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Comprehensive Analysis of *TNXB*-correlated Expressed Genes in Metastatic Lymphoid Tumor Cells Based on a Bioinformatic Approach

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Analyses of expression levels of tenascin-X gene（*TNXB*）in primary and metastatic tumor cells from 23 cancer tissues showed that *TNXB* expression is significantly downregulated in metastatic lymphoid tumor cells. Correlation analysis of *TNXB* expression with the expression of 19,193 genes in 22 metastatic lymphoid tumor cells showed that 4,654 genes have significant correlated expression. Among genes exhibiting correlated expression with *TNXB*, 62 genes showed upregulated expression in metastatic lymphoid tumor cells. Metascape analysis of the 62 upregulated genes revealed enrichment of genes related to immune response. TRRUST analysis of the 62 upregulated genes revealed 8 transcription factors with comprehensive transcriptional networks. Among them, *STAT1* was predicted to regulate expression of *CD40* and *CD86*, while *RELA* was predicted to regulate expression of *CD40*, *CD83*, *CD86*, *ALOX5* and *SLC25A27*. Downregulation of *TNXB* and associated upregulation of these target genes may play a role in certain characteristics of metastatic lymphoid tumor cells.

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INTRODUCTION

The tumor microenvironment consists of the extracellular matrix（ECM）along with tumor cells, immune cells, and stromal cells. The ECM plays pivotal roles in tumor progression, metastasis and immune suppression［1］. Tenascins（tenascin-C, -R, -XB, -W）are a family of ECM glycoproteins that modulate various cellular properties including adhesion, proliferation, migration and differentiation［2］. Tenascins have a common molecular organization, namely, a cysteine-rich segment at the amino terminus followed by epidermal growth factor（EGF）-like repeats, fibronectin type III（FNIII）-like repeats, and a fibrinogen-related（FBG）domain at the carboxy terminus［3, 4］. Tenascin-C（TNC）［5］ and tenascin-W（TNW）［6］ have been shown by many studies since their discoveries to have intricate links to tumor progression. TNC is highly expressed in tumor cells as well as stromal cells. Many data indicate a supportive role of TNC in tumor growth, metastasis, angiogenesis and suppression of immune surveillance［5］. Murdamoothoo *et al*.［7］ showed that retention of $CD8^+$ tumor-infiltrating T lymphocytes by TNC/CXCL12/CXCR4 in TNC-rich stroma in breast cancer prevents these cells from reaching and killing the tumor cells, resulting in tumor growth and subsequent metastasis. Similarly, TNC

also provides an immune-suppressive lymphoid stroma via TNC/CCL21/CCR7 in oral squamous cell carcinoma [8]. Similar to TNC, TNW is prominently expressed in the tumor stroma and is often found to be localized in the perivascular stroma [9], but the amounts of TNC and TNW vary depending on the cancer, indicating that their expression is regulated independently [10].

In contrast to TNC and TNW, there have been few reports on TNXB in the cancer field. TNXB is expressed at relatively high levels in malignant mesothelioma [11, 12] and ovarian cancer [13], indicating that the possibility of its use as a tumor marker. In most cancers, however, it has been shown by large pan-cancer analyses that the expression of *TNXB* is significantly downregulated [14, 15] and that a high level of *TNXB* expression in cancers is correlated with a good prognosis [15]. Consistent with these observations, *Tnxb*-deficient mice in which B16-BL6 melanoma cells were grafted showed promotion of invasion and metastasis due to enhanced activities of matrix metalloproteinases (MMPs) compared with those in wild-type mice [16]. In addition, Yang *et al*. [17] showed in esophageal squamous cell carcinoma (ESCC) that efficient suppression of *TNXB* expression can significantly enhance cell proliferation and that silencing of *TNXB* expression significantly increases colony formation. These results indicated that TNXB functions as a tumor suppressor [18].

So far, there have been studies in which the expression level of *TNXB* in cells in normal tissues was compared with that in cells in tumor tissues including tumor cells, stromal cells and immune cells. On the other hand, there has been no report on a comparison of *TNXB* expression levels in primary and metastatic tumor cells. The aim of this study was first to characterize tumor tissues that show a significant difference between *TNXB* expression levels in primary and metastatic tumor cells comprehensively by using a gene expression database of pan-cancer cells. The second aim was to identify genes that show both correlated expression with *TNXB* expression and significantly differential expression in metastatic tumor cells and primary tumor cells in the characterized tumor tissues. The third aim was to elucidate the biological function and transcriptional regulatory networks of the genes identified in the metastatic tumor cells.

