The analysis of doxorubicin resistance in human breast cancer cells using antibody microarrays

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Abstract

Doxorubicin is considered to be the most effective agent in the treatment of breast cancer patients. Unfortunately, resistance to this agent is common, representing a major obstacle to successful treatment. The identification of novel biomarkers that are able to predict treatment response may allow therapy to be tailored to individual patients. Antibody microarrays provide a powerful new technique, enabling the global comparative analysis of many proteins simultaneously. This technology may identify a panel of proteins to discriminate between drug-resistant and drug-sensitive samples. The Panorama Cell Signaling Antibody Microarray was exploited to analyze the MDA-MB-231 breast cancer cell line and a novel derivative, which displays significant resistance to doxorubicin at clinically relevant concentrations. The microarray comprised 224 antibodies selected from a variety of pathways, including apoptotic and cell signaling pathways. A standard ≥2.0-fold cutoff value was used to determine differentially expressed proteins. A decrease in the expression of mitogen-activated protein kinase–activated monophosphotyrosine (phosphorylated extracellular signal-regulated kinase; 2.8-fold decrease), cyclin D2 (2.5-fold decrease), cytoskeleton 18 (2.5-fold decrease), cyclin B1 (2.4-fold decrease), and heterogeneous nuclear ribonucleoprotein m3-m4 (2.0-fold decrease) was associated with doxorubicin resistance. Western blotting was exploited to confirm results from the antibody microarray experiment. These results suggest that antibody microarrays can be used to identify novel biomarkers and further validation may reveal mechanisms of chemotherapy resistance and identify potential therapeutic targets. [Mol Cancer Ther 2006;5(8):2115–20]

Introduction

Doxorubicin is an anthracycline antibiotic, which remains an important agent in many chemotherapy regimens. Although doxorubicin is currently considered to be one of the most effective agents in the treatment of breast cancer, resistance leads to an unsuccessful outcome in many patients. The identification of novel biomarkers, which correlate with treatment response, would allow therapy to be tailored on an individual patient basis. Ultimately, those patients unlikely to respond to doxorubicin would be spared from serious life-threatening side effects for no therapeutic gain. Biomarkers may also provide information on new drug targets for future therapeutic intervention. Overcoming resistance to doxorubicin would represent a major advance in the effective management of breast cancer. Although the mechanisms remain unclear, it is accepted that the development of drug resistance is a multifactorial phenomenon (1) and the use of global analytic techniques is becoming increasingly appreciated in this field. Such techniques offer the advantage of simultaneously analyzing whole genomes, transcriptomes, or proteomes. Changes in mRNA levels are not absolutely indicative of alterations in the corresponding levels of cellular proteins; therefore, proteome analysis provides a complementary approach to microarray gene expression profiling. Two-dimensional gel electrophoresis, in combination with mass spectrometry, is a state-of-the-art method for elucidating global variations in protein expression; however, the technique is laborious to do and reproducibility remains a challenge (2). In addition, two-dimensional gel electrophoresis has a limited ability to detect low abundance proteins and is not readily amenable to high-throughput applications. Antibody microarrays provide a powerful new technology for the simultaneous analysis of known proteins. A high precision robot is used to spot hundreds of different antibodies at high density onto a platform, such as a glass slide; a format that is compatible with existing hardware and software tools for gene expression microarrays (3). The surface of the slides is chemically modified to present functional groups for the covalent binding of antibodies, which allows the antibodies to maintain their activity despite immobilization. A fluorescence-based detection procedure analogous to that exploited in gene expression microarray profiling can be used to measure the relative protein expression levels between different samples. Two samples are labeled with a different fluorescent dye and are hybridized simultaneously to the antibody microarray. The immobilized antibodies capture their fluorescently labeled complementary proteins, and the ratio of fluorescence at each spot can be used to determine relative protein abundance in each sample (4). This new technique is simple, fast, and highly reproducible. Although still in
its infancy, antibody microarray technology has previously been exploited in cancer research (4–6). We aimed to investigate the use of an antibody microarray to identify differentially expressed proteins in a novel breast cancer cell line displaying significant resistance toward doxorubicin when compared with parental cells.

