Immunological Disorders and Immunotherapy

TMEM4 is Highly Expressed in Plasma Cell Neoplasms and Associated with B Lymphocyte Differentiation

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Abstract

Aiming to identify biomarkers for plasma cell neoplasms, we analyzed gene expression profile and proteomic characteristics of mouse models of plasmacytomas along with other types of B-cell neoplasms. We found that transmembrane protein 4 (Tmem4) was highly expressed in plasmacytomas in comparison with early-stage B-cell neoplasms. The serum levels of Tmem4 were also significantly greater in mice with plasmacytoma comparing to those with early-stage B-cell neoplasms ($P<0.01$). Mechanistically, increased Tmem4 expression in B cells led to increased levels of IL-10, a cytokine that promotes the growth of malignant plasma cells and participates in the terminal differentiation of B cells into plasma cells. In addition, over-expression of Tmem4 promoted B cell terminal differentiation as evidenced by the increase in expression of XBP1, CD38, and CD138. Furthermore, we found that TMEM4 was highly expressed in plasma cells of multiple myeloma patients. These findings suggested that Tmem4 plays an important role in plasma cell differentiation and has a potential to serve as a biomarker for plasma cell neoplasms.

Keywords: Neoplasms; Plasma cell; TMEM4; CNPY2; Lymphoma

Introduction

Plasma cells, as the terminally differentiated form of B lymphocytes, play a key role in humoral immunity by producing and secreting immunoglobulin (Ig). The development of plasma cells from mature B cells is precisely regulated, with a distinct gene expression program consisting of up- and down-regulated expression of genes. The unique gene expression program determines the differentiation and proper function of plasma cells. For instance, during plasma cell differentiation, the levels of transcription factor B lymphocyte-induced maturation protein 1 (BLIMP1) increase [1]. BLIMP1 inhibits the expression of genes required for B cell receptor signaling and germinal center (GC) B cell function, while it promotes the expression of genes important for plasma cell function such as XBP1 [1]. XBP1 encodes X-box binding protein 1 (XBP1), a highly expressed transcription factor in plasma cells. XBP1 plays a key role in the unfolded protein response which is critical to the process of Ig production, thus maintaining the survival and function of plasma cells [2,3].

Like other lymphoid neoplasms, the uniquely expressed molecules in plasma cells could serve as biomarkers for plasma cell neoplasms such as multiple myeloma (MM). MM is an incurable disease largely due to the difficulty in early detection and ineffectiveness of treatment. Recent studies showed that XBP1 could be used as a predictive biomarker for the efficacy of thalidomide-based treatment for MM [4]. Nevertheless, there is still a strong need for additional biomarkers for disease stratification, drug efficacy prediction, prognoses, and druggable targets for plasma cell malignancy.

In our effort to identify novel molecules specific for normal and malignant plasma cells, we took advantage of our mouse models which develop a variety of B-cell lineage neoplasms [5]. By comparing the gene expression profile between plasmacytomas and neoplasms derived from earlier-stage B cells, we found that Tmem4 expression was significantly increased in plasmacytomas. Tmem4 encodes transmembrane protein 4 (Tmem4), also called MIR-interacting saposin-like protein (Msap) or canopy FGF signaling regulator 2 (CNPY2). Studies showed that TMEM4 enhanced cell spreading and motility through increasing the

phosphorylation of myosin regulatory light chain (MRLC) in neuron cells [6]. Recently, it has been reported that Tmem4 was a target protein of the Wnt/GSK3 signaling pathway. Wnt increased the intracellular concentration Tmem4, which was likely through its inhibitory effect on GSK3-mediated proteasome degradation, thus stabilizing Tmem4 [7]. Several proteomic profiling studies revealed that Tmem4 was one of the prominent proteins expressed in stem cells including mouse embryonic stem cells [8], mouse spleen stem cells [9], and human embryonic stem cells [10]. Furthermore, it was shown that stem-cell factors OCT4 and NANOG regulated the expression of TMEM4 via undetermined mechanisms [11]. Most recently, it was reported that TMEM4 mediated the regulatory effect of fibroblast growth factor-21 (FGF21) on low-density lipoprotein receptor (LDLR) [12]. However, the role of TMEM4 in B cell function remains unclear.

In this study, we report that Tmem4 was highly expressed in mouse plasma cells and plasma cell neoplasms, and increasing levels of Tmem4 drove mature B cell differentiation into plasma cells and enhanced cell motility.

