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Original article:

Expression of Integrins, Anchorage Dependent Apoptosis and Invasiveness of Multidrug Resistant Human Breast Carcinoma Cells

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ABSTRACT

The aim of the study was to investigate the role of integrins in anchorage dependent apoptosis (anoikis) and in vitro invasion of human breast cancer cell line MCF-7 and its multidrug resistant subline MCF-7Dox. Acquisition of MDR was associated with markedly decreased expression of collagen specific α2β1 and αvβ3 integrins, laminin specific α3β1 and α6β1 receptors and dramatic up-regulation of fibronectin specific α5β1 integrin. The MDR subline was substantially more resistant to anoikis than their wild type counterparts. Furthermore, MCF-7Dox cells secreted MMP-9 collagenase and invaded Matrigel. We demonstrate for the first time that stimulation of β1 integrin signaling strongly sensitizes MCF-7 cells to anoikis.

Key words: integrins, anoikis, multidrug resistance, tumor invasion

INTRODUCTION

Irresponsiveness of tumor cells to chemotherapeutic drugs is a major obstacle for therapeutic success in cancer patients. Multidrug resistance (MDR), the most well documented phenotype of clinically relevant tumor resistance, is defined as long-term survival of cells in the presence of different agents (Roninson et al. 1984; Roninson 1997; Barzilay et al. 1997). This phenotype is mediated by a 140-170 kDa P-glycoprotein (Pgp), a transmembrane ATP dependent transporter capable of effluxing a variety of natural metabolites and xenobiotics out of the cell (Roninson et al. 1984; Roninson 1997; Barzilay et al. 1997)

However, mechanisms of tumor cell resistance are not confined to drug transporters. Recently, Dalton and co-workers have introduced the term 'cell adhesion mediated drug resistance' indicating a Pgp-independent fashion of cellular protection from chemotherapeutic drug induced apoptosis (Damiano et al. 1999; Elliott and Sethi 2002; Hazlehurst et al. 2003). This mechanism is triggered upon adhesion of cells to extracellular matrix, implicating the interaction of cell surface receptors called integrins with their cognate ligands in altered drug response (Barzilay and Hickson 1997; Damiano et al. 1999; Elliott and Sethi 2002; Hazlehurst et al. 2003). These results, together with their role in proliferation signaling, broaden the

significance of integrins in cell survival (Rintoul and Sethi 2002; Berman et al. 2003).

Integrins are a superfamily of heterodimers with covalently linked α- and β-subunits. The majority of β-subunits can dimerize with different α-subunits, thereby forming integrin subfamilies β1, β2, β3 etc. (Berman et al. 2003; Eble 1997). The ligand specificity of integrins is normally redundant, so that each particular receptor can bind more than one extracellular matrix protein, and one matrix protein can interact with different receptors (Berman et al. 2003; Eble 1997).

One paradigm demonstrating an essential role of integrins in cell survival and perhaps drug resistance can be death caused by detachment of cells from solid support (anoikis) (Frisch and Screaton 2001; Cheresh and Stupack 2002). The integrin mediated signaling is thought to mediate higher resistance of adherent cells compared with cells maintained in suspension (Elliott and Sethi 2002; Chrenek et al. 2001; Damiano 2002). Indeed, integrins are critical regulators of anti-apoptotic signaling; in particular, this function is important for countering the cytotoxicity of chemotherapeutic drugs (Truong et al. 2003; Yeh et al. 2004). Sensitivity of tumor cells to cytosine arabinoside, cisplatin and camptothecine depends, at least in part, on integrin mediated activation of pro-apoptotic p53 and c-Abl (Truong et al. 2003). The α4β1 receptor signaling confers the resistance of B-cell leukemia to fludarabine through activation of pro-apoptotic Bcl-xL and inhibition of p53 (Fuente et al. 2003).

