PSMB7 is associated with anthracycline resistance and is a prognostic biomarker in breast cancer

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BACKGROUND: To date individual markers have failed to correctly predict resistance against anticancer agents in breast cancer. We used gene expression patterns attributable to chemotherapy-resistant cells to detect potential new biomarkers related to anthracycline resistance. One of the genes, PSMB7, was selected for further functional studies and clinical validation.

METHODS: We contrasted the expression profiles of four pairs of different human tumour cell lines and of their counterparts resistant to doxorubicin. Observed overexpression of PSMB7 in resistant cell lines was validated by immunohistochemistry. To examine its function in chemoresistance, we silenced the gene by RNA interference (RNAi) in doxorubicin-resistant MCF-7 breast cancer cells, then cell vitality was measured after doxorubicin treatment. Microarray gene expression from GEO raw microarray samples with available progression-free survival data was downloaded, and expression of PSMB7 was used for grouping samples.

RESULTS: After doxorubicin treatment, 79.8 ± 13.3% of resistant cells survived. Silencing of PSMB7 in resistant cells decreased survival to 31.8 ± 6.4% (P > 0.001). A similar effect was observed after paclitaxel treatment. In 1592 microarray samples, the patients with high PSMB7 expression had a significantly shorter survival than the patients with low expression (P < 0.001).

CONCLUSION: Our findings suggest that high PSMB7 expression is an unfavourable prognostic marker in breast cancer.

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The major cause of cancer therapy failure is either primary drug resistance or the development of secondary resistance against currently available antineoplastic agents. Many different mechanisms of drug resistance have been identified, including the overexpression of members of the family of adenosine triphosphate-binding cassette (ABC)-transporters such as P-glycoprotein (ABCB1), the multidrug resistance-associated proteins MRP1 and MRP2 and the mitoxantrone resistance protein/breast cancer resistance protein (Gottesman et al, 2002). Drug resistance is also mediated by defects in intrinsic cellular safeguard mechanisms capable of activating apoptosis. The loss of programmed cell death induced by antitumour drugs can be achieved through disruption of regulators of DNA damage signalling, such as p53 and bcl-2. Moreover, alterations in cell cycle checkpoints, repair of damaged cellular targets and various additional more or less well-characterised mechanisms can contribute to the resistance.

The anthracycline antibiotic doxorubicin (adriamycin) is widely used for treatment of breast, ovarian, bronchogenic and gastric solid tumours, lymphomas and leukaemias (Martin et al, 2003).

Triple negative (ER-, PR-, HER2-) breast patients receive anthracycline-based chemotherapeutic agents in monotherapy or in combination protocols. Although a number of different mechanisms have been proposed for the cytotoxic effect of anthracyclines, the primary mechanism of drug action is likely to be the inhibition of DNA biosynthesis through topoisomerase II binding and consequently conferring an S/G2 cell cycle arrest (Gewirtz, 1999). As a topoisomerase inhibitor, it potentially induces apoptosis in normal cells as well (Sabourin and Osheroff, 2000).

In several reports, authors applied DNA array technologies to find genes correlated with doxorubicin resistance (Ayers et al, 2004; Kang et al, 2004; Folgueira et al, 2005; Gianni et al, 2005; Cleator et al, 2006; Györffy et al, 2006; Hess et al, 2006). In our earlier study, we used gene expression patterns of closely related chemotherapy-resistant and -sensitive parental cancer cell lines to identify discriminatory genes associated with drug resistance. The use of a set of different cell lines for the identification of discriminating genes allows a tissue-independent application for predicting therapy response. We applied our model to predict sensitivity in a set of 44 breast cancer samples. The patient group characterised by the gene expression profile similar to that of drug-sensitive cell lines exhibited over 50% longer survival than the group exhibiting the profile characteristic of resistant cells (Györffy et al, 2005). Overall, in these gene expression signatures, a considerable number of genes were associated with doxorubicin resistance protein (Gottesman MR or the mitoxantrone resistance protein/breast cancer resistance protein/ABCB1), the multidrug resistance-associated proteins MRP1 and MRP2 and the mitoxantrone resistance protein/breast cancer resistance protein (Gottesman et al, 2002).
MATERIALS AND METHODS