Methods

Animal model

Plasmacytoma was induced in BALB/c mice by intraperitoneal

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injection of pristane which caused chronic granulomatous peritonitis, as described previously [13]. The tumors developed in granulomas and were dependent on IL-6. In majority of cases, the tumors had spontaneous IgH-Myc translocation t(12;15) involving Myc on chromosome 15 and IgH on chromosome 12 [13]. All animal studies were performed under NIAID IACUC approved protocol LIP6.

Human bone marrow biopsy

Bone marrow was aspirated from patients with MM or idiopathic thrombocytopenic purpura (ITP) during diagnosis process. The protocol was approved by the Ethics Committee of the First Municipal Hospital of Ningbo. Written informed consent was obtained from each patient.

Cell culture and gene transfection

Murine B-cell lymphoma cell lines were created from the mouse models as previously described [14]. The B-cell leukemia line BCL1-3B3 was obtained from American Type Culture Collection (ATCC, VA, USA) and the plasmacytoma cell line PCT-AP was kindly provided by Dr. M. Potter (National Cancer Institute, NIH, Bethesda, MD, USA). Mouse plasma cells were prepared from the spleen using magnetic beads conjugated with CD138 antibody (Miltenyi Biotec, CA, USA). The cells were maintained at 37°C with 5% CO_2 in RPMI 1640 (Invitrogen Life Technologies, CA, USA) with 10% fetal bovine serum (FBS) (Hyclone, IL), 10 mM sodium pyruvate, 2 mM glutamine, 1x non-essential amino acids, 0.5 μM β-mercaptoethanol, and 0.1 M HEPES buffer (pH 7.4).

Tmem4 cDNA was inserted into mammalian expression vector pAcGFP1C1 (Invitrogen, Carlsbad, CA, USA) and verified by sequencing. Plasmid DNA (2 μg) of pAcGFP1C1-Tmem4 and pAcGFP1C1 (control vector) were used to transfect 2×10^6 cells using Amaxa transfection with program X-001and solution V (Gaithersburg, MD, USA).

Oligonucleotide microarray and quantitative real-time RT-PCR (qPCR)

Gene expression profile of mouse B-cell lymphoma tissues were analyzed using oligonucleotide microarrays as described previously [15]. Briefly, 500ng high quality RNA was isolated and reverse transcribed into cDNA using a SuperScript III reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA). cDNA from samples and from a reference pool derived from mouse hematopoietic cell lines were hybridized to a 70-mer oligonucleotide microarray chip (Microarray Research Facility at National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA). The quality of RNA extracted from tumor tissues were examined by Agilent Bioanalyzer. Microarray data were uploaded to the GEO database (accession no. GSE1908, www.ncbi.nih.gov/geo). For qPCR, cDNA was prepared and analyzed using the SYBR Green method on an ABI 7900 system (Applied Biosystems, CA, USA). Gapdh was used as reference gene. The ΔCt (difference in the cycle of threshold) values were calculated and compared among samples.

Two-dimensional gel electrophoresis and mass spectrometry analysis

Tissues for two-dimensional gel electrophoresis (2DGE) were dissolved in extraction buffer containing 8M urea, 4% (w/v) Bio-Lyte 4/7, and 2 mM tributyl phosphine (Bio-Rad, Hercules, CA, USA) and vigorously vortexed. After centrifugation, the supernatant was combined with a buffer mixture containing rehydration buffer (8 M urea, 2% CHAPS, 50mM DTT, and 0.2% (w/v) Bio-Lyte 4/7

ampholytes), IPG buffer, and bromophenol blue, and subsequently rehydrated overnight with Immobiline Drystrips (pH 4/7, 11cm) on a Reswelling Tray (all from Amersham Biosciences, NJ, USA). The first dimension electrophoresis of 2DGE was performed using a Multiphore II Electrophoresis System, and the second dimension was performed using precast ExcelGel SDS gels on a Multiphor II Flated System (Amersham Biosciences, NJ, USA). Protein gels were stained using a silver staining kit (Amersham Biosciences). The protein spots were detected, quantified, and matched using Proteomweaver software (Definiens, Munich, Germany). Protein spots of interest were excised from the gel and subjected to in-gel digestion. Peptides from in-gel digests were analyzed using a ProteomeX LC-MS/MS system (ThermoElectron, San Jose, CA, USA). MS/MS spectra were searched against database utilizing the BioWorks and SEQUEST programs (Thermo Fisher, Waltham, MA, USA). A protein ID was assigned when MS/MS spectra of at least two peptides from the same protein exhibited at a minimum the default Xcorr vs. charge values set by the program.

