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Use of comparative proteomics to identify potential resistance mechanisms in cancer treatment

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Abstract

Drug resistance is a major problem in successful cancer chemotherapy. Many molecular mechanisms that are responsible for drug resistance are known whereas others have yet to be discovered. Determining the exact mechanism activated in a particular case (clinical or laboratory) is a difficult task. Recently, proteomics has been applied to investigate drug resistance mechanisms in model cancer cell lines. As a result, novel mechanisms of resistance have been discovered and known mechanisms of resistance confirmed. In this paper, we wish to review recent developments and progresses in the application of proteomic tools to identify known and novel drug resistance mechanisms in drug-selected model cancer cell lines. Our combined analyses of multiple proteomic studies of various drug resistant cancer cell lines revealed that many mechanisms of resistance likely exist in any given drug-selected cancer cell line and that common mechanisms of resistance may be selected in a spectrum of cancer cell lines. These observations suggest that combination therapies targeting multiple mechanisms to sensitize drug resistant cancers may be necessary to eradicate cancers in the future.

Keywords

proteomics; 2-dimensional gel electrophoresis; mass spectrometry; drug resistance; cancer chemotherapy

Introduction

Drug resistance is a major problem in successful cancer chemotherapy. Cancer cells do not respond sufficiently to a spectrum of plural structurally and functionally unrelated anticancer agents. The resistance can be primary (intrinsic) where tumor cells do not respond to anticancer drugs from the beginning of the treatment or secondary (acquired) where tumor cells develop resistance during chemotherapy. There are many known mechanisms that are responsible for acquired drug resistance such as increased drug efflux, DNA repair, and altered survival and apoptotic signaling pathways. Many model cell lines selected by anticancer drugs are available for investigating the mechanisms of acquired drug resistance. However, determining the exact mechanism activated in a particular case is a difficult task. Furthermore, novel and multiple mechanisms may exist which cannot be studied using the tools targeting any one known

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mechanism of resistance. The recent development of genomic and proteomic approaches has helped greatly in addressing these issues. In this paper, we review recent developments and progresses in the application of comparative proteomic tools to identify potential resistance mechanisms in drug-selected model cancer cell lines. We also performed collective analyses of up-to-date proteomic data and discovered potential, common mechanisms of drug resistance which should help to guide future studies and cross validate findings of various proteomic profiling.

Proteomics

Conventional proteomics refers to the use of traditional two-dimensional gel electrophoresis (2-DE) to separate proteins, combined with mass spectrometry (MS) and database search to identify proteins. Recently, more advanced methods and technologies have been developed including surface-enhanced laser desorption/ionization (SALDI), shot gun proteomics, isotope-coded affinity tagging (ICAT) coupled with MS, and protein microarray. Comparative proteomics uses these technologies, but only to identify the proteins with differential levels between two samples of different disease stages or treatment conditions (for a review of proteomics, see ¹).

Although 2-DE was used to study protein profiles in drug-resistant human KB carcinoma cells by the Gottesman's group as early as 1986 ², the first systematic comparative proteomic profiling and analysis of the drug-selected cancer cell lines was performed years later with the advancement of the MS technology ³. Currently, many laboratory-derived drug resistant cancer cell lines have been analyzed using the comparative proteomic approach and groups of proteins potentially contributing to the selected drug resistance have been identified. Due to limited space, we will focus our review on studies that used the conventional comparative proteomics (see Table 1 for proteomic approaches used in the studies reviewed here).

Drug resistance-associated proteins identified using comparative proteomic profiling.

In all up-to-date comparative proteomic profiles of various drug resistant cancer cell lines, multiple proteins in numerous processes or pathways were found to have altered expressions in any given cell line (Table 2 and Table 3). Some of these proteins were identified in multiple studies of various drug resistant cancer cell lines, whereas others were found only in some particular cases (Table 3). While some of the identified proteins have known roles in drug resistance, the others do not and may represent a new set of molecules that are important in drug resistance. The following text describes representative proteins that have alterations in each functional group as discovered in more than one proteomic profiling with a focus on proteins that were subsequently verified to likely represent novel drug resistance mechanisms (annexin I, annexin IV, TCTP, 14-3-3 σ , γ -synuclein, PKM2, and mGluR4). We will also discuss representative proteins that have not been verified functionally (e.g., RAN), have been known to cause drug resistance (e.g., HSP27, MGr1-Ag, ABCG2, and Prx 3), or apparently contradict expression changes from previous findings (e.g., tubulin α and Prx 2). Readers are directed to the references for other proteins of interest listed in Table 3. It should be noted, however, that many proteins listed in Table 3 have not been verified and that known proteins that cause drug resistance in the cell lines listed in Table 2 may not have been found using proteomics due to technical limitations (see Concluding Remarks). Furthermore, the functional mechanisms of some proteins with verified roles in drug resistance have yet to be fully elucidated.

