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The *IL-12Rβ2* gene functions as a tumor suppressor in human B cell malignancies

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Article **Oncology**

The *IL-12Rβ2* gene is expressed in human mature B cell subsets but not in transformed B cell lines. Silencing of this gene may be advantageous to neoplastic B cells. Our objective was to investigate the mechanism(s) and the functional consequence(s) of *IL-12Rβ2* gene silencing in primary B cell tumors and transformed B cell lines. Purified tumor cells from 41 patients with different chronic B cell lymphoproliferative disorders, representing the counterparts of the major mature human B cell subsets, tested negative for *IL-12Rβ2* gene expression. Hypermethylation of a CpG island in the noncoding exon 1 was associated with silencing of this gene in malignant B cells. Treatment with the DNA methyltransferase inhibitor 5-Aza-2′-deoxycytidine restored IL-12Rβ2 mRNA expression in primary neoplastic B cells that underwent apoptosis following exposure to human recombinant IL-12 (hrIL-12). hrIL-12 inhibited proliferation and increased the apoptotic rate of *IL-12Rβ2*–transfected B cell lines in vitro. Finally, hrIL-12 strongly reduced the tumorigenicity of *IL-12Rβ2*–transfected Burkitt lymphoma RAJI cells in SCID-NOD mice through antiproliferative and proapoptotic effects, coupled with neoangiogenesis inhibition related to human IFN-γ–independent induction of hMig/CXCL9. The *IL-12Rβ2* gene acts as tumor suppressor in chronic B cell malignancies, and […]

The *IL-12Rβ2* gene functions as a tumor suppressor in human B cell malignancies

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Introduction

IL-12 is a heterodimeric cytokine bridging innate resistance and antigen-specific adaptive immunity (1). IL-12 is produced predominantly by professional APCs and serves as an important regulator of T cell and NK cell function (2, 3). IL-12 has a powerful antitumor activity related to both IFN-γ–dependent and –independent mechanisms (4-9).

Numerous effects of IL-12 on human B cells from normal individuals have been reported, such as induction of proliferation and differentiation to Ig-secreting cells (10), upregulation of IL-18R (11), and induction of IFN-γ mRNA and protein (11–13).

The biological functions of human IL-12 are mediated by the heterodimeric IL-12R composed of two subunits, the β1 and the β2 chains, conferring high-affinity binding of and responsiveness to IL-12 (14, 15). The β1 chain participates in the formation of the IL-23 receptor, whereas the β2 chain is an unique component of IL-12R (1). The *IL-12Rβ2* gene maps on chromosome 1p31.2 and is composed of 16 introns and 16 exons, the first of which is not transcribed (15).

We have previously shown that human lymphoblastoid B cell lines (LCLs) and Burkitt lymphoma (BL) cell lines express constitutively IL-12Rβ1 mRNA but lack IL-12Rβ2 mRNA (16). In contrast, normal human naive, germinal center (GC), and memory B cells express constitutively the transcripts of both *IL-12R* genes (11). These findings led us to speculate that malignant B cells could benefit from the silencing of the *IL-12Rβ2* gene.

Here we first investigated *IL-12R* gene expression in primary tumor cells from patients with different chronic B cell lymphoproliferative disorders representing the postulated counterparts of the major mature B cell subsets, as no information was available on this issue. IL-12Rβ2 mRNA expression was not detected in any sample. Second, we studied the mechanism(s) involved in and the functional consequences of the silencing of this gene in human primary neoplastic B cells, as well as in transformed B cell lines. Our findings support the conclusion that the *IL-12Rβ2* gene functions as tumor suppressor in a wide spectrum of human B cell malignancies.

Results

Expression of IL-12R in normal tonsil B cell subsets and in malignant B cells from different human chronic lymphoproliferative disorders. Figure 1A shows one experiment, representative of the ten performed with identical results, in which the IL-12Rβ1 and β2 transcripts were detected in naive, GC, and memory B cells isolated from tonsil, consistently with a previous study (11). As is apparent, these B cell fractions expressed CD19, but not CD3γ, CD56, or CD68, mRNA.

In subsequent studies, frozen tonsil tissue sections were stained sequentially with phycoerythrin-conjugated (PE-conjugated) anti–IL-12Rβ2 and FITC-conjugated CD19 mAb's or isotype and fluorochrome matched control mAb's and then with DAPI. Images were acquired using either a filter selective for fluorochrome (Figure 1, C, G, K and D, H, L) or a triple-band — i.e., DAPI, PE, and FITC–specific — filter (Figure 1, E, I, M and F, J, N).

Figure 1B shows a DAPI staining with the follicular mantle (FM), the GC, and the subepithelial (SE) area in boxes. Figure 1, C, G, K and D, H, L, shows staining with CD19 (green) or anti–IL-12Rβ2 (red) mAb's, respectively. As is apparent, the large majority of cells positioned in the FM, GC, and SE areas expressed CD19

Nonstandard abbreviations used: B chronic lymphocytic leukemia (B-CLL); Burkitt lymphoma (BL); follicular lymphoma (FL); follicular mantle (FM); germinal center (GC); green fluorescent protein (GFP); human IFN-γ (hIFN-γ); human recombinant IL-12 (hrIL-12); lymphoblastoid cell line (LCL); mantle cell lymphoma (MCL); marginal zone lymphoma (MZL); methylation-specific PCR (MSP); phycoerythrin (PE); subepithelial (SE).

Conflict of interest: The authors have declared that no conflict of interest exists.

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