Western blot analysis

Selective tissue dissection was performed prior to protein analysis to avoid procurement of normal tissue or areas of necrosis, hemorrhage, or inflammation, as previously described [13]. Tissues for western blot analysis were homogenized in T-PER tissue protein extraction reagent (Pierce, IL, USA) with protease inhibitor cocktail (Roche Molecular Biochemicals, IN, USA). Cultured cells were lysed using RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP-40, 0.25% Na-deoxycholate) containing 1 mM PMSF and 1x Roche complete mini protease inhibitor (Roche, IN, USA). Protein samples were loaded onto a 4-12% gradient SDS-PAGE, and run at a constant current. Following electrophoresis, proteins were transferred to a nitrocellulose membrane. The membrane was blocked with 4% fat-free milk powder in PBS and incubated with appropriate primary and secondary Abs. Anti-Tmem4 antibody was developed in house. Primary antibody against MRLC2, phorphorylated MRCL2 (pMRLC2), XBP1, and β-ACTIN were all purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Protein signals were detected using ECL reagents (Pierce, IL, USA).

Flow cytometry analysis

Cells were incubated with FITC-labeled antibodies against CD138, CD38, B220, or PC-1, and analyzed using FACSCalibur as previously described [15].

ELISA and cytokine analysis

The wells of a 96-well plate were coated with a polyclonal anti-Tmem4 antibody developed in-house, followed by adding mouse serum (100 μL). After washing, wells were incubated with biotin-labeled monoclonal anti-Tmem4 antibody (#12929, Cell Signaling, CA, USA). Subsequently, wells were washed and incubated with streptavidin-conjugated horseradish peroxidase followed by incubating with TMB chromogen solution (Life Technologies, CA, USA). The absorbance was detected at 650 nm. The levels of IL-10 and other cytokines in B lymphocytes were analyzed using Cytokine Array (RayBiotech, GA, USA).

Immunohistochemical analysis

Immunohistochemical (IHC) staining of bone marrow biopsy was performed as described previously [15]. Briefly, bone marrow biopsy was fixed in formalin and embedded in paraffin. Serial sections were incubated with Abs specific to TMEM4 (14635-1-AP, Proteintech Group, Chicago, USA) or CD138 (clone MAB-0200, Maxim, Fuzhou, China). Subsequently, the sections were incubated with appropriate

secondary Abs. The signals were developed using diaminobenzidine tetrahydrochloride (Sigma-Aldrich, MO, USA) as chromogen.
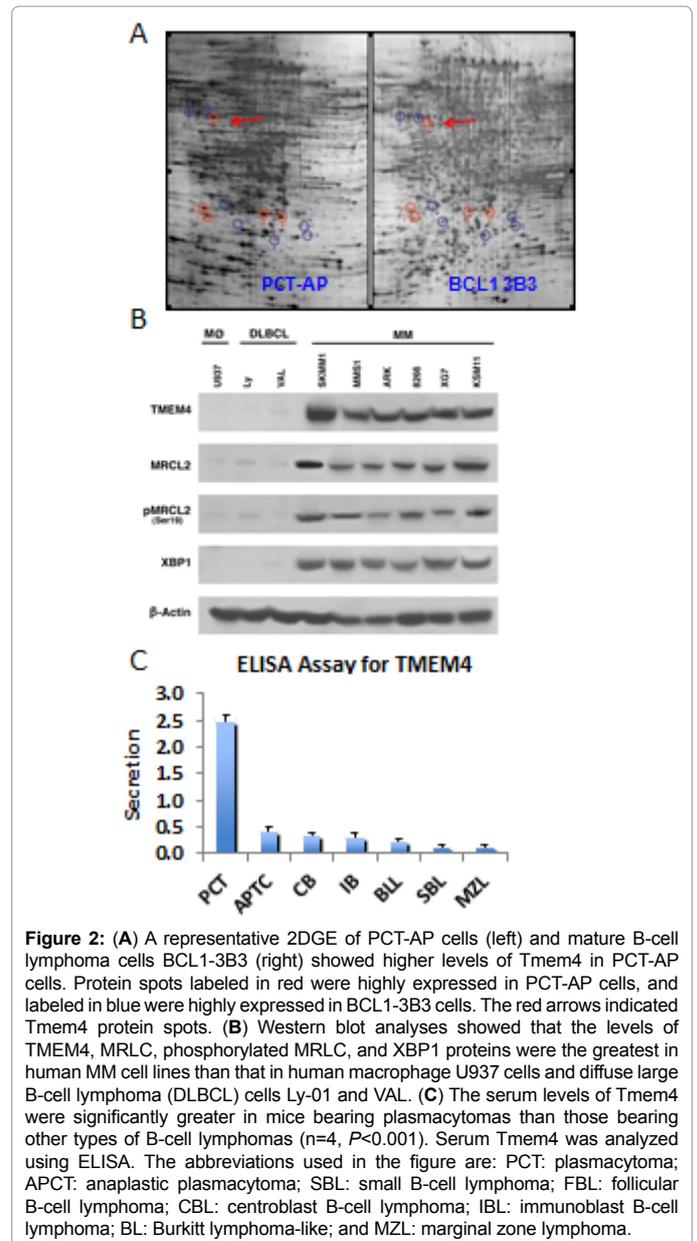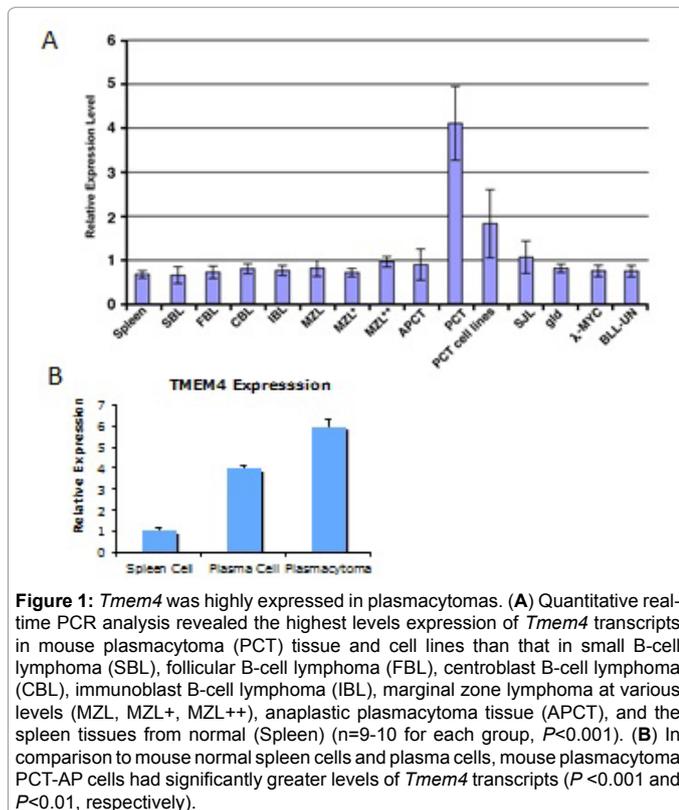
Statistical analysis