We showed that *TNXB* expression is significantly downregulated in metastatic lymphoid tumor cells compared with its expression in primary lymphoid tumor cells. Enrichment analysis of the genes exhibiting correlated expression with *TNXB* and upregulation in metastatic lymphoid tumor cells showed that the genes are related to immune response and inflammatory response. Furthermore, some transcriptional regulatory networks were revealed to coordinate the expression of *TNXB*-correlated genes in metastatic lymphoid tumor cells.

MATERIALS AND METHODS

DepMap database analysis

Dependency Map (DepMap) (https://depmap.org/portal/) is a tumor cell line database that integrates and provides existing cell line databases such as Cancer Cell Line Encyclopedia (CCLE) (https://sites.broadinstitute.org/ccle/). Transcriptional expression data for each of 19,193 genes in a total of 1,228 cell lines that were defined as primary and metastatic cells from 23 types of tumor tissues were downloaded from DepMap. The expression data file (OmicsExpressionProteinCodingGenesTPMLogp1.csv) from DepMap Public 23Q2 was downloaded on August 8, 2024. The expression levels of *TNXB* in primary and metastatic tumor cells were then analyzed. Cervix tumors include cervical adenocarcinoma, cervical squamous cell carcinoma, glassy cell carcinoma of the cervix, mixed cervical carcinoma, and small cell carcinoma of the cervix, while lymphoid tumors include Hodgkin lymphoma, non-Hodgkin lymphoma, B-lymphoblastic leukemia/lymphoma, and T-lymphoblastic leukemia/lymphoma, categorized into Oncotree Primary Disease in DepMap.

Genes with correlated expression with TNXB in metastatic lymphoid tumor cells

In the 22 metastatic lymphoid tumor cell lines, the Pearson correlation coefficient (r) of expression levels between *TNXB* and each of 19,193 genes was explored using Data Explorer in DepMap portal and Rstudio (version 4.2.2). We considered $r > 0.30$ as

a positive correlation, and $r < -0.30$ as a negative correlation.

Analysis of differentially expressed genes by iDEP

Analysis of differentially expressed genes (DEGs) in 19,193 genes between 86 primary and 22 metastatic lymphoid tumor cell lines in DepMap database was performed by integrated Differential Expression & Pathway analysis (iDEP) 2.01 (http://bioinformatics.sdstate.edu/idep/). The values of $\log_2$ (TPM+1) of each gene in the primary and metastatic lymphoid tumor cells were used for the expression levels of the genes by iDEP. The cutoff value of false discovery rate (FDR) was set at 0.1. Minimal fold change (FC) was set at 2.0.

Gene enrichment analysis by Metascape

Metascape is a tool developed for gene annotation and gene set enrichment analysis (https://metascape.org/gp/index.html#/main/step1) [19]. Among genes showing correlated expression with *TNXB*, 62 upregulated intersection DEGs and 81 downregulated intersection DEGs in metastatic lymphoid tumor cells were delved for the Metascape analysis, conducted on August 14, 2024.

Search for transcriptional regulation networks by TRRUST

Transcriptional regulation networks of the 62 upregulated *TNXB*-correlated expressed genes were investigated by utilizing Transcriptional Regulatory Relationships Unraveled by Sentence-based Text mining (TRRUST) version 2 (https://www.grnpedia.org/trrust/) [20].

Statistical analysis

A box-and-whisker plot was used to compare the expression levels of *TNXB* in primary and metastatic cell lines from tumors. A P value of < 0.05 was considered statistically significant based on the *t*-test following the F-test. Statistical analysis was preformed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Comparison of TNXB expression levels in primary and metastatic cell lines from 23 tumor tissues

The expression levels of *TNXB* were compared in primary and metastatic cell lines from 23 tumor tissues in DepMap database, as shown in Table 1. Among the 23 tumor tissues analyzed, *TNXB* expression was significantly decreased in metastatic cells compared with that in primary cells of cervix ($P < 0.01$) and lymphoid ($P < 0.05$) tumors (Fig. 1). On the other hand, *TNXB* expression levels were not significantly different in primary and metastatic cells of other tumors (Table 1). These results indicate that *TNXB* expression would be decreased, inversely correlating with the metastatic characteristic of cervix and lymphoid tumor cells.

Identification of genes that show correlated expression with TNXB expression in metastatic lymphoid tumor cells

It has been reported that TNXB functions as a tumor suppressor [18]. However, it is yet to be determined which pathways and factors are involved in this process.

