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DFNA5 (ICERE-1) contributes to acquired etoposide resistance in melanoma cells

Hermann Lage[a,*], Heike Helmbach[b], Claudia Grottke[a], Manfred Dietel[a], Dirk Schadendorf[b]

[a] *Institute of Pathology, Charité, Campus Mitte, Humboldt University Berlin, Schumannstr. 20/21, D-10117 Berlin, Germany*
[b] *Skin Cancer Unit at the DKFZ and University Hospital Mannheim, University Heidelberg, Theodor Kutzer Ufer 1, D-68135 Mannheim, Germany*

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Abstract Resistance to drug treatment is a common observation in malignant melanoma. In order to analyze alterations in mRNA expression profiles associated with drug resistance in melanoma cells we previously established a panel of various drug-resistant cell variants derived from the human melanoma line MeWo and compared the mRNA expression profiles by a differential display technique. By that approach it could be demonstrated that the expression level of a mRNA encoded by a gene found to be mutated in non-syndromic hearing impairment, DFNA5 (ICERE-1), was distinctly decreased in the 33-fold etoposide-resistant melanoma cell line MeWo ETO 1. To evaluate the hypothesis that a decrease in DFNA5 mRNA expression level contributes to the acquired etoposide resistance phenotype exhibited by MeWo ETO 1 cells, this drug-resistant line was stably transfected with the DFNA5-encoding cDNA. Transfected clones showed a 30–35% reduced etoposide susceptibility by comparing the IC_{25}, IC_{50} and IC_{75} values of these clones with those displayed by the non-transfected, etoposide-resistant melanoma cell line MeWo ETO 1 and controls. Furthermore, etoposide exposure of stable DFNA5 transfectants resulted in an increase of caspase-3-mediated apoptotic events in DFNA5-transfected clones in comparison to MeWo ETO 1 cells and controls. The data therefore demonstrate that a decrease in DNFA5 mRNA expression level is associated with an increased etoposide resistance in melanoma cells due to an elevated cellular susceptibility to trigger a caspase-3-depending signal pathway leading to programmed cell death.

Key words: Drug resistance; Malignant melanoma; MeWo; Apoptosis

1. Introduction

Resistance to antineoplastic agents is a major obstacle to successful treatment of metastatic malignant melanoma. Although moderate response rates of 20–50% of limited duration can be obtained, relapses occur with high frequency and second-line therapies are largely ineffective leading to a 5-year survival rate of 2%. Several mechanisms have been described mediating drug resistance in cancerous cells, but in malignant melanoma the processes leading to chemoresistance are largely unknown [1,2]. Consequently, the identification of factors involved in the high intrinsic drug resistance of malignant melanoma is of urgent importance in order to develop new treatment modalities and improve response rates in advanced melanoma. A significant correlation between treatment response in melanoma patients and the level of drug resistance in vitro of fresh single cell suspensions derived from metastases of these patients was observed [1]. This suggests an important role of cellular mechanisms leading to drug resistance in melanoma.

In order to get further insights into the mechanisms conferring cellular drug resistance to malignant melanoma, we previously established a model system in vitro consisting of various drug-resistant sublines derived from the human melanoma cell line MeWo [3]. The rationale behind this strategy is the notion that clinically relevant mechanisms of drug resistance could be de novo induced or could be amplified in the case of intrinsic resistance. In that way, the mechanisms become accessible for detailed and reproducible analysis. This panel of drug-resistant melanoma cells was analyzed subsequently with regard to known mechanisms causing drug resistance in various tissues. In that way, alterations in the activity of enzyme systems involved in DNA repair [4,5] and of DNA topoisomerase II [6] could be observed in antitumor drug-resistant melanoma cells. Analyses of changes in the mRNA expression profiles in drug-resistant MeWo derivatives by the differential display technique [7] revealed various candidate factors potentially involved in the drug-resistant phenotype [8]. By this approach, a distinct down-regulation of the mRNA encoding DFNA5 was detected in the MeWo-derived cell line MeWo ETO 1, characterized by a 33-fold increased resistance level against the clinically relevant DNA topoisomerase II-inhibiting epipodophyllotoxin etoposide.