The statistical significance of experimental data was analyzed using the general linear model procedure of SAS software (SAS Institute, NC, USA).

Results

Tmem4 was highly expressed in mouse plasmacytoma cells

As a discovery-driven approach, using oligonucleotide microarray, we analyzed gene expression profiles of mouse B-cell lineage lymphomas derived from various developmental stages of B cells, including small B-cell lymphoma (SBL), follicular B-cell lymphoma (FBL), centroblast B-cell lymphoma (CBL), immunoblast B-cell lymphoma (IBL), marginal zone lymphoma (MZL), anaplastic plasmacytomas (APCT), and plasmacytomas (PCT). Of these B-cell malignancies, IBL, SBL, and CBL were GC-derived neoplasms. Among these lymphomas, we found that the levels of Tmem4 transcripts were higher in PCT, even in comparison to that in APCT (Supplementary Figure S1). We used real-time quantitative PCR to verify the microarray data, and confirmed that PCT expressed higher Tmem4 than any other types of B-cell neoplasms (P<0.001) (Figure 1A). In addition, we also examined the levels of Tmem4 mRNA in mouse splenic cells, plasma cells, and PCT cell line PCT-AP. The results showed that PCT-AP cell line expressed significantly higher level of Tmem4 mRNA than spleen cells (P<0.001) and plasma cells (P< 0.01) (Figure 1B).

Moreover, we used 2DGE coupled with mass spectrometry to systematically analyze the proteome of B-cell lymphoma cell lines. Similar to Tmem4 transcript, Tmem4 protein level was also higher

Figure 1: *Tmem4* was highly expressed in plasmacytomas. (**A**) Quantitative real-time PCR analysis revealed the highest levels expression of *Tmem4* transcripts in mouse plasmacytoma (PCT) tissue and cell lines than that in small B-cell lymphoma (SBL), follicular B-cell lymphoma (FBL), centroblast B-cell lymphoma (CBL), immunoblast B-cell lymphoma (IBL), marginal zone lymphoma at various levels (MZL, MZL+, MZL++), anaplastic plasmacytoma tissue (APCT), and the spleen tissues from normal (Spleen) (n=9-10 for each group, *P*<0.001). (**B**) In comparison to mouse normal spleen cells and plasma cells, mouse plasmacytoma PCT-AP cells had significantly greater levels of *Tmem4* transcripts (*P* <0.001 and *P*<0.01, respectively).

