論　文　内　容　要　旨

BMP and activin membrane-bound inhibitor (BAMBI) regulates mesothelioma cell proliferation and clinical outcome

(BAMBI の発現による悪性中皮腫細胞の細胞増殖制御)

主指導教員：藤井　万紀子 教授
(医系科学研究科　ゲノム口腔腫瘍学)
副指導教員：加藤　功一 教授
(医系科学研究科　生体材料学)
副指導教員：谷本　幸太郎 教授
(医系科学研究科　歯科矯正学)

NGUYEN TRUONG DUC HOANG

(医歯薬保健学研究科　医歯薬学専攻)

Mesothelioma is a rare malignant tumor originating from the mesothelial surface of the pleura or, more rarely, at other locations such as the peritoneum. Most patients are diagnosed late-stage, and the prognosis is poor with a 12–20-month median survival period. The current first-line chemotherapy for mesothelioma has relied on the combination of cisplatin and pemetrexed. Despite numerous clinical trials over the last two decades, there is a lack of treatment options in the second line and beyond setting. Therefore, it is urgently required to understand the carcinogenicity to develop a molecularly targeted therapy for mesothelioma. Connective tissue growth factor (CTGF), a member of the CCN cysteine-rich family, is a multifunctional secretory protein and known to be involved in biological and pathological processes. Our previous studies have suggested that tumor-cell-derived CTGF plays an essential role in the proliferation of malignant mesothelioma cells. By selecting the genes affected by knockdown of CTGF expression, we found that the expression of Bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI) gene significantly reduced. BAMBI was initially known as a transmission inhibitor of the TGF-β/BMP pathway because of the homologous structure to TGF-β family type I receptors but lacking the intracellular serine/threonine kinase module required for signaling. In colorectal cancer, elevated BAMBI expression correlates with poor prognosis. TGF-β signaling is upregulated upon BAMBI inhibition resulting in reduced cell viability and motility *in vitro* and *in vivo*. The recent studies so far suggest BAMBI as a potential target of treatment for various diseases. However, the roles of BAMBI in mesothelioma are not studied yet. In this study, we explore the interaction between CTGF and BAMBI, and the effect of CTGF and BAMBI expression on proliferation and cell cycle progression in mesothelioma cells. We further examined the characteristics of BAMBI in mesothelioma cells and carried out survival analysis of BAMBI expression on mesothelioma patients.

The experiments were performed in 7 mesothelioma cell lines (MSTO-211H, Y-MESO-8D, Y-MESO-14, Y-MESO-27, NCI-H28, NCI-H2052, NCI-H2452) and SV40 transfected mesothelial cell line MeT-5A. Mesothelioma cells were inhibited the expression of BAMBI (or CTGF) by BAMBI (or CTGF) siRNA transfection compared to negative control siRNA transfection. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and western blot analysis were used to examine the mRNA and protein expression. The function of CTGF and BAMBI expression in the proliferation of mesothelioma cells was evaluated by cell viability assay. Cells were treated with PrestoBlue 10% and measured fluorescence at 546nm/590nm wavelength. For localization of BAMBI protein in mesothelial and mesothelioma cells, we performed immunofluorescence staining on MeT-5A cells and Y-MESO-27 cells. Survival analysis of BAMBI mRNA expression on mesothelioma patients was explored by the log-rank test in Gene Expression Profiling

Interactive Analysis (GEPIA), http://gepia.cancer-pku.cn/.

Results: First, the expression of CTGF protein was examined on seven mesothelioma cell lines. Western blot results showed that CTGF protein was unevenly detected in all cell lines. Strong CTGF expression was observed in MSTO-211H, Y-MESO-14, and Y-MESO-27 cell lines. Therefore, Y-MESO-14 and Y-MESO-27 cell lines were selected for further analysis. We investigated the effect of CTGF expression on cell growth and BAMBI expression. qRT-PCR and western blot results confirmed successful knockdown of CTGF expression in Y-MESO-14 and Y-MESO-27 cells transfected with CTGF siRNA. CTGF siRNA transfected Y-MESO-14 and Y-MESO-27 cells showed significantly reduced cell growth compared with negative control siRNA Y-MESO-14 and Y-MESO-27 cells. Knockdown of CTGF expression inhibited mRNA and protein level of BAMBI in Y-MESO-14 and Y-MESO-27. We then investigated the effect of BAMBI expression on cell growth and CTGF expression. BAMBI siRNA transfection effectively inhibited mRNA and protein BAMBI expression in Y-MESO14 and Y-MESO-27 cells. Inhibition of BAMBI suppressed the growth of Y-MESO-14 and Y-MESO-27 cells, suggesting that CTGF might regulate the proliferation of mesothelioma cells via BAMBI expression. Furthermore, mRNA and protein expression of CTGF significantly increased in BAMBI-inhibited Y-MESO-27 cells. It suggested that BAMBI works not only as a downstream target but also works as a negative feedback regulator of CTGF.

To explore the mechanism of BAMBI and CTGF, the expressions of cell cycle proteins were investigated on BAMBI- or CTGF-knockdown cells. The mRNA and protein levels of Cyclin D1, Cyclin D3, CDK2 were significantly decreased in BAMBI-inhibited Y-MESO-14 and Y-MESO-27 cells. In CTGF-knockdown Y-MESO-14 and Y-MESO-27 cells, the mRNA and protein expressions of Cyclin D3 and CDK2 were significantly reduced. The similar effects of BAMBI and CTGF on the expression of cell cycle proteins supported their relationship and suggested that BAMBI may play a crucial role in the proliferation of mesothelioma cells.

To examined BAMBI characteristics, we analyzed the expression and localization of BAMBI in mesothelioma cells and mesothelial MeT-5A cells. Western blot analysis showed strong BAMBI protein expression in all the mesothelioma cells while a little in MeT-5A cells. BAMBI protein was mainly observed in cytosol and a little in membranes of MeT-5A and Y-MESO-27 cells. N-cadherin staining was used to show the location of the cell membrane. Analyzing the TCGA datasets by GEPIA, the Kaplan Meyer Curve showed that high mRNA expression of BAMBI was significantly associated with poor prognosis of MM patients in overall survival (hazard ratio 1.9, 95% confidence interval, p= 0.011)

In summary, our results demonstrate that CTGF controls mesothelioma cell growth

via BAMBI expression by regulating cell cycle progression. BAMBI works not only as a downstream target but also as a negative feedback regulator of CTGF. Our study also provides initial knowledge of BAMBI in mesothelioma cells. BAMBI may play an essential role in cell proliferation and be able to a novel molecular target for mesothelioma treatment.