5 P

IS P53 EXPRESSION, DETECTED BY IMMUNOHISTOCHEMISTRY, AN IMPORTANT PARAMETER OF RESPONSE TO TREATMENT IN TESTIS CANCERS?

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The impact of P53 expression on response to anticancer treatment is still a matter of debate. P53 expression was assessed by IHC using DO7-Dako clone mAb in 50 untreated patients with germ cell testicular tumors (GCTTs). High positive expression of P53 in the tumor cells was significantly associated with favorable clinical outcome P=0,01. Our findings show that, contrary to what is seen in most other neoplasias, P53 overexpression is associated with better response to anticancer treatment. They argue against the hypothesis of chemoresistance for patients with P53 overexpression and suggest P53 accumulation to be a marker indicative of tumor sensitivity, at least in GCTTs.

6 O

INTRINSIC MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN IN HUMAN MALIGNANT MELANOMA CELL LINES

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Disseminated malignant melanoma is characterized by unresponsiveness to chemotherapy without overexpressing corresponding P-glycoprotein. Another ABC-transporter protein functioning as transmembrane drug-efflux pump and involved in multidrug resistance (MDR), is the multidrug resistance-associated protein (MRP). We investigated wether MRP plays a role in malignant melanoma chemoresistance. Using the RT-PCR (two different oligonucleotid primer sets) we determined mRNA-expression in 33 cell lines established from primary tumors (n=11), metastatic lesions (n=17) and malignant effusions (n=5). Protein expression was determined by Western blot analysis (MRPm6, QCRL-1) and confirmed by immunocytochemistry. All cell lines scored positiv for MRP mRNA at different levels (5 high, 13 intermediate, 15 low). Levels of MRP mRNA were significantly higher in primary tumors as compared to metastases and malignant effusions. The respective protein was detectable in Western blot and in immunocytochemistry. Accumulation studies using ^{3}H-daunomycin as MRP substrate and genistein as specific MRP modulator were performed and revealed - in contrast to the control cell models GLC4/ADR and HL60/AR - no drug efflux activity of MRP. In conclusion melanoma cell lines express MRP mRNA and protein at different levels. The biological role has to be investigated in further studies.

7 O

RELATIONSHIP TO MULTIDRUG RESISTANCE OF THE TRANSPORTER ASSOCIATED WITH ANTIGEN PRESENTATION TAP.

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Multidrug resistance (MDR) has been related to two members of the ABC-superfamily of transporters, P-glycoprotein (Pgp) and Multidrug Resistance-associated Protein (MRP). We investigated the relationship of another ABC-transporter, the Transporter-associated with Antigen Presentation TAP, with MDR. TAP translocates peptides from the cytosol to the ER-lumen where association with MHC class I molecules occurs. Using an anti-TAP antiserum on immunocytochemstry and Western blot, we found TAP overexpression in 5 of 8 MDR lines of different histogenetic origin. Reversion to drug sensitivity was associated with a decrease in TAP levels. Antiserum specificity was confirmed by using T1 (carrying TAP genes) and T2 (T1 mutant TAP-deficient) cells as controls. T1/T2 relative resistance to VP16, vincristine, and doxorubicin, was 2.33 ± 0.33, 1.7 ± 0.10, and 1.65 ± 0.07, respectively ($p<0.05$ for the 3 drugs). Transfection of the TAP genes into TAP-deficient T2 cells conferred resistance to VP16, vincristine, and doxorubicin (2- to 2.5-fold). Furthermore, in a competition assay, VP16 and vincristine inhibited TAP-dependent peptide translocation to the ER. Collectively, our results support that TAP genes may contribute to MDR. TAP may transport drugs into the ER, thus influencing intracellular drug distribution.

