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c-Jun AND GST-π EXPRESSION IN HUMAN PLASMA CELLS

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ABSTRACT

Bone marrow samples from 33 patients affected by MM and MGUS, and 8 patients not affected by lymphoproliferative diseases were studied for expression of c-Jun (a component of the transcription factor AP-1) and glutathione-S-transferase π (GST-π) using immunocytochemical methods. A high and frequent expression of these two proteins was found both in MM and MGUS patients (31/33 patients positive for c-Jun and 29/33 patients positive for GST-π) and in controls not affected by monoclonal gammopathy (7/8 patients positive for both c-Jun and GST-π). No statistically significant correlation was found between c-Jun- and GST-π-positive plasma cells. The expression of these two proteins was not related to clinical or laboratory data. Our results seem to confirm a possible role of the transcriptional complex AP-1 in activating GST-π promoter in human plasma cells.

Key words: c-Jun, AP-1 transcription factor, glutathione-S-

C-Jun is a protein product of the Jun proto-oncogene family which is contained in mammalian transcription factor AP-1 (activator protein-1). The AP-1 complex includes some members of the Jun (c-Jun, Jun B and Jun D) and Fos (c-Fos, Fos B, Fra 1 and Fra 2) protein families[1] that exhibit DNA binding activity. c-Jun/AP-1 is able to bind a specific heptameric consensus sequence in DNA (TGACTCA) and stimulate transcription from an adjacent promoter.[2]

The AP-1 binding site has been identified as the TPA responsive element (TRE) of several genes whose transcription is induced in cultured cells treated with TPA.[3] TPA is able to activate protein kinase C, which probably serves as a receptor for tumor promoters.[4] Angel *et al*.[3] suggested a function for the AP-1 complex in transmitting the effects of phorbol esters (and possibly growth factors) from the membrane to the nucleus.

The AP-1 transcription factor is induced by IL-6,[5] a plasma cell growth factor whose role has been frequently emphasized in the etiopathogenesis of multiple myeloma. Moreover, in a recent paper[6] we demonstrated a high expression of glutathione S-transferase (GST-π) isoenzyme, which has been related to resistance to alkylating agents,[7] in patients affected by multiple myeloma.

A sequence containing a typical heptameric AP-1 binding site has been shown in GST-π gene promoter, and the involvement of Jun and Fos proteins in regulating transcriptional activation of GST-π gene has been demonstrated in both human and animal cells.[8]

These data induced us to search for possible expression of c-Jun/AP-1 on plasma cells from patients affected by multiple myeloma using immunocytochemical methods.

Patients and Methods

We evaluated 33 patients (14 male and 19 female, median age 67 years, range 52-76) with MGUS or MM between December 1994 and October 1995. The staging of the patients and the isotype of the monoclonal component are reported in Table 1. Ranges of β2-microglobulin, thymidine kinase and protein C reactive were, respectively: 1.78-18.8 mg/L; 4.27-40 U/L; 0.2-10.8 mg/dL. Eighteen patients had been treated with VAD or alkylating agents; 15 patients had not been treated before. We also evaluated 8 patients affected by sideropenic anemias or non hematological diseases as normal controls.

The percentage of bone marrow plasma cells was determined by immunofluorescence using fluorescein-conjugated goat anti-human immunoglobulin subtypes.

Expression of Jun and GST-π were studied by immunocytochemical methods.

In order to study c-Jun/AP-1 expression, after fixation we incubated cells (1 h. at 37°C) with a polyclonal rabbit antibody (cat# sc-45, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) (1:30 in PBS-BSA 0.5%) as a first layer, and with biotinylated swine anti-rabbit immunoglobulins in PBS as secondary antibody.

The specificity of the antibody raised against c-Jun/AP-1 was assayed using its control peptide (sc-45 P, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). For neutralization, we reacted antibody with a tenfold (by weight) excess of peptide antigen in PBS.

GST-π expression was detected as previously described.[6] Counting of positive plasma cells was performed on 200 cells independently by two researchers. The significance of the correlation between the percentages of positivity for c-Jun and GST-π was evaluated by using the Wilcoxon test. The intensity of positivity was classified as follows: +, only a few granules spread in the cytoplasm; ++, diffuse cytoplasmic granules; +++, granules covering the whole cell.

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Results

Thirty-one out of 33 (94%) samples were positive for c-Jun expression (Figure 1) and 16/18 (89%) treated patients and all untreated patients showed positive plasma cells ranging from 2% to 100%. In 20 samples, the percentage of positive plasma cells was over 90%, whereas plasma cells from patients not affected by monoclonal gammopathy were all positive for c-Jun, except for one negative sample.

No difference in the percentage or intensity of plasma cell positivity was observed between the patients affected by MM and those affected by MGUS, or among treated and untreated patients and those treated with different cytotoxic agents.