Cell lines

The doxorubicin/daunorubicin-resistant derivatives of human gastric carcinoma cell line EPG85-257P, pancreatic carcinoma cell line EPP85-181P, colon carcinoma cell line HT-29 and breast cancer cell line MCF-7 were established in our laboratory as described earlier (Lage and Dietel, 2002). Cells were cultured in Leibovitz L-15 medium (Sigma-Aldrich, Budapest, Hungary) supplemented with 10% foetal calf serum (Life Sciences, Grand Island, NY, USA), 1 mm l-glutamine, 80 IE/l insulin, 2.5 mg l–1 dglucose, 1.1 g l–1 NaHCO3, 1% minimal essential vitamins and 20 000 kIE l–1 trisylol in a humidified atmosphere containing 5% CO2 at 37°C. Culture media of resistant cell lines were supplemented with daunorubicin for HT-29 (0.125 µg ml–1), EPP85-181 (2.5 µg ml–1) and EPG-257 (2.5 µg ml–1) and doxorubicin (0.05 µg ml–1) for MCF-7 cells (Farmatia Carlo Erba, Freiburg, Germany). The 24 h treatment of drug-sensitive cells was performed at the identical drug concentrations.

Sensitivity of cells to doxorubicin and paclitaxel

Cell proliferation assay (Cell Proliferation Kit I (MTT) Roche, IN, USA) was used to monitor sensitivity of doxorubicin-resistant cell lines. Protocol was carried out as directed by the manufacturer. Briefly, 5000 cells per well in a microplate were plated, and drug was added after an overnight incubation in increasing grade of 0.0001x–100x of the clinically administered dose (doxorubicin: 0.02 µg ml–1, paclitaxel: 0.025 µg ml–1) in triplicates. Six days later, MTT (3-[4, 5-dimethylthiazolyl-2]-2, 5-diphenyltetrazolium bromide) was added to each well for 4 h. After this incubation period, the formed purple formazan salt crystals were solubilised and quantified 1 day later with the use a BioTek PowerWave XS Microplate Spectrophotometer.

Autofluorescence of doxorubicin

A total of 50 000 sensitive MCF-7 cells per well were plated in triplicate in a 96-well plate for four different conditions: (1) cells treated by doxorubicin, (2) untreated cells, (3) doxorubicin in L15 medium without cells and (4) L15 medium alone. Doxorubicin treatment (0.2 µg ml–1) was carried out on the second day for 1.5 h. MTT staining and reading the results were performed as described above. Differences were assessed by using a t-test.

RNA isolation and cDNA synthesis

RNA was isolated from 1 × 106 cells in logarithmic growth phase using the Qiagen RNeasy Mini Kit following the manufacturer’s protocol (Qiagen GmbH, Hilden, Germany). Isolated total RNA was quantified by UV-spectroscopy and quality checked by analysis on a LabChip (BioAnalyzer, Agilent Technologies, Santa Clara, CA, USA). RNA samples were stored at –80°C. cDNA was synthesised from 5 µg total RNA by firstly annealing to 5 pmol µl–1 HPLC purified T7- (dT)24 primer (MWG-Biotech, Ebersberg, Germany) at 70°C for 10 min. Second, reverse transcription, second-strand synthesis and cleanup of double-stranded cdNA was performed according to the protocols provided by Affymetrix (http://www.affymetrix.com/index.affx). Synthesis of biotin-labeled cRNA was performed using the BioArray High Yield RNA Transcription kit (Enzo Diagnostics, Farmingdale, NY, USA). cRNA concentration was determined by UV-spectroscopy and the distribution of cRNA fragment size was checked on a LabChip (BioAnalyzer, Agilent Technologies).

