23

MODULATION OF MDR1, c-erb-B2 AND p53 EXPRESSION BY CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER

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The purpose of this study was to investigate how chemotherapeutic treatment may affect the expression detected in the basal state of P-glycoprotein (MDR1), c-erb-B2 and p53 in avanced breast cancer.

Materials and Methods: Tumor samples were obtained from 17 patients with inoperable breast cancer prior to induction chemotherapy. In 10 cases, samples could also be studied after combined treatment with 4-Epirubicin, Cyclophosphamide and 5-FU (FEC), the number of administered cycles varying between 1 and 5. Fresh-frozen 6 μM cryostat sections were studied by means of immunohistochemistry using the c219 and JSB-1 (P-glycoprotein), NCL-CB11 (c-erb-B2) and pb 1801 (p53) monoclonal antibodies and the streptavidin-biotin-peroxidase technique.

Results: In the basal state, P-glycoprotein was expressed in 9/17 tumors (52.9%), c-erb-B2 in 6/17 tumors (35.2%) and p53 in 4/17 tumors (23.5%). Of the 10 tumors studied before and after chemotherapeutic treatment, P-glycoprotein was expressed in 5/10 before and in 10/10 (100%) after treatment; c-erb-B2 was expressed in 2 before and in none after treatment, p53 was expressed in 3 before and in 4 after treatment.

P-glycoprotein expression was induced by chemotherapy in all tumors studied, whereas cells expressing c-erb-B2 and p53 seem to be variably affected.

25

WT1 FUNCTION IN OVARIAN TUMORIGENESIS: POSSIBLE ROLE OF WT1/p53 INTERACTION.

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The frequent allelic deletions of the short arm of chromosome 11 in ovarian tumors suggest the possibility that the WT1 gene, a proposed tumor suppressor gene located on chromosome 11p13 and expressed in the human fetal genitourinary system, may contribute to the development of ovarian neoplasias. Structural and sequence analysis of the entire coding portions of the WT1 gene did not reveal any abnormalities in 20 ovarian tumor specimens (13 of them with 11p13 allelic deletion) and 5 cell lines we have analyzed. These findings disprove the hypothesis that the WT1 gene functions as a classical tumor suppressor gene in ovarian tumorigenesis and suggest that a different recessive oncogene may be exposed by the observed 11p13 allelic deletions. However, we considered the possibility that other molecular events might exert an effect on the WT1 function and we analyzed WT1 mRNA expression by Northern blotting in 19 epithelial ovarian tumors and in 5 ovarian carcinoma cell lines. The analysis showed that the WT1 gene is transcriptionally active in all the tested samples, but considerable variations in the expression levels were found. The possible functional effects of such variability have been evaluated in relation to the potential WT1/p53 protein interaction (Maheswaran *et al.*, 1993). The observed concordance between high WT1 mRNA expression and high percentage of p53 positive cells leads us to hypothesize that, in a subset of ovarian tumors, the presence of a mutated p53 might alter the transcriptional control exerted by WT1 protein on some growth-related genes.

27

PROLIFERATION ARREST BY EXOGENOUS ^{wt} *p53* IS ABROGATED BY MUTATION OR LACK OF EXPRESSION IN ^{m} *p53* CELL LINES

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Mutations in the *p53* tumor suppression gene are frequent in a wide spectrum of human tumors, including colon and breast carcinomas.To evaluate the role of exogenous wild-type *p53*, we transfected the MDA-MB-468 breast carcinoma cell line (hemizygous for codon 273 *p53* mutation) and the highly metastatic colon carcinoma cell line KM12SM (heterozygous for a mutation at codon 179) with a full-length cDNA ^{wt}*p53* in the pCDM8-neo expression vector having a CMV promoter. Transfection efficiency with the ^{wt}*p53* revealed a 3-4 fold decrease in the number of colonies as compared to the control vector only (containing the neo gene). The integration and the expression of exogenous ^{wt}*p53* was assessed in the early passages by PCR, Southern blot, RT-PCR, Northern blot and Western blot. Proliferation rate was unchanged in two of the six selected clones. When MDA-MB-468 clones were implanted orthotopically (i.m.f.p) in female Balb/c nude mice only one of them showed a prolonged latency on tumor growth. Indeed, the tumorigenic phenotype is not fully suppressed by exogenous wt p53.Our results demonstrated that the exogenous ^{wt} *p53* acquires new mutations or is not always expressed when is introduced in mutated p53 cell lines. The failure of exogenous ^{wt} *p53* to reverse the progression -in our systems- , is due to the inactivation of the ^{wt} *p53* through the same mechanisms that inactivated endogenous gene (mutation, deletion or lack of expression).

24

N-RAS & C-MYC EXPRESSION INDUCES CDDP RESISTANCE IN HUMAN MELANOMA CELL LINES.

