* mRNA was increased in lung cancer tissues. On the other hands, northern blot analysis showed that *TMEM209* was not expressed in normal tissues except testis, indicating TMEM209 to be a cancer-testis antigen. I also confirmed the increased expression of TMEM209 protein in lung cancer cell lines. Immunocytochemical analysis revealed that TMEM209 protein is localized in the nuclear envelope, the golgi apparatus and the cytoplasm of lung cancer cells. Transient expression of TMEM209 protein by using expression plasmid (pCAGGS/TMEM209) significantly enhanced cell growth. Concordant with this result, suppression of TMEM209 expression by small interference RNA (siRNA) against TMEM209 inhibited the growth of cancer cells. Thus, TMEM209 is likely to play significant roles in cancer cell growth and/or survival. These data suggest that TMEM209 could be a good target for lung cancer treatment.

To further investigate the role of TMEM209 in lung tumorigenesis, I tried to identify interacting protein(s) of TMEM209 in cancer cells. Immunoprecipitation assay followed by mass spectrometric analysis identified NUP205 (Nucleoporin 205 kD) as a candidate of TMEM209 interacting proteins. I validated the interaction between TMEM209 and NUP205 by combination of immunoprecipiation and western blot analysis. Semi-quantitative RT-PCR indicated that *NUP205* was highly expressed in lung cancer tissues. In addition, the result of northern blot analysis showed that *NUP205* was not expressed in normal tissues except testis, demonstrating that NUP205 could be a cancer-testis antigen. When I suppressed TMEM209 expression by siRNA, NUP205 protein expression was significantly reduced without affecting NUP205 mRNA level. These results suggested that TMEM209 could stabilize NUP205 protein. I also found the interaction between NUP205 and c-Myc which function as an oncogenic transcriptional factor in several malignancies including lung cancer. Knockdown experiments by siRNA against TMEM209 or NUP205 showed that c-Myc protein level was increased in the cytoplasm and decreased in the nucleus of lung cancer cells, although NUP205 protein level in whole cell lysate was not affected under treatment with proteasome inhibitor, MG132. These results revealed that TMEM209-NUP205 complex could be important for nuclear transport of c-Myc. In addition,

treatment with siTMEM209 reduced expression of CDC25A (Cell Division Cycle 25 Homolog A) and CDK1 (cyclin-dependent kinase 1) those are well known targets of c-Myc. Since CDC25A and CDK1 are involved in cell cycle progression and highly expressed in lung cancers, TMEM209 might regulate c-Myc target genes and promote cancer cell growth.

In conclusion, my experimental results suggested that TMEM209 would interact with NUP205 in nuclear pore, promote the nuclear transport of c-Myc and activate c-Myc transcription that contributes to cell growth and tumorigenesis. Our findings suggest that TMEM209 overexpression as well as TMEM209-NUP205 interaction could be involved in lung carcinogenesis, and be a promising molecular target for lung cancer therapy.