Hybridization protocol

The fragmented cRNA was hybridised to the HG-U133A arrays (Affymetrix, Santa Clara, CA, USA) in a hybridization oven at 45°C for 16 h. Subsequent washing and staining of arrays was performed using the GeneChip fluidics station protocol EuGene-WS2. Finally, probe arrays were scanned using the GeneChip System confocal scanner (Hewlett-Packard, Santa Clara, CA, USA). For each cell line triplicates (sensitive, resistance and treated), hybridization was made once, so altogether 12 hybridizations were made. The microarray data can be downloaded from GEO (accession no. GSE3926).

Pre-processing of microarray data

Quality control analysis was performed according to the suggestions of The Tumor Analysis Best Practices Working Group (Hoffman et al., 2004). Scanned images were inspected for artefacts. All RNA targets included in the analysis exhibited present calls of >25% and were not degraded. We have applied RMA (Irizarry et al., 2003) for the normalisation of hybridization intensities. The pre-processing was performed using Bioconductor packages in the R statistical environment. RMA is one of the most common array normalization methods supplying cross-project normalization with good specificity and excellent sensitivity.

Feature selection

We arranged the complete data set into three classes according to the resistance and treatment properties of the cell lines. We
compared drug-sensitive parental, doxorubicin/daunorubicin-treated parental and resistant cell lines. For identifying discriminating genes, the Prediction Analysis for Microarrays (PAM v.1.23) package was used as described earlier (Tibshirani et al., 2002). PAM uses soft thresholding for producing a shrunken centroid, which allows the selection of genes with high discriminative potential. An overview of the applied statistical approach is presented in Figure 1. We decided to pick the top 100 genes from each comparison for the selection of the best discriminatory group of genes. Then genes were identified, which discriminate between parental and treated parental cell lines (reflecting effect of the drug treatment) and resistant and parental cell lines (reflecting genes of resistance).

**Additional analyses**

Hierarchical clustering was performed using the Genesis software (Sturm et al., 2002). Before clustering, we performed a second normalization at the gene level to set the average expression of each transcript to 0 to present gene inductions or repressions of identical magnitude as numerically equal. Gene annotations were performed using the NetAffx Analysis Centre (http://www.affymetrix.com/analysis/index.affx).

**Immunohistochemistry**

Cells were grown on microscopic slides and fixed in ice-cold methanol–acetone mixture (1:1). Activity of endogenous peroxidase was blocked by incubation in 1% H₂O₂. Immunohistochemical stainings were carried out in triplet with primary antibody polyclonal antiserum against PSMB7 (dilution 1:200; GenWay Biotech, Inc., CA, USA). Subsequently, slides were incubated with secondary biotinylated antibodies anti-mouse, rabbit and goat; optimally prediluted from LSAB +, HRP Kit (DakoCytomation, Glostrup, Denmark); followed by optimally prediluted streptavidin–biotinylated peroxidase complex (LSAB +, HRP, DakoCytomation) and the chromogen NovaRed (Vector Laboratories, Peterborough, UK) at room temperature. The intensity of antigen expression was measured using the digital imaging system Lucia-G/F (Nikon, Tokyo, Japan). Mean saturation feature was determined in four microscopic fields at 200-fold magnification. Nucleus staining was performed by DAPI (4’,6-diamidino-2-phenylindole).

**Oligos for RNAi**

Target of siRNA oligos for PSMB7 were designed using siRNA Target Finder (http://www.ambicon.com) and siDESIGN Center (http://www.dharmacon.com) softwares. DNA oligonucleotide templates for three target mRNAs were ordered (Csertex Kft, Budapest, Hungary), then were synthesised by Silencer siRNA Construction Kit (Applied Biosystems, Darmstadt, Germany) using the manufacturer’s instruction. Synthesised siRNA duplex concentrations were measured by NanoDrop ND-1000 spectrophotometer (BCM, Houston, TX, USA) then diluted as needed. PCR primers were designed by Primer3 software (http://frodo.wi.mit.edu/) and their binding site was verified by NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Out of three synthesised siRNA oligos, one silenced effectively PSMB7 better than 80%. Figure 3 shows binding location of every synthesised oligos and PCR primers (effective siRNA is framed by continuous line). Effectiveness of siRNA oligos was validated by RT–PCR (Figure 3B).