Materials and Methods

Establishment of a Doxorubicin-Resistant Cell Line

The doxorubicin-resistant variant MDA-MB-231DR was established from the surviving population of MDA-MB-231 breast cancer cells after treatment of the parental population with a single dose of 200 nmol/L doxorubicin (D1515, Sigma-Aldrich, Dorset, United Kingdom) for 24 hours. The concentration of doxorubicin used to select out the resistant cell line was based on reported clinically achievable plasma concentrations in the range of 1.4 to 34.4 μmol/L (7, 8) to apply a degree of clinical relevance to this in vitro study.

Assessment of In vitro Doxorubicin Resistance

The relative resistance of MDA-MB-231 and MDA-MB-231DR to doxorubicin was measured using a modified colony-counting assay. Cells were plated at a concentration of $1 \times 10^5$ cells per well in triplicate wells of six-well tissue culture plates. Three hours after plating, the triplicate wells were treated with doxorubicin (range, 0–400 nmol/L). Twenty-four hours after treatment, the doxorubicin-containing medium was aspirated from each well and replaced with drug-free medium. The cells were cultured for 12 days on which the resulting cell colonies were fixed, stained with eosin, and counted. A colony was defined as a group of $\geq 50$ cells, and plating efficiency was calculated as described previously (9). ANOVA was used to assess the statistical significance of differences in the dose-response curves. The extraction of protein samples coincided with the colony-counting assessment of resistance.

Antibody Microarrays

The Panorama Cell Signaling Antibody Microarray kit (CSAA1-1KT, Sigma-Aldrich; ref. 10) was exploited, which detects a wide variety of proteins, representing a broad range of biological functions, including apoptotic and cell cycle proteins. The antibody microarray was composed of 224 highly specific antibodies spotted in duplicate on nitrocellulose-coated glass slides. Each antibody microarray contained 32 subarrays each with duplicate spots of seven antibodies as well as a single positive control for Cy3 and Cy5 and a single negative control. The complete list of antibody pairs can be found at the Sigma-Aldrich web site.1

Protein Extraction

Cells were harvested for protein extraction at $\sim 80\%$ confluence. Protein was extracted using cells from two T-75 tissue culture flasks ($\sim 2.0 \times 10^6$ cells in total) for each cell line, yielding $\geq 1$ mg/mL protein. Cells were washed twice in 5 mL of cold PBS. Buffer A (1 mL; antibody microarray kit component) was added directly to one flask of cells and incubated in ice on an orbital shaker for 5 minutes. Cells were collected using a cell scraper, and the buffer/cell mix was transferred to the second flask and again incubated in ice on an orbital shaker for 5 minutes. The cells were again collected using a cell scraper, and the buffer/cell mix was transferred to a microfuge tube. Samples were centrifuged at 10,000 rpm in a microfuge for 10 seconds, and the supernatant was transferred to a fresh tube. Protein concentrations were determined using the Bradford Assay (B6916, Sigma-Aldrich) in accordance with the manufacturer’s instructions.

Fluorescent Labeling of Proteins

Extracts with protein concentrations $\geq 1.0$ mg/mL were used for fluorescent labeling. The range of concentrations used in this study was 1.0 to 2.99 mg/mL. All extracts were diluted to a concentration of 1 mg/mL in buffer A before labeling. A dual fluorescent-labeling assay analogous to that exploited in cDNA microarray experiments was used. Extracts were labeled separately using either NHS ester-activated monofunctional reactive Cy3 or Cy5 dyes (PA23001 and PA25001, respectively, Amersham Biosciences, Buckinghamshire, United Kingdom). Each protein sample (1 mL) was added directly to one vial of either Cy3 or Cy5 and mixed thoroughly by inversion. The labeling reaction was allowed to proceed for 30 minutes at room temperature in the dark. During this period, samples were mixed every 10 minutes. Labeled samples were purified from free excess Cy dye using the SigmaSpin columns supplied with the antibody microarray kit. The columns were centrifuged for 2 minutes at 4,000 rpm before 150 μL of labeled protein sample were pipetted onto the center of the column. A further centrifugation at 4,000 rpm was done for 4 minutes. The eluate was retained, and protein concentration was estimated using the Bradford assay as above. Labeled protein was stored at $-20^\circ$C until hybridization. For the purpose of this study, four biological replicates were created, in which MDA-MB-231 was labeled with Cy3 and MDA-MB-231DR was labeled with Cy5. Two reverse color-labeled replicates were also created, in which MDA-MB-231 was labeled with Cy5 and MDA-MB-231DR was labeled with Cy3. This allowed compensation for any potential bias in binding of Cy3 or Cy5 dyes to the protein samples.

