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TSPAN33 is a novel marker of activated and malignant B cells

Van Phi Luu[a,b], Peter Hevezi[a,b], Felipe Vences-Catalan[c], José Luis Maravillas-Montero[a,b], Clayton Alexander White[b,d], Paolo Casali[b,d], Luis Llorente[e], Juan Jakez-Ocampo[e], Guadalupe Lima[e], Natalia Vilches-Cisneros[f], Juan Pablo Flores-Gutiérrez[f], Leopoldo Santos-Argumedo[c], Albert Zlotnik[a,b,*]

[a] Department of Physiology and Biophysics, University of California Irvine, Irvine, CA, USA
[b] Institute for Immunology, University of California Irvine, Irvine, CA, USA
[c] Department of Molecular Biomedicine, CINVESTAV-IPN, México, D.F., Mexico
[d] Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine, CA, USA
[e] Department of Immunology, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubiran, México, D.F., Mexico
[f] Pathological Anatomy Service, University Hospital, Universidad Autónoma de Nuevo León, Monterrey, NL, México

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Abstract We have identified *Tspan33* as a gene encoding a transmembrane protein exhibiting a restricted expression pattern including expression in activated B cells. TSPAN33 is a member of the tetraspanin family. TSPAN33 is not expressed in resting B cells, but is strongly induced in primary human B cells following activation. Human 2E2 cells, a Burkitt's lymphoma-derived B cell model of activation and differentiation, also upregulate TSPAN33 upon activation. TSPAN33 is expressed in several lymphomas including Hodgkin's and Diffuse large B cell lymphoma. TSPAN33 is also expressed in some autoimmune diseases where B cells participate in the pathology, including rheumatoid arthritis patients, systemic lupus erythematosus (SLE), and in spleen B cells from MRL/Fas$^{lpr/lpr}$ mice (a mouse model of SLE). We conclude that TSPAN33 may be used as a diagnostic biomarker or as a target for therapeutic antibodies for treatment of certain B cell lymphomas or autoimmune diseases.

Abbreviations: BCMA, B cell maturation antigen; BIGE, body index of gene expression (database); TSPAN33, tetraspanin 33; BL, Burkitt's lymphoma; RA, rheumatoid arthritis; NHL, non-Hodgkin's lymphoma; DLBCL, Diffuse large B cell lymphoma; HL, Hodgkin's lymphoma; SLE, systemic lupus erythematosus.

* Corresponding author at: Department of Physiology and Biophysics and Institute for Immunology, 3034, Hewitt Hall, University of California Irvine, Irvine, CA 92697-4120, USA. Tel.: +1 949 824 0876.
E-mail address: azlotnik@uci.edu (A. Zlotnik).

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1. Introduction

The discovery and characterization of lineage specific markers have been instrumental for the identification of cell subsets that underlie the complexity of the immune system. Cell surface markers, such as CD3ε (pan T cell marker), CD4 (helper T cells), CD8 (cytotoxic T cells), and B220/CD45R (B cells), are routinely used to differentiate lymphocyte populations [1,2]. Advances in flow cytometry labeling techniques led to the characterization of CD4 subtypes (Th1, Th2, Th17 and Treg cells) based on the detection of lineage-specific transcription factors [3]. The discovery of regulatory 'B10 cells' was based on the identification of a small subset of B cells that are $CD1d^{hi}CD5^{+}$ and secrete IL-10 [4–6]. In addition, lineage specific surface markers (such as the B cell marker CD20), represent useful targets for the development of therapeutic mAbs that have proven effective against various lymphomas as well as autoimmune diseases like rheumatoid arthritis (RA) through their ability to delete pathogenic B cells [7,8].