**SIRNA transfection of MCF-7 cell lines**

Transfection was carried out with 5 μl SiPORT NeoFX Transfection Reagent (Applied Biosystems, Budapest, Hungary) in six-well plates. A total of 230,000 cells were plated into each well. The final concentration of siRNA was 10 nM in 2.5 ml serum and antibiotic-free medium. After 24 h of incubation, medium was washed twice with sterile PBS, then normal growth medium was added. Total RNA was extracted in the 48th h after transfection with Qiagen RNeasy Mini Kit as described earlier, CDNA synthesis and DNA amplification were carried out by OneStep RT–PCR Kit (Qiagen GmbH) with gene-specific PCR primers. GAPDH was used as internal control. We used 24 amplification cycles for amplification. Amplification products were separated on 2% agarose gel stained with ethidium-bromide. The effect of silencing was calculated by Adobe Photoshop CS2 software.

**Combination of RNAi and chemotherapy treatment**

To investigate the function of selected gene in chemoresistance, we combined RNAi and drug treatment. PSMB7 overexpressed in resistant cells was silenced in doxorubicin-resistant MCF-7 cell lines (MCF-7-RAdr); 24 h after transfection, the reaction was stopped by replacing the medium with normal growth medium containing drug in concentration of 0.2 μg ml⁻¹. Cells were trypsinised on the 72nd h and counted by CASY DT Cell Counter (Innovatis AG, Reutlingen, Germany). We used negative siRNA-treated (AllStars Negative Control siRNA, Qiagen GmbH) MCF-7-RAdr cells, siRNA-untreated MCF-7-RAdr cells and siRNA-untreated MCF-7 cells for controls with their doxorubicin-treated analogue. For a statistical analysis, the number of living cells was counted in each well. Experiments were carried out three times, and each well was measured three times in each experiment. The number of drug-treated cells was normalised to untreated cells in siRNA-treated, negative siRNA-treated and siRNA-untreated wells. The t-test was used for analysis of difference between groups. Significance level was set at P = 0.05.

**Validation on clinical samples**

For in silico validation of the genes, a database containing processed GEO microarray samples was established as described earlier (Györffy and Schafer, 2009). Dataset includes GSE12276.
RESULTS
Identification of differentially regulated genes
To identify genes associated with the resistance to doxorubicin/daunorubicin, we contrasted gene expression profiles of closely related drug-resistant and -sensitive cell lines derived from breast, pancreatic, colon and gastric cancer. For microarray analysis, RNA was prepared from non-treated parental cells, from parental cells treated with doxorubicin for 24 h and from resistant derivatives cultured continuously in the presence of doxorubicin at a concentration that completely eliminated the sensitive cells. The complete data set comprising raw data and Affymetrix .CEL files is available in the GEO database (http://www.ncbi.nlm.nih.gov/geo/) using the GEO accession number GSE3926. The prediction analysis of microarrays was performed to pick the top 100 genes differentially expressed in each sample set independent of tissue origin. To visualise discriminatory expression changes, we clustered the top genes (see Figure 2A and B). In these, three probe sets representing proteasome subunits were expressed stronger in doxorubicin-resistant cell lines, but not in doxorubicin-treated parental cells (see arrows in Figure 2).

Autofluorescence of doxorubicin
Examining the effect of doxorubicin autofluorescence on MTT results, there was no difference between doxorubicin-treated and -untreated cells ($P = 0.38$). Fluorescence of doxorubicin supplemented L15 medium differed significantly from doxorubicin-treated cells ($P < 0.01$), but did not differ from untreated L15 medium ($P = 0.065$).

Prognostic potential of 64 proteasome subunits
The normalised gene expression of the GEO-downloaded 1592 samples was filtered to include only the proteasome subunits present on the HG-U133A microarrays. The prognostic potential for all 64 probe sets was computed by dividing the patients into over- and underexpressed group as compared with the median (thus, 531 patients were in each group). The analysis resulted in 12 significant genes below an FDR of 0.05. Of these, only one was also detected by the microarray analysis (PSMB7), which was further investigated. Immunocytochemistry confirmed the overexpression of PSMB7 as observed on the microarrays (Figure 2C and D).