The survival pathways governed by integrins require further investigation. It remains to be understood whether integrins transduce solely protective (anti-apoptotic) signaling whose interruption leads to anoikis of detached cells. Do integrins generate pro-apoptotic signals when cell-matrix interaction is disassembled? We have shown that vitronectin specific αvβ3 integrin is capable of promoting apoptosis in colon carcinoma cells detached from culture plastic (Kozlova et al. 2001). Similar pro-apoptotic activity has been reported for other integrins (Cheresh and Stupack 2002; Marco et al. 2003). Therefore, cells that lack these receptors should have an advantage to survive under certain stress conditions. One may hypothesize that down-regulation of these particular integrins can benefit for selection of cell variants with altered cytotoxic stress response. This hypothesis has been proven by our recent data on dramatic decrease of αvβ3 integrin expression in fibroblasts selected for a Pgp-mediated MDR (Kozlova et al. 2004).

In this report we show for the first time that the MCF-7Dox cells, a subline of human breast adenocarcinoma cell line MCF-7 that acquired a Pgp-mediated MDR in the course of selection with the chemotherapeutic agent doxorubicin, express low-to-null amounts of collagen specific integrins α2β1 and αvβ3, laminin specific integrins α3β1 and α6β1 but much higher amount of fibronectin specific receptor α5β1 compared with wild type cells. Furthermore, MCF-7Dox cells are more resistant to anoikis and possess higher ability to migrate through Matrigel (in vitro invasiveness). Secretion of MMP-9 collagenase was significant in the MDR subline but negligible in wild type cells. Finally, stimulation of β1 integrin signaling with specific antibody significantly sensitizes MCF-7 cells to anoikis.

MATERIALS AND METHODS

Chemicals

Monoclonal antibodies BHA2.1 to α2β1 integrin, P3G8 to αv subunit, and polyclonal antibodies to cytoplasmic domains of α1, α2, α4, α5, β1, β3 and β5 subunits were purchased from Chemicon Int. (USA). Monoclonal antibodies P1B5 to α3β1 integrin were from Dako Corp. (USA). Monoclonal anti- HLA-ABC antibodies ICO-53 were the gift of A.Baryshnikov (N.Blokhin Cancer Center, Moscow). (N-hydroxysuccine imidobiotin amidocaproate

(NHS-biotin), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT), polyhydroxyethyl metacrylate (poly-HEM), poly-L-lysine, Rhodamine 123 (Rh 123) and doxorubicin were from Sigma (UDA). Oligonucleotides were synthesized by Syntol (Moscow). Reagents for reverse transcription and polymerase chain reaction (RT-PCR) were obtained from Invitrogen (USA).

Cell lines

The MCF-7 human breast carcinoma cell line was obtained from American Type Culture Collection, (ATCC) (USA). The MCF-7Dox subline (gift of T.Ignatova, University of Illinois at Chicago, USA) was established in the course of stepwise selection for survival in the continuous presence of doxorubicin. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (HyClone, USA), 2 mM L-glutamine, 100 U/ml penicillin and 100 γ/ml streptomycin at 37^0C, 5% CO_2 in humidified atmosphere. The MCF-7Dox cells were propagated in the presence of 1.7 μM doxorubicin. Cells in logarithmic phase of growth were used in all experiments.

Protein detection

Labeling of cell surface proteins with biotin, cell lysis, immunoprecipitation of integrins, electrophoresis and immunoblotting were performed as described earlier (Kozlova et al. 1997). For detection of Pgp mouse anti-Pgp antibody (1:1000) was used; for detection of β-actin (internal standard) a murine monoclonal antibody was used (1:5000). Secondary antibodies were horseradish peroxidase-conjugated anti-mouse IgG (1:2500) (all antibodies from Sigma Chemical Co., St. Louis, MO). Proteins were detected with Enhanced Chemiluminescence System (ECL; Amersham, UK).

RT-PCR

RT-PCR analysis of expression of genes coding for integrin subunits were performed as in (Kozlova et al. 2001). Primer sequences and reaction conditions are presented in Table 1.

Pgp-mediated transport

The MCF-7 and MCF-7Dox cells ($5x10^5$ cells in 2 ml of medium) were loaded with 300 nM Rh 123, a fluorescent dye transported by Pgp (Zubercova and Babushkina 1998) for 1 hour at 37^0C). A portion of MCF-7Dox cells was exposed to Rh 123 in the presence of 20 μM verapamil, an inhibitor of Pgp transport (Zubercova and Babushkina 1998). After the completion of incubation cells were detached from plastic with 0.02% EDTA, resuspended in saline. Cell associated fluorescence was measured on a flow cytometer (FACSCalibur, Becton Dickinson, USA). Five thousand events were collected for each sample. Data were analyzed using CellQuest™ software.

