学位論文内容の要旨
（Summary of dissertation）

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学 位 論 文 題 名
（Title of dissertation）

Enhancement of malignant properties of human malignant pleural mesothelioma cells under hypoxia
（低酸素環境下におけるヒト悪性胸膜中皮腫細胞の悪性化に関する研究）

【Background and Objectives】

Malignant pleural mesothelioma (MPM) is an intractable tumor of mesothelial cells lining the visceral and parietal pleura. Often MPM progresses to advanced stages without clinical signs or symptoms, and has involved other organs by the time of diagnosis; its prognosis is very poor even after multi-modality treatments. Unfortunately MPM incidence is increasing and the peak of MPM mortality is apprehended to be in the near future. To date, there are only few pieces of information of molecular mechanisms for development and malignant progression of MPM. In solid tumors, the microenvironment often becomes hypoxic, a condition that the oxygen supply to tissues decreases, during the expansion of a tumor mass. It is known that intratumoral hypoxia acts as both tumor-suppressive and –progressive factors. In this study, I aimed to determine (a) whether hypoxia enhances malignant behaviors such as cell growth, motility and invasiveness of MPM cells, and if it does, (b) by which mechanism(s) hypoxia involves the malignancy.

【Materials and Methods】

Six MPM cell lines including the 2 newly established cell lines (established in this study) were used. These cell lines were cultured under hypoxia (1% O_2) or normoxia (21% O_2) for 24-48 h. Cell growth was analyzed by assays for doubling time, colony and spheroid formation. Cell motility and invasiveness were analyzed by

phagokinetic track assay and by an assay using type I collagen gel, respectively.

【Results】

Firstly, I examined whether the MPM cells showed adaptive cellular responses to hypoxia. All the cell lines showed promoter activity through hypoxia-responsive elements under hypoxia. Hypoxia upregulated the expressions of GLUT-1, VEGFA and HK2, which are downstream genes of hypoxia-inducible factor (HIF). Hypoxia reduced saturation density and sizes of spheroids whereas it enhanced colony-forming ability. And hypoxia enhanced their cell motility and invasivenesss. Knockdown analyses by using siRNA targeting HIF-1α and HIF-2α, which are main transcription factors responding to hypoxic stress, revealed that hypoxia-enhanced motility and invasiveness were mediated through activation of HIF-1α but not HIF-2α. RT-PCR analysis suggested that MUC1 gene which encoded a highly glycosylated membrane protein could be a candidate of downstream genes of HIF-1α. To determine whether HIF-1α activated MUC1 transcription, I performed promoter analysis by using reporters with serial deletions of MUC1 promoter. As a result, it was found that a part (-1,473/-763 which contains two putative hypoxia-responsive elements (HRE) sites) of the promoter region was necessary for MUC1 transactivation under hypoxia. Flow cytometric and immunoblot analyses revealed that hypoxia increased the expression of sialylated MUC1 on the cell surface of MPM cells. To analyze the roles of MUC1 in hypoxia-enhanced motility and invasiveness, I performed knockdown experiments of the gene by using siRNAs, which reduced motility and invasiveness of the MPM cells under hypoxia.

【Discussion】

These findings on cell growth suggest the complexity of tumor microenvironment where additive factors such as changes of cell density alter the responses of MPM cells to hypoxia. The results from the experiments of motility and invasion indicated that the increased expression of MUC1 through activation of HIF-1α pathway likely played an important role in the enhancement of cell motility and invasiveness of MPM cells under hypoxia.

【Conclusion】

From these results, I conclude that hypoxia promoted *in vitro* malignant behaviors of MPM cells, especially motility and invasiveness, through HIF-1α -MUC1 pathways.