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PTX3 is constitutively active in CLL cells

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Abstract

The pentraxin-related PTX3, commonly produced by myeloid and endothelial cells, is a humoral pattern-recognition protein of the innate immune system. Because CLL patients' PTX3 plasma levels are high and most circulating cells in patients with chronic lymphocytic leukemia (CLL) are CLL cells, we reasoned that CLL cells produce PTX3. Western immunoblotting revealed that low-density cells from 7 out of 7 CLL patients produce high levels of PTX3, flow cytometry analysis revealed that the PTX3-producing cells are B lymphocytes co-expressing CD19 and CD5, and confocal microscopy demonstrated that PTX3 co-localized with STAT3. Because signal transducer and activator of transcription (STAT)-3 is constitutively activated in CLL cells and we identified putative STAT3 binding sites within the PTX3 gene promoter, we postulated that phosphorylated STAT3 triggers transcriptional activation of PTX3. Immunoprecipitation analysis of CLL cells' chromatin fragments showed that STAT3 antibodies precipitated PTX3 DNA. STAT3 knockdown induced a marked reduction in PTX3 expression, indicating a STAT3-induced transcriptional activation of the PTX3 gene in CLL cells. Using an electromobility shift assay that we established and a dual-reporter luciferase assay, we confirmed that STAT3 binds the PTX gene promoter. Downregulation of PTX3 induced apoptotic disassembly of CLL cel3ls, suggesting that inhibition of PTX3 might benefit patients with CLL.

Background

Pentaxin related 3 (PTX3) is a humoral factor that is upregulated in CLL.

An activated isoform of the transcription factor STAT3 ($_{ser}$727-STAT3) is constitutively present in CLL cells.

STAT3 activates pro survival and antiapoptotic programs in these cells.

The promoter of the PTX3 gene has several STAT3 binding sites.

Hypothesis

STAT3 induces the expression of PTX3.

PTX3 provides CLL cells with a survival advantage.

Materials & Methods

To determine levels of PTX3 we used ELISA and observed its presence by confocal microscopy.

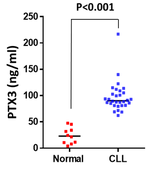
We applied Western blot to determine levels of STAT3 and levels of STAT3 CD5 and CD19 by flow cytometry.

We used chromatin immunoprecipitation (ChIP) assay to identify STAT3-STAT3 promoter sites binding and verified the results by electromobility shift assay (EMSA).

Promoter activity was assessed by luciferase assay.
We transfected the cells with STAT3-shRNA or with PTX3-siRNA and assessed apoptosis by flow after staining with annexin-PI.

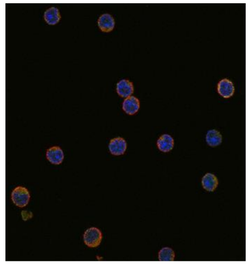
qRT-PCR was used to determine levels of STAT3-dependent transcripts.

Results



A. PTX3 is upregulated in CLL cells

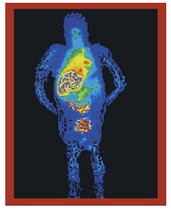
Levels of PTX3 in cells that were harvested from healthy volunteers and from patients with CLL were determined by ELISA



B. STAT3 and PTX3 are present in the cytosol of CLL cells

Confocal microscopy: Dapi staining for nucleus (blue), PTX3 (yellow) STAT3 (red).

Summary



Discussion

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