学 位 論 文 の 要 旨

氏名　GONG　AO

学　位　論　文　名	Tenascin-XB Plays a Role in the Infiltration of Immune Cells in Tumor Microenvironment
発　表　雑　誌　名 （巻，初頁～終頁，年）	Biomedical Research (Tokyo) (in press)
著　　者　　名	Ao Gong, Yuichi Iida, Kohei Kawakami, Kazuo Yamada, Yasuyuki Saito, Ken-ichi Matsumoto

論 文 内 容 の 要 旨

INTRODUCTION

The tumor microenvironment (TME) consists of various cell types besides tumor cells, including immune cells, fibroblasts, blood vessels, and extracellular matrix (ECM), which play crucial roles in tumor progression, metastasis, and immune suppression by secreting tumor-specific ECMs. Tenascins are a family of large, multifunctional glycoproteins of the ECM consisting of four members (tenascin-C, -R, -XB, and -W). Tenascin-C (TNC) and Tenascin-W (TNW) are particularly present in various tumor stroma and have been proposed as targets for anti-cancer therapies. Tenascin-XB/tenascin-X (TNXB) is involved in collagen deposition, stability, and fibrillogenesis. Deficiency of TNXB causes classical-like Ehlers-Danlos syndrome type 1 (clEDS1), characterized by hyperflexible skin, joint hypermobility, and easy bruising, without atrophic scarring. However, in tumor-related areas, there are few reports on TNXB except for its expression patterns in various tumor tissues. We previously reported that TNXB plays a tumor suppressor role, in which TNXB-deficient (*Tnxb*$^{-/-}$) mice exhibit enhanced tumor cell proliferation, invasion, and metastasis that were caused by increased matrix metalloproteinases (MMPs) activities and expression. However, the role of TNXB in TME remains obscure. This study aimed at elucidating the tumor suppressive mechanism of TNXB, especially focusing on the infiltration of immune cells in the TME.

MATERIALS AND METHODS

Six-week-old C57BL/6 (WT), BALB/c, and *Tnxb*$^{-/-}$ female mice were used for the experiment. B16-OVA melanoma cells (MO5) were inoculated subcutaneously into WT and *Tnxb*$^{-/-}$ mice. Tumor volume was measured every two days using calipers after 12 days of tumor inoculation. Survival rates were estimated using the Kaplan-Meier method. Mixed lymphocyte reactions (allo-MLR) were conducted by co-culturing splenocytes from WT or *Tnxb*$^{-/-}$ mice with mitomycin C-treated splenocytes from BALB/c mice. Additionally, T cell activation assay was also performed using splenocytes stimulated with phorbol myristate acetate (PMA) and ionomycin from WT and *Tnxb*$^{-/-}$ mice. These assays were followed by flow cytometry analysis (CytoFLEX, Bechman). RT-qPCR (TP900, Takara) was performed to assess gene expression of T cell activation-related cytokines and chemokines in naïve spleens and spleens and tumor tissues from tumor-bearing WT and *Tnxb*$^{-/-}$mice. IFN-γ concentration in the splenocytes supernatants from WT and *Tnxb*$^{-/-}$mice after stimulation with PMA and ionomycin was measured by ELISA. CD4^{+} and CD8^{+} T cell populations infiltrating into the tumor tissues and their activations were examined by flow cytometry analysis. Immunofluorescence staining was performed using the tumor tissue sections of WT and *Tnxb*$^{-/-}$ mice by confocal microscope (FV3000, Olympus). All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

After inoculation, tumor growth was significantly increased in *Tnxb*$^{-/-}$ mice than that in WT mice. Overall survival rates were reduced in *Tnxb*$^{-/-}$ mice than those in WT mice.

In vitro allo-MLR experiments showed that CD4^{+} and CD8^{+} T cells from *Tnxb*$^{-/-}$ mice exhibited significantly lower proliferation than those from WT mice after co-culture with splenocytes from BALB/c mice. However, the activation of CD4^{+} T cells from *Tnxb*$^{-/-}$ mice was significantly increased compared with that from WT mice, but not CD8^{+} T cells. Additionally, in T cell activation assay, the frequency of CD4^{+} and CD8^{+} effector memory T cells (T_{EM}) from *Tnxb*$^{-/-}$ mice was significantly higher than that from WT mice after stimulation with PMA and ionomycin for 20 h.

Gene expression of T cell activation-related cytokines and chemokines in WT and *Tnxb*$^{-/-}$ mice showed that *Tnf* and *Cxcl9* were significantly decreased in the naïve spleens, indicating that TNXB deficiency intrinsically contributes to poor immune response. After tumor inoculation, spleens of tumor-bearing mice showed similar results except for *Cxcl9* expression. In the tumor

tissues after tumor inoculation, the gene expression of T cell activation-related cytokines and chemokines *Il2, Il7, Cxcl9,* and *Cxcl10* and *Tnf* was significantly decreased in the *Tnxb*$^{-/-}$ mice. These results indicate that TNXB deficiency contributes to the downregulation of T cell activation-related cytokines and chemokines, particularly within tumor tissues, which may facilitate tumor progression. Similarly, IFN-γ concentration in the splenocyte supernatant of *Tnxb*$^{-/-}$ mice was significantly lower than that of WT mice after stimulation with PMA and ionomycin for 24 h and 48 h, indicating that the anti-tumor effect of the immune cells from *Tnxb*$^{-/-}$ mice by IFN-γ was weaker than that from WT mice *in vitro*.

Furthermore, analysis of T cell infiltration in tumor tissues by flow cytometry revealed a significant reduction of $CD8^+$ T cell population and its reduced activation in *Tnxb*$^{-/-}$ mice compared with those in WT mice *in vivo*, while $CD4^+$ T cell population and its activation were not significantly different. Immunofluorescence staining revealed reduced infiltration of $CD4^+$ and $CD8^+$ T cells in the tumor tissues of *Tnxb*$^{-/-}$ mice compared with that of WT mice. These results indicate that TNXB deficiency reduced the population of infiltrating $CD8^+$ T cells and impaired the activation of $CD8^+$ T cells in the tumor tissues.

CONCLUSION

This study revealed that although $CD4^+$ and $CD8^+$ T cells from *Tnxb*$^{-/-}$ mice tend to be activated more than those from WT mice, the infiltration population and overall activation level of $CD8^+$ T cells were attenuated in the tumor tissues of *Tnxb*$^{-/-}$ mice. This indicates that TNXB deficiency causes tumor progression by a significant reduction of $CD8^+$ T cell infiltration and its reduced activation in the tumor tissues. In addition, the poor immune response traits of *Tnxb*$^{-/-}$ mice are intrinsic and present even before tumor inoculation. This may be attributed to the reduced level of T cell activation-related cytokines and chemokines in *Tnxb*$^{-/-}$ mice. Our study provides new insights relevant to tumor control and immune response mediated by TNXB. Further studies are required to elucidate the precise molecular mechanisms by which TNXB influences immune cell infiltration and its activation. Evidence that TNXB suppresses tumor progression warrants further investigation.