The human DFNA5 (DFNA is used as the code for dominant hearing impairment genes, and the numerals are assigned in order of discovery; overview in [9]) gene encodes a non-syndromic hearing impairment protein [10] also designated ICERE-1 [11]. Transcription of the gene revealed a predicted 496-amino acid protein of unknown physiological function. Mutations in the DFNA5 gene, resulting in premature termination of the open reading frame, were found in an extended family with autosomal dominant deafness [12]. The expression pattern of DFNA5 indicated that this gene might participate in the tumor biology of breast cancers [11]. However, definite proof that DFNA5 is involved in the pathogenesis of breast carcinoma is lacking. In addition, a putative role of DFNA5 in the development of drug resistance in cancer cells is fully unclear.

To verify the hypothesis that a down-regulation of the DFNA5-encoding transcript contributes to the development

*Corresponding author. Fax: (49)-30-450 536 900.
E-mail: hermann.lage@charite.de

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of an etoposide resistance phenotype in malignant melanoma cells, the etoposide-resistant human melanoma cell line MeWo ETO 1 was transfected with a DFNA5-encoding expression vector construct. These experiments demonstrated that overexpression of DFNA5 increased the susceptibility of the drug-resistant melanoma cell line MeWo ETO 1 to the cytotoxic action of etoposide mediated by an enhancement of the cellular susceptibility to trigger caspase-3-dependent apoptotic events.

2. Materials and methods

2.1. Cell lines

Establishment of the etoposide-resistant subline MeWo ETO 1 from the drug-sensitive human melanoma cell line MeWo [13], derived from a lymph node metastasis, has been described earlier [3]. Cells were grown in Leibovitz L-15 medium (Biowhittaker, Walkersville, MD, USA) supplemented with 10% fetal calf serum (Gibco/BRL, Grand Island, NY, USA), 1 mM L-glutamine, 6.25 mg/l fetuin, 80 IU/l insulin, 2.5 mg/ml transferrin, 1 g/l glucose, 1.1 g/l $NaHCO_3$, 1% minimal essential vitamins, and 20 000 kIU/l trasylol in a humidified atmosphere of 5% CO_2 at 37°C. In order to ensure maintenance of the etoposide-resistant phenotype, medium for MeWo ETO 1 was supplemented with 1.0 μg/ml etoposide (Bristol-Myers, Munich, Germany). In addition, the medium used for cultivation of transfected melanoma cell clones contained 400 μg/ml G418 (Invitrogen, San Diego, CA, USA).

2.2. Northern blot analysis

Total RNA was prepared using RNAzol reagent (Gibco/BRL) according to the manufacturer's instructions. 20 μg of total cellular RNA was fractionated on 1% agarose-formaldehyde gels and transferred onto a Hybond-N membrane (Amersham, Aylesbury, UK). Blots were hybridized with a 635-bp DFNA5-encoding cDNA fragment [8] labeled with [^{32}P]dCTP by random primed labeling (Amersham). Blots were incubated with 25 ng labeled cDNA probe in 6×SSPE, 10% dextran sulfate, 7% SDS and 0.5% BLOTTO at 65°C, for 16 h. Finally, the membrane was washed under high stringency conditions (0.1×SSC, 1% SDS at 60°C). To check that equivalent amounts of RNA were analyzed, the membranes were rehybridized with a 1.2-kb phosphoglycerate kinase (PGK) cDNA probe.

2.3. Transfection and enforced expression of DFNA5 in MeWo ETO 1

An eukaryotic expression construct containing a full-length DFNA5-encoding cDNA was kindly provided by D.A. Thompson (Stanford University School of Medicine, Stanford, CA, USA). The 2168-bp cDNA, designated ICERE-1 [11], was cloned into the *Eco*RI (5′-end) and *Xho*I (3′-end) sites of the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA, USA). Sequence analysis was performed to confirm that the insert was in a sense orientation to the cytomegalovirus (CMV) promoter of the vector. The expression vector construct was transfected into the etoposide-resistant human melanoma cell line MeWo ETO 1 using liposome transfer (Lipofectin, Gibco/BRL). Stably transfected cell clones were obtained by using G418 as selecting agent. As controls, MeWo ETO 1 cells were also transfected with empty expression vectors.