8 O

TUMOR CELLS TRANSFECTED WITH HSP27 HEAT SHOCK PROTEIN SHOW MODIFICATION IN PROLIFERATION AND TUMORIGENICITY

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The 27-kD heat shock protein (hsp27) is a member of the small heat shock protein (HSP) family.HSP 27 may play a role in the regulation of cell growth, differentiation as well as in the transformation processes.

We examined the role of hsp27 in tumorigenicity. Stable transfectants of a melanoma cell line (A375) and an epidermal squamous carcinoma cell line (A431) , isolated by co-transfection of a hsp27 expression vector (pSG-2711) and a neomycin-resistant plasmid, were obtained. Clones expressing high levels of hsp27 were analysed by immunohistochemistry and immunoblotting. Growth analysis of transfectants in A375 and A431 tumor cells showed in vitro a lower proliferation rate than control cells of both lines. To investigate the correlation of hsp27 expression and tumorigenicity, transfectants of each cell type as well as control cells were injected into nude mce. A delay in tumor development was detected in animals inoculated with cells overex-pressing hsp27. However, after this delay, in some of these animals tumor appeared and no difference could be observed in their growth dynamics compaired to control tumors. Tumors showed no hsp27 expression (immunohistochemistry and PCR) implicating instability of the transduced DNA, when maintained under not selective conditions.

The present study shows that genetic manipulation of tumor cells may provide valuable information on the role of hsp27 in tumor growth.

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9 P

A POSSIBLE MECHANISM OF P-GLYCOPROTEIN REGULATION IN P-388 LEUKAEMIA CELLS

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The aim of the present study is to try exercising resistant leukaemia cells (LC) regulation by means of glycoconjugates synthesis stimulation using Dolichyl Phosphate (DP) *in vitro*.P-388 (LC) with induced resistance to Doxorubicin (Dox) (P-388/Dox) were used.DP and P-GP fractions were analysed by HPLC methods. It is confirmed that plasmatic membrans of P-388/Dox LC contain 5,6 - 6,4% of P-GP as a resistance marker. Resistant P-388/Dox LC differ from sensitive ones (P-388/0) in P-GP content by 10-12 times. The study showed 3,5-fold DP decrease in P-388/Dox cells. The investigations demonstrate that the situation can be changed by resistant cells treatment with DP. The DP concentration in P-388/Dox cells was returned to the normal level. It is established that DP in the concentration 10^{-6} M aid 7-9-fold reducing P-GP in membranes of P-388/Dox cells. The P-388/Dox cells cultivation in medium with DP proceeded to give lowered P-GP content in membranes no over 0,4-0,6 %, which amount was consistent with the level of P-GP in P-388/0 cells.These results indicate that the appearence of multidrug resistance in LC can be regulated using DP.

10 O

EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE mRNA IN HUMAN TUMOR-ASSOCIATED MACROPHAGES

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The enzyme inducible Nitric oxide synthase (iNOS) is known to catalyse the release of Nitric oxide from L-arginin. Expression of iNOS is readily induced in macrophages of various mammals, but the presence of this enzyme in human monocytes and macrophages has not been entirely clarified. In this study we tested whether iNOS mRNA and protein can be detected in human tumor-associated macrophages (TAMs). TAMs were isolated from malignant effusions of cancer patients (n=14) by gradient separation. The reverse transcription polymerase chain reaction (oligonucleotide primers derived from human hepatocyte iNOS mRNA) and immuncytochemistry (anti-macNOS) were used. Stimulation of TAMs was achieved by incubating the cells with rh-GM-CSF and rh-IFN-g and a combination of these cytokines. iNOS specific mRNA as well as iNOS protein could be detected in TAMs.The mRNA signal was induced in 10/14 samples and increased in 4/14 following stimulation with the cytokines GM-CSF and IFN-g. This stimulation correlated with enhancement of TNF-a production and cell mediated cytotoxicity of TAMs against tumor targets. Our finding that iNOS is detectable and can be stimulated in human TAMs supports the notion that iNOS/NO are important molecules in antitumoral effector mechanisms.