Cellular resistance

Cellular resistance to chemotherapeutic drugs was determined in an MTT-test (Damiano et al. 1999). $5x10^3$ cells in 190 μl medium were plated into a 96-well plate (Corning, the Netherlands) overnight and then left untreated (control) or treated with anticancer drugs (5-10 μl, each concentration in duplicate) for 72 hrs. Twenty μl of 5 mg/ml aqueous MTT solution were added for the last 2 hrs of treatment. Formazan was dissolved in dimethyl sulfoxide, and optical density (OD) was measured on a spectrofluorimeter (LKB, Sweden) at 540 nm. Cell viability was calculated as mean OD of wells with drug treated cells to OD of control wells (100%). Resistance index was calculated as IC_{50} (a concentration that resulted in survival of 50% cells) for MCF-7Dox cells to that of MCF-7 cells.

Invasion

Invasion through Matrigel was studied as described (Morozevich et al. 2004).

Table 1. Conditions of PCR-based detection of integrin subunit expression.

Integrin	Forward primer	Reverse primer	Conditons
α1	5'-catgcggggctcgttttggaa-3'	5'-cggccacatctcgggaccaga-3'	94^0 C, 45 sec. 60^0 C, 60 sec. 72^0 C, 60 sec. 35 cycles
α2	5'-tggggtgcaaacagacaagg-3'	5'-gtaggtctgctggttcag-3'	94^0 C, 45 sec. 58^0 C, 60 sec. 72^0 C, 60 sec. 30 cycles
α3	5'-tacgtgcgaggcaatgaccta-3'	5'-tttgggggtgcaggatgaagct-3'	94^0 C, 45 sec. 60^0 C, 60 sec. 72^0 C, 60 sec. 30 cycles
α5	5'catttccgagtctgggccaa-3'	5'-tggaggcttgagctgagctt-3'	94^0 C, 60 sec. 58^0 C, 60 sec. 72^0 C, 60 sec. 30 cycles
α6	5'-tggaggtacagttgttggcg-3'	5'-ctccgttaggttcagggagt-3'	94^0 C, 45 sec. 58^0 C, 60 sec. 72^0 C, 60 sec. 35 cycles
αv	5'-gttgggagattagacagagga-3'	5-'caaaacagccagtagcaacaa-3'	94^0 C, 45 sec. 56^0 C, 45 sec. 72^0 C, 60 sec. 30 cycles
β1	5'-tgttcagtgcagagccttca-3'	5'-cctcatacttcggattgacc-3'	94^0 C, 60 sec. 56^0 C, 60 sec. 72^0 C, 60 sec. 30 cycles
β3	5'-ggggactgcctgtgtgactc-3'	5'-cttttcggtcgtggatggtg-3'	94^0 C, 60 sec. 58^0 C, 60 sec. 72^0 C, 60 sec. 30 cycles
β5	5'-cgagcttgggataaagcaag-3'	5'-tcaacaggcatctcaacagc-3'	94^0 C, 60 sec. 56^0 C, 60 sec. 72^0 C, 60 sec. 30 cycles
β-actin	5'-gtggggcgccccaggcacca-3'	5'-ctccttaatgtcacgcacgatttc-3'	94^0 C, 30 sec 60^0 C, 30 sec. 72^0 C, 30 sec. 30 cycles

Secretion of metalloproteinases
Secretion of metalloproteinases (MMPs) by cells after conditioning the culture medium for 24 h was studied by the method of enzymography (Morozevich et al. 2004).

Anoikis
Anoikis was determined by assaying the internucleosomal DNA degradation after plating of cells on a non-adhesive substrate poly-HEM (Frisch and Francis 1994). Quantitative measurement of DNA fragmentation was performed using Cell Death Detection ELISA Kit (Roche Diagnostic GmbH, Germany) as recommended by the manufacturer. This ELISA-based method detects histones associated with mono- and oligonucleosomes accumulated in apoptotic cells.

