# 論　文　内　容　要　旨

Aminopeptidase N/CD13 as a Potential Therapeutic Target in Malignant Pleural Mesothelioma

(アミノペプチダーゼ N/CD13 は悪性胸膜中皮腫における有望な治療標的である)

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Malignant pleural mesothelioma (MPM) is a rare, but aggressive malignant tumor with poor prognosis. Only a minority of patients with MPM is eligible for multimodality therapy, including surgery and radiotherapy, and effective therapeutic strategies for patients with inoperable MPM are limited; therefore, novel systemic therapies are needed. Angiogenesis is a crucial factor in the progression of MPM, indicating that antiangiogenic strategies might be beneficial for MPM treatment. Aminopeptidase N (APN/CD13) is associated with tumor progression through the promotion of both tumor cell invasion and angiogenesis. The expression of APN/CD13 in various types of tumors, including lung, pancreatic, and colon cancers as well as acute lymphoblastic leukemia, has been reported to be associated with poor prognosis; however, the clinical significance of its expression in MPM remains to be elucidated. On the basis of these findings, we hypothesized that APN/CD13 is associated with MPM progression and is an attractive molecular target for the treatment of MPM by inhibiting angiogenesis. To investigate the clinical significance of APN/CD13 in MPM, we immunohistochemically evaluated APN/CD13 expression in resected tumors and analyzed its association with survival in MPM patients. The median survival time of patients with high levels of APN/CD13 expression was significantly shorter than that of patients with low levels of APN/CD13 expression. Moreover, a multivariate analysis demonstrated that both high APN/CD13 expression and histology (non-epithelioid) were independently associated with poor prognosis. To determine the efficacy of APN/CD13 as a molecular target in MPM, we examined the antitumor effects of MT95-4, a fully humanized anti-APN/CD13 mAb, in an orthotopic implantation mouse model of MPM, using the MPM cell lines EHMES-10 (abundantly expressing APN/CD13) and MSTO-211H (scarcely expressing APN/CD13). MT95-4 treatment reduced tumor growth and angiogenesis in mice harboring EHMES-10, but not MSTO-211H cells. Furthermore, in mice harboring EHMES-10 cells, MT95-4 combined with cisplatin more effectively suppressed tumor progression than cisplatin alone. Further to investigate the mechanism underlying the anti-angiogenic effect of MT95-4, we evaluated whether MT95-4 directly affect the expression of angiogenic factors produced by tumor cells by ELISA and angiogenesis antibody array and found that MT95-4 did not affect the expression of angiogenic factors. Given that the angiogenic factors did not play a key role in the

antiangiogenic effect of MT95-4, we hypothesized that the antiangiogenic effect of MT95-4 *in vivo* was achieved by the inhibition of extracellular matrix degradation. A matrix degradation assay showed that the invasiveness of vascular endothelial cells was enhanced in the presence of APN/CD13 and MT95-4 suppressed extracellular matrix degradation by tumor cells by inhibiting APN/CD13 activity. Consequently, we suggest that the antiangiogenic effect of MT95-4 treatment was achieved by the inhibition of APN/CD13 activity, which is involved in the degradation of extracellular matrix elements, thereby inhibiting the release and migration of vascular endothelial cells into the perivascular stroma. In conclusion, we showed that APN/CD13 expression was potentially associated with poor prognosis in MPM patients. Moreover, our results revealed that MT95-4 suppressed tumor progression likely by inhibiting angiogenesis in an orthotopic implantation mouse model of MPM exhibiting high levels of APN/CD13 expression. Additionally, we demonstrated that MT95-4 in combination with cisplatin could be a promising therapeutic strategy for the treatment of MPM-specific tumors exhibiting high levels of APN/CD13 expression.