Due to the small number of datasets in which cervix tumor cells are deposited (primary: n = 14, metastatic: n = 6), we focused on analyses of lymphoid tumor cells (primary: n = 86, metastatic: n = 22). To elucidate the features of metastatic lymphoid tumor cells that are relevant to *TNXB* expression, we investigated the genes for which expression is correlated with expression of *TNXB* in 22 metastatic lymphoid cell lines. Analyses of the correlation of *TNXB* expression with that of 19,193 genes in the metastatic lymphoid tumor cells revealed a total of 4,654 genes with substantial correlation, namely 3,723 genes with positive correlations ($0.30 < r < 0.83$) and 931 genes with negative correlations ($-0.75 < r < -0.30$) (Fig. 2). These results indicated that nearly 80% of the genes correlated with *TNXB* expression show positive correlations.

Identification of DEGs between metastatic and primary lymphoid tumor cells and overlapped genes with correlated expression with TNXB expression

The iDEP analysis revealed DEGs in metastatic

Table 1. *TNXB* expression levels in primary and metastatic cells from various of tumor tissues.

Tumor		Number of tumor cell lines used	Average[a]	Standard deviation (SD)	*P* value[b]
Biliary tract	Primary	30	1.17	1.03	0.91
	Metastatic	8	1.21	1.47	
Bladder/Urinary tract	Primary	30	1.08	1.10	0.91
	Metastatic	7	1.05	0.56	
Bone	Primary	30	1.16	1.14	0.19
	Metastatic	6	2.46	2.10	
Bowel	Primary	56	0.68	0.56	0.29
	Metastatic	20	0.88	0.76	
Breast	Primary	32	0.89	0.72	0.15
	Metastatic	35	1.19	0.97	
Cervix	Primary	14	1.20	0.87	0.01
	Metastatic	6	0.43	0.26	
Central Nervous System (CNS)/Brain	Primary	83	1.16	1.14	0.28
	Metastatic	2	0.28	0.25	
Esophagus/Stomach	Primary	33	1.10	1.10	0.64
	Metastatic	44	0.98	1.19	
Eye	Primary	8	0.49	0.61	0.91
	Metastatic	4	0.45	0.58	
Head and neck	Primary	40	0.80	0.89	0.10
	Metastatic	9	0.42	0.51	
Kidney	Primary	29	0.75	0.69	0.20
	Metastatic	10	1.37	1.36	
Lung	Primary	91	1.12	0.90	0.48
	Metastatic	99	1.04	0.73	
Lymphoid	Primary	86	1.07	0.88	0.03
	Metastatic	22	0.79	0.38	
Myeloid	Primary	42	1.21	0.99	0.06
	Metastatic	6	2.07	1.36	
Ovary/Fallopian tube	Primary	29	0.80	0.88	0.63
	Metastatic	35	0.90	0.75	
Pancreas	Primary	29	0.82	0.60	0.08
	Metastatic	24	1.30	1.20	
Peripheral nervous system	Primary	13	0.92	1.42	0.93
	Metastatic	20	0.88	1.24	
Pleura	Primary	13	2.42	1.87	0.49
	Metastatic	5	3.26	3.08	
Prostate	Primary	6	1.43	0.95	0.43
	Metastatic	5	1.02	0.61	
Skin	Primary	33	0.62	0.71	0.39
	Metastatic	56	0.50	0.44	
Soft tissue	Primary	10	1.25	1.57	0.31
	Metastatic	12	0.69	0.57	
Thyroid	Primary	10	0.70	0.67	0.79
	Metastatic	6	0.61	0.42	
Uterus	Primary	35	0.99	1.01	0.38
	Metastatic	5	0.58	0.46	

[a]Data for expression levels of *TNXB* in primary and metastatic cells from tumor tissues in DepMap database were collected, and then their averages were calculated.
[b]Statistic analysis, primary group vs. metastatic group.

