学位論文内容の要旨
(Summary of Dissertation)

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学 位 論 文 題 名（Dissertation Title）
Studies on the functional roles of biglycan in tumor microenvironment
(腫瘍微小環境におけるbiglycan の役割に関する研究)

Background and Purpose:

Tumor growth and metastasis depend on tumor blood vessels. Tumor vasculature is structurally and functionally abnormal. Tumor blood vessels are immature and leaky, and cannot sufficiently transport anticancer drugs and immune cells that attack cancer cells. In addition, tumor is excessively fibrotic, which is a barrier to the migration of immune cells leading to an insufficient response of immunotherapy and anticancer drug treatment. Normalization of tumor blood vessels benefits tumor microenvironment by promoting tumor blood flow, reducing hypoxia and increasing drug delivery. Thus, normalization of tumor vasculature enhances the effects of anticancer drugs and immunotherapy. Previously we have found biglycan is a proteoglycan in the extracellular matrix is highly expressed in tumor endothelial cells. Biglycan secreted from tumor endothelial cells induces tumor angiogenesis and facilitates tumor metastasis. Furthermore, biglycan expressed tumor blood vessels in lung cancer patients are associated with a poor prognosis in our recent findings. The antitumor effect by targeting biglycan was expected, but the inhibitory effect in tumor mouse model was unknown. In the current study, we focused on the functional role of stromal biglycan in breast cancer microenvironment using biglycan-knockout (*Bgn* KO) mice, especially in tumor angiogenesis and immune responses.

Materials and Methods:

By bioinformatics analysis, we compared and analyzed Biglycan expression and patient prognosis in human breast cancer patients using TCGA database. Murine breast cancer model was created using *Bgn* KO mice and wild-type mice. Tumor growth and metastasis were evaluated. The structure of tumor blood vessels was examined by immunostaining using tumor tissue specimens by comparing blood vessel density, the number of pericyte-covered blood vessels lectin-positive blood vessels and hypoxic regions. We also evaluated the number of CD8-positive T cell infiltrations and cancer fibrosis. Furthermore, we analyzed the amount of drug delivery to cancer tissue via blood vessels and compared the therapeutic effects of the anticancer drug paclitaxel between *Bgn* KO and wild tumors.

Results:

Breast cancer patients with high Biglycan expression had shorter progression-free survival, suggesting that biglycan expression is a poor prognostic factor. Furthermore, biglycan expression was higher in

the tumor stromal compartment compared to the epithelial compartment. Knockout of biglycan in the stroma in E0771 tumor-bearing mice inhibited metastasis to the lung without affecting tumor growth. Histologically, *Bgn* KO was impaired tumor angiogenesis, the number of blood vessels covered with pericytes increased, and blood vessels were normalized by repressing tumor necrosis factor-α/angiopoietin 2 signaling. Moreover, fibrosis was suppressed and CD8+ T-cell infiltration was increased in tumor-bearing *Bgn* KO mice. Furthermore, chemotherapy drug delivery and efficacy were improved *in vivo* in *Bgn* KO mice.

Discussion:

Biglycan has been extensively investigated in cancer cells. Biglycan has bidirectional roles modulating tumor growth and progression. However, the expression and function of stroma biglycan in tumor microenvironment required to be elucidated. By bioinformatics analysis, biglycan was only expressed in tumor blood vessels of mouse breast cancers, and not in normal mammary gland tissue. Furthermore, co-expression analysis by cBioPortal showed that BGN was positively associated with PECAM1 and ANGPT2 expression in human breast cancers. Both PECAM1 and ANGPT2 genes are encoding the angiogenesis related molecules, which means that BGN is involved in regulating angiogenesis. In the current study, we found that stromal biglycan inhibition enhanced chemotherapeutic efficacy through normalization of not only the vasculature by downregulation of Angpt2 expression resulting in increased oxygen perfusion and the delivery of chemotherapy agents. To our knowledge, this is the first report demonstrating that stromal biglycan mediates the abnormality of the tumor vasculature, suggesting that biglycan may be regarded as a promising candidate to normalization of tumor vasculature in breast cancer. Furthermore, biglycan depletion in stroma suppressed tumor fibrosis as well as downregulated collagen I expression. Furthermore, α -SMA + fibroblasts were fewer in biglycan depleted tumors. Biglycan may activate CAFs via upregulating α-SMA expression, thus enhancing fibrosis. Increasing ECM stiffness can enhance cancer progression and metastasis.

Thus, a novel target (biglycan) has been discovered to normalize the tumor microenvironment by normalization of tumor vasculature, suppressing cancer fibrosis and improvement immune cell recruitment. In addition, biglycan is expected to enhance the therapeutic effect of immunotherapy and anticancer drugs and reduce side effects. Normalization of blood vessels that carry drugs and suppression of fibrosis of cancer tissues are expected to lead to enhanced effects of anticancer drug treatment and immunotherapy. The therapeutic efficacy of targeting biglycan combined with immunotherapy will be needed to be discovered. To target biglycan in specific cells, biglycan conditional knock-out mice will be used in the future study. Research is also expected to establish the development of inhibitors targeting Biglycan and effective administration methods.

Conclusion:

Our results showed that inhibition of biglycan enhances the effects of anticancer drugs and the effects of cancer immunity through normalization of the cancer microenvironment including tumor blood vessels. Targeting stromal biglycan may yield a potent and superior anti-cancer effect in breast cancer.