Survival of cells after gene silencing and treatment with doxorubicin and paclitaxel
To examine the function of the PSMB7 gene in doxorubicin resistance, we combined gene silencing with drug treatment to assess its effect on cell survival. Viability of cells without doxorubicin treatment was better in both siRNA-untreated and siRNA-treated wells compared with cells with doxorubicin treatment. A specific cytotoxic effect of negative control siRNA was negligible as compared with the effect of gene-specific siRNA. Data after normalization are presented in Figure 4; 79.8 ± 13.3% of resistance cells survived after doxorubicin treatment. Combined with gene silencing, only 31.8 ± 6.4% of the MCF-7-RAdr cells survived. The significance between siRNA-treated and siRNA-untreated MCF-7-RAdr cells after doxorubicin treatment was $P > 0.001$. After doxorubicin treatment, 48.3 ± 8.1% of sensitive cells survived. A total of 73.3% of negative control siRNA-treated cells survived. After combination of paclitaxel treatment and gene silencing, 22.6 ± 4% of the MCF-7-RAdr cells survived compared with siRNA-untreated cells, whereas 43.8 ± 6% of sensitive cells survived (data not shown). Relative cell vitality of siRNA-treated and siRNA-untreated MCF-7-RAdr cells after paclitaxel treatment differed significantly ($P = 0.03$).

Biomarker potential of PSMB7 in clinical samples
To assess the prognostic power of PSMB7 gene in resistance, clinical samples were used to validate the results of cell culture model. We grouped the 1592 breast cancer patients on the basis of the expression of PSMB7 using the Affymetrix HGU133A probe set 200786. at. Of these, 963 out of 1220 patients were ER positive and 187 out of 1156 lymph node positive. The average relapse-free survival was 6.42 years. Unfortunately, detailed treatment history was generally not available. Patients above the median had a significantly shorter survival than patients below the median ($P < 0.001$) (Figure 5).

DISCUSSION
In our study we examined the function of the proteasome subunit PSMB7 gene in drug resistance in breast cancer cell line and in breast cancer patients. So far, no earlier study has risen up concerning drug resistance neither for PSMB7 gene nor any of proteasome subunits. In a cell culture model with a combination of RNAsi and drug treatment, we validated the causative function of this gene in chemoresistance. The examination of 1592 breast cancer patient showed that overexpression of this gene is associated with poor prognostic outcome.

At the preliminary phase of this study, we contrasted the expression profiles of four pairs of different human tumour cell lines of gastric, pancreatic, colon and breast origin, their counterparts resistant to the topoisomerase inhibitors daunorubicin or doxorubicin and the sensitive parental cells after a 24h-chemotherapy treatment using Affymetrix HG-U133A microarrays. We also performed additional analyses to exclude the influence of doxorubicin autofluorescence (Karukstis et al, 1998) on our results. Finally, we identified the top transcripts associated with doxorubicin/daunorubicin resistance, but not with treatment response. So far, the majority of microarray-based chemoresistance-associated gene sets concentrated on finding genes associated with resistance rather than on the difference between drug response and drug resistance. Surprisingly, we have found genes such as proteasome subunits coding PSMB7 and PSMD13 having function in the mechanism of resistance that earlier have not been described. Of these, only the expression of PSMB7 was able to predict survival in clinical samples and was, therefore, further investigated using RNAsi.

