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| 論文題目 | Beta-1,4-galactosyltransferase-3 deficiency suppresses the growth of immunogenic tumors in mice<br>（ガラクトース転移酵素-3 欠損マウスは高免疫原性腫瘍の増殖を抑制する） | | |

（論文内容の要旨）


Beta-1,4-galactosyltransferase-3 (B4GALT3) is a member of the beta-1,4-galactosyltransferases (B4GALTs) family, involved in the transfer of UDP-galactose to terminal *N*-acetylglucosamine residues in glycoproteins. Although B4GALT3 is differentially expressed in tumors and adjacent normal tissues and has a significant correlation with the clinical prognosis in a range of cancers such as neuroblastoma, cervical, and bladder cancer, the precise role of B4GALT3 in the tumor immune microenvironment (TIME) remains largely unexplored. The aim of this study is to unravel the functions of B4GALT3 in the TIME.

The subcutaneous transplantation of either weakly or strongly immunogenic tumor cells into wild-type (WT) and *B4galt3* knockout (KO) mice revealed that tumor cell growth was suppressed in KO mice. Bone marrow transplantation and $CD8^+$ T cell depletion experiments elucidated the pivotal role of immune cells in suppressing tumor growth in *B4galt3* KO mice. Analyses of the cell types and gene expression in the TIME was undertaken through flow cytometry and RNA sequencing. The results showed that *B4galt3* KO mice manifested suppressed growth of strongly immunogenic tumors, a phenomenon accompanied by a marked increase in $CD8^+$ T cell infiltration within the tumors. Other family members, B4GALT1 nor B4GALT4, were not involved in the reduction of immunogenic tumors, suggesting differences in substrates and activity among B4GALT family members. *N*-glycosylated proteins from WT and *B4galt3* KO mice were compared using liquid chromatography tandem mass spectrometry (LC-MS/MS)-based glycoproteomic analyses to identify proteins that are glycosylated by B4GALT3. *N*-glycan modification of several proteins were altered, including the pivotal integrin alpha L (ITGAL), which plays a crucial role in T cell infiltration, activity and proliferation. The *B4galt3* deficiency showed that KO $CD8^+$ T cells exhibited heightened susceptibility to activation, showing enhanced downstream phosphorylation of focal adhesion kinase (FAK), a phenomenon intricately linked to ITGAL signaling. It has been reported that changes in the conformation of ITGAL can influence downstream signal transmission. Although there are antibodies capable of identifying these specific conformations, unfortunately, they are only applicable to humans, rendering it impossible to analyze the conformational changes in *B4galt3* KO mice. This represents a limitation of this research.

The study indicates that the potential of *B4galt3* deficiency in amplifying anti-tumor immune responses, predominantly through higher $CD8^+$ T cell influx into the TIME. It suggests that B4GALT3 might have suppressive roles in cancer immunity by modulating the glycan structure of molecules present on the $CD8^+$ T cell surface, a hypothesis supported by the observed alterations in the glycan structures in immune cells. An important finding was the absence of adverse effects on growth, development, or reproduction in *B4galt3* KO mice, suggesting B4GALT3 as a promising and safe therapeutic target against cancer.


（論文審査の結果の要旨）

本研究は、β1,4-ガラクトース転移酵素の１つである *B4galt3* が生合成に関与する糖鎖の役割に注目し、その欠損ががん免疫微小環境における免疫細胞に与える影響を解析した。

B4GALT3 のがん免疫微小環境中の役割を解明するため、*B4galt3* 欠損マウスを作製し、免疫原性が異なる６種のがん細胞をマウスに移植したところ、*B4galt3* の欠損マウスが免疫原性の強いがん細胞の増殖だけを抑制することがわかった。FACS 解析および RNA-seq 解析により、この抑制は $CD8^+$ T 細胞の浸潤とその細胞障害活性の増強に関連していることを明らかにした。

また、質量分析装置（LC-MS/MS）を用いたグライコプロテオミクス解析により、*B4galt3* の欠損が $CD8^+$T 細胞のインテグリンαL (ITGAL) の *N* 型糖鎖修飾に影響を与えていた。糖鎖修飾の変化は ITGA 下流の FAK のリン酸化を増強し、$CD8^+$T 細胞の活性化と浸潤能力の増強に関連していることが示唆された。

以上の研究は B4GALT3 による糖鎖付加ががん免疫微小環境における免疫応答を制御することを明らかにし、糖鎖を標的とした新しいがん治療法の開発への応用の可能性が期待できる。

したがって、本論文は博士（　医科学　）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、令和５年１１月２２日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日：　　年　　月　　日　以降