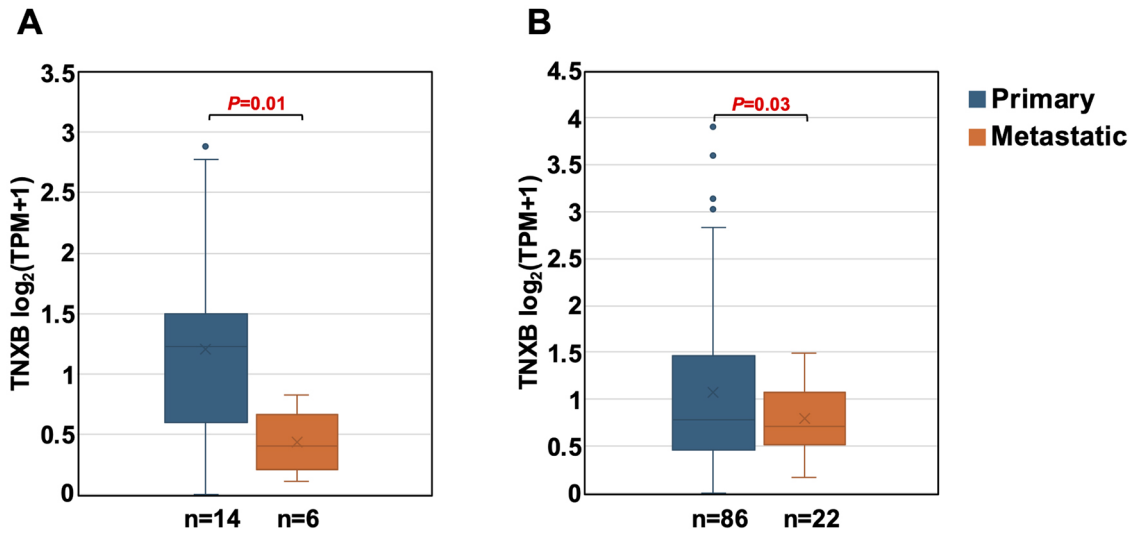

Fig. 1. Expression levels of *TNXB* in primary and metastatic tumor cells from DepMap database. (A) Cervix tumor cells. The numbers of primary and metastatic cervix tumor cell lines used were 14 and 6, respectively. (B) Lymphoid tumor cells. The numbers of primary and metastatic lymphoid tumor cell lines used were 86 and 22, respectively.

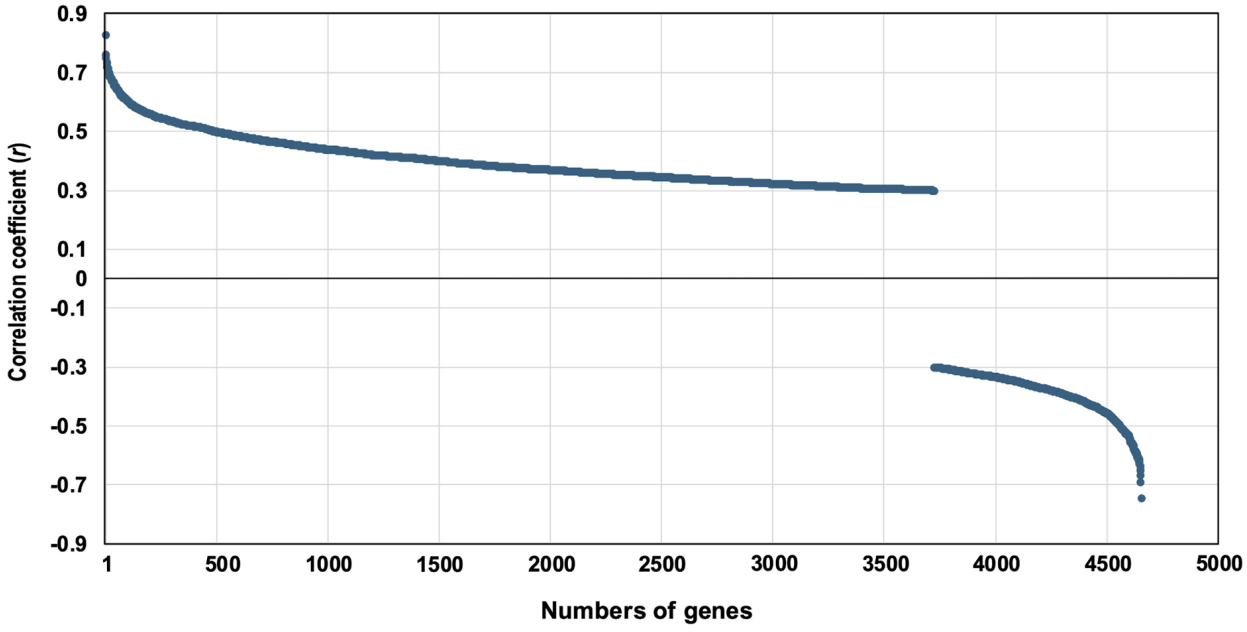

Fig. 2. Correlation coefficients of expression levels between *TNXB* and each of 19,193 genes in the 22 metastatic lymphoid tumor cell lines by using Data Explorer in DepMap portal and Rstudio. This figure shows the correlation coefficients (r values) of 3,723 genes with positive correlations ($0.30 < r < 0.83$) and 931 genes with negative correlations ($-0.75 < r < -0.30$) (totally 4,654 genes).