In the experiments with regulation of anoikis by immobilized anti-integrin antibodies cell death was determined by [^{14}C]-thymidine incorporation into DNA (McGahon et al. 1995; Rozzo et al. 1997). Cells were incubated in the medium supplemented with [^{14}C]-thymidine (2 μCi/2·10^5 cells) for 17 hrs at 37^{0}C, washed with saline, incubated for an additional 1 h, detached from plastic, resuspended in the medium containing 2,5% fetal calf serum and incubated on a support coated with anti-integrin antibodies (see below) at 37^{0}C for 3.5 h. Then cells were transferred to the medium with 10% serum, plated on poly-HEM and further incubated at 37^{0}C for 24 h. After the completion of incubation cells were lysed in buffer containing 20 mM Tris-HCl, pH 7,4, 4 mM EDTA and 0.4% Triton X-100, and centrifuged at 12,000 g. The pellet was dissolved in the above buffer containing 1% sodium dodecylsulfate. Radioactivity of the supernatant (fragmented DNA) and pellet (intact DNA) was measured. Percent apoptosis was calculated as counts of supernatant /counts of the pellet+counts of the supernatant.

For immobilization of antibodies 24-well plates were coated with rabbit antibodies to mouse IgG (25 μg/ml) for 2 h at 37^{0}C, rinsed with saline, blocked with albumin (10 mg/ml in saline, 1 h), washed with medium and incubated with anti-β1, anti-αv or anti-HLA-ABC antibodies (1:100 each) for 18 h at 4^{0}C. Then plates were washed three times with medium and were used for cell plating (see above).

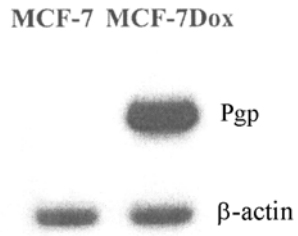
Statistical analysis
Statistical analysis was carried out using Student' s *t*-test. Differences between groups with $p<0.05$ were considered statistically significant.

RESULTS

Pgp-mediated MDR in MCF-7Dox cells
As shown in Figure 1a, MCF-7Dox cells express Pgp (as determined by immunoblot analysis), whereas in MCF-7 cells its expression is below the level of detection. Furthermore, efflux of Rhodamine 123 by MCF-7Dox cells is substantially more pronounced compared with that by MCF-7 cells, and this effect is reversible by verapamil (Figure 1b). These data indicate that MCF-7Dox cells express functionally competent Pgp.

These cells are resistant not only to the selective agent doxorubicin but also to vincristine, taxol and mitoxantrone, drugs known to be transported by Pgp (Table 2). Thus, MCF-7Dox cells possess Pgp-mediated MDR phenotype.

A



B

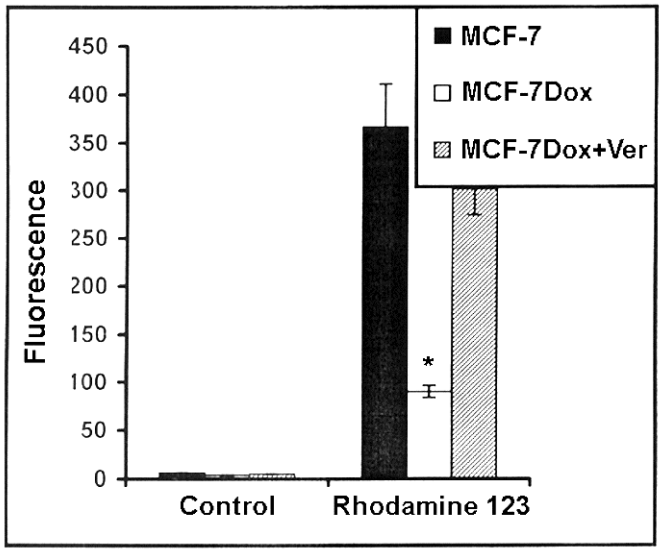


Figure 1: MCF-7Dox cells express functional Pgp. *a* – Westerm blot analysis of Pgp expression. *b* – effect of verapamil on Pgp mediated transport. Shown are data of three experiments (mean ± s.e.m.) $^{*}P < 0,05$ (MCF-7Dox compared with MCF-7Dox + verapamil).

Table 2. Cellular resistance to chemotherapeutic drugs.