The Homo sapiens gene PSMB7 encodes proteasome (also called prosome or macropain) subunit β-type, 7. In the Haloarchaeon Halofaxia volcanii proteasomal 20S, components are required for cell growth (Zhou et al, 2008). Proteasome and tRNA modification genes are co-transcribed, revealing that a number of additional enzymes are co-regulated with proteasomes at the transcriptional level in the same species (Gil et al, 2007). Other animal experiments related to proteasome and immunology were made on Japanese pufferfish (Clark et al, 2001) and zebrafish (Michalova et al, 2000). The importance of monitoring proteasomes has emerged in the last couple of years as it has an important function in the degradation of many proteins involved in cell cycle...
**Figure 2** Discriminating transcripts: (A) hierarchical clustering of all three groups (parental, treated parental, resistance cell line) against each other and (B) hierarchical clustering of parental vs treated parental cell lines. Red arrows show appearance of three probe sets measuring proteasome subunits on resistance-associated gene list. Immunohistochemical localization of PSMB7 expression in sensitive (C) and resistant cells (D). Reactions of cytoplasmic localization were obtained for PSMB7 (green arrows).
regulation, apoptosis and angiogenesis. As these pathways are fundamental for cell survival and proliferation, particularly in cancer cells, the inhibition of proteasome might deliver an attractive potential anticancer therapy. Proteolysis is conducted by 20S proteasomes, complexes of 28 subunits arranged as a cylinder in four heteroheptameric rings: α-1 to -7, β-1 to -7, β-1 to -7 and α-1 to -7. The catalytic subunits are β-1 (PSMB6), β-2 (PSMB7) and β-5 (PSMB5). Three additional subunits, β-1i (PSMB9), β-2i (PSMB10) and β-5i (PSMB8), are induced by γ-interferon (IFNG) and are preferentially incorporated into proteasomes to make immunoproteasomes. PSMB11, or β-5t, is a catalytic subunit expressed exclusively in cortical thymic epithelial cells (Murata et al., 2007).

The function of one of the catalytic subunits, the PSMB7 gene in cancer is still an undiscovered spot of molecular biology. Its overexpression is described in colorectal carcinomas, in both cytoplasmic and nuclear region (Rho et al., 2008). After adaptation of proteasome inhibitor expression of PSMB5 increased, but other components of proteasome such as PSMB7 did not (Oerlemans et al., 2008). Looking for tissue-specific alteration in proteasome units, researchers treated mice with 3H-1,2-dithiole-3-thione (D3T), which functions as a cancer preventive agent proved both in animal and human studies; 24 h later, expression of the 20S catalytic core subunits PSMB5, PSMB6 and PSMB7 were increased in liver, lung, small intestine and colon of mice (Kwak et al., 2007a).

Elevated expression of proteasome catalytic subunits led to increase in proteasomal peptidase activities in these tissues. Oral administration of D3T also exerted a pharmacodynamic action in some brain regions of these mice and proteasomal peptidase activities were significantly elevated in the cerebral cortex–hippocampus. These results indicate that increased proteasome expression by inducers may have a function in protection/attenuation of protein aggregate-mediated disorders (Kwak et al., 2007b).

After in vitro inspection, we further validated the discriminating function of differential PSMB7 expression in 1592 publicly available microarrays. By dividing the patients as having over- or underexpressed PSMB7 as compared with the median, we achieved high significance. However, our approach is limited...
because of inadequate treatment information. A future study for fine tuning of PSMB7 expression with RT–PCR in a large clinical sample collection with detailed clinical follow-up could identify exact cut offs for thresholds, which could validate and enhance its discriminative potential in clinical use.

We might consider the idea to silence overexpressed PSMB7 as part of a chemotherapy. However, despite promising in vitro results, the establishment of effective RNAi conditions is still complicated. A major impediment to the clinical use of RNAi therapy is the need to deliver these macromolecules to each and every cancer cell to trigger a direct and specific effect. Several studies have shown in vivo efficacy in the delivery of siRNAs using various strategies such as complexing siRNAs with cationic lipids, nanoparticles, melittin, melittycin, histone-thiosemicarbazone, histone-thioneins, melittin and collagen (Nguyen et al, 2008). Xenograft mouse model was also effectively used in several studies (Xiao et al, 2008; Xie et al, 2008; Zhang et al, 2008, 2009; Shi et al, 2009). In human beings, RNAi is already in clinical use in therapy of macula degeneration and respiratory syncytial virus infection, but so far no effective RNAi-based inhibition of tumour progression is available.