2.4. Cell growth inhibition assay

Chemoresistance was tested using a cell proliferation assay basing on sulforhodamine B (SRB), a protein binding reagent [14]. Melanoma cells (population doubling time for each cell line including each of the transfectant clones is 46 h in the absence of any anticancer drug) were distributed into 96-well plates at a concentration of 5000 cells/well. For adhesion and to attain logarithmic growth phase cells were incubated for 48 h prior to drug application. After 6 days chronic drug incubation the assay was terminated by removing the medium and adding chilled 10% trichloroacetic acid. After 1 h incubation at 4°C wells were washed five times with tap water and cell-associated protein was stained by adding 0.4% SRB in 1% acetic acid for 10 min at room temperature. Absorbance was measured at 540 nm after drying and re-solubilization in 20 mM Tris–HCl (pH 10). For determination of IC_{25}, IC_{50} and IC_{75} values, cells were incubated with increasing concentrations of etoposide; the absorbance difference of control cells without drug was set at 100%. Graphs of cell survival against dose of antineoplastic agent were plotted, and the IC_{25}, IC_{50} and IC_{75} values were calculated from multiple (at least three) independent experiments for each cell clone.

2.5. Determination of apoptosis

Twenty-four hours prior to chemotherapeutic treatment, cells from each line were plated into 6-well tissue culture dishes. To induce apoptosis, cells were exposed to etoposide for 72 h at 37°C. For etoposide treatment etoposide-resistant melanoma cells MeWo ETO 1, two independent DFNA5-expressing MeWo ETO 1 clones (MeWo ETO 1/DFNA5 clone 1 and MeWo ETO 1/DFNA5 clone 2) and a control clone containing an empty expression vector (MeWo ETO 1/control clone 1) were either untreated or treated with etoposide 10 or 40 μg/ml. Sensitive MeWo cells were either unexposed or exposed to etoposide 1 or 5 μg/ml, respectively. Live adherent and non-adherent cells were collected, counted to calculate the survival rate, and used for the determination of the DNA fragmentation assay. Histone-associated DNA fragments released from nuclear fractions of chemotherapeutically treated cells were quantified using the Cell Death Detection ELISA (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. DNA fragmentation is presented as the nucleosome enrichment factor compared to the respective untreated control.

2.6. Determination of caspase-3 activity

For the specific quantitative in vitro determination of caspase-3-like activity a fluorometric immunosorbent enzyme assay (Roche Diagnostics) was used according to the manufacturer's recommendations. Briefly, active caspase-3 was bound by a monoclonal mouse anti-caspase-3 antibody to a microtiter plate. After washing the fluorogenic caspase-3-specific substrate peptide DEVD-AFC was added and the caspase-3-catalyzed cleavage rate was measured by fluorescent microplate reader after 1 h with a 409-nm excitation filter and a 500-nm emission filter. Data were normalized against the untreated control and interpreted as fold induction. The relative caspase-3 activity values are means of at least three independent triplicate experiments. The specificity of the assay was confirmed using the specific caspase-3-inhibiting peptide DEVD-CHO (Calbiochem, San Diego, CA, USA) (data not shown).

3. Results

3.1. Enforced expression of DFNA5-encoding transcripts in MeWo ETO 1

Since a decreased expression level of DFNA5-encoding mRNA was detected in the etoposide-resistant melanoma cell line MeWo ETO 1 [8], a mammalian expression vector was used to overexpress DFNA5-specific mRNA in a CMV promoter-dependent manner in this cell line. After G418 selection, several stable DFNA5 cDNA-transfected clones were obtained. These transfectants demonstrated no gross morphological changes in comparison to the original cell line MeWo ETO 1. Starting from these clones, 30 G418-resistant DFNA5 cDNA-transfected clones and 30 G418-resistant control clones containing an empty expression vector were analyzed for etoposide resistance using a SRB-based cell proliferation assay. Eleven of 30 DFNA5 cDNA-transfected clones showed an increased sensitivity to etoposide exposure when compared to the etoposide-resistant cells MeWo ETO 1. None of the control clones transfected with an empty expression vector showed any alteration in etoposide sensitivity. Two of the DFNA5-overexpressing clones (MeWo ETO 1/DFNA5 clone 1 and MeWo ETO 1/DFNA5 clone 2) showing an elevated etoposide sensitivity were chosen to examine the underlying mechanisms in more detail. Fig. 1 demonstrates that both DFNA5 cDNA-transfected clones had an increased DFNA5 mRNA expression level. The extent of DNFA5 expression in the transfectants was similar to the expression level detected in

