論文の内容の要旨

獣医学 専攻

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Understanding the functional role of selected bioactive compounds and altered genes on gastric carcinogenesis of the differentiated subtype using *A4gnt*-deficient mice model

（*A4gnt* 欠損マウスを用いた分化型胃がんに対する特定の生理活性物質と関連遺伝子の機能的役割の研究）

An emerging frontier in gastric cancer (GC) research has unveiled the role of a defective glycosylation in the gastric gland mucin function. Loss of the *O*-glycan terminal, α1, 4-linked *N*-acetylglucosamine residues (αGlcNAc) on the MUC6 scaffold protein has connoted a strong malignant repercussion with differentiated subtype orientation. Similarly in mice, deletion of the glycosyltransferase gene- α1, 4-*N*-acetylglucosaminyltransferase (gene symbol: *A4gnt*) was tightly associated with a concomitant abolition of α1GlcNAc expression leading to a well-recapitulated stepwise GC progression. In the present dissertation, I took advantage of this *A4gnt* knockout (KO) mice model and established whether it could facilitate both bioactivity testing and gene function studies.

In the first chapter, I clarified the efficacy of a brown seaweed-derived soluble-type β-glucan (Laminaran) on the premalignant cancer progression using 12-15 week-old *A4gnt* KO mice that specifically display low-grade gastric dysplasia. Following three consecutive weeks of treatment, Laminaran caused a marked attenuation of the gross mucosal lesion, which corresponded histologically to a substantial inhibition of the pyloric mucosal thickness and polymorphonuclear lymphocytes (PMNLs) infiltration. Moreover, as opposed to the untreated control group, Laminaran effectively counterbalanced the increased induction in both proliferative and angiogenic processes and modulated the gene transcripts of several inflammation-associated cytokine showing significant restoration of *Il10* expression and considerable repression of *Il11* gene transcription. These findings altogether indicate that Laminaran can exert a remarkable restraint on the progressive development of gastric dysplasia.

In the second chapter, the preferential utility of the 60-week old *A4gnt* KO mice that exhibit full-blown differentiated-type GC has assisted crucially in illuminating the antitumor property of the crude ethanolic extract of propolis (EEP) from the Philippine stingless bees (*Tetragonula biroi* Friese). In this study, I highlighted the first indication of a significant subtype specificity of EEP towards differentiated subtype of GC but not diffuse-type as ascribed from the results derived on five different human gastric cancer cell lines. Mechanistically, this was found to involve a profound modulation of several cell cycle related gene transcripts, which notably correlated with cell cycle arrest at the G0/G1 phase. To reinforce these observations, EEP-treated *A4gnt* KO mice demonstrated a significant regression of the gross and histological lesions of gastric pyloric tumors in contrast with the corresponding KO control animals. Also, the specific gene expression regulation of selected cell cycle components and the considerable augmentation of p21 protein coupled with the marked reduction of actively dividing S-phase cells irrevocably supported these observations. Together, these findings alluded to the significant tumor suppressive activity of the Philippine stingless bee propolis, which can be potentially exploited as an adjunct therapeutic option in cases of non-invasive differentiated-type GC.

Lastly for the third chapter, I explored the plausibility of harnessing the capacity of *A4gnt* KO mice to shed light on the apparent obscurity in the contribution of the gastrointestinal tract-tropic galactose binding lectin, galectin-4 (gene symbol: *Lgals4*) in gastric carcinogenesis. Here, I initially validated the strong affinity of galectin-4 towards differentiated-type orientation in different established models of GC including canine cases of gastric mucosal lesions, human gastric cancer cell lines, and *A4gnt* KO mice. A novel *A4gnt/Lgals4* double KO (DKO) mouse was subsequently generated to comprehensively ascertain the stage-specific *in vivo* action of galectin-4. As clearly shown by the combined morphological and gene expression analyses, these DKO mice exemplified two distinct phenotypic traits. At 10- and 30 weeks of age, the relative increase in pyloric mucosal thickness was accompanied by a heightened proliferative and angiogenic processes and upregulated transcription of few pro-inflammatory cytokines and chemokine ligands. Contrastingly, at 50-week old, these DKO mice exemplified a profound tumor growth restriction, depressed proliferation and angiogenesis, and down-expressed gene expression of pro-inflammatory cytokines and chemokines. Interestingly, irrespective of the age (or cancer stage), the magnitude of the distribution of the infiltrating T-lymphocytes and macrophages in the *A4gnt/Lgals4* DKO animals were

significantly dampened as opposed to those of the *A4gnt* KO counterpart. Furthermore, using an additional novel model, *A4gnt/Il11* DKO and *A4gnt/Lgals4/Il11* triple KO (TKO) mice, I later proposed that in these 50-week old *A4gnt/Lgals4* DKO mice, IL-11/STAT3 signaling appeared to be a critical regulatory pathway as divulged by the profound decrement in the phosphorylated-STAT3 (P-STAT3) profile and the pronounced improvement in all the above-mentioned parameters in comparison with the corresponding *A4gnt* KO control.

Based on the account of these above-mentioned studies, the two-fold objective of (1) probing the efficacy of selected bioactive compounds and (2) delimiting the function of an altered genes in GC was greatly realized following the conscious decision to employ this unique *A4gnt* KO mice. Truly, the utility of this *in vivo* animal model served as a powerful tool in acquiring a more holistic view of GC and in advancing the field of biomedical science.