Figure 2: (**A**) A representative 2DGE of PCT-AP cells (left) and mature B-cell lymphoma cells BCL1-3B3 (right) showed higher levels of Tmem4 in PCT-AP cells. Protein spots labeled in red were highly expressed in PCT-AP cells, and labeled in blue were highly expressed in BCL1-3B3 cells. The red arrows indicated Tmem4 protein spots. (**B**) Western blot analyses showed that the levels of TMEM4, MRLC, phosphorylated MRLC, and XBP1 proteins were the greatest in human MM cell lines than that in human macrophage U937 cells and diffuse large B-cell lymphoma (DLBCL) cells Ly-01 and VAL. (**C**) The serum levels of Tmem4 were significantly greater in mice bearing plasmacytomas than those bearing other types of B-cell lymphomas (n=4, *P*<0.001). Serum Tmem4 was analyzed using ELISA. The abbreviations used in the figure are: PCT: plasmacytoma; APCT: anaplastic plasmacytoma; SBL: small B-cell lymphoma; FBL: follicular B-cell lymphoma; CBL: centroblast B-cell lymphoma; IBL: immunoblast B-cell lymphoma; BL: Burkitt lymphoma-like; and MZL: marginal zone lymphoma.

in PCT-AP cells than in BCL1-3B3 cells (Figure 2A). We further examined TMEM4 protein levels in human B-cell neoplasm cell lines, including several human MM cell lines (SKMM1, MMS1, ARK, 8266, XG7, and KSM11), two diffuse large B-cell lymphoma (DLBCL) cell lines (OCI-LY01, VAL), and one histyocytic lymphoma cell line (U937), and found that MM cell lines express higher levels of TMEM4 protein than any other B cell lines (Figure 2A). In addition, as a validation to the proteomic study results shown in Figure 2A, the levels of MRCL2, phosphorylated MRCL2, and XBP1 were all significantly higher in MM cell lines than that in DLBCL and U937 cell lines (Figure 2B). Furthermore, we used public database GEO Profiles to analyze the levels of TMEM4 transcripts in human B lymphocytes. In dataset GDS56 (http://www.ncbi.nlm.nih.gov/geo/tools/profileGraph. cgi ID=GDS3516:202857_at), it was reported that in comparison to normal naïve B cells, centroblasts, centrocytes, and memory B cells, normal plasma cells had the highest levels of TMEM4 transcripts (Supplementary Figure S2).
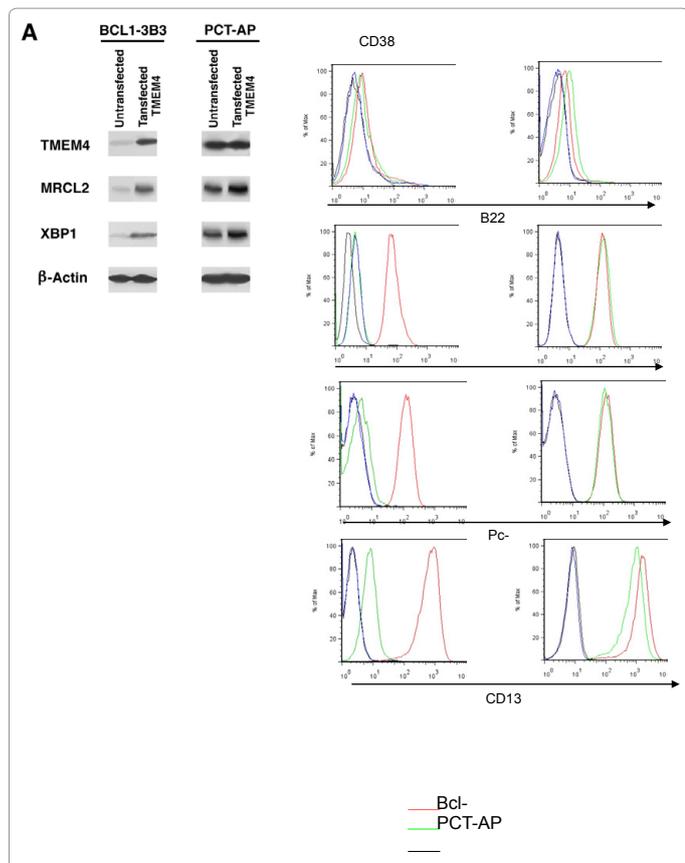
Figure 3: Tmem4 promoted the differentiation of plasma cells. (**A**) Western blot analyses showed that forced expression of *Tmem4* (*Tmem4+*) in BCL1-3B3 cells increased the levels of Mrlc and Xbp1 proteins. In PCT-AP cells, forced expression of Tmem4 (*Tmem4+*) slightly increased the levels of Mrlc and Xbp1 proteins. (**B**) Flow cytometry analyses showed that forced expression of *Tmem4* (red) increased the levels of CD38, CD138, and PC-1 in BCL1-3B3 cells but not in PCT-AP cells. Vectors without *Tmem4* gene (empty vector) served as control for Tmem4. Cells were also stained using Ig isotype of each antigen for control.
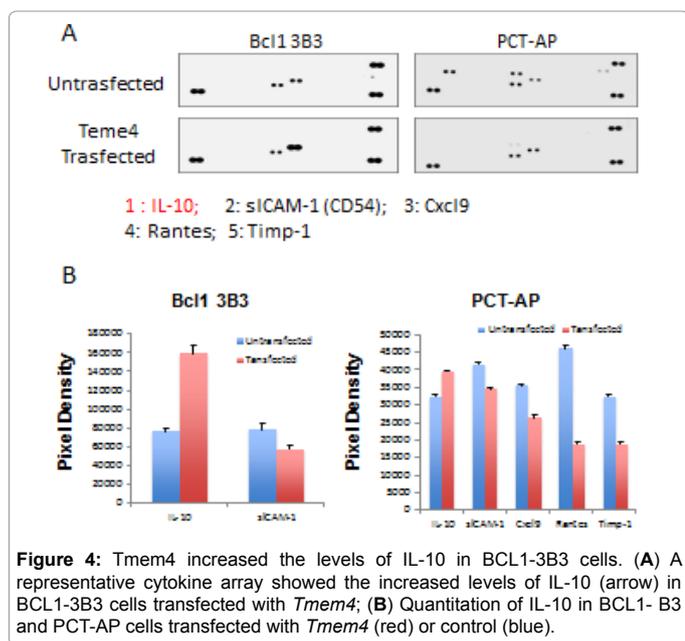
Figure 4: Tmem4 increased the levels of IL-10 in BCL1-3B3 cells. (**A**) A representative cytokine array showed the increased levels of IL-10 (arrow) in BCL1-3B3 cells transfected with *Tmem4*; (**B**) Quantitation of IL-10 in BCL1- B3 and PCT-AP cells transfected with *Tmem4* (red) or control (blue).