Cell line	Doxorubicin	Vincristine	Taxol	Mitoxantrone	
MCF-7	248 ± 29	38 ± 11	112 ± 24	138 ± 13	
MCF-7Dox	2465 ± 109 (9,9)	5410 ± 135 (14,2)	8120 ± 412 (72,5)	7465 ± 181 (54,1)	

Shown are IC_{50} (nM; mean ± s.e.m.) of three independent experiments. In parentheses: resistance index (see Materials and Methods for details).

Anchorage dependent apoptosis (anoikis)

As noted above, integrin mediated signaling has been causatively implicated in cell survival, including altered response to chemotherapeutic drugs (Truong et al. 2003; Yeh et al. 2004; Fuente et al. 2003). These results link the roles of integrins in anoikis and drug resistance. We therefore investigated whether MDR cells are cross resistant to anoikis. Cell death was determined by assaying the internucleosomal degradation of DNA down to ~180 bp fragments, a hallmark of apoptosis. Incubation of MCF-7 and MCF-7Dox cells on non-adhesive substrate poly-HEM for 24 h resulted in the characteristic apoptotic DNA ladder which was clearly detectable in wild type cells and barely visible in their MDR counterparts (data not shown). Quantitative analysis of DNA degradation (by ELISA detection of histones in cytoplasmic lysates) demonstrated ~4-fold increase of fragmented DNA in non-adherent MCF-7 cells compared with non-adherent MCF-7Dox cells (Figure 2), indicating that MDR cells are also resistant to anoikis.

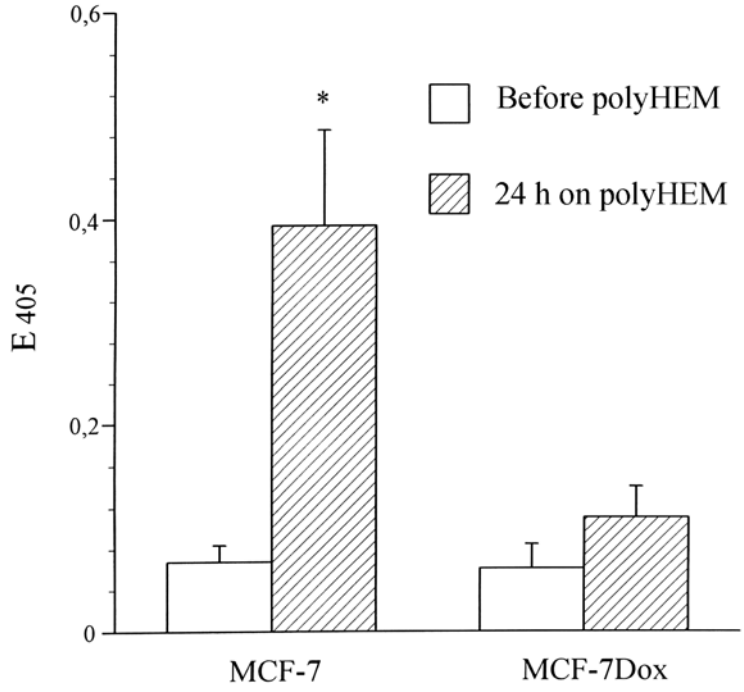


Figure 2: Sensitivity of MCF-7 and MCF-7Dox cells to anoikis. Lysates from 3000 cells of each type were used for analysis. Shown are data of four independent experiments (mean ± s.e.m.) $^{*}P < 0,001$ (MCF-7 cells after incubation on polyHEM compared with cells before plating on polyHEM).

Integrin expression in MCF-7 and MCF-7Dox cells

Because integrins are key regulators of anoikis (Frisch and Screaton 2001; Cheresh and Stupack 2002), one can hypothesize that difference in cell susceptibility to detachment from extracellular matrix can be due to altered expression of integrins in wild type and MDR cells. Indeed, MCF-7 and MCF-7Dox cells show dramatic differences in cell surface expression of these receptors (Figure 3). The MCF-7Dox cells lack collagen specific receptor $\alpha 2\beta 1$, vitronectin specific integrin $\alpha v\beta 5$ and collagen/laminin specific integrin $\alpha 3\beta 1$. The collagen/vitronectin specific integrin $\alpha v\beta 3$ was found in neither cell line. The MCF-7Dox cells carry much

more fibronectin specific integrin α5β1 and, to a minor extent, collagen specific α1β1 compared with wild type counterparts (Figure 3). In contrast, MCF-7 cells expressed α2β1, α3β1 - almost 100% positive cells as determined by FACScan (not shown) and αvβ5 integrins (40-60% positive cells). To confirm the data on cell surface integrin expression by gene expression analysis, RT-PCR analysis was performed.