In most of the proteomic studies reviewed here, the commonly investigated anticancer drugs include, but are not limited to, Adriamycin, cisplatin, carboplatin, vinblastine, vincristine,

etoposide (VP-16), daunorubicin, and mitoxantrone. While vinblastine and vincristine are vinca alkaloids that exert their cytotoxicities by targeting microtubules, the remaining therapeutics are DNA-damaging agents. The anthracycline antibiotics, Adriamycin (doxorubicin) and daunorubicin, etoposide and mitoxantrone are all thought to cause cytotoxicity by inhibiting topoisomerase and, thus, double strand DNA breaks. Both cisplatin and carboplatin cause DNA damage by forming inter- and/or intra-strand DNA adduct lesions and, thus, cytotoxicity.

1. Calcium-binding proteins

As shown in Table 3, several Ca^{++} -binding and control proteins were found to have altered expression in the drug resistant cells compared with their corresponding parental sensitive cells. The overall trend of elevation in expression of Ca^{++} -binding proteins in drug resistant cells suggest that Ca^{++} signaling may be a very important factor for cancer cells to survive drug treatment. Annexins are a family of calcium-dependent phospholipid-binding proteins including 13 members.

Annexins appear to be involved in several cellular processes such as exocytosis, endocytosis and ion channel activity. In a proteomic analysis, it was found that the expression of annexin IV was increased in A204197-resistant human colon cancer cell line HCT-15/A204197 and paclitaxel-resistant lung cancer cell line H460/T800⁴. Enforced ectopic expression of annexin IV in 293T cells subsequently confirmed a 3-fold increase in paclitaxel resistance in the same study, validating its role in drug resistance⁴. Interestingly, the expression of Annexin IV was decreased in the cisplatin-resistant ovarian cancer cell lines IGROV-1/CP⁵ and IGROV1-R100⁶ as well as in a cisplatin-resistant SKOV3/CDDP cell line⁷. It is currently unknown if the decreased annexin IV expression contributes to cisplatin resistance. Proteomic profiling of Adriamycin-resistant breast cancer cell line MCF7/Adr showed that the expression of annexin I, III, and V was increased^{8,9}. An earlier study of Adriamycin-resistant multidrug resistant small cell lung cancer cell line, H69AR, using molecular cloning technology, also showed that annexin II was increased in this atypical drug resistant cell line¹⁰. While Annexin III was over-expressed in cisplatin and carboplatin-resistant human ovarian cancer cell lines SKOV3/CDDP, SKOV3/CBP, A2780/CDDP, and A2780/CBP, annexin I was over-expressed only in the carboplatin-resistant SKOV3/CBP cells⁷. In a recent study, ectopic over-expression of annexin I in MCF7 increased its resistance to Adriamycin, melphalan, and etoposide, whereas knocking down its expression in SKOV3 increased its sensitivity to these drugs¹¹. Thus, these findings validated the functional role of annexin I in drug resistance. Interestingly, annexin III expression was decreased in a colorectal cancer cell line HTR-18, which is resistant to EGFR-blocking antibody C225 compared with a sensitive cell line Caco-2,¹² although it is not yet known if the differential expression of annexin III contributes to the C225 resistance in HTR-18 cells.

The mechanism of annexin-mediated drug resistance is currently unknown. However, because annexins appear to be involved in several cellular processes such as exocytosis, its over-expression may confirm drug resistance through the enhanced exocytosis of drug filled vesicles¹¹. Alternatively, annexins may be stress proteins and play important roles in protecting cells from stress signals and cytotoxic agents and, thus, cause drug resistance by preventing cellular apoptosis¹³.

Proteomic profiling of melanoma cell lines MeWo selected for resistance to etoposide, cisplatin, vindesin, or fotemustine revealed that the expression of translationally controlled tumor protein 1 (TCTP, also named p23, histamine-releasing factor, and fortilin) was up-regulated in all four resistant sublines¹⁴. TCTP possesses a calcium-binding property¹⁵ and has later been shown to cause resistance to etoposide-induced apoptosis by transient ectopic over-expression in HeLa cells¹⁶, validating its potential role in etoposide resistance. However,

it is unknown as to how the altered expression of such a Ca^{++} -binding protein cause drug resistance.

2. Cell cycle and checkpoint proteins

Several proteins known to be important regulators of cell cycle and checkpoint have been observed to have changes in their expression in the drug resistant cancer cell lines (Table 3). One of the frequently observed molecules with increased expression in drug resistant cancer cell lines is 14-3-3 σ . These cell lines with increased 14-3-3 σ expression include that of the breast selected with Adriamycin in the presence (MCF7/AdrVp, MCF7/AdVp3000)¹⁷ or absence (MCF7/Adr)^{8,9} of verapamil, pancreas selected with mitoxantrone (EPP85-181RNOV)¹⁸, and stomach resistant to thermal treatment¹⁹.