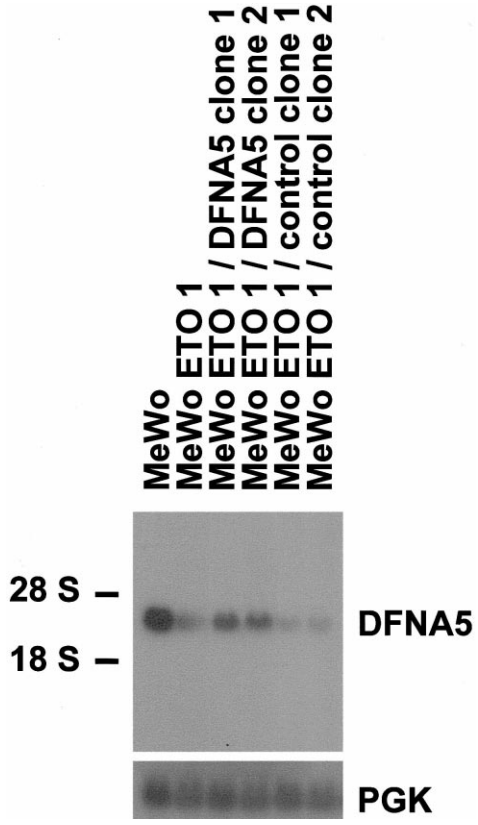

Fig. 1. Northern blot analyses of mRNA expression in DFNA5 cDNA-transfected etoposide-resistant melanoma MeWo ETO 1 cell lines. Total cellular RNA was size-fractionated, transferred to a nylon membrane and hybridized with a ^{32}P-labeled 635-bp DFNA5-encoding cDNA probe. As control, the blots were probed with a cDNA encoding PGK. Cells used were: MeWo, non-resistant human melanoma cell line; MeWo ETO 1, etoposide-resistant derivative of MeWo; MeWo ETO 1/DFNA5 clone 1, DFNA5 transfected MeWo ETO 1 clone; MeWo ETO 1/DFNA5 clone 2, DFNA5-transfected MeWo ETO 1 clone; MeWo ETO 1/control clone 1, empty vector-transfected MeWo ETO 1 clone; MeWo ETO 1/control clone 2, DFNA5-transfected MeWo ETO 1 clone showing no alteration in etoposide resistance.

the non-resistant, parental melanoma cell line MeWo. Two controls, MeWo ETO 1 transfected with an empty expression vector (MeWo ETO 1/control clone 1) and a DFNA5-transfected MeWo ETO 1 clone (MeWo ETO 1/control clone 2) exhibiting no modulation in the etoposide-resistant phenotype, demonstrated no alteration in DFNA5 mRNA expression compared to the expression in the non-transfected counterparts.