Furthermore, we also analyzed the levels of Tmem4 in the sera of mice bearing PCT or other types of B-cell neoplasms including APCT, CBL, IBL, BL, SBL, and MZL. The results showed that Tmem4 was detectable in serum, and the serum levels of Tmem4 were significantly higher in mice bearing PCT than that in mice bearing other types of B-cell neoplasms (P<0.001) (Figure 2C).

Over-expression of Tmem4 promoted terminal differentiation of B cells

Because of the high level of Tmem4 in plasma cells, we reasoned if Tmem4 was associated with plasma cell differentiation. We over-expressed Tmem4 in BCL1-3B3 cell line, a mature B cell line with few endogenous expression of this protein. The transfection efficiency was monitored by a control transfection using GFP construct. Only the experiments with at least 70% transfection efficiency were used for this study. As a result, the expression of XBP1 protein was increased, suggesting that Tmem4 may drive the differentiation of mature B cells to plasma cells (3A). In PCT-AP cells, exogenous expression of Tmem4 further increased the XBP1 protein level (Figure 3A). Furthermore, using flow cytometry analysis, we found that over-expression of Tmem4 in BCL1-3B3 cells led to acquisition of a phenotype of terminally differentiated plasma cells as evidenced by the increased levels of CD138 and XBP1, two markers of plasma cells (Figure 3B).

Tmem4 increased IL-10 levels

Because IL-10 is a cytokine involved in the terminal differentiation of B cells into plasma cells as well as a growth factor for malignant plasma cells [16], we examined the levels of IL-10 in BCL1-3B3 cells with forced Tmem4 expression. The results showed that Tmem4 expression significantly increased the levels of IL-10 (P<0.01) (Figure 4). This result suggested a possible involvement of IL-10 in the effect of Tmem4 on plasma cell differentiation.