lymphoid tumor cells compared with those in primary lymphoid tumor cells. Genes with more than a 2-fold difference in expression level between metastatic and primary lymphoid tumor cells were selected as significant DEGs: 163 upregulated genes and 202 downregulated genes (Fig. 3A). We also examined overlapping of the significant DEGs with the 4,654 *TNXB*-correlated genes. As a result, among the 4,654 genes showing correlated expression with *TNXB*, 62 DEGs (Fig. 3B) and 81 DEGs (Fig. 3C) showed upregulated expression and downregulated expression, respectively, in metastatic lymphoid tumor cells. Intriguingly, the expression of 58 of the 62 upregulated genes showed a negative correlation

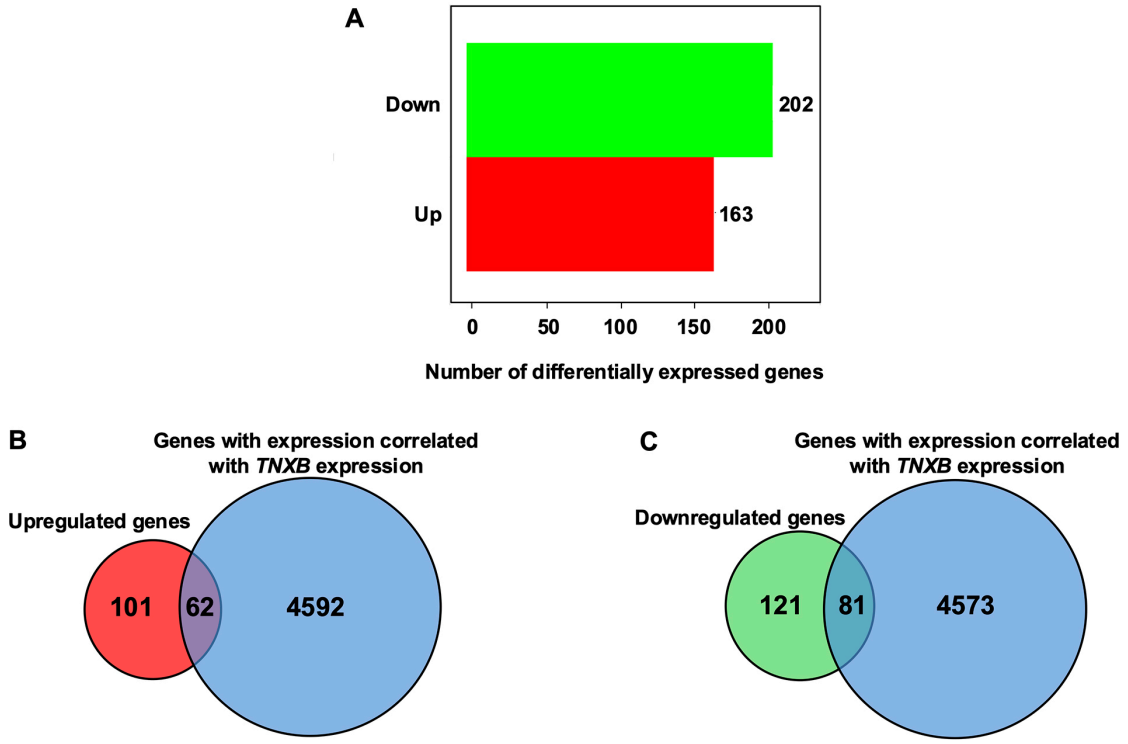

Fig. 3. (A) Numbers of upregulated and downregulated genes in metastatic lymphoid tumor cells compared with those in primary lymphoid tumor cells. (B) Venn diagram depicting the overlap between the 163 upregulated genes shown in (A) versus the 4,654 correlated expressed genes with *TNXB* in metastatic lymphoid tumor cells. (C) Venn diagram depicting the overlap between the 202 downregulated genes shown in (A) versus the 4,654 correlated expressed genes with *TNXB*.

with the expression of *TNXB*, while the expression of only four genes (*FCGR2B*, *FCRLB*, *FKBP4*, and *HLA-DQA2*) showed a positive correlation with the expression of *TNXB*. Meanwhile, the expression of only three genes (*CYP2R1*, *NREP* and *ZSCAN18*) out of the 81 downregulated genes exhibited a negative correlation with the expression of *TNXB*, but the expression of the remaining 78 genes showed a positive correlation with the expression of *TNXB*. These tendencies suggested a possible link between the DEGs and *TNXB* in metastatic lymphoid tumor cells.