As it was examined in the last decades, overexpression of individual genes can be associated with resistance against given agents. ABCB1 gene strongly correlates to chemoresistance (Clarke et al 1992; Kamata et al, 2008; Larbcharoensub et al, 2008; Shi et al, 2008), TOP2A is a potential gene for predicting anthracyclin resistance (Molina et al, 2005; Tanner et al, 2006; Harris et al, 2007; Mano et al, 2007). Expression of metallothioneins is linked to tamoxifen resistance (Surowiak et al, 2004), and the gene Tau is a predictor of resistance against neoadjuvant paclitaxel therapy (Rouzier et al, 2005; Andre et al, 2007). The favourable outcome of this study suggests that PSMB7 gene has an important function in predicting both doxorubicin and paclitaxel resistance.

In summary, our findings support the function of the proteasome in the development of chemotherapy resistance. High PSMB7 expression is an unfavourable prognostic marker in breast cancer.

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REFERENCES

Adams J, Palombella VJ, Saussville EA, Johnson J, Destree A, Lazarus DD, Maas J, Pien CS, Prakash S, Elliott PJ (1999) Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 59: 2615 – 2625
André F, Hatzis C, Anderson K, Sotiriou C, Mejia JA, Wang B, Hortobagyi GN, Symmans WF, Pusztai L (2007) Microtubule-associated protein-tau is a bifunctional predictor of endocrine sensitivity and chemotherapy resistance in estrogen receptor-positive breast cancer. Clin Cancer Res 13: 2061 – 2067
Ayers M, Symmons WF, Stec J, Damakos AL, Clark E, Hess K, Lecocq M, Metivier J, Booser D, Ibrahim N, Valero V, Royce M, Arun B, Whitman G, Ross J, Snieg N, Hortobagyi GN, Pusztai L (2004) Gene expression profiles predict complete pathologic response to neoadjuvant paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide chemotherapy in breast cancer. J Clin Oncol 22: 2284 – 2293
Ciolli S, Leoni F, Casini G, Breschi C, Santini V, Bosi A (2008) The addition of liposomal doxorubicin to bortezomib, thalidomide and dexamethasone significantly improves clinical outcome of advanced multiple myeloma. Br J Haematol141: 814 – 819
Clark MS, Shaw L, Kelly A, Snell P, Elgar G (2001) Characterization of the MHC class I region of the Japanese pufferfish (Fugu rubripes). Immunogenetics 52: 174 – 185
Clarke R, Currier S, Kaplan O, Love lace E, Boully V, Gottesman MM, Dickson RB (1992) Effect of P-glycoprotein expression on sensitivity to hormones in MCF-7 human breast cancer cells. J Natl Cancer Inst 84: 1506 – 1512
Cleator SJ, Bowles TJ, Dexter T, Fullford L, Mackay A, Smith IE, Valgeirsson H, Ashworth A, Dowsett M (2006) The effect of the stromal component of breast tumours on prediction of clinical outcome using gene expression microarray analysis. Breast Cancer Res 8: R32
Folgueira MAAK, Carraro DM, Brentani H, Patra DFD, Barbosa EM, Netto RB (2008) Based inhibition of tumour progression is available. British Journal of Cancer (2010) 102(2), 361 – 368
Gil MA, Sherwood KE, Maupin-Furlow JA (2007) Transcriptional linkage of Halofax volcanii proteasomal genes with non-proteasomal gene neighbours including RNome P, MOSC domain and SAM-methyltransferase homologues. Microbiology 153: 3009 – 3022
Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2: 48 – 58
Gyorffy B, Serra V, Juracht K, bdul-Ghani R, Garber M, Stein U, Petersen I, Lage H, Dietel M, Schafer R (2005) Prediction of doxorubicin sensitivity in breast tumors based on gene expression profiles of drug-resistant cell lines correlates with patient survival. Oncogene 24: 7542 – 7551
Gyorffy B, Surowiak P, Kiesslich O, Denkert C, Schafer R, Dietel M, Lage H (2006) Gene expression profiling of 30 cancer cell lines predicts resistance towards 11 anticancer drugs at clinically achieved concentrations. Int J Cancer 118: 1699 – 1712
Gyorffy B, Schafer R (2009) Meta-analysis of gene expression profiles related to relapse-free survival in 1,079 breast cancer patients. Breast Cancer Res Treat 118: 433 – 441
Harris L, Fritsch H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast Jr RC (2007) American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer. J Clin Oncol 25: 5287 – 5312
Hess KR, Anderson K, Simmans WF, Valero V, Ibrahim N, Mejia JA, Booser D, Theriault RL, Budzar AU, Dempsey P, Rouzier R, Snieg N, Ross JS, Vidaurre T, Gomez HL, Hortobagyi GN, Pusztai L (2006) Pharmacogenomic predictor of sensitivity to preoperative chemotherapy with paclitaxel and fluorouracil, doxorubicin, and cyclophosphamide in breast cancer. J Clin Oncol 24: 4236 – 4244
Hoffman EP, Awad T, Palma J, Webster T, Hubbell E, Warrington JA, Nakamoto JHL, Camargo LP, Vencio RZN, Snitcovsky IML, Makdissi FBA, Silva PJDE, Goes JGCS, Brentani MM (2005) Gene expression profile associated with response to doxorubicin-based therapy in breast cancer. Clin Cancer Res 11: 7434 – 7443
Gewertz DA (1999) A critical evaluation of the mechanisms of action for the proposed antitumor effects of the anthracycline antibiotics daunorubicin and doxorubicin. Biochem Pharmacol 57: 727 – 741
Gianni L, Zambetti M, Clark K, Baker J, Cronin M, Wu J, Mariani G, Rodriguez J, Carcangiu M, Watson D, Valagussa P, Rouzier R, Symmans WF, Ross JS, Hortobagyi GN, Pusztai L, Shrik S (2005) Gene expression profiles in paraffin-embedded core biopsy tissue predict response to chemotherapy in women with locally advanced breast cancer. J Clin Oncol 23: 7265 – 7277