Tmem4 increased the expression of cytoskeletal regulatory protein Mrlc and the levels of neurite-like structure in B cells

It has been reported that TMEM4 regulates cytoskeletal regulatory protein MRLC in neuron cells [6]. To determine if Tmem4 has a similar function in B cells, in ectopically expressed Tmem4 in BCL1-3B3 cells, we found that Tmem4 over expression up-regulated the Mrlc protein level (Figure 3A). Furthermore, we examined the levels of total and phosphorylated forms of Mrlc in B cells at various developmental stages. As shown in Figure 5A, the levels of total and phosphorylated Mrlc proteins were low or very minimal in B cells derived from GC lymphomas where Tmem4 protein levels were also low or very minimal; on the other hand, the levels of total and phosphorylated MRLC proteins were higher in B cells derived from PCT where Tmem4 levels were also higher (Figure 5A). These results revealed a positive correlation between Tmem4 and Mrlc levels.

Morphologically, we observed that, compared to the control cells (Figure 5B), over-expression of Tmem4 in BCL1-3B3 cell line gained sharp protrusions (Figure 5C) and neurite-like outgrowth (Figure 5D), similar to the results obtained from studies in neuron cells [6].

TMEM4 was highly expressed in plasma cells of MM

To determine the levels of TMEM4 in human plasma cell neoplasms, we examined four bone marrow biopsy samples collected from MM patients by IHC. The results showed that TMEM4 was located on cell membrane of plasma cells as CD138 did. And TMEM4 was highly expressed in plasma cells in MM bone marrow. Whereas as
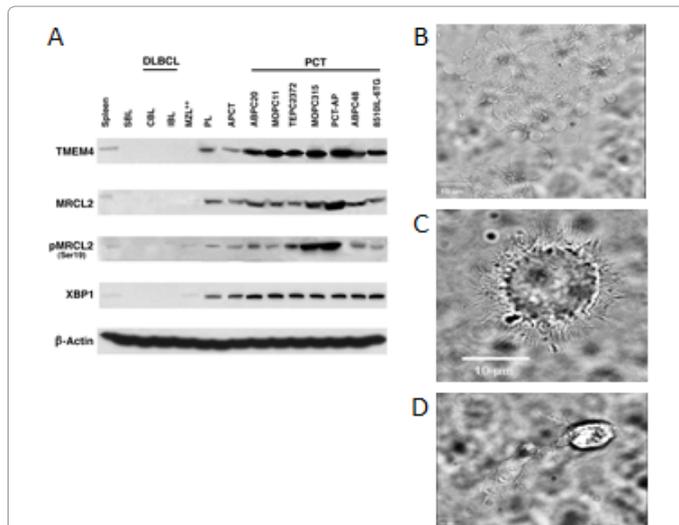
Figure 5: Tmem4 was highly expressed in plasmacytoma (PCT) cells and increased neurite-like structure in B cells. (A) Western blot analysis showed that *Tmem4*, together with Mrlc and Xbp1, were highly expressed PCT cell lines comparing to B cells derived from GC lymphomas and the spleen. (B, C, D) Compared to the control cells (B, bar: 10 μm), forced expression of Tmem4 in BCL1-3B3 cells increased sharp protrusions (C, bar: 10 μm) and the levels of neurite-like structure (D, bar: 10 μm).
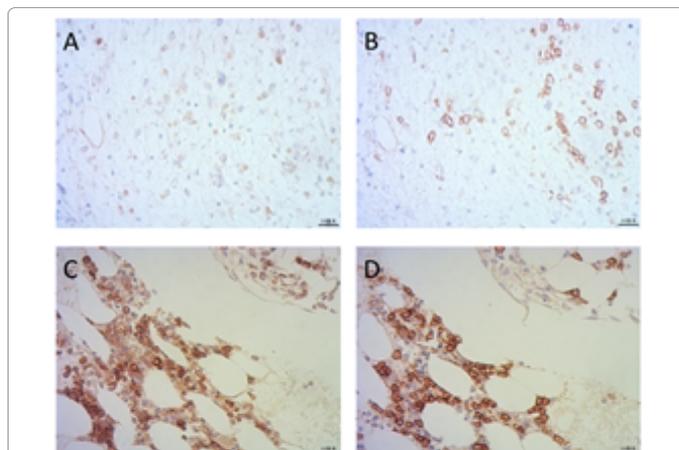

Figure 6: TMEM4 was highly expressed in plasma cells in bone marrow from multiple myeloma patients. Bone marrow biopsies collected from patients with idiopathic thrombocytopenic purpura (ITP) (A,B) or multiple myeloma (MM) (C,D) were used for immunohistochemical (IHC) analysis for TMEM4 (A,C) or CD138 (B,D). (A, C) Comparing to plasma cells in bone marrow from the non-neoplastic disease ITP (A), plasma cells in bone marrow from MM expressed high levels of TMEM4 (C). (B,D) CD138 staining was used as control for plasma cells in the bone marrow. Representative photographs from one IHC patient and one MM patient are shown. The bar in the photographs represents 20 μm.

control, plasma cells in bone marrow from ITP expressed very low level TMEM4 (Figure 6).

