Discrimination of biclonal B-cell chronic lymphoproliferative neoplasias by tetraspanin antigen expression

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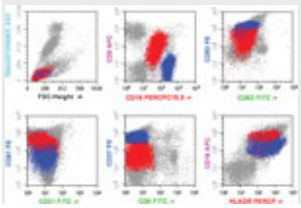
The adhesion and migration properties of a cell and its interaction with stroma or other cells are modulated, among others, by tetraspanin proteins.[1] Tetraspanin antigens are a family of 28 membrane proteins widely expressed in different cell types. Each cell type displays a specific pattern of expression of several tetraspanins that interact among themselves forming a tetraspanin complex. This complex forms a new type of membrane microdomain[1, 2] that interacts with other membrane proteins, such as integrins, CD19, CD21, HLA-class II antigens and some growth factor receptors.[2] The specific function of the tetraspanin proteins is not well understood, but they clearly modulate the biological function of the associated molecules in the complex.[3] Therefore, the pattern of expression of tetraspanin and tetraspanin-associated molecules can modulate the adhesion and migration characteristics of both normal and tumor cells. In the hematopoietic system, B-cell maturation is associated with changes in the pattern of expression of tetraspanins.[4] Also in, B-cell malignancies, the tetraspanin phenotype appears to reflect the differentiation stage of the cell of origin, and thus can be of use in tumor phenotyping.[4]

Here, we report a case of an adult female diagnosed with a biclonal B-cell lymphoproliferative disorder, with coexistence of B-chronic lymphocytic leukemia (B-CLL) and splenic marginal zone B-cell lymphoma (SMZL)[5] – two tumors closely related with respect to their B-cell maturation stage, – and attempted their discrimination by the determination of the pattern of tetraspanin antigen expression. For this purpose, five tetraspanin antigens were simultaneously analyzed. Discrimination between the two B-cell clones could be achieved based on conventional markers because of their different reactivity for CD5 and CD19 (B-CLL cells were $CD5^{+}/CD19^{dim}$ and SMZL cells were $CD5^{-}/CD19^{++}$) as illustrated in Figure 1 (panel b). The following tetraspanin antigens were studied: CD37 (B-cell specific), CD53 (restricted to lymphoid-myeloid lineages), CD9 and CD81 (expressed in most cell types, including hematopoietic and epithelial cells), and CD63 (mostly present in endosomes). In addition, the reactivity for the CD19, CD21 and HLA-DR tetraspanin-associated molecules was determined by multicolor flow cytometry, as previously described.[5]

Figure 1.



Illustrative bivariate dot plots of a peripheral blood (PB) sample from a patient with two distinct neoplastic B-cell clones, one corresponding to a $CD19^{+}/CD5^{+}$ B-cell chronic lymphocytic leukemia (red dots, with lower light scatter characteristics) and the other to a $CD19^{++}/CD5^{-}$ splenic marginal zone lymphoma (blue dots) top right and middle. Note that clearly different patterns of expression for the CD53 (top right), CD81 (bottom left), CD37 (bottom middle) and CD19 (bottom right) antigens were observed in each of the two B-cell clones. Immunophenotypic studies were performed on erythrocyte-lysed (PB) samples using well-established stain, lyse and then wash procedures.[8] Finally, cells were resuspended in 0.5 ml of PBS and analyzed in a FACSCalibur flow cytometer using the CellQuest software program (BDB). The source of the MoAb was as follows: CD19, CD38 and anti-HLA-DR were purchased from Becton Dickinson Biosciences (–BDB- San José, CA, USA), CD53 and CD81 were from PharMingen (San Diego, CA), CD9 and CD63 from Immunotech (Marseille, France), CD21 from DakoCytomation (Glostrup, Denmark) and CD37 from DIATEC.com AS (Oslo, Norway).

Full figure and legend (207K)

Interestingly, the two distinct B-cell clones showed a different pattern of expression of several tetraspanin antigens and tetraspanin-associated molecules (Figure 1). Accordingly, three of the five tetraspanin antigens studied (CD53, CD81 and CD37) could be used to clearly discriminate between the two clonal B-cell populations in a similar way to the CD19 and CD5 markers, especially if the CD53/CD81 and CD37/CD81 combinations were used in double-staining with different fluorochomes; B-CLL cells were $CD53^{-}$, $CD81^{+}$ and $CD37^{-}$ while SMZL cells were $CD53^{+}$, $CD81^{-}$ and $CD37^{+}$. In turn, the CD19 tetraspanin-associated molecule was also differentially expressed by the two distinct B-cell clones, while CD21 and HLADR could not be used to discriminate between the two tumor clones. It should be noted that in B cells, CD19 forms part of a signaling complex of which tetraspanins are a major component; however, until now such complexes have been characterized only for CD81[6] and CD81 forms complexes with other tetraspanins.[1, 2] The varying composition of the tetraspanins-CD19-CD21 complexes on the two neoplastic B-cell populations suggests that the complex composition, and therefore the signaling, are different in each of the two coexisting B-cell clones.[7] Functionally, it is interesting to note that CD53 is an antigen whose engagement protects cells from apoptosis, and thus could be related to the prolonged survival of B-CLL, but not SMZL, neoplastic B-cells.[3] In addition, the observation of the existence of a different pattern of tetraspanin expression could also reflect the existence of different adhesion properties for each tumor cell population, and thus affect their migration characteristics.

From a practical point of view, our results suggest that determination of tetraspanin antigens can contribute to improving the characterization and discrimination of different B-cell malignancies, especially once diagnosis of biclonal B-lymphoproliferative disorder is suspected.