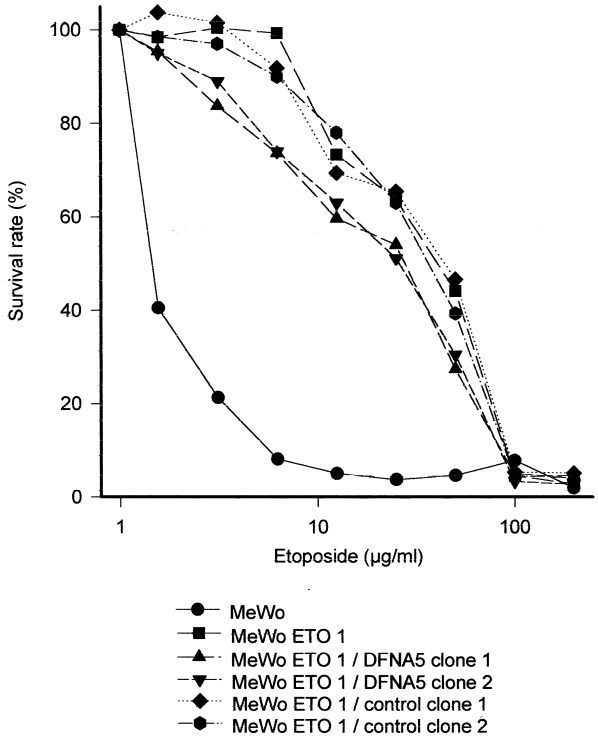

Fig. 2. Determination of growth inhibition in DFNA5-specific transcript-overexpressing melanoma clones after cellular exposure to etoposide. Points represent the mean values obtained from at least three independent experiments. Calculated IC_{75}, IC_{50} and IC_{25} values are given in Table 1.

3.2. Drug sensitivity of DFNA5 cDNA-transfected clones

Examination of etoposide sensitivity of DFNA5 cDNA-transfected MeWo ETO 1 clones (MeWo ETO 1/DFNA5 clone 1 and MeWo ETO 1/DFNA5 clone 2) revealed a 30–35% reduced drug susceptibility by comparing the IC_{50} values of these clones with the IC_{50} values exhibited by the non-transfected, etoposide-resistant melanoma cell line MeWo ETO 1 and the controls (Fig. 2 and Table 1). The IC_{25} values indicating the etoposide resistance of DFNA5 transfectants were reduced by 54–57% compared to the controls (Table 1). Likewise, at the level of IC_{75} values, a reduction in etoposide sensitivity could be observed in the range of 18–23% (Table 1).

3.3. Induction of apoptosis by etoposide

Fig. 3 demonstrates that exposure of the non-resistant human melanoma cell line MeWo to increasing concentrations of etoposide resulted in an augmentation of apoptotic events determined by measuring the apoptosis-dependent emergence

Table 1
Etoposide sensitivity of DFNA5-overexpressing melanoma clones

Melanoma clone	IC_{75} (μg/ml)	RR[a] (fold)	RS[b] (%)	IC_{50} (μg/ml)	RR (fold)	RS (%)	IC_{25} (μg/ml)	RR (fold)	RS (%)
MeWo	2.7 ± 0.4	1	−100	1.2 ± 0.2	1	−100	0.27 ± 0.05	1	−100
MeWo ETO 1	70 ± 1	25.9	0	40 ± 1	33.3	0	13 ± 1	48.1	0
MeWo ETO 1/DFNA5 clone 1	54 ± 1	20	−22.9	28 ± 1	23.3	−30	5.6 ± 0.6	20.7	−56.9
MeWo ETO 1/DFNA5 clone 2	58 ± 1	21.5	−17.1	26 ± 1	21.7	−35	6 ± 0.5	22.2	−53.8
MeWo ETO 1/control clone 1	72 ± 1	26.7	+2.8	37 ± 1	30.8	−7.5	11 ± 2	40.7	−15.4
MeWo ETO 1/control clone 2	67 ± 3	24.9	−4.3	44 ± 1	36.7	+10	15 ± 2	55.6	+15.4

[a]RR, relative resistance (fold) compared to the non-resistant melanoma cell line MeWo.
[b]RS, relative sensitivity (%) compared to the etoposide-resistant melanoma cell line MeWo ETO 1.