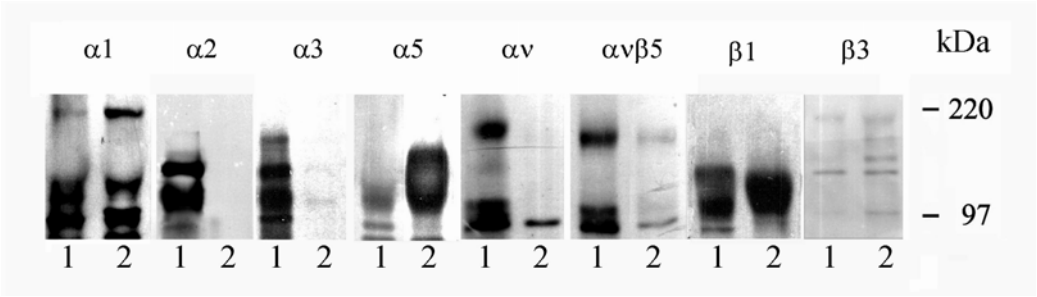


Figure 3: Cell surface expression of integrins in MCF-7 (1) and MCF-7Dox (2) cells. Biotinylated cell surface proteins (see 'Materials and Methods') were precipitated with anti-integrin antibodies, separated in PAGE and transferred onto a nitrocellulose membrane. Blots were processed as indicated in 'Materials and Methods'.

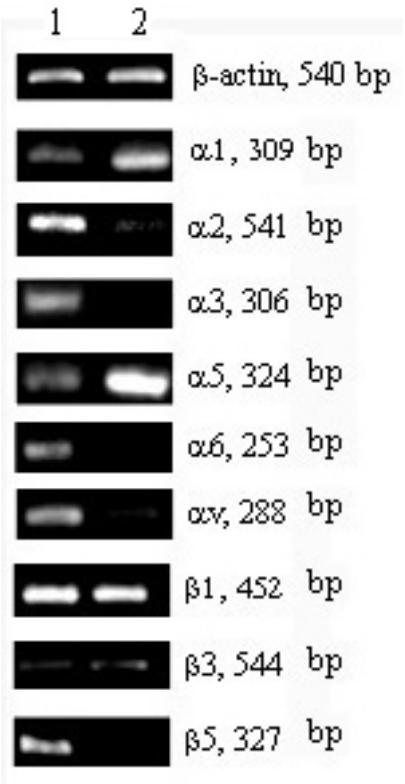


Figure 4: Expression of genes coding for integrin subunits (RT-PCR analysis). Primer sequences and reaction conditions are given in Materials and Methods. 1, MCF-7; 2, MCF-7Dox. Right, sizes of respective PCR products are indicated.

As shown in Figure 4, abundance of integrin mRNAs is in excellent agreement with data on cell surface expression. In MCF-7Dox cells, the most active are the genes coding for α5 and β1 subunits whereas in MCF-7 cells α2, α3, αv, β1 and β5 genes are expressed, confirming higher amounts of α2β1, α3β1 and αvβ5 dimers in these cells determined by protein analysis.

We next hypothesized that integrins of β1 and/or αv families can be critical for cell survival. If that holds true, down-regulation of these receptors could provide an explanation for higher resistance of MCF-7Dox cells to anoikis. On the other hand, resistance to anoikis could be an additional mechanism of stress resistance in MDR cells. To test this hypothesis, we plated MCF-7 cells on anti-β1 or anti-αv antibodies immobilized on plastic. Adhesion of cells to immobilized anti-integrin antibodies causes clustering of these receptors within the plasma membrane and thereby triggers integrin-mediated signal transduction.

Table 3. Activation of β1 integrin signaling stimulates anoikis in MCF-7 cells.