The subsequent validation experiments showed that ectopically over-expressing 14-3-3 σ in MCF7 cells caused resistance to Adriamycin and mitoxantrone whereas silencing its expression using siRNA drastically enhanced the sensitivity of the drug resistant breast cancer cell line MCF7/AdVp3000 to these drugs¹⁷. Ectopic over-expression of 14-3-3 σ in HEK293 cells also caused resistance to mitoxantrone. Similar results were also observed with prostate cancer cell lines²⁰. It is also noteworthy that 14-3-3 σ expression is up-regulated early during the stepwise Adriamycin selection of the breast cancer cell line, suggesting that 14-3-3 σ may be an early respondent gene to drug attack, and physiologically relevant in cancer chemotherapy. Because 14-3-3 σ is a critical G2/M regulator by binding to and sequestering Cdc2/cyclin B1 complex in cytoplasm^{21,22}, cancer cells with elevated expression of 14-3-3 σ have the advantage of surviving drug treatment by efficiently causing G2/M arrest for DNA repair rather than causing mitotic catastrophe and cell death. 14-3-3 σ has also been suggested to bind to Bax and Bad and, thus, inhibit drug-induced apoptosis mediated by these molecules²⁰.

It was found recently that 14-3-3 σ is an independent prognosis factor for the poor survival of patients with colorectal, pancreatic, lung, endometrial, and breast cancers in clinical studies^{23–27}. It was also found that the expression of 14-3-3 σ increased as prostate tumors progress²⁸. Adenocarcinomas of the prostate with high Gleason scores had significantly higher staining and percentages of 14-3-3 σ immunoreactive cells than that of low Gleason scores. These observations suggest that the advanced prostate adenocarcinomas are likely drug resistant. Indeed, our recent studies showed that the androgen-independent prostate cancer cell lines expressed more 14-3-3 σ than the androgen-dependent ones and were more resistant to anticancer drugs²⁰. Thus, 14-3-3 σ is a prognosis factor for poor survival likely by causing drug resistance in chemotherapy of human cancers of multiple origins.

However, ectopic over-expression of 14-3-3 σ in pancreatic cancer cell line EPP85-181P did not cause resistance to mitoxantrone²⁹. The reason for this discrepancy is currently unknown. It was suggested that other factors may be needed for a putative multi-model mechanism of drug resistance which is lacking in the EPP85-181P cell line²⁹.

3. Chaperones

As shown in Table 3, many proteins with chaperone activity had differential expressions between drug resistant and their corresponding sensitive parental cell lines. In most proteomic analyses of various drug resistant cancer cell lines, members of heat shock proteins (HSP) were found to be expressed differently. The most frequently observed protein is HSP27 with increased expression in melanoma cell line MeWo selected with vindesine, cisplatin, fotemustine, or etoposide³⁰; breast cancer cell line MCF7 selected with Adriamycin in the absence³¹ or presence of verapamil¹⁷; etoposide-resistant neuroblastoma cell line SH-SY5Y³²; vincristine-resistant stomach cancer cell line SGC7901/VCR³³; and cisplatin-selected SKOV3/CDDP⁷.

As stress response chaperone proteins, HSPs act to protect cells by inhibiting apoptosis and enhancing protein and DNA damage repairs. Previously, it has been found that ectopic over-expression of HSP27 in breast cancer cells increased resistance to Adriamycin, and down-regulating endogenous HSP27 expression sensitized the resistance³⁴. It was later shown that the elevated expression of HSPs, including HSP27, caused increased efficiency in repair of Adriamycin-induced DNA damages and, thus, resistance to Adriamycin³⁵. It was also found that HSP27 expression was increased in a colorectal cancer cell line HTR-18, which is resistant to EGFR-blocking antibody C225 compared with a sensitive cell line Caco-2¹². In the latest proteomic study, down-regulating HSP27 expression in gastric cancer cells SGC7901/VCR sensitized these cells also to vincristine, validating its role in vincristine resistance³³. Furthermore, the high expression level of HSP27 has been thought to cause drug resistance and poor prognosis in breast³⁶ and ovarian^{37,38} cancer patients in clinical studies. Proteomic analyses of 24 B-cell chronic lymphocytic leukemia patients also showed that HSP27 was up-regulated in patients with shorter survival compared with patients of longer survival³⁹. Thus, HSP27 over-expression likely causes resistance to various anticancer drugs and clinically shorter survival (for a detailed review on HSPs, see⁴⁰). However, it has also been found in some other clinical studies that HSP27 expression has no prognostic value⁴⁰. Although the reason for the discrepancy between these studies is not yet clear, it may be disease specific. Further clinical studies are needed to solve this dispute.