of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates, a 11.6-fold increase using 1 μg/ml and a 29.9-fold increase using 5 μg/ml etoposide. However, etoposide treatment of the etoposide-resistant cell line MeWo ETO 1 did not lead to such a high programmed cell death rate even using still higher drug concentrations. Treatment of MeWo ETO 1 cells with 10 μg/ml etoposide increased the relative factor of nucleosome enrichment by approximately 2.2-fold, whereas exposure using 40 μg/ml etoposide increased the quantity of apoptotic events 2.1-fold. Similar data were obtained using a stably transfected cell clone with an empty expression vector (MeWo ETO 1/control clone 1). Etoposide treatment using 10 μg/ml etoposide resulted in a 1.1-fold increase, exposure to 40 μg/ml etoposide led to a 1.7-fold enhancement of nucleosomes. In contrast to those non-significant effects, both stably DFNA5 cDNA-transfected melanoma clones (MeWo ETO 1/DFNA5 clone 1 and MeWo ETO 1/DFNA5 clone 2) demonstrated a much more pronounced increase of apoptotic events due to etoposide treatment. Etoposide exposure using 10 μg/ml caused a 2.7-fold (MeWo ETO 1/DFNA5 clone 1) or 2.3-fold (MeWo ETO 1/DFNA5 clone 2) nucleosome enhancement, drug exposure using 40 μg/ml resulted in an 8.5-fold (MeWo ETO 1/DFNA5 clone 1) or an 8.2-fold (MeWo ETO 1/DFNA5 clone 2) increase of nucleosomes.

3.4. Activation of caspase-3 after etoposide treatment

To define the apoptotic pathway that is influenced by DFNA5, the catalytic activity of caspase-3 was measured. Caspase-3 is one death protease, among others, that can be activated in consequence of an upstream apoptotic signal transduction and catalyzes the cleavage, and therefore the breakdown, of several cellular components related to DNA repair and regulation [15]. The synthetic fluorogenic peptide DEVD-AFC was used to determine caspase-3-like activity. As demonstrated in Fig. 4, the caspase-3-mediated cleavage activity increased dramatically in drug-sensitive MeWo cells following a 72-h treatment with etoposide (26.5-fold using 1 μg/ml and 44.7-fold using 5 μg/ml), while no detectable induction of caspase-3-like activity could be measured in the etoposide-resistant cell variant MeWo ETO 1 and the empty expression vector-transfected melanoma cell clone MeWo ETO 1/control clone 1, even using higher etoposide concentrations (40 μg/ml). In contrast, both DFNA5 cDNA-transfected MeWo ETO 1-derived clones, MeWo ETO 1/DFNA5 clone 1 and clone 2, restored the cellular capability to activate caspase-3 due to etoposide treatment. Drug exposure using 10 μg/ml mediated a 1.9-fold (MeWo ETO 1/DFNA5 clone 1) or 2.5-fold (MeWo ETO 1/DFNA5 clone 2) enhancement of the caspase-3-like catalytic activity, whereas an etoposide concentration of 40 μg/ml even resulted in a 15.0-fold (MeWo ETO 1/DFNA5 clone 1) or 14.7-fold (MeWo ETO 1/DFNA5 clone 2) increase of caspase-3-mediated peptide cleavage.

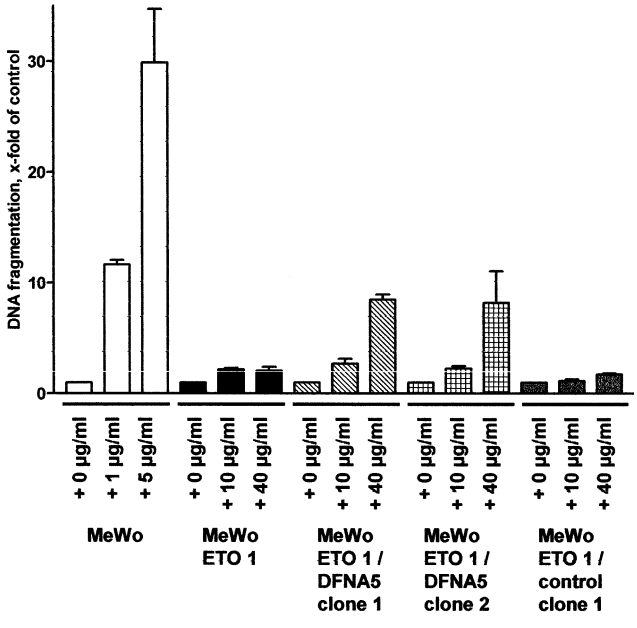

Fig. 3. Determination of etoposide-dependent induction of apoptosis in DFNA5 cDNA transfectants as measured by the emergence of mono- and oligonucleosomes in cytoplasmic fractions of cell lysates by an ELISA approach. Columns represent the values obtained from at least three independent experiments, bars represent S.D.