1.1. TSPAN33 is a novel B cell activation marker

We sought to identify novel markers of human leukocytes. To this end, we analyzed a comprehensive database of human gene expression from 105 different human tissues including cells of the immune system (known as the body index of gene expression (BIGE) database) [9,10]. This database is useful for the identification of novel genes associated with specific organs or cells [11]. We identified a gene (*Tspan33*) that encodes a transmembrane protein not previously associated with B cells. The tetraspanin superfamily is defined by a conserved domain structure (Pfam00335) with a cysteine-rich long extracellular loop (LEL) containing a highly conserved cysteine–cysteine–glycine (CCG) motif [12]. These features facilitate the formation of large molecular complexes with other proteins, such as integrins or other tetraspanins and mediate diverse functions including proliferation, adhesion, motility, and differentiation. Some tetraspanins are widely expressed in adult tissues while others, (including CD82, CD151 and CD37), exhibit a more limited expression profile and are highly expressed in specific cell lineages of the immune system [13].

1.2. Previous reports on TSPAN33

TSPAN33 has been previously reported as Penumbra (proerythroblast nu membrane), since it was originally detected in a subpopulation of erythrocyte progenitors in murine bone marrow suggesting that it was involved in hematopoiesis [14]. *Tspan33* expression in the mouse bone marrow was detected in the TER 119^{+} fraction of bone marrow cells (erythroblasts), but not in neutrophils, T cells, monocytes, NK cells, or (resting) B cells [14]. Indeed, it is expressed in mouse pre-CFU erythroid cells and in mouse bone marrow [15]. These results may be explained by the small contribution that these $Tspan33^{+}$ erythrocyte progenitors make to total bone marrow RNA. Interestingly, Heikens et al. [14] generated a $Tspan33^{-/-}$ mouse, and some of these mice displayed abnormal erythropoiesis within 3 months and splenomegaly at 1 year of age. However, as we show here, the expression of TSPAN33 in normal human bone marrow is very low (Fig. 1) and is instead specifically and strongly expressed by activated B lymphocytes.

1.3. Approach

We have confirmed the expression of TSPAN33 in both mouse and human B cells. Taken together, these results indicate that TSPAN33 is a novel marker of activated B cells. In contrast to other B cell specific antigens (i.e. CD20, CD19) that are present on both resting and activated B cells, TSPAN33 is only expressed by activated B cells. We next sought to determine if TSPAN33 was also expressed in human diseases that involved activated malignant B cells. To this end we measured TSPAN33 expression in Hodgkin's lymphoma (HL), various types of non-Hodgkin's lymphoma (NHL), and in two autoimmune diseases, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

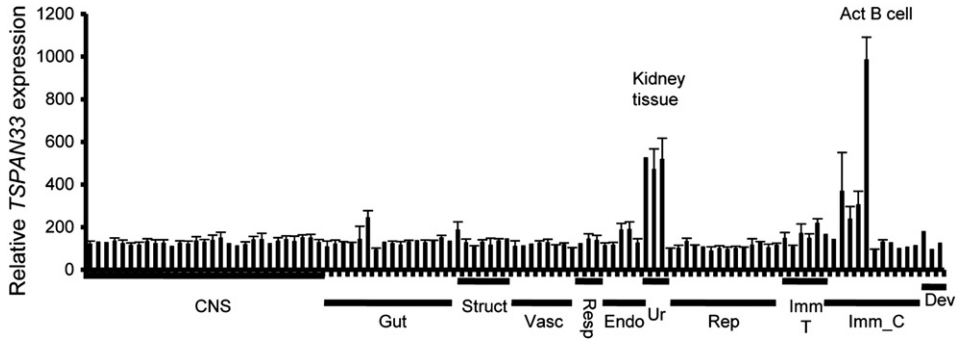

Figure 1 TSPAN33 expression is restricted to activated B cells in normal human tissues. Affymetrix gene array (U133 plus 2.0) data compiled from the human body index of gene expression database observing *TSPAN33* expression in normal human tissue (n = 8) and immune cells. X axis is organized by organ systems: CNS (central nervous system), Gut (gastrointestinal), Struct (structural), Vasc (vasculature), Resp (respiratory), Endo (endocrine), Ur (urinary), Rep (reproductive), Imm_T (immune tissue), Imm_C (immune cells), and Dev (developmental).