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Dissertation Abstract

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Mac-2-binding protein glycan isomer enhances the aggressiveness of hepatocellular carcinoma by activating mTOR signaling

(Mac-2結合蛋白糖鎖修飾異性体はmTORシグナル伝達経路を活性化することにより肝細胞癌の悪性度を増悪させる)

Background: Liver cancer is the fourth leading cause of cancer-related death worldwide. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, mostly occurs in people with liver cirrhosis. For HCC patients with severe liver fibrosis, treatment option is limited. Therefore, deeper understanding of HCC in fibrotic liver is important for clinical management of HCC. Wisteria floribunda agglutinin $(WFA)^+$ Mac-2 binding protein (M2BPGi) is a novel serum marker for liver fibrosis. Clinical studies reported that an elevated serum level of M2BPGi can predict development of HCC in cirrhotic liver and poor prognosis of HCC patients after hepatic resection. By experimental study, M2BPGi is produced by hepatic stellate cell in the patients with liver fibrosis. However, no studies explained relationship between M2BPGi and aggressiveness of HCC. Galectin-3 in the most known binding partner of M2BP, and high expression of galectin-3 in HCC is associated with poor prognosis. Therefore, this study aimed to clarify the localization of M2BPGi, galectin-3 and *M2BP mRNA* on the HCC tissue, further to analyze effect of M2BPGi against HCC.

Materials and method: The protein expression of M2BPGi and galectin-3 and the *mRNA* expression of *M2BP* were evaluated in surgically resected human HCC samples. Localization of proteins were evaluated using immunofluorescence method, while *M2BP mRNA* were detected using RNAscope assay. Recombinant M2BPGi (rM2BPGi) were used to analyze the function on the human HCC cell lines (PLC/PRF/5, HepG2 and Huh7) using proliferation, invasion and wound healing assay. In addition, effect of M2BPGi on HCC were evaluated in-vivo on the subcutaneous xenograft using subcutaneous mini-osmotic pump for constant administration of treatment. To check importance of M2BP ligand, we downregulated galectin-3 in the HCC cells by siRNA transfection, further the cells are treated with rM2BPGi and underwent functional assessment. Galectin-3 and M2BP binding were evaluated HCC cell lysate with and without rM2BPGi using co-immunoprecipitation method. Transcriptome analysis were performed on HCC cell lines with and without rM2BPGi treatment. Rapamycin (mTOR inhibitor) used to confirm the result of transcriptome analysis.

Results: By immunofluorescence study, M2BPGi and galectin-3, as a ligand of M2BP, were co-localized on the HCC cells in the tumor area of the tissue. In the adjacent non-tumor area, M2BPGi and galectin-3 were co-localized on the CD68 positive cells (Kupffer cell). *M2BP mRNA* were expressed on stroma of the cirrhotic liver and HCC. rM2BPGi enhanced the proliferation and invasion of the HCC cells. In-vivo experiment confirmed that treatment of M2BPGi promoted tumor growth in HCC-bearing mouse model. In addition, the ki-67 labeling index was higher in M2BPGi-treated tumors than in PBS-treated tumors. Proliferation invasion ability of M2BPGi treated HCC cell were decreased due to galectin-3 downregulation. Co-immunoprecipitation analysis showed cellular galectin-3 binds with rM2BPGi on the HCC cell line. In addition, rM2BPGi treatment enhanced the membranous galectin-3 expression on the HCC cell. Transcriptome analysis revealed that activation of mTOR signaling due to M2BPGi treatment on HCC cell line. Activation of mTOR signaling in the HCC cell were analyzed after M2BPGi treatment by western blotting. Proliferation of M2BPGi-treated cells were decreased after treatment of mTOR inhibitor. Moreover, M2BPGi-induced mTOR signaling were cancelled by galectin-3 downregulation in the HCC cell line.

Discussion: In this study, we focused on the liver fibrosis marker, M2BPGi and its binding partner galectin-3 in the HCC. Our result suggested that M2BPGi is derived from stromal cells of the cirrhotic liver and may be interact with HCC cells by galectin-3. We further showed that M2BPGi could bind to and induce the membrane expression of galectin-3 on the HCC cells. Moreover, M2BPGi promoted HCC malignancy in vitro by binding with galectin-3, which consequently activated mTOR signaling. This was confirmed by the observation that galectin-3 knockdown significantly inhibited the effect of M2BPGi on HCC cells. Our in vivo findings were consistent with the *in vitro* data and further demonstrated the importance of M2BPGi-induced mTOR signaling on HCC growth. Other researchers has reported that activation of mTOR signaling is potential indicator of poor prognosis in patients with HCC however mTOR inhibitor treatment did not improve the clinical efficacy in patients with advanced HCC. Therefore our findings suggests that mTOR inhibitor treatment might be helpful for the treatment of mTOR-activated HCC patients with high level of M2BPGi.

Conclusion: M2BPGi is produced by stromal cells in the cirrhotic liver and interact with HCC cells, further it enhances the progression of HCC by activating mTOR signaling in the presence of galectin-3. M2BPGi may serve as a link between HCC and stromal cells and may thus explain the fundamental mechanism underlying HCC progression. Accordingly, M2BPGi may be a promising therapeutic target in patients with liver cirrhosis who develop HCC. (Word count: 765)