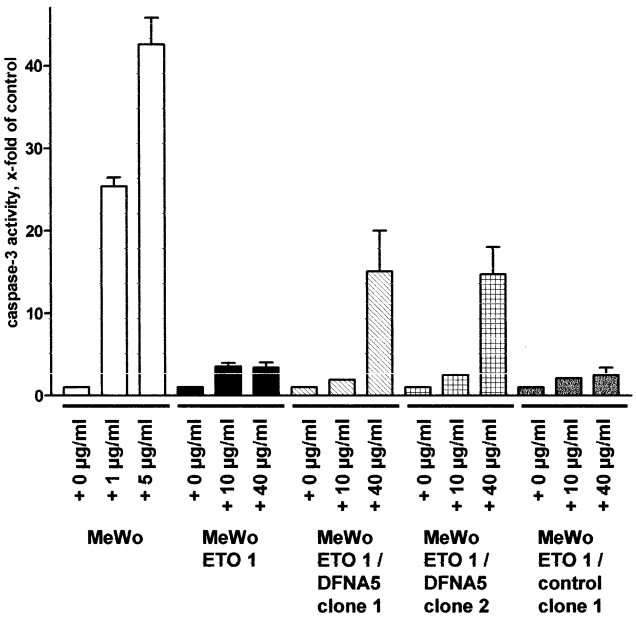

Fig. 4. Activation of caspase-3-like activity in DFNA5 cDNA-transfected MeWo ETO 1 cell variants after exposure to increasing concentrations of etoposide. Columns represent the values obtained from at least three independent experiments, bars represent S.D.

4. Discussion

A previously performed evaluation of the mRNA expression profiles in a panel of drug-resistant melanoma cell lines derived from the MeWo line by the differential display technique [7] demonstrated that the expression level of the mRNA encoding a non-syndromic hearing impairment protein, DNFA5, is distinctly decreased in the etoposide-resistant melanoma cell line MeWo ETO 1 [8]. In this study, we investigated the hypothesis that a decreased expression of the DFNA5-specific transcript might be involved in the acquired drug resistance phenotype exhibited by MeWo ETO 1 cells by affecting the cellular susceptibility to trigger programmed cell death. Hence, we transfected the etoposide-resistant cell line MeWo ETO 1 with a CMV promoter-driven DFNA5-encoding cDNA expression vector construct. By this approach it

was possible to enhance the DFNA5 mRNA expression level to an extent similar to that observed in the original, non-resistant cell line MeWo. DFNA5 mRNA-overexpressing clones derived from the 33-fold etoposide-resistant melanoma cell line MeWo ETO 1 showed a 30–35% reduced degree of etoposide resistance at the IC_{50} level compared to non-transfected MeWo ETO 1 cells and controls assessed by cell proliferation assays. The fact that approximately one third of DFNA5-transfected clones and none of the controls reduced their vulnerability to etoposide treatment supports the idea that the new phenotype exhibited by transfected clones is indeed caused by DFNA5 overexpression and not by unspecific clonal effects.

Sparse data are available concerning the physiological function of the gene designated DFNA5. At first, a linkage analysis identified this gene as causing hearing loss in an extended Dutch family with autosomal dominant progressive hearing loss starting in the high frequencies, and mapped the gene to chromosome 7p15 [16]. In that family suffering from that type of non-syndromic hearing impairment an insertion/deletion mutation was identified in intron 7 of the DNFA5 gene [10]. This mutation causes skipping of exon 8, resulting in premature termination of the open reading frame. To identify possible functionally relevant motifs on the basis of the cDNA and amino acid sequences of human DFNA5 and its murine counterpart Dfna5h, several databases were searched, but no significant results were obtained. Furthermore, no evidence was provided that Dfna5h is mutated in deaf mouse mutants.

