Multiple Myeloma

The expression of PRDI-BF1β isoform in multiple myeloma plasma cells

The *PRDM1* gene, a master regulator of plasma cells (PC), can generate two transcription factor isoforms: PRDI-BF1α and PRDI-BF1β. The present study shows that purified human normal PC have a significantly lower level of PRDI-BF1β expression than that in tumoral PC isolated from multiple myeloma (MM) (0.06±0.01 and 0.25±0.05, respectively; $p<0.001$). The role of this finding in MM is discussed.

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Human positive regulatory domain I binding factor 1 (PRDI-BF1 or BLIMP-1) is a transcription factor that has been demonstrated to act as a master regulator required and sufficient for the generation and for the prolonged maintenance of plasma cells (PC).[1] PRDI-BF1 essentially functions as a repressor, causing exit from cell cycling and the extinction of the expression of several genes critical for B-cell development at earlier stages.[2] The *PRDM1* gene, which codifies for PRDI-BF1, contains an alternative promoter capable of generating a PRDI-BF1 deleted protein (called PRDI-BF1β), which lacks 101 amino acids comprising most of the regulatory domain (Figure 1A). PRDI-BF1β has been detected in relevant quantities in multiple myeloma (MM) cell lines (U266 and NCI-H929).[3] Since this molecule contains the DNA-binding domain but bears a disrupted regulatory domain, PRDI-BF1β might behave as an inhibitor of functional PRDI-BF1, called PRDI-BF1α. We decided to compare, using real time polymerase chain reaction (RT-PCR), the occurrence of PRDI-BF1α and PRDI-BF1β in human MM cell lines (U266 and NCI-H929, from ECACC, Salisbury, UK), MM patients` tumoral PC (n=17) freshly purified by immunomagnetic separation with monoclonal anti-CD138–coated microbeads (Miltenyi-Biotec, Auburn, CA, USA) and normal human PC (n=11) purified from either bone marrow, tonsil or colon lamina propria, as previously described.[4] In order to perform a relative quantitative RT-PCR analysis of both factors, two different oligonucleotides pairs were used, one detecting both the α- and β-isoforms, and the other recognizing only the PRDI-BF1β isoform (Figure 1B). Total RNA was extracted and reverse transcribed from purified PC using RNA isolation and transcriptor kits from Roche (Barcelona, Spain). Primer mixes and TaqMan probes, assay references Hs00153357_m1 for PRDI-BF1(α and β) and

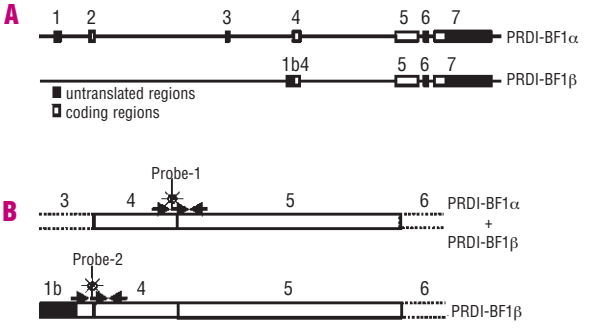

Figure 1. *PRDM1* gene organization and primer localizations. A. Schematic exon-intron distribution of the *PRDM1* gene showing the composition of the PRDI-BF1α and PRDI-BF1β isoform exons. **B.** Locations of probes and of primer pairs on cDNA used for detecting the two transcripts. Probe-1 hybridizes on the sequence junction between exon-4 and exon-5 (common to both isoforms) and does not discriminate between the two transcript isoforms. Probe-2 (5´-(FAM)-CTCTGGAATAGATCTTTTC-(TAMRA)-3´) is a specific probe for detecting the PRDI-BF1β isoform which hybridizes on the sequence junction between exon-1 and exon-2 for the PRDI-BF1β isoform. The forward primer sequence was 5´-CCGAACATGAAAAGACGATAAAACTGA-3´ and the reverse primer sequence 5´-CCGTCAATGAAGTGGTGAAGCT-3´.