Hybridization

Before hybridization, the dye-to-protein molar ratio was determined following the calculations supplied with the antibody microarray kit. Only samples with a dye-to-protein molar ratio $> 2$ were applied to the antibody microarray as recommended in the antibody microarray kit protocol. Antibody microarray slides were washed by a brief submersion in PBS. The Cy3 and Cy5 labeled sample pairs were added at equal protein concentrations (10 μg/mL each) to 5 mL of Array Incubation buffer (antibody microarray kit component). The resulting solution was mixed by inversion and added to a well of the quadriferm Cell Culture Vessel supplied with the antibody microarray kit. The antibody microarray slide was added to the well containing the labeled samples. The Vessel was protected from exposure to light with aluminum foil and incubated on an orbital shaker (30 rpm) at room temperature for...

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1 www.sigmaaldrich.com/sigma/general%20information/csaa1inf.pdf.
45 minutes. Subsequently, 5 mL of wash buffer (antibody microarray kit component) was added to the remaining wells of the Vessel and the slide was washed on an orbital shaker for 5 minutes in each well. The wash buffer was decanted from well 4 and replaced with 5 mL of proteomics grade water (Bio-Rad, Hemel Hempstead, United Kingdom) for a final wash for 2 minutes. The hybridized antibody microarray slide was removed from the well and air dried in the dark overnight.

**Image Acquisition and Analysis**

The hybridized antibody microarray slides were scanned using a GenePix Personal 4100A Microarray Scanner (Axon Instruments, Union City, CA) with 532 and 635 nm lasers. Primary analysis was done with the GenePix Pro (version 4.1) software package (Axon Instruments). Images of scanned antibody microarrays were gridded and linked to a protein print list. Absent spots were flagged automatically by GenePix Pro; however, all spots were manually reviewed. Further analysis was done using Acuity (version 4.0) software (Axon Instruments) for the identification of differentially expressed proteins. Microarrays were normalized based on the Lowess method due to a slight skew in data distribution. The log ratios of Cy5 to Cy3 were determined for each spot to estimate the relative concentration of each protein in the two independently dye-labeled samples of each experiment. Unreliable data were removed from analyses by applying quality control criteria. Such criteria were set to include only those spots with a small percentage (<3%) of saturated pixels, spots that were not flagged as absent, spots with relatively uniform intensity and uniform background, and those spots that were detectable above background levels. The Student’s t test was done to identify those spots that were significantly (P < 0.05) dye swapped, and from this group, those proteins showing a ≥2-fold difference in expression in ≥50% of individual microarrays were deemed significant. This cutoff was selected because it is the standard in expression microarray analysis and has also been used previously in antibody microarray experiments (6).

**Western Blot Analysis**

Cells were harvested by trypsinization, washed twice in PBS, and resuspended directly in 1 mL of Laemml buffer [62.5 mmol/L Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 1% protease inhibitor mix (Amersham Biosciences), 0.00125% bromphenol blue]. Samples were placed on an end-over-end rotator for 15 minutes at 4°C, and cellular debris was removed by centrifugation at 13,000 rpm for 15 minutes at 4°C. The supernatant was collected, and the protein concentration was determined using the RC-DC Protein Assay kit (Bio-Rad) in accordance with the manufacturer’s instruction. Equal amounts of protein (15–20 μg/lane) from both cell lines were loaded onto a 12% Tris-glycine Novex gel (Invitrogen Life Technologies, Paisley, United Kingdom). Cruz Marker molecular weight standards (Santa Cruz Biotechnology, Santa Cruz, CA) and Precision Plus Dual Color molecular weight standards (Bio-Rad) were electrophoresed simultaneously at 200 V for 60 minutes. Proteins were transferred to a nitrocellulose membrane (Bio-Rad) at 100 V for 90 minutes at 4°C. Nonspecific binding sites were blocked by incubating the membrane in blocking buffer (5% nonfat dry milk, 0.05% Tween 20 in TBS) for 60 minutes on an orbital shaker. Primary antibodies [cycin B1 (MS-338; NeoMarkers, Fremont, CA), glyceraldehyde-3-phosphate dehydrogenase loading control (Ab9485; Abcam, Cambridge, United Kingdom), β-actin loading control (Ab8227; Abcam), cycin D2 (SC-181; Santa Cruz Biotechnology), phosphorylated extracellular signal-regulated kinase (p-ERK; SC-7383; Santa Cruz Biotechnology), ERK2 (SC-154; Santa Cruz Biotechnology), and cytokeratin 18 (SC-6259; Santa Cruz Biotechnology)] were added at concentrations of 1:100 (cycin B1, cycin D2, p-ERK, ERK2, and cytokeratin 18) or 1:1,000 (glyceraldehyde-3-phosphate dehydrogenase and β-actin) and incubated at room temperature for 2 hours on an orbital shaker. The membrane was subsequently washed with 0.05% Tween 20 in TBS. The appropriate horseradish peroxidase–conjugated secondary antibody (anti-mouse or anti-rabbit; Santa Cruz Biotechnology) was diluted in blocking buffer (5% nonfat dry milk, 0.05% Tween 20 in TBS) for 60 minutes on an orbital shaker. The membrane was washed with 0.05% Tween 20 in TBS before protein detection. Proteins were detected using the SuperSignal West Fico Chemiluminescent Substrate (Fierce, Northumberland, United Kingdom) according to the manufacturer’s instructions. Films were scanned using a GS-800 laser densitometer (Bio-Rad), and protein expression was measured using Quantity One software version 4.6.1 (Bio-Rad). Bands were normalized against glyceraldehyde-3-phosphate dehydrogenase or β-actin, and the fold change was calculated using the mean value of fold changes from three replicates.

