# 学位論文審査結果の要旨

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（学位論文審査結果の要旨）

CD44v9 Induces Stem Cell-Like Phenotypes in Human Cholangiocarcinoma

【主論文審査結果の要旨】

著者らは論文において下記の内容を述べている。

**Background:** Our previous study demonstrated an overexpression of CD44 variant 9 (CD44v9) in human cholangiocarcinoma (CCA) tissues that was associated with inflammation-related tumor development. However, the participation of CD44v9 in cholangiocarcinogenesis remains poorly understood. Therefore, in this study, we examined the potential roles of CD44v9 in CCA cells to understand the carcinogenic mechanism.
**Methods:** Using normal cholangiocytes (MMNK1) and CCA cells (KKU213), the expression levels of CD44v9 and its related molecules were quantified through RT-qPCR and immunofluorescence (IF) staining. To evaluate its biological functions, we performed CD44v9 (exon 13) silencing using siRNA transfection, and assessed cell proliferation through MTT assay, cell migration and invasion by transwell technique, and carried out cell cycle analysis by flow cytometry. *In vivo* tumor growth was assessed by nude mouse xenografts, and histological and molecular changes were determined.
**Results:** KKU213 exhibited higher protein expression levels of CD44v9 than those of MMNK1 through IF staining. RT-qPCR analysis revealed that the mRNA expression level of CD44v9 was predominantly elevated in CCA cells along with its neighboring exons such as variant 8 and 10,

minimally affecting the standard form of CD44. CD44v9 silencing could regulate redox system in CCA cells by reducing the expression levels of SOD3 and cysteine transporter xCT. CD44v9 silencing suppressed the CCA cell proliferation by induction of apoptosis and cell cycle arrest. Migration and invasion were decreased in CD44v9 siRNA-treated CCA cells. CD44v9 downregulation inhibited CCA tumor growth in mouse xenografts. IF analysis demonstrated the histological changes in xenograft tissues such as an increase in connective tissues through collagen deposition and reduction of hyaluronic acid synthesis through CD44v9 silencing. CD44v9 knockdown *in vitro* and *in vivo* increased E-cadherin and reduced vimentin expression levels, resulting in reduction of epithelial-mesenchymal transition (EMT) process. Moreover, CD44v9 modulated Wnt10a and β-catenin in tumorigenesis.

**Conclusion:** Our results indicate that CD44v9 plays a potential role in CCA development by the regulation of cell proliferation and redox balancing. CD44v9 silencing may suppress tumor growth, migration and invasion through EMT: a finding that could potentially be applied in the development of targeted cancer therapy.

不死化正常胆管細胞株 MMNK1 に比べ、ヒト由来胆管癌細胞株 KKU213 において CD44v9 が高発現し、その抑制により細胞増殖や遊走能・浸潤能を低下し得ることを明らかにした。また、CD44v9 が化学療法抵抗性に関与することが示され、CD44v9 が胆管癌の治療標的分子となる可能性を示した論文であり、学術上極めて有益であり、学位論文として価値あるものと認めた。

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