British Journal of Cancer (2010) 102(2), 361 – 368
drug-resistant gastric cancer cells using high-density oligonucleotide microarrays. Clin Cancer Res 16: 272 – 284.
Karuktsis KK, Thompson EH, Whites JA, Rosenfeld RJ (1998) Deciphering the fluorescence signature of daunomycin and doxorubicin. Biophys Chem 73: 249 – 263.
Kwak MK, Huang B, Chang H, Kim JA, Kensler TW (2007a) Tissue specific increase of the catalytic subunits of the 26S proteasome by indirect antioxidant dithiothreitol in mice: enhanced activity for degradation of abnormal protein. Life Sci 80: 2411 – 2420.
Kwak MK, Huang B, Chang H, Kim JA, Kensler TW (2007b) Tissue specific increase of the catalytic subunits of the 26S proteasome by indirect antioxidant dithiothreitol in mice: enhanced activity for degradation of abnormal protein. Life Sci 80: 2411 – 2420.
Lage H, Dietel M (2002) Multiple mechanisms confer different drug-resistant phenotypes in pancreatic carcinoma cells. Cancer Res Clin Oncol 128: 349 – 357.
Larbcharoensub N, Leopairat J, Sirachainan E, Narkwong L, Bhongmakapat M, Thai K, Khunrattana A, et al (2008) The expression of CYP1A1 and CYP1B1 genes in patients with oral squamous cell carcinoma. Life Sci 83: 1029 – 1038.
Larsson K, Wahlestedt C, Schake K, Gogarten J (1983) The proteasome explicitly catalyzes a specific N-end rule degradation pathway in mammalian cells. J Mol Biol 165: 463 – 473.
Larsson K, Wahlestedt C, Schake K, Gogarten J (1983) The proteasome explicitly catalyzes a specific N-end rule degradation pathway in mammalian cells. J Mol Biol 165: 463 – 473.
Larsson K, Wahlestedt C, Schake K, Gogarten J (1983) The proteasome explicitly catalyzes a specific N-end rule degradation pathway in mammalian cells. J Mol Biol 165: 463 – 473.