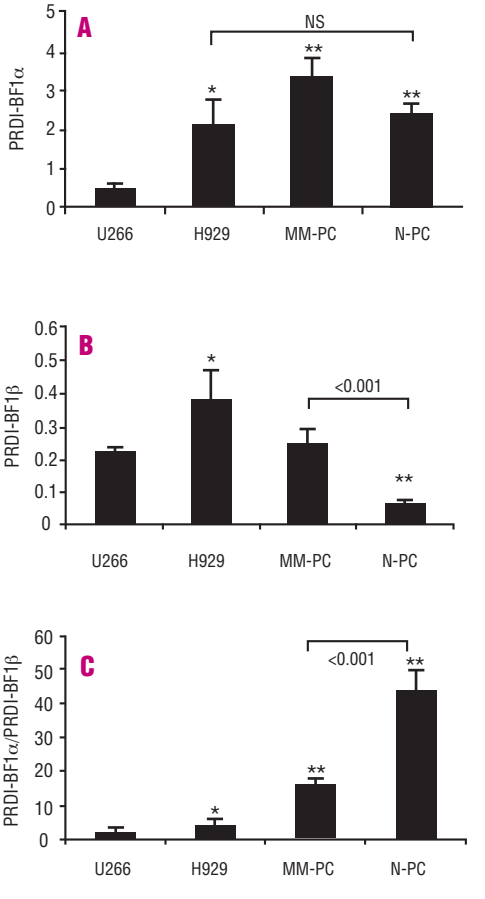

Figure 2 (right). Expression of PRDI-BF1 transcripts measured by real-time PCR. All transcript expression results are calculated relative to β-actin and are expressed in arbitrary units. The Mann-Whitney U test was used to determine the statistical differences. A. PRDI-BF1α transcript expression in MM cell lines (U266 and NCI-H929), in human normal PC (N-PC) and in MM patients` PC (MM-PC). The data were obtained by subtracting the value observed with Probe-2 (specific for the β isoform) from values observed with Probe-1 that detected α and β isoforms in each experiment. B. PRDI-BF1β transcript expression levels obtained with Probe-2. C) Ratio of PRDI-BF1α/PRDI-BF1β considering the results of expression levels obtained from A) and B). The results represent the mean±s.e.m. of 4, 3, 11 and 17 different experiments for U266 and NCI-H929 cell lines, N-PC and MM-PC, respectively. *=$p<0.05$ and **=$p<0.01$ compared to U266. NS: not *significant.*

Hs99999903_m1 for β-actin (used as an internal reference), were obtained from Applied Biosystems (Foster City, CA, USA). For PRDI-BF1β specific detection, a probe was synthesized using the Assays-by-Design Service (Applied Biosystems). The quantity of PRDI-BF1α was obtained by subtracting the PRDI-BF1β value from the quantity obtained for both isoforms together. The relative quantitative values were calculated using the $2^{-\Delta\Delta CT}$ method.[5]

Figure 2A shows that PRDI-BF1α was expressed at considerable levels in all PC samples tested and no significant differences were found in the PRDI-BF1α expression between the MM cell line NCI-H929, normal PC and MM patients` PC (2.10±0.66; 2.38±0.26; 3.32±0.52, respectively: arbitrary units, mean±s.e.m.). The reduced level of PRDI-BF1α observed in the U266 cell line (0.5±0.11; mean±s.e.m.) has been previously reported in a study using a ribonuclease protection assay.[3] The relatively high expression of the α-isoform in all cell types tested could be expected bearing in mind that its protein product, PRDI-BF1α, is strictly required for commitment to the PC fate.[1,6-8] When the expression level of the PRDI-BF1β isoform was tested and compared in the same PC populations (Figure 2B), significantly greater differences were observed. Interestingly, normal human PC had a markedly lower transcript level for this factor (0.06±0.01) than did the other three MM PC populations (0.23±0.01, 0.38±0.09 and 0.25±0.05, for U266 and NCI-H929 MM cell lines, and MM patients` PC, respectively; mean±s.e.m.). These data suggest that the level of PRDI-BF1β expression could be a feature distinguishing between malignant and normal PC. For this reason we decided to examine the ratio of PRDI-BF1α/PRDI-BF1β transcript levels in the different PC under study. As shown in Figure 2C, MM cell lines had very low ratios (2.26±0.56 and 5.28±0.35 for U266 and NCI-H929, respectively), a result that was due to the high level of PRDI-BF1β expression. In contrast, normal PC had a ten-fold higher PRDI-BF1α/PRDI-BF1β ratio (43.68±5.89). The ratios obtained in normal PC were more than three times higher than those of MM patients` PC (16.37±1.04). The physiological significance of the differential expression of the two PRDI-BF1 isoforms by PC remains to be elucidated. In this regard, other PRDM family members have also been shown to be expressed in two different isoforms, the deleted one acting as a negative regulator of the other.[9] In addition, the tumorigenic capacity of a defective PRDI-BF1α has been recently observed in human diffuse large B-cell lymphoma.[10] Therefore, it is conceivable that the overexpression of PRDI-BF1β detected in MM patients` PC could contribute to progression into the tumoral state.[3]

Esther Ocaña,[†] Inés González-García,[†]* Norma C. Gutiérrez,° Francisco Mora-López,* José A. Brieva,* Antonio Campos-Caro**

**Unidad de Investigación, Hospital Universitario Puerta del Mar, Cádiz; °Servicio de Hematologia, Hospital Universitario de Salamanca, Salamanca, Spain*

[†]These authors contributed equally to this work.

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Correspondence: Antonio Campos-Caro, Unidad de Investigación, 9ª Planta, Hospital Universitario Puerta del Mar, Avenida Ana de Viya, 21, 11009 Cádiz, Spain.
E-mail address: antonio.campos.exts@juntadeandalucia.es

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