主　論　文

β-1,3-galactosyl-*O*-glycosyl-glycoprotein β-1,6-*N*-acetylglucosaminyltransferase 3 increases MCAM stability, which enhances S100A8/A9-mediated cancer motility

（β-1,3-galactosyl-*O*-glycosyl-glycoprotein β-1,6-*N*-acetylglucosaminyltransferase 3は、MCAM タンパク質の安定性を上昇させ、S100A8/A9 誘導性のがん細胞走化性を促進する）

[緒言]

A single glycosyltransferase can induce glycosylation of many different proteins, especially in integrated membrane proteins and secreted proteins, through an endoplasmic reticulum (ER)/Golgi pathway. S100A8 and S100A9 are small EF-hand calcium-binding proteins belonging to the S100 family and physiologically form a heterodimer complex, simply termed S100A8/A9 or calprotectin, a major functional form reported to be in close association with cancer metastasis. Our search for other unknown receptors for S100A8/A9 resulted in the discovery of novel S100A8/A9 receptors (S100 Soil Sensor Receptors (SSSRs)) in cancer cells, EMMPRIN, MCAM and ALCAM, which play a critical role in cancer metastasis. These SSSRs are highly glycosylated, owing to their cell surface location as receptors utilizing the ER/Golgi pathway.

In this study, we found that the β-1,3-galactosyl-O-glycosyl-glycoprotein β-1,6-N-acetylglucosaminyltransferase 3 (GCNT3) was efficiently up-regulated in malignant melanoma cells, lung cancer cells and mesothelioma cells compared to their non-malignant immortalized cell counterparts. GCNT3 induced a marked increase in S100A8/A9-mediated cell migration and invasion through the functional activation of MCAM. Interestingly, we also found that GCNT3-mediated glycosylation of MCAM is elevated in cancer cells that are linked to an increase of stability between the engagement of S100A8/A9 and MCAM.

[材料と方法]

The following two human melanoma cell lines established from the same patient were used WM-115 and WM-266-4 (ATCC, Rockville, MD, USA) throughout the experiments. Cells were transiently transfected with the plasmid vectors using FuGENE-HD (Promega BioSciences, San Luis Obispo, CA, USA). siRNAs (Ambion, Austin, TX, USA) were transfected using Lipofectamin RNAiMAX reagent (Invitrogen, Carlsbad, CA, USA). The stable clones were established by a convenient electroporation gene delivery method. Western and Northern blot analysis was performed under conventional conditions for detection of protein and mRNA respectively. Quantitative real-time PCR was performed on a LightCycler rapid thermal cycler system. Cell invasion or migration was assayed using the Boyden chamber method. The studies using patient-derived tissue sections were approved by the research ethics committees of Niigata University Medical and Dental Hospital, the specimens only used at Niigata University. Written informed consent was obtained from each patient for use of the materials.

[結果]

To identify a glycosyltransferase(s) that is associated with metastatic signatures via S100A8/A9-SSSRs signaling, first we compared the expression levels of key glycosyltransferase-encoding genes in non-metastatic WM-115 and in highly metastatic WM-266-4 cells. The results showed three genes (GALNT12, GCNT3 and MGAT4A) that outstandingly displayed high expression levels. Next, we examined whether the candidate genes show any correlation to S100A8/A9-mediated cancer metastasis. Interestingly, the highest activity was provided by the forced expression of GCNT3. We also found that MCAM was the only protein required for acceleration of S100A8/A9-mediated migration and invasion in WM-115 cells. To examine the clinical relevance of expression patterns of GCNT3 and MCAM using tissues obtained from melanoma patients. We found that MCAM was strongly stained with membrane localization and GCNT3 was also clearly positive in the intracellular area of melanoma regions. These results indicate that GCNT3 expression is positively correlated with MCAM expression in melanoma tissue.