Discussion

In the present study, a discovery-driven approach using proteomic and functional genomic technologies led us to identify a novel protein, Tmem4 which was highly expressed in late-stage B cells including plasma cells and PCT cells. Importantly, high levels of Tmem4 protein were also detected in sera of mice bearing PCT. Mechanistically, we showed that Tmem4 was able to switch B cell phenotypes from GC-B cells to terminally differentiated plasma cells. These data strongly

suggested an important role for Tmem4 in plasma cell development as well as its potential as a biomarker for plasma cell neoplasms.

The differentiation of B lymphocytes to plasma cells represents a critical step in the development of humoral immunity. This process is accompanied by a unique gene expression program which underlies the distinct biological functions of plasma cells as Ig-secreting, terminally differentiated B cells [17]. On the other hand, the growth of PCT and MM cells is dependent on a complex interplay between growth factors, adhesion molecules, and other factors in the tumor microenvironment. Specific biomarkers for plasma cells and their malignant counterparts would provide help in effective management of plasma cell neoplasms such as MM. Currently the primary biomarker for MM is M protein. M proteins are monoclonal Ig molecules excessively produced by clonal neoplastic plasma cells, and their presence in urine or blood constitutes an important diagnostic criterion for MM [18]. Nevertheless, M protein can hardly serve as a biomarker for early stage MM, and a small portion of MM patients lack the high levels M proteins in their blood [19]. In addition to M proteins, CD138 can also serve as a prognostic biomarker for MM [20,21]. XBP1 is another B-cell differentiation-associated protein, and its expression is mainly restricted to plasma cells [22,23]. Study shows that XBP1 can be used to predict MM patients' responsiveness to thalidomide-based chemotherapy [4]. Similarly, in the present study, we found that the levels of Tmem4 were significantly greater in plasma cells than that in earlier-stages of B cells, and that Tmem4 could be detected in the serum of PCT mice, thus strongly suggesting a potential value of Tmem4 as a novel biomarker and as a therapeutic target for plasma cell neoplasms.

In the present work, we found that, comparing to early-stage B cells, plasma cells expressed high levels of TMEM4. This may suggest a role of this protein in plasma cell differentiation. Indeed, we found that forced expression of Tmem4 in mature B cells increased the expression levels of XBP-1 and IL-10 proteins. XBP1 is a well-characterized B-cell differentiation-associated protein [23,24], and IL-10 is a cytokine which potently promotes mature B cell differentiation and also stimulates the growth of neoplastic plasma cells [25,26]. These data revealed a novel pathway by which Tmem4 regulates plasma cell differentiation and neoplasm development. Previously it was reported that B-cell-activating factor (BAFF), a transcription factor promoting B lymphocyte survival and differentiation, mildly increased gene expression levels of TMEM4 in human hairy cell leukemia cells [24]. This was in agreement with our current data about the function of Tmem4 in promoting B cell differentiation.

TMEM4 was shown to increase the migration of glioma cells through its stimulatory effect on the phosphorylation of cytoskeleton protein MRLC, an important event leading to increasing the motility of mammalian non-muscle cells [27]. Similarly, in the present study, we found that the phosphorylated MRLC level in B cells was increased by expression of Tmem4, and concomitantly, the migration of plasma cells increased. This suggested that, in addition to its stimulatory effect on B cell differentiation, Tmem4 was also able to promote the migration of plasma cells. Cell migration is important for both plasma cell biology and the dissemination of neoplastic plasma cells. Thus, the present data suggest that Tmem4 may contribute to the dissemination and metastasis of plasma cell neoplasms.

The high levels of TMEM4 expression in plasma cells and their neoplastic counterparts are also suggestive that this protein could serve as a therapeutic target for plasma cell neoplasms. Previous studies have shown that antibodies against plasma-cell surface molecules such as CD38 and CD138 have certain cell killing effects on myeloma cells

while an antibody against DDK1 exhibited inhibitory effects on tumor-induced osteolysis [28,29]. Because TMEM4 is also a cell surface molecule, it is possible that specifically targeting this molecule would provide a new avenue for treating plasma cell neoplasms.

Taken together, the present work showed that Tmem4 was highly expressed in plasma cells, PCT tissues, and the sera of mice bearing PCT. Mechanistically, Tmem4 can promote plasma cell differentiation in a B cell line. These data suggest that Tmem4 as a potential biomarker for diagnostic evaluation and targeted therapy in plasma cell neoplasms is worthy of further investigation and exploration.

Acknowledgments

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