By comparing estrogen receptor (ER)-positive and ER-negative breast carcinoma cell lines using a differential display approach the DFNA5- (in that study designated ICERE-1) encoding mRNA was found to be overexpressed in the ER-negative breast cancer cell lines [11]. However, alignment of the original DFNA5 cDNA sequence and the ICERE-1 cDNA sequence revealed two important differences leading to alternative initiation and stop codons [10]. On the basis of various considerations, e.g. sequencing of the genomic DNA prepared from the breast carcinoma cell line MDA-MB-231 that was used to isolate ICERE-1 cDNA, Van Laer et al. [10] concluded that DFNA5 and ICERE-1 represent the same gene and that the start and stop codons had been identified incorrectly in ICERE-1. Although it was hypothesized that DFNA5 may be involved in tumor biology specific to hormonally unresponsive breast cancers [11], definite proof is lacking that DFNA5 is involved in the pathogenesis of breast carcinoma. Nevertheless, since this study revealed that enhanced expression of the DFNA5-encoding mRNA increases the cellular susceptibility to drug-induced apoptosis in malignant melanoma cells, further evidence is provided that DFNA5 may indeed be involved in cancer biology and that the status of the DFNA5 mRNA expression level could lead to consequences in therapeutic strategies.

Analyses of the expression pattern of DFNA5-encoding mRNA in various human tissues revealed a high expression level in the placenta while no or very low expression could be detected in other tissues [10,11]. Moreover, in embryonic cell lines, breast cancer cell lines, carcinoma lines derived from human tissue other than breast, including melanoma lines [8], and various primary human breast tumor specimens, transcription of the DFNA5-encoding gene could be detected. These data indicate that DFNA5 mRNA expression is particularly pronounced in embryonic, fetal and cancerous cells. Especially in these cell types the regulation, or in the case of cancer cells the dysregulation of differentiation and development by many factors involved in the control of programmed cell death is of prominent importance. Thus, a putative activity of DFNA5 in the influence of apoptotic cell death appears not to be astonishing.

The exact molecular events by which the DFNA5-mediated alterations in etoposide susceptibility are effected are not clear. However, it could be demonstrated that the enhanced drug sensitivity of DFNA5 cDNA-transfected melanoma clones is accompanied by an increased cellular disposition for programmed cell death mediated by activation of caspase-3 following etoposide exposure. This observation gives evidence that the product encoded by the DFNA5 gene might be involved in a cellular pathway leading to apoptosis. This pathway should be triggered by drug treatment or alternative cellular stress. Thus, a reduced expression level of the DFNA5-encoding transcript would cause a decrease of apoptotic events which would manifest themselves as an increase in drug tolerance.

The available data did not explicitly clarify whether the DFNA5 gene product has a direct effect on apoptosis in melanoma cells. Hence, it should be taken into consideration that the DFNA5-dependent cellular events involved in triggering programmed cell death might be mediated indirectly, e.g. by a DFNA5-dependent modulation of alternative drug resistance mechanisms being active in the etoposide-resistant melanoma cells. At least it was demonstrated that the DNA mismatch repair system, which is modulated in the etoposide-resistant melanoma cells [4,5], plays an important role in antineoplastic drug resistance by affecting cellular pathways leading to apoptosis (overview in [17]).

The 30–35% extent of change in drug susceptibility in this cellular in vitro model is not dramatic. However, a different result is not to be expected since it was demonstrated that alternative mechanisms such as alterations in DNA mismatch repair [4,5] and DNA topoisomerase II activity [6] were associated with the acquired etoposide resistance phenotype exhibited by MeWo ETO 1 cells. Furthermore, additional candidate factors were identified [8] which might play a potential role in the etoposide resistance phenotype of these cells. On the other hand, it is important to note that the effect of change in drug tolerance associated with alterations in DFNA5 mRNA expression level has implications and is of clinical significance. In the clinical situation, a two-fold increase in drug resistance is sufficient to circumvent a successful chemotherapeutic treatment of malignant cells. Thus, for the oncologist it is critical to take into consideration that changes in DFNA5 mRNA expression level may be involved in a multimodal mechanism leading to therapy resistance of malignant melanoma.

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