We next investigated the physiological role of GCNT3 in S100A8/A9-MCAM axis-mediated cancer motility using siRNA in WM-266-4 cells. Higher expression of GCNT3 was confirmed in WM-266-4 cells than in WM-115 cells at the protein level. We found that MCAM was upregulated in WM-266-4 cells. Interestingly, we found that the level of MCAM was significantly decreased by downregulation of GCNT3 using siRNA and by talniflumate. The GCNT3 siRNA and talniflumate had no appreciable effect on MCAM expression at the mRNA level in WM-266-4 cells. In addition, we found that both GCNT3 siRNA-mediated GCNT3 suppression and talniflumate-mediated GCNT3 suppression significantly attenuated the basal ability of in vitro migration of WM-266-4 cells. The same phenomena were also observed by using WM-115 GCNT3 stable clones. Collectively, the results indicate that GCNT3 plays a pivotal role in maintenance of MCAM protein at a high level, resulting in acquisition of strong responsiveness to S100A8/A9 that is linked to increased cellular migration and invasion.

To gain an insight into the mechanisms by which GCNT3 maintains MCAM protein at a high level, we examined the half-life of intrinsic MCAM protein in WM-115 cells using cycloheximide. Interestingly, we found that MCAM degradation was significantly delayed in cells transfected with GCNT3. The difference was obvious at 12 and 24 hours after treatment with cycloheximide. To determine how GCNT3 was able to have a prolonged effect on MCAM stability, we attempted to detect specific glycosylation on MCAM. We found that the immunoprecipitated MCAM showed higher levels of GlcNac when WM-115 cells were transfected with aberrant GCNT3. We also confirmed that GlcNAc modification level was greatly attenuated by the expression of the catalytic dead mutGCNT3 but not wtGCNT3. Similar phenomenon was also observed in the WM115-derived clone. Finally, we found that forced expression of mutGCNT3 significantly downregulated migration activity of WM-266-4 cells under either the presence or absence of S100A8/A9 in culture. Taken as a whole, our results indicate that GCNT3 controls MCAM stability by its catalytic activity-mediated glycosyl modification that correlates with a greater ability for cancer cell motility and invasion in response to extracellular S100A8/A9

[考察]

It is well known that some organs are more prone than others to metastasis from certain types of cancer. This phenomenon was first discussed by Stephen Paget et al. as the "Seed and Soil" theory over a century ago in 1889. Cell surface proteins including receptors are commonly glycosylated, and such glycosylation patterns are usually altered during the process of cancer formation and its subsequent progression. However, the glycosyltransferases that regulate the glycosyl modification of cell surface proteins are still not well understood in cancer metastasis involved in the S100A8/A9-SSSRs axis. To our knowledge, this report is the first report showing that GCNT3, a glycosyltransferase dominantly expressed in aggressive melanoma, increased the stability of only MCAM among the SSSRs via GlcNac modification that lead to an enhanced responsiveness to S100A8/A9.

Rao and colleagues reported interesting data regarding GCNT3 and pancreatic cancer and they suggested clinical relevance between GCNT3 overexpression and malignancy in pancreatic cancers. Through an in vitro approach, they found that forced expression of GCNT3 was associated with cancer aggressiveness and that GCNT3-KO as well as GCNT3 inhibition with talniflumate, significantly reduced cell viability and spheroid formation. These findings corresponded well to our results for melanoma with the possible inclusion of other cancers, such as skin, lung and breast cancers and mesotheliomas, that showed higher expression levels of GCNT3 consistently or partially.

Next, we focused on how GCNT3 is involved in increasing the half-life of MCAM. We showed that MCAM was glycosylated by GCNT3 through its catalytic activity, resulting in extra attachments of GlcNAc. It has been reported that the addition of β1-6 GlcNAc branching on integrin β1 caused a more fully glycosylated and mature form of integrin β1. The addition of β1-6 GlcNAc branching also inhibited the β1 protein degradation in human hepatocellular carcinoma cells, which was linked to the promotion of integrin-dependent migration and invasion. This process was mediated by another glycosyltransferase, MGAT5. It is reasonable to assume that a similar process may apply to MCAM.

[結論]

We demonstrated in this study that GCNT3 has critical role in cancer migration and invasion through positive regulation of MCAM stability. Further analysis of this new axis between GCNT3 and MCAM may provide a better understanding of cancer metastasis that is affected by S100A8/A9. The result of this study may also be useful for the design a promising strategy for therapy of various cancer types.