Next, to investigate the functions and pathways of the 62 upregulated DEGs, Metascape-based gene enrichment analysis of gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) was conducted. The top 13 enriched GO functional annotation terms ($P < 0.01$) for the 62 upregulated DEGs are presented in Fig. 4A. They include B cell activation, regulation of leukocyte activation, negative regulation of cell adhesion, positive regulation of immune response, inflammatory response, regulation of peptide hormone secretion, antigen processing and presentation, humoral immune response, inorganic ion homeostasis, regulation of epithelial to mesenchymal transition, regulation of MAPK cascade, cell-matrix adhesion, and leukocyte chemotaxis. The pathway enrichment includes allograft rejection and transcriptional misregulation in cancer ($P < 0.01$) as shown in Fig. 4A. These results indicated that most of the enriched 62 upregulated DEGs are related to immune response and inflammatory response. On the other hand, the top 8 GO terms ($P < 0.01$) enriched for the 81 downregulated DEGs were secondary alcohol metabolic process, response to acid chemical, cellular response to cytokine stimulus, regulation of hydrolase activity, negative regulation of intracellular signal transduction, viral process, negative regulation of cellular component organization and positive regulation of lipid metabolic process as shown in Fig. 4B. In addition, the top 12 enriched pathways ($P < 0.01$) included apoptosis, clathrin-mediated endocytosis, photodynamic therapy induced unfolded protein re-

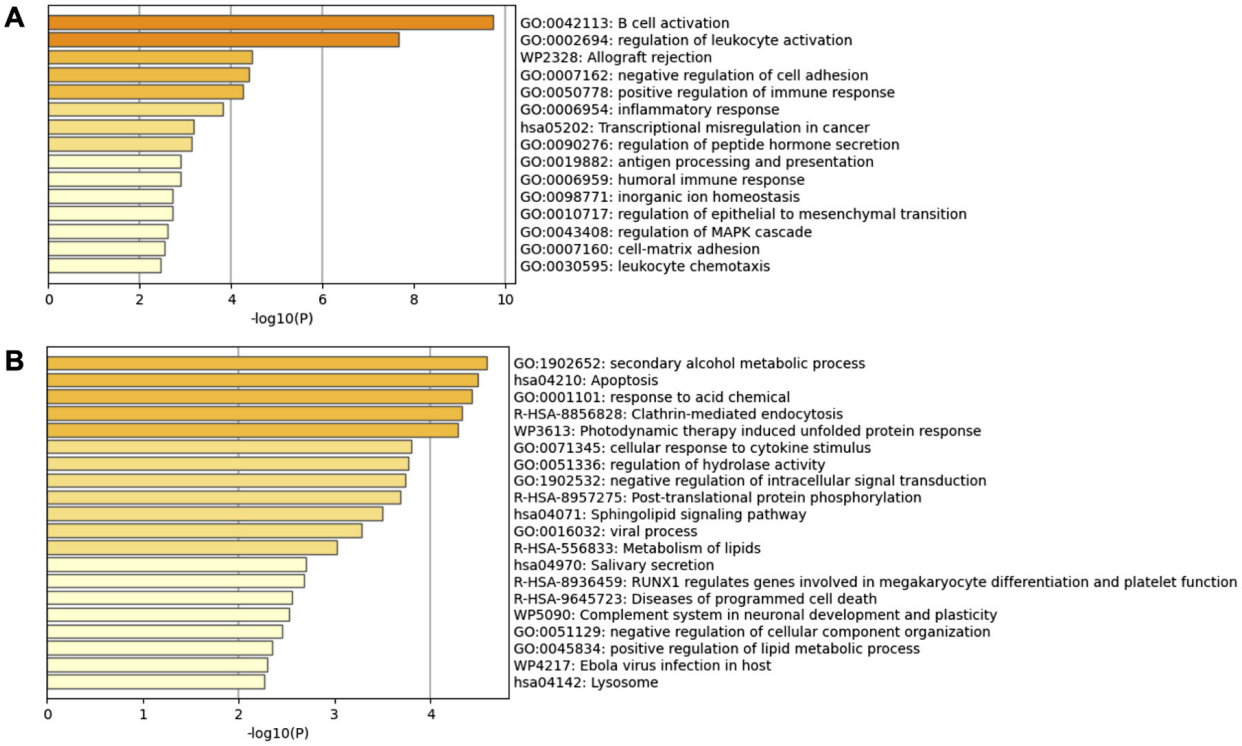

Fig. 4. Metascape enrichment analysis. Bar chart of enriched terms relevant to the 62 upregulated genes in metastatic lymphoid tumor cells compared with those in primary lymphoid tumor cells (A) and the 81 downregulated genes (B) with correlated expression with *TNXB* in metastatic lymphoid tumor cells, respectively. Each term was sorted according to its *P*-value significance.