Overall, 29/33 (88%) samples from MM and MGUS patients were positive for GST-π expression, showing diffuse brown granules in the cytoplasm (Figure 2). The percentage of GST-π-positive plasma cells ranged from 8% to 100%, with no relationship with the type of monoclonal gammopathy (MM or MGUS) or any previous treatment. All plasma cells from patients not affected by monoclonal gammopathy were positive for GST-π, with the exception of one case that was negative for both c-Jun and GST-π expression.

No significant correlation was found between the percentage of positivity for c-Jun and GST-π in monoclonal plasma cells according to the Wilcoxon test.

Comment

Jun is a component of the AP-1 complex that plays an important role in controlling transcription of several target genes in response to a variety of stimulations of cell surface receptors by growth factors and cytokines, such as PDGF, EGF, TNF-α, IL-1.[9] The AP-1 complex may also be responsible for neoplastic transformation; transfection of c-Jun without any structural change of the protein is able to induce cell transformation in rat fibroblasts.[10]

Our study shows that normal as well as MGUS and MM plasma cells frequently express Jun and GST-π. We did not find any significant correlation between the percentage of plasma cells reactive to antibodies directed against c-Jun and that of GST-π-positive cells. Nevertheless, it is interesting to note that there is concordant positivity for the two proteins in both samples from patients and normal controls.

Our observation appears to confirm the suggested role for AP-1 in activating GST-π promoter. Given their frequent expression of Jun and GST-π, drug resistance could be considered a constitutive characteristic of plasma cells, as shown in a previous study[7] reporting co-expression of P-170 and GST-π on plasma cells from patients affected by multiple myeloma, even if the disease was untreated.

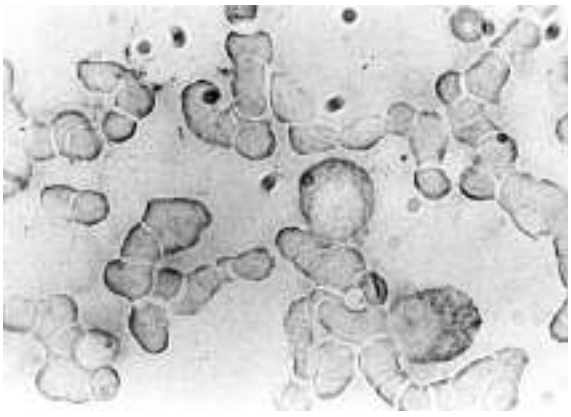

Figure 1. Immunocytochemical detection of c-Jun. Positivity is revealed by the dark granules covering both the cytoplasm and nucleus of plasma cells.

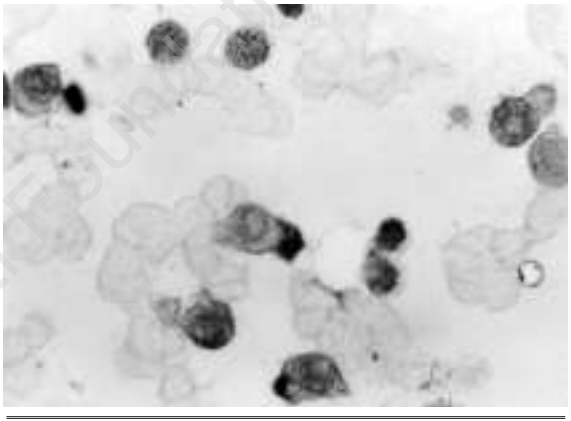

Figure 2. Immunocytochemical detection of GST-π. Note the dark staining in the cytoplasm of positive plasma cells.

References

1. Curran T, Franza BR Jr. Fos and Jun: the AP-1 connection. Cell 1988; 55:395-7.
2. Nakabeppu Y, Ryder K, Nathans D. DNA binding activities of three murine Jun proteins: stimulation by Fos. Cell 1988; 55:907-15.
3. Angel P, Imagawa M, Chiu R, et al. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell 1987; 49:729-39.
4. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumour promotion. Nature 1984; 308:693-8.
5. Hsu W, Kerppola TK, Chen P-L, Curran T, Chen-Kiang S. Fos and Jun repress transcription activation by NF-IL6 through association at the basic zipper region. Mol Cell Biol 1994; 14:268-76.
6. Petrini M, Di Simone D, Favati A, Mattii L, Valentini P, Grassi B. GST-π and P-170 co-expression in multiple myeloma. Br J Haematol 1995; 90:393-7.
7. Johnston JB, Israels LG, Goldenberg GJ, et al. Glutathione S transferase activity, sulfhydryl group and glutathione levels and DNA crosslinking activity with chlorambucil in chronic lymphocytic leukemia. J Natl Cancer Inst 1990; 82:776-9.
8. Bergelson S, Pinkus R, Daniel V. Intracellular glutathione levels regulate Fos/Jun induction and activation of glutathione S-transferase π gene expression. Cancer Res 1994; 54:36-40.
9. Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1991; 1072:129-57.
10. Schutte J, Viallet J, Nau M, Segal S, Fedorko J, Minna J. jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. Cell 1989; 59:987-97.