Substrate	Anoikis, %
Anti-HLA	16,4 ± 2,2
Poly-L-lysine	16,7 ± 1,3
Anti-β1	27,2 ± 4,4*
Anti-αv	16,5 ± 2,1

[^{14}C]-Thymidine-labelled cells were incubated on the indicated substrates and then plated on poly-HEM for 24 h at 37^{0}C. Anoikis was determined as indicated in "Materials and Methods". Shown are the mean ± s.e.m of three independent experiments. *P < 0,05 relative the controls (anti-HLA and poly-L-lysine).

Table 3 demonstrates that activation of β1-mediated signaling led to ~70% increase of percentage of cells undergoing anoikis whereas control treatments such as integrin-unrelated anti-HLA antibodies or poly-L-lysine were without the effect. Activation of αv signaling did not alter the viability of MCF-7 cells devoid from solid support.

Invasiveness of wild type and MDR cells

Capability to survive in suspension allows cells to invade surrounding tissues and metastasize (Frisch and Screaton 2001; Yawata et al. 1998). We therefore suggested that invasiveness could be more pronounced in MCF-7Dox cells. Indeed, the experiments with migration through Matrigel showed that invasiveness of MDR cells was significantly increased compared with wild type cells (Table 4).

To get insight into the mechanisms of this effect, we studied the activity of metallproteinases (MMPs), the enzymes known to degrade extracellular matrix proteins (Egeblad and Werb 2002) . The role of integrins in regulation of MMPs, in particular, collagenases, has been established (Heino 1996; Riikonen et al. 1995). We have previously demonstrated that Rous sarcoma virus transformed hamster fibroblasts with high invasive and metastatic potential produce elevated amounts of MMP-2 compared with isogenic low invasive/low metastatic counterparts (Morozevich et al. 2004). As shown in Figure 5, MCF-7Dox cells secreted substantial amount of MMP-9 (by zymographic assay) whereas this enzyme activity was undetectable in MCF-7 cells. Neither cell line produced MMP-2.

Table 4. Invasion of MCF-7 and MCF-7Dox cells through Matrigel.

Cell lines	Number of migrated cells
MCF-7	257 ± 175
MCF-7Dox*	3818 ± 335

Cells ($5x10^4$ in 300 μl of DMEM+0.5% fetal calf serum) were plated into the upper chamber of Transwell in which the separating membrane was pre-coated with 150 μg Matrigel. After incubation for 120 h at 37^0 C cells migrated to the bottom chamber were counted. Data of three experiments (mean± s.e.m.). *P < 0,001 (MCF-7Dox cells compared with MCF-7 cells).

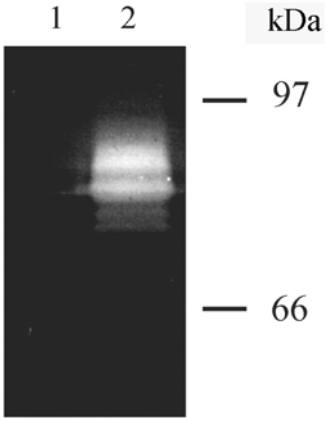


Figure 5: Secretion of MMP-9 by MCF-7 (1) and MCF-7Dox (2) cells. Cells were grown in the medium containing 10% fetal calf serum until subconfluence, washed and further incubated in serum free medium for 24 h at 37^0C. Enzymography of conditioned medium was performed as described (Morozevich et al 2004).

DISCUSSION

The role of integrins in cell survival, drug resistance and tumor progression remains not fully understood. Multidrug resistant ovary carcinoma cells express more α2β1 and α6β1 and are more invasive *in vitro* (Sedlak et al. 1996). Elevated amounts of α2, α5, α6, β1, β4, MMP- 2 and MMP-9, as well as higher *in vitro* invasiveness were reported for melphalan-resistant subline of nasopharyngeal carcinoma cells; in contrast, taxol-resistant subline showed lower α2β1 expression and attenuated invasiveness (Liang et al. 2001). Our data corroborate the results of other groups that the expression of α5β1 receptor is up-regulated (Nista et al. 1997), and α2β1 integrin is down-regulated (Narita et al. 1998) in doxorubicin selected MDR subline of MCF-7 cells. However, α5β1 expression in a renal cell carcinoma line with Pgp-mediated resistance to vinblastine was decreased (Meyer et al. 1999).