**Results**

**Establishment of Doxorubicin-Resistant Cells**

Figure 1 indicates the relative resistance of MDA-MB-231 and MDA-MB-231DR to doxorubicin over the concentration range of 0 to 400 nmol/L. The IC50 values for MDA-MB-231 and MDA-MB-231DR were 25 and 35 nmol/L, respectively. MDA-MB-231DR was significantly less sensitive to doxorubicin than MDA-MB-231 at 50, 100, and 200 nmol/L concentrations (P < 0.01, ANOVA) with a maximal 9-fold increase in resistance seen at 100 nmol/L. Western blot, immunocytochemistry, or real-time quantitative PCR analysis revealed that both parent and derivative cells showed similar expression profiles for p53, P-glycoprotein, multidrug resistance protein 1, breast cancer resistance protein, and lung resistance protein (data not shown).

**Antibody Microarrays**

The Panorama Cell Signaling Antibody Microarray kit was exploited to analyze the MDA-MB-231 breast cancer cell line and the novel derivative displaying significant resistance to doxorubicin (MDA-MB-231DR). The antibody microarray was composed of 224 different antibodies spotted in duplicate on nitrocellulose-coated glass slides. Data from biological replicates were used, in which...
MDA-MB-231 protein extracts were labeled with Cy3 and MDA-MB-231DR protein extracts were labeled with Cy5. Also included in the analysis were the data from two dye-swapped replicates, in which the samples were reverse color labeled. To identify those proteins that showed no bias in Cy3 and Cy5 dye incorporation, a Student’s t test was done (with significance set at \( P < 0.05 \)). Following this selection, those proteins showing a \( \geq 2 \)-fold difference in expression between the cell lines in \( \geq 50\% \) of individual antibody microarray experiments were deemed as significantly differentially expressed. A decrease in the expression of mitogen-activated protein kinase (MAPK)–activated monophosphotyrosine (p-ERK; 2.8-fold decrease), cyclin D2 (2.5-fold decrease), cytokeratin 18 (2.5-fold decrease), cyclin B1 (2.4-fold decrease), and heterogeneous nuclear ribonucleoprotein m3-m4 (2.0-fold decrease) was associated with doxorubicin resistance. Interestingly, no proteins were found to be significantly overexpressed in the MDA-MB-231DR cell line compared with the parental cell line.

**Western Blot Analysis**

Four differentially expressed proteins identified in the antibody microarray experiments were further validated using Western blot analysis in three biological replicates using glyceraldehyde-3-phosphate dehydrogenase or \( \beta \)-actin as a control to ensure equal protein loading. Using this technique, the decreased expression of p-ERK (particularly p-ERK1), cyclin D2, and cyclin B1 was confirmed in the MDA-MB-231DR cell line when compared with the parental cell line (Fig. 2). The decreased expression of cytokeratin 18 was not confirmed in all replicates.

