Altered expression of p53 and MDM2 proteins in hematological malignancies*

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Received September 12, 2002

In order to define the possible role of the MDM2 gene in the pathogenesis of human leukemia, the expression of MDM2 protein was examined in samples of fixed-permeabilized peripheral blood (PB) or bone marrow (BM) cells of leukemic patients by using flow cytometry. The present study showed, that normal PB and BM cells expressed low levels of MDM2. Overexpression of this protein was more frequently found in leukemic cells, namely in samples of patients with advanced, than those in incipient clinical stage of disease at examination. Of the 34 leukemias tested in our laboratory 24 (70%) showed abnormal expression of the MDM2 protein. This include 8/12 (66%) ALL, 10/13 (76%) B-CLL, and 6/9 (66%) AML. Since MDM2 and p53 are functionally related and overexpression of MDM2 can abrogate wild (wt)-p53 tumor suppressive function, we examined simultaneously with MDM2 protein expression also the expression of both wt-p53 and mutant (mt)-p53 with two MoAbs (Ab5 and Pab240). As measured by flow cytometry only a small part of the observed wt-p53 protein was in true wt-conformation (Ab5+), while most was in mt-conformation (Pab240+), which could mean, that most of the p53 protein in the cells was not functional, as in its usual role as a suppressor of the cell cycle. The MDM2 positive cases were negative for p53 (Pab240-) in hematopoietic cells of patients with B- and T-ALL at diagnosis and in relapsed disease. Samples of patients in remission with immunophenotype of normal cells were p53 and MDM2 negative. The expression of Ki67 antigen a nuclear protein associated with cell proliferation was used to verify the proliferative activity of the leukemic cells. Results of the two-color flow cytometric assay, which allows better definition of pathologic cell populations and nuclear fluorescence data for p53, MDM2 or Ki67 on a population of cells expressing only a given surface blast marker, confirmed their coexpression in the same cell.

Our preliminary results supported the view that the expression of p53 is very probably involved in the regulation of leukemic hematopoiesis and that the inhibition of p53 expression could modulate the proliferation of leukemic cells. It appears, that MDM2 overexpression, which may be p53-dependent, or also p53-independent plays an important role in leukemogenesis and/or disease progression.

Key words: p53, MDM2, flow cytometry, leukemia cells.

Inactivation by mutations is the most commonly detected abnormality in the p53 tumor suppressor gene. The MDM2 oncogene encodes a 90 kilodalton nuclear phosphoprotein that is induced by wild type (wt) p53 after DNA damage and inactivates p53 function, functioning as a p53 negative feedback regulator [1, 4, 8, 17]. Therefore, MDM2 may function both to enhance cell survival and to induce cell proliferation [22]. Although p53 alterations are common in human solid tumors, they are infrequent in hematological malignancies [19, 21]. Conversely, overexpression of MDM2 protein is frequently observed in hematological malignancies, particularly in patients with poor prognosis and advanced disease [3, 9, 10, 22, 26, 28, 29]. Importantly, MDM2 overexpression is not always related to alterations of p53, suggesting that MDM2 can impact on the growth and survival of tumor cells independent of p53 [15, 21, 23]. Although considerable attention has been focused on the structural changes in p53 and their biological consequences, the functional significance of MDM2 overexpression in human cancers is not well understood.

We have previously documented overexpression of p53 protein in some leukemia cells by flow cytometry [12, 13]. The present study may contribute to elucidation of some alternative mechanisms to p53 mutation for their functional

*This work was supported by the Grant No. 2/1109/21 from the Slovak Grant Agency.