These discrepancies can be due to redundancy of integrin signaling and lack of strict ligand specificity of integrins. Therefore, different integrins can interact with the same matrix protein and transduce signals with similar physiological consequences (Berman et al. 2003; Morozevich et al. 2004). One can assume that tumor progression is not associated with changes in a single integrin but it is rather a spectrum of these receptors that ensures the optimal adaptation of cells to microenvironment. In this scenario the resulting phenotype – MDR, invasiveness etc. – can be mediated by an array of integrins. In other words, each particular integrin can participate in establishment of particular phenotype (including stress resistance) depending on extracellular matrix content.

Here we show for the first time that β1 family integrins can stimulate anoikis of cultured breast carcinoma cells whereas the αvβ5 receptor cannot. However, we have found that, in colon carcinoma cells, the αvβ3 integrin that is functionally close to the αvβ5 promoted anchorage dependent apoptosis (Kozlova et al. 2001). This cell type dependent effect has been demonstrated for α6β4 (Bachelder et al. 1999; Dowling et al. 1996) and α4β1 (Marco et al. 2003; Matsunaga et al. 2003) receptor.

Stimulation of anoikis by engagement of β1 integrins in MCF-7 cells sheds light on the mechanism of anti-anoikis in MDR counterparts with down-regulated β1 receptors. Importantly, in primary mammary gland tumors the abundance of collagen- and laminin specific α2β1 and α3β1 integrins (involved in cell differentiation) was found to be decreased but vitronectin- and fibronectin specific receptors αvβ3 and α5β1 (involved in cell migration and proliferation) were elevated (Chrenek et al 2001). Several groups have reported on positive correlation of tumor cell resistance to anoikis and chemotherapeutic drugs (Fuente et al. 2003; Matsunaga et al. 2003; Schmidt et al. 2002).

Mechanisms of integrin mediated apoptosis remain poorly understood. In endothelial cells αvβ3 and β1 integrins generated pro-apoptotic signaling given that one of these receptors was not bound to its cognate ligand; the non-ligated integrin can interact with caspase 8 (Cheresh and Stupack 2002). This phenomenon was termed integrin-mediated death. The authors suggested that this mode of cell death differ from anoikis.

An alternative mechanism of integrin-mediated death could be due to altered balance between active and inactive forms of p53 in the cell. Exogenous expression of the β4 gene in α6β4-negative RKO cells stimulated apoptosis via α6β4 dependent p53 activation. In contrast, in p53-deficient cells α6β4 plays an anti-apoptotic role by activation of Akt/protein kinase B (Bachelder et al. 1999). Significantly, cell adhesion to extracellular matrix leads to elevation of p53 and increased sensitivity to DNA-interacting drugs doxorubicin and etoposide (Truong et al. 2003). Detachment of cells caused a decrease of p53 abundance and concomitant acquisition of drug resistance. However, these effects seem to be limited to certain cell types. If our models fall into this category, one can hypothesize that down-regulation of β1 integrins in MCF-7Dox cells is likely to mediate the resistance to anoikis and anticancer drugs.

The α5β1 integrin can play an important role in the complex phenotype of MCF-7Dox cells (i.e., multifactorial stress resistance and increased invasiveness). We hypothesize that up-regulation of this receptor can provide a dual effect:

1. The protective effect of α5β1 in anoikis has been documented (Lee and Juliano 2000; Matter and Ruoslahti 2001); therefore, this integrin may have impact on survival of MCF-7Dox cells upon detachment from extracellular matrix and contribute to their resistance to chemotherapeutic drugs.

2. The α5β1 mediated signaling up-regulates MMPs and thereby stimulates invasiveness (Werb et al. 1989). It is feasible to suggest that overexpression of this receptor in MCF-7Dox cells is not a mere correlate of elevated production of MMP-9 and migration through Matrigel but is a mechanistic factor of these phenomena. Thus, α5β1 integrin can be a molecule that provides both survival and invasion signals to ensure advanced malignancy of these cells. In turn, α5β1 integrin emerges as a target for therapeutic intervention to counter survival and invasiveness of tumor cells.

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