論　文　概　要

論 文 題　目 : Mechanism for suppression of metastasis activity in cancer cells by expression of core 3 *O*-glycan
（Core 3 *O*-グリカンの発現による癌細胞転移能抑制メカニズムの研究）

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目的:

The mucin-type *O*-glycans attached to the serine and threonine residues of proteins are diverse in structure and function. Alterations can be occurred in various mucin type *O*-glycan structures during tumor progression. Core 3 structure is a mucin-type *O*-glycan that plays important roles in differentiation and mucosal barrier formation in digestive organs. Core 3 structure is synthesized by the core 3 synthase or B3GNT6. B3GNT6 was cloned by Iwai et al, then B3GNT6-expressing cells and B3GNT6 antibody (G8-144) were established for revealing the role of B3GNT6. Various studies of B3GNT6 and its tumor suppression activities have been reported, for instance, the expression of B3GNT6 in prostate cancer cell lines decreased migration and invasion of the cells, and intravascularly injected B3GNT6-expressing cells showed decreased metastasis to the lung in nude mice. B3GNT6 expression was reported to suppress ~~of~~ tumor growth and metastatic activities in pancreatic cancer cell lines. The immunohistochemistry (IHC) study using G8-144 found that the expression of B3GNT6 was depleted in gastric and colon cancer tissues. Recently, it has been proposed that B3GNT6 expression on MUC1 regulates EMT-MET plasticity via the MUC1/p53/miR-200c signaling cascade, suggesting that the expression of B3GNT6 correlates with prevention of cancer progression. However, the mechanism and role of B3GNT6 in cancer cells are not understood. Therefore, in this study I aimed to elucidate the role of B3GNT6 in cancer and to evaluate B3GNT6 expression levels in cancer and its possibility to use as diagnosis and prognosis marker.

対象と方法:

This study has been divided into two parts, which are biochemical studies on the B3GNT6-expressing cells and clinicopathological analysis using G8-144 antibody. The B3GNT6-expressing cells were established and the effects of B3GNT6 on glycan structures, migration activity, EMT induction, and gene expression profiles were evaluated in these cells. For the clinicopathological part, G8-144 antibody was subjected to IHC on extrahepatic cholangiocarcinoma (eCCA) tissues, which are perihilar (pCCA) and distal (dCCA) type tissues. Moreover, the clinicopathological correlation of B3GNT6 expression of eCCA patients was investigated in comparison with MECA-79 expression.

結果:

Immunocytochemistry was done using peanut agglutinin (PNA) and jacalin for determining the expression of core-1 and core-3 structures respectively. I found that core-1 structures were decreased while jacalin staining showed high expression of core-3 glycan structures in B3GNT6-expressed cells but not mock cells. Besides, the transwell migration assay showed that B3GNT6-expressed A549 cells were decreased by 36.48% of migration activity than control cells. From the staining of eCCA specimens, we found that patients with B3GNT6 positive in dCCA were significantly correlated with a long-life span than B3GNT6-negative patients, while intrahepatic and extrahepatic perihilar types were not.

考察:

The alteration of the *O*-glycan structures in cancer is one of the characteristics responsible for its aggressiveness. B3GNT6 expression has been reported to suppress tumor progression and inhibit metastasis in sarcoma, pancreatic, and prostate cancer cells, while aberrant *O*-glycans, such as Tn and STn, are increased in various carcinomas. As the results from glycan structures, migration activity, EMT induction, and gene expression profile studies of B3GNT6-expressing cells, I suggested that the cancer cells expressing B3GNT6 are less malignant than those with negative expressions. Additionally, stable expression of B3GNT6 altered expression of proteins carrying *O*-glycans, such as reduced core 1 synthesis via a competition to utilize 3' position of GalNAc on the glycoproteins. Furthermore, the trans-well migration assay showed that the number of migrated B3GNT6-expressing cells were decreased. Therefore, B3GNT6 would be a favorable marker for some cancer diagnoses and B3GNT6 or its product, core 3 *O*-glycans, could inhibit cancer migration and metastasis. In the present study, B3GNT6 was found to be positive in eCCA and was mainly observed in the non-invasive area. Importantly, the detection of B3GNT6 in dCCA patients was significantly associated with a longer survival rate than B3GNT6-negative patients. Further, the MECA-79 epitope was identified to be increased upon cancer progression and is associated with poor prognosis in gastric cancers. MECA-79 was thus identified to be associated with a shorter survival rate of dCCA, especially when expressed in invasive cancer areas. Based on the statistical analyses, MECA-79 positivity was found to be significantly correlated with the cancer metastasis factors.

結論:

Induction of B3GNT6 reduced core-1 synthesis probably due to a competition to utilize 3’ position of GalNAc on the glycoproteins and decreased migration activity in A549 and MCF7 cells. B3GNT6-expressing cells seem to exhibit reduced EMT induction, this experiment will be further elucidated. Expression of B3GNT6 in CCA extrahepatic distal type were significantly correlated with a long-life span of patients, in contrast with MECA-79. These findings imply that these two antibodies could be employed to analyze the *O*-glycan pathways to predict the prognosis of patients with dCCA.