**Discussion**

Resistance to doxorubicin represents a major obstacle to the successful treatment of patients with breast cancer. The identification of novel biomarkers, which correlate with treatment response, would allow treatment to be tailored on an individual patient basis. Global analytic techniques, such as gene expression microarray technology, have generated data in the field of chemotherapy resistance. However, changes in mRNA levels are not absolutely indicative of alterations in the corresponding levels of cellular protein; thus, analysis of the proteome represents a complementary approach to gene expression profiling. Novel antibody microarray technology offers the potential to investigate the differential expression of hundreds of known proteins simultaneously. In this study, the antibody microarray technique was assessed in the analysis of the MDA-MB-231 breast cancer cell line and a novel derivative displaying significant resistance to doxorubicin (MDA-MB-231DR).

Development of a doxorubicin-resistant cell line required only a single treatment of 200 nmol/L doxorubicin and 1 month of culture after treatment. The low level change in resistance was statistically significant. The MDA-MB-231DR cell line showed a \( IC_{50} \) of 35 nmol/L doxorubicin (compared with 25 nmol/L for the parent cell line) with a maximal 9-fold increase in resistance at 100 nmol/L. This is comparable with other studies, which have established in vitro resistance to doxorubicin (11, 12). In terms of the clinical relevance of this cell line, recent evidence suggests that the concentrations of doxorubicin found in the plasma of patients treated with this drug range from \( 1.4 \) to 34.4 \( \mu \)mol/L (7, 8). This, together with the fact that doxorubicin has a short (24–48 hours) terminal half-life and is rapidly metabolized by the liver (13), means that the concentration of doxorubicin actually taken up by the tumor is potentially within a nanomolar concentration range. This novel model, derived using a clinically relevant drug dose, can be used to assess expression changes associated with subtle, low level changes in tumor resistance. This model
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may more closely represent the in vivo situation than drug-resistant cell lines derived using doses that are in excess of the clinical range or that show an increased resistance of 100- to 1,000-fold.

The antibody microarray results suggest that the decreased expression of p-ERK (−2.8-fold), cyclin D2 (−2.5-fold), cytokeratin 18 (−2.5-fold), cyclin B1 (−2.4-fold), and heterogeneous nuclear ribonucleoprotein m3-m4 (−2.0-fold) may be associated with resistance to doxorubicin. The cutoff level used to determine significance (≥2.0-fold change in expression between samples) was chosen because this is standard for expression microarray analysis and has also been used previously for up-regulated proteins in the antibody microarray analysis of mantle cell lymphoma (6). The overexpression of cyclin D1, a protein known to be involved in the pathogenesis of mantle cell lymphoma, was readily detected by Western blotting and immunohistochemistry, and this protein was used to calibrate their antibody microarray experiments. Although cyclin D1 overexpression only became significant if their cutoff value was reduced from 2.0-fold to 1.3-fold, some of the targets identified using this approach could not be validated using alternative techniques (6). Loss of expression of low-abundance proteins may be subtle; however, it may still exert a causative effect and the importance of low-level changes in expression (<2-fold) will require further investigation. We identified five significant targets using a 2.0-fold change as a cutoff and further assessed four of these proteins in confirmatory Western blot experiments. False positives, which are a potential problem with gene expression microarray experiments, will also be a factor in the antibody microarray technique, but the cutoff level must represent a balance to encompass subtle changes in expression levels, which may still constitute important functional changes while also maximizing the specificity of the antibody microarray technique. As with expression microarrays, a confirmatory technique must be used to validate the antibody microarray data. Using Western blotting as a confirmatory method, we were unable to identify significantly reduced expression of cytokeratin 18 in the MDA-MB-231DR cells and this may represent a false discovery. However, the reduced expression of p-ERK1, cyclin D2, and cyclin B1 in MDA-MB-231DR cells was confirmed by Western blotting in three biological replicates, suggesting that these are potential targets worthy of further studies into doxorubicin resistance.