sponse, post-translational protein phosphorylation, sphingolipid signaling pathway, metabolism of lipids, salivary secretion, *RUNX1* regulates genes involved in megakaryocyte differentiation and platelet function, diseases of programmed cell death, complement system in neuronal development and plasticity, and Ebola virus infection in host and lysosome as shown in Fig. 4B. Unexpectedly, it was found that the enriched functions and pathways of the 81 downregulated DEGs are extensively diverged. Thus, we focused on the 62 upregulated *TNXB*-correlated expressed genes in further analysis.

Transcriptional regulation networks of the 62 upregulated TNXB-correlated expressed genes

Next, we examined the comprehensive transcriptional regulation networks of the 62 upregulated *TNXB*-correlated expressed genes by TRRUST. The analysis of the 62 upregulated genes showed 8 transcription factors (TFs), *STAT1*, *RELA*, *TRERF1*, *IRF4*, *STAT6*, *YY1*, *NFKB1* and *SPI1*, as key regulators ($P < 0.05$). *STAT1* regulates the expression of *CD40* and *CD86*. *RELA* regulates the expression of *CD40*, *CD83*, *CD86*, *ALOX5* and *SLC25A27*. *TRERF1* regulates the expression of *CD40* and *CD86*. *IRF4* regulates the expression of *BCL6* and *MS4A1*. *STAT6* regulates the expression of *CD40* and *CNR1*. *YY1* regulates the expression of *FCGR2B* and *WDFY4*. *NFKB1* regulates the expression of the same genes for which expression is regulated by *RELA*. *SPI1* regulates the expression of *CD40*, *MS4A1* and *BCL6*. Subsequently, the correlations of these TFs with *TNXB* expression in metastatic lymphoid tumor cells was investigated. Two TFs, *STAT1* ($r = 0.45$) and *RELA* ($r = 0.30$) (Fig. 5), were found to have substantial correlations ($r > 0.30$ and $r < -0.30$) with *TNXB* expression.

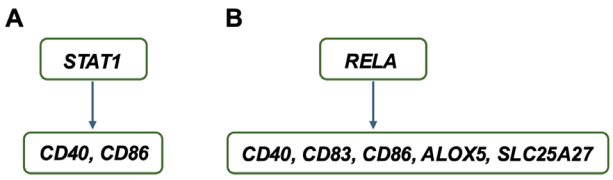

Fig. 5. Possible transcriptional regulation networks based on TRRUST. (A) *STAT1*-regulated gene network. (B) *RELA*-regulated gene network.

DISCUSSION

This study is the first study in which the expression of *TNXB* in 23 cancer tissues was compared in primary and metastatic tumor cells. Among them, a significant difference with decreased expression in the metastatic tumor cells was only found in cervix tumors and lymphoid tumors. This coincided with our argument that TNXB functions as a tumor suppressor in these tumors. Also, 62 genes were identified as genes exhibiting correlated expression with *TNXB* expression in metastatic lymphoid tumor cells and higher expression levels in metastatic tumor cells than in primary tumor cells. Furthermore, we found the possibility that the expression of *CD40* and *CD86* among the 62 genes is regulated by both *RELA* and *STAT1*, while the expression of *CD83*, *ALOX5*, and *SLC25A27* is regulated by *RELA* in metastatic lymphoid tumor cells.

We performed JASPAR analysis (https://jaspar.elixir.no) with the relative score threshold set at 0.85 to examine whether STAT1 and RELA bind to the 1,957-bp region of *TNXB* promoter [21]. Consequently, there were one binding site each for STAT1 and RELA in the promoter region (data not shown). Thus, it is likely that STAT1 and RELA regulate not only the expression of the corresponding upregulated *TNXB*-correlated expressed genes but also the expression of *TNXB*.

CD86 is prominently found on the surface of antigen-presenting cells and tumor cells and is involved in the costimulatory signals or coinhibitory signals essential for T cell proliferation and cytokine production by binding to CD28 or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expressed on T cells, respectively [22]. The higher levels of CD86 and soluble CTLA-4 in patients with acute lymphoblastic leukemia (ALL) indicate a poor prognosis [23]. CD86 is involved in immune infiltration in acute myeloid leukemia (AML) and has been shown to be a crucial factor for the tumor microenvironment in AML [24].