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Background: The importance of N-ras and c-myc expression, individually and in cooperation, in carcinogenesis and development of resistance is well documented. Sklar[1] and Isonishi[2] described the induction of CDDP resistance by *ras* in transfected cells. The c-myc expression correlated directly with the degree of CDDP resistance in transfection experiments (Sklar[3], Niimi[4]). A major disadvantage of the cells after transfection is the genetic instability.

Purpose: The use of 8 unmanipulated human melanoma cell lines (patient material) with several known genetic characteristics allows the determination of N-ras and c-myc involvement in CDDP resistance.

Methods: A colorimetric test (MTT assay) was used to assess dose/survival curves for the different cells. The sensitivity is quantified by ID_{50} (inhibitory dose at 50% survival) and RF (resistance factor) values.

Results: Elevated c-myc expression in these melanoma cells is correlated with high CDDP resistance. ID_{50} of cells with high c-myc expression was 7μM CDDP, while cells with low c-myc expression had an ID_{50} of 1 μM CDDP. The presence of WT *vs* mutated N-ras sequence did not influence significantly the CDDP sensitivity.

Conclusions: Among the large pool of possible underlying mechanisms for resistance development, c-myc expression could be a genetic determinant for CDDP resistance. Our experiments prove the c-myc involvement in CDDP sensitivity of unmanipulated human malignancies. In our human melanoma cells, we confirm that c-myc expression is directly correlated with CDDP resistance (Sklar[3] transfection experiments with NIH3T3 cells).

[1] Sklar MD, Cancer Res 48: 793 (1988) [2] Isonishi S, et.al., Cancer Res 51: 5903 (1991) [3] Sklar MD, Prochownik EV, Cancer Res 51: 2118 (1991) [4] Niimi S, et.al., Br. J. Cancer 63: 237 (1991).

26

ALTERING TUMORIGENICITY AND INVASIVE PROPERTIES BY TREATMENT WITH 5-AZACYTIDINE

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Changes in the methylation pattern of DNA sequences are known to selectively affect the gene expression and in consequence the tumor phenotype and metastatic behavior.

In order to study whether it influences the invasive properties of the murine c-26 colon carcinoma, we treated these cells with the hypomethylating agent 5-azacytidine (5-aza-C). Treatments were made for 24 hours with increasing concentrations of drug (5 to 100 μM) and then cells were allowed to recover for 7days in medium without 5-aza-C. The isolated clones were studied for murine type IV collagenase expression at protein and mRNA level and it showed an enhancement of 72-kDa gelatinase (MMP-2) in a drug concentration-dependent-manner. The methylation pattern of the MMP-2 locus were performed with the genomic DNA of the control and 5-aza-C clones after digestion with Hpa-II or Msp-I enzymes. Our results suggested that the expression of this gene could be influenced by hypomethylation.

In spite of these results, the metastatic potential was not increased in Balb/c mice, showing a prolonged latency on tumor growth depending of drug concentration treatments. Nevertheless, we could not detect differences in cell proliferation "in vitro" between the clones and the parent untreated cell line. On the other hand, a reduction in chromosome number also occurred progressively with the increasing drug concentration.

Our results provide the evidence that the hypomethylation induced by 5-aza-C treatment can affect specific molecular pathways involved in tumorigenicity and metastasis in a positive and negative way.

28

MONOCYTE FUNCTIONS FOLLOWING TREATMENT WITH COLONY STIMULATING FACTORS IN PATIENTS WITH ADVANCED TESTICULAR CANCER UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION.

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The influence of colony-stimulating factors (CSF) on monocyte functions of 34 patients with relapsed or refractory testicular cancer receiving high dose chemotherapy followed by autologous bone marrow transplantation (ABMT) was tested. 8 patients were treated as control group without CSF therapy, whereas 12 patients received recombinant human granulocyte macrophage colony-stimulating factor (GM-CSF) and 12 patients received recombinant human granulocyte colony-stimulating factor (G-CSF). For the assessment of monocyte activation induced by the colony stimulating factors, monocyte mediated cytotoxicity, production of tumor necrosis factor a (TNF-a) and the expression of major histocompatibility complex (MHC) class I & II antigens were chosen.Monocyte- mediated cytotoxicity directed against tumor cells and production of (TNF-a) was significantly increased in patients receiving GM-CSF, as compared to patients from the control group, while no such effect was detectable in patients under G-CSF therapy. Furthermore, monocytes from patients with GM-CSF therapy showed a significant increase in MHC-Class I and II antigen expression, as compared to patients without CSF treatment ($p<0.001$). No change in spontaneous expression of MHC Class I or II antigens was seen in patients under G-CSF treatment. However, monocytes derived from the latter patients were more likely to be induced to express MHC Class II antigens by interferon gamma. In conclusion our results show that monocytes derived from patients, who underwent treatment with GM-CSF exhibited a series of activation parameters, while no such effect was exerted by G-CSF.