MAPK-Activated Monophosphotyrosine (p-ERK)

The MAPK superfamily of enzymes plays a key role in widespread signaling pathways and serves to coordinate key cellular processes, such as cell proliferation and cell death. MAPKs have been classified into three subfamilies: the ERK family, the c-Jun NH2-terminal kinase family, and the p38 MAPK family. The ERK subfamily consists of two closely related MAPKs, ERK1 and ERK2 (p44 and p42, respectively). These proteins are terminal kinases, which exist in a kinase cascade (14). Each kinase phosphorylates and, thereby, activates the next member in sequence. The phosphorylation of ERK1 and ERK2 is regulated by MAPK/ERK kinase 1 and MAPK/ERK kinase 2, which in turn are phosphorylated and activated by Raf-1 in response to Ras stimulation. MAPK enzymes, including ERK, require the dual phosphorylation of regulatory tyrosine and threonine residues to become fully activated; however, partially phosphorylated forms exist (15). In the present study, the down-regulation of the tyrosine-phosphorylated form of ERK was associated with doxorubicin resistance. The expression of unphosphorylated isoforms (ERK1 and ERK2) remained unchanged between the cell lines. Further studies will be required to evaluate the other phosphorylated forms of ERK (phosphothreonine and diphosphorylated forms) and the upstream regulators before postulating any role for this specific p-ERK form in chemotherapy resistance. MAPK pathways are involved with the transmission of many different signals, including the apoptotic signal. The down-regulation of key players within these pathways, including p-ERK, could be associated with chemotherapy resistance due to the attenuation of proapoptotic signaling.

Cyclins

Doxorubicin cytotoxicity has previously been linked to the up-regulation of cyclin B1 (16). Cyclin B1 and D2 are key cell cycle regulators. Cyclin D2 is required for the G1-S phase transition, and cyclin B1 regulates the progression of cells through G2 into the M phase of the cell cycle. Apoptosis is intricately linked with the cell cycle, and although the mechanisms remain elusive, there is accumulating evidence to suggest that cyclins also play a role in apoptosis. In particular, cyclin B1 has been implicated with apoptosis induced by several methods, including the use of DNA-damaging agents (17–19). An increase in the level of cyclin B1 protein was required for γ-radiation-induced apoptosis in hematopoietic cells, and antisense inhibition was found to abrogate apoptosis (19). Cyclin B1 may induce apoptosis through activation of the cdc2 kinase, a protein that may also be regulated by p-ERK (19, 20). The cdc2 kinase was not present on the antibody microarray; therefore, the expression level of this protein requires investigation. Cyclin B1 has also been implicated in clinical studies of response to chemotherapy. High expression levels of cyclin B1 were found to predict a good clinical outcome in follicular lymphoma patients treated with the doxorubicin-containing cyclophosphamide-Adriamycin-vincristine-prednisone (CHOP) chemotherapy regimen (21). The down-regulation of cyclin B1 expression was detected in a lung cancer cell line (A549/UCN) selected for resistance to a novel anticancer compound, UCN-01 (22). This suggests that the decreased expression of cyclin B1 seen in the present study could be directly associated with an attenuated apoptotic response in the MDA-MB-231DR cells, manifesting as doxorubicin resistance. Cyclin D members have previously been associated with apoptosis (23, 24); however, there is considerably less evidence to suggest that the expression of cyclin D2 plays a role in apoptotic pathways. The level of cyclin D2 protein, possibly via the induction of p27, is up-regulated in cells committed to apoptosis (25).
The antibody microarray technology offers a high-throughput approach to study protein expression; however, the study is limited to the number of antibodies printed on the slide. The Panorama Cell Signaling Antibody Microarray kit exploited in this study allowed the analysis of a selected group of 224 proteins, which is a relatively small number when taking into consideration that the whole proteome may consist of >1,000,000 different proteins. In fact, several potential proteins of interest, including MAPK/ERK kinase and cdc2 kinase, were not represented on this antibody microarray. This pilot antibody microarray study has provided several novel candidate proteins and pathways for further evaluation, and although it remains to be seen if there is a mechanistic link of these targets with doxorubicin resistance in these cells, we believe that antibody microarray analysis is a promising new technique. Antibody microarray technology may offer several advantages over traditional proteomic technologies in terms of ease of use and rapid quantitative data generation, especially with the availability of slides containing several hundred antibodies. The analysis of low-abundance and membrane-associated proteins may be more suited to an antibody microarray strategy. This study has shown that antibody microarray technology can be exploited to identify potential biomarkers, which may be useful in the prediction of response to doxorubicin in breast cancer. If the functional significance of these, or other, targets is confirmed in the mechanism of doxorubicin resistance, then the possibility of using a customized antibody microarray as a screening tool for clinical samples would seem to be an attractive option to consider.

References

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