CD40, a member of the tumor necrosis factor receptor superfamily, is strongly expressed on Hodgkin lymphoma Reed-Sternberg (HRS) cells [25], while CD40L, its cognate ligand, is expressed on activated T cells. CD40 engagement by CD40L-expressing T cells leads to HRS proliferation and survival, NF-κB activation, increased interferon regulatory factor 4 (IRF4), and production of cytokines and chemokines such as chemokine ligand 5 (CCL5), contributing to tumor microenvironment formation by recruiting $CD4^+$ T cells, enosinophils and mast cells [26]. It is known that the upregulation of CD86 and CD40 expression relies on NF-κB and STAT1 [27-29].

CD83 is highly expressed in Hodgkin lymphoma cell lines and Hodgkin and HRS cells. CD83 is transferred to surrounding T cells by trogocytosis, which leads to increased expression of programmed death-1 (PD-1). This process causes immunosuppressive functions of surrounding T cells expressing PD-1. CD83 works as a potential biomarker and therapeutic target in Hodgkin lymphoma [30].

Arachidonate 5-lipoxygenase (ALOX5) is highly expressed in mantle cell lymphoma (MCL) [31]. It is involved in the leukotriene biosynthetic pathway in MCL and other B cell malignancies [32].

Uncoupling protein-4 (UPC4/SLC25A27) in the mitochondrial inner membrane is predominantly expressed in the brain, and it has been suggested to play a role in the modulation of energy production and levels of mitochondrial reactive oxygen species (ROS) [33]. However, there has been no study on the role of SLC25A27 in cancer. As an exception, a study using data about *SLC25A27* expression in The Cancer Genome Atlas (TCGA) tumor database showed that *SLC25A27* expression is downregulated in most tumors [34].

The correlation coefficients of expression levels of *TNXB* vs. *CD86*, *CD83*, *CD40*, *ALOX5* and *SLC25A27* in metastatic lymphoid tumor cells were all negative, namely, -0.52, -0.42, -0.39, -0.36, and -0.31, respectively. Since it is known that *CD86*, *CD83*, *CD40* and *ALOX5* facilitate the promotion of lymphoid tumor growth as mentioned above, negative correlated expression of *TNXB* vs. *CD86*, *CD83*, *CD40* and *ALOX5* would reflect the properties of TNXB as a tumor suppressor.

It has been reported that overexpression of *STAT1* induces apoptosis in the classical Hodgkin lymphoma (cHL) cell line L1236 [35], indicating that *STAT1* also has aspects of a tumor suppressor. Our finding that expression of *STAT1* has a positive cor-

relation with that of *TNXB* (r = 0.45) suggests a close relationship between *STAT1* and *TNXB* in lymphoid metastasis.

The expression of *STAT1* and *RELA* in metastatic lymphoid tumor cells tends to be downregulated just a little compared with that in primary lymphoid tumor cells (data not shown), consistent with their positive correlation with *TNXB*. Thus, in the tumor microenvironment, the tumor suppressive function of TNXB may be weakened in correlation with the downregulation of *STAT1* and *RELA*. Although the tumor suppressive function of TNXB remains elusive, weakening of it may lead to the upregulation of *CD86*, *CD83*, *CD40* and *ALOX5* in metastatic lymphoid tumor cells, which would also be somehow regulated by STAT1 and/or RELA as suggested by the TRRUST analysis.

CONCLUSION

We identified 62 upregulated *TNXB*-correlated expressed genes in metastatic lymphoid tumor cells and investigated transcriptional networks for the 62 genes. The coordinated expression of the *TNXB*-correlated expressed genes would be involved in characteristics of metastatic lymphoid tumor cells such as proliferation, apoptosis, migration and adhesion. In the future, we will disclose the relevance between *TNXB*-correlated gene expression networks affected by the administrations of various types of therapeutic agents and their pharmacological efficacy.

Author contribution

TG and KM conceived the study. TG conducted all data curation and analyses and edited and reviewed the manuscript. TG also created the program with Rstudio for calculating correlation coefficients. KM organized the data collection, interpreted the data, wrote the manuscript, and edited and reviewed the manuscript. HK validated the R program made by TG, performed data analyses, and edited and reviewed the manuscript. All authors have read and approved the published version of the manuscript.

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Conflict of Interest

All authors declare no conflict of interests.

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