4. Cytoskeletal proteins

Cytoskeletal proteins are important for structural integrity and dynamic remodeling of cells during the development of neoplastic phenotype and execution of apoptosis. These proteins include the ones involved in forming microfilaments, intermediate filaments, microtubules, and their associated proteins.

Many anticancer drugs, such as vinblastine and vincristine, are anti-microtubule agents. Thus, the expression of tubulin and microtubule-associated proteins may play some role in resistance to these anticancer drugs. Verills et al.⁴¹ performed proteomic analyses of human leukemia cell lines selected for resistance to vincristine (CEM/VCR R) or vinblastine (CEM/VLB100) compared with the parental CEM cells. They found that the expression of both tubulin α and β was decreased in both CEM/VCR R and CEM/VLB100 cell lines (see Table 3). However, it has been found previously that enforced over-expression of tubulin α causes resistance, whereas its down-regulation reduces resistance in H460 cells to anti-mitotic drugs including vinblastine⁴². Thus, the observed decrease of tubulin α expression in CEM/VCR R and CEM/VLB100 cell lines may not be a cause of drug resistance. Similarly, knocking down the tubulin β expression using antisense oligonucleotide also increased the sensitivity of breast cancer cell lines to paclitaxel⁴³. A retrospective correlation study also showed that breast cancer patients with lower tubulin β expression had better prognosis than those with a higher expression level⁴³. These observations suggest that functional validations are required to verify the potential roles of proteins in drug resistance as revealed by proteomic approaches.

Proteomic analyses also revealed that the expression of γ -synuclein was up-regulated in mitoxantrone resistant HT-29RNOV and daunorubicin resistant HT-29RDB colon cancer cell lines⁴⁴. γ -synuclein is a centrosome-associated protein in interphase cells, and localized to the spindle poles during mitosis⁴⁵. It was later shown that over-expression of γ -synuclein causes drug resistance by modulating MAPK pathways⁴⁶, thus, validating the role of γ -synuclein in drug resistance predicted by proteomic profiling⁴⁴.

5. Metabolic enzymes

Many metabolic enzymes have been found to have differential levels between drug resistant and sensitive parental cancer cells (Table 3). One good example is pyruvate kinase M2 (PKM2),

which is involved in converting ADP to ATP. Proteomic profiling analyses showed that the expression of PKM2 was decreased in three different drug resistant cancer cell lines: SGC7901/VCR ³³, SNU-638/CP ⁴⁷, and IGROV-1/CP ⁵. Yoo et al. also found that the activity of pyruvate kinase was decreased in the cisplatin-resistant SNU-638 cell line ⁴⁷, confirming the decreased expression of PKM2. Analysis of an additional 11 different gastric cancer cell lines revealed that the PKM2 activity negatively correlates with cisplatin resistance. Furthermore, suppressing PKM2 expression by antisense oligonucleotide resulted in cisplatin resistance in the gastric SNU638 cell line ⁴⁷. Thus, it is likely that the decreased PKM2 expression contributes to cisplatin resistance. However, the mechanism as to how the decreased PKM2 expression causes cisplatin resistance is currently unknown. Its decreased expression may also contribute to vincristine resistance which needs to be verified functionally ³³.

It should also be noted that the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was increased in the Adriamycin-resistant MCF7/Adr cells ^{8,31}. In a metabolic marker-focused proteomic profiling analysis of 101 breast cancer tissues, Isidoro et al. ⁴⁸ also found that the expression of GAPDH was significantly increased in patients with a poor survival rate. However, it is currently unknown whether GAPDH expression contributes to Adriamycin resistance. These findings also suggest that caution should be taken for the use of GAPDH as a control in determining expression changes of other proteins in western blot analyses.

6. Protein synthesis and ribosomal proteins

As shown in Table 3, the expression of several ribosomal proteins was altered in various drug resistant cancer cell lines. One interesting protein is the 40S ribosomal protein SA, which was also named 37-kDa laminin receptor precursor (37LRP) and multidrug-resistance-associated protein MGr1-Ag. Proteomic profiling revealed that the expression of this protein increased in three different drug resistant breast cancer cell lines or xenografts (MaCa3366/Tam 49, MCF7/VP ⁵⁰ and MCF7/MX ⁵⁰). Previously, it has also been found that the MGr1-Ag expression is increased in a vincristine-resistant gastric cancer cell line, SGC7901/VCR; and down-regulating MGr1-Ag expression with antisense cDNA significantly reduced the resistance of SGC7901/VCR to vincristine, Adriamycin, and 5-FU ^{51,52}. Thus, the elevated expression of MGr1-Ag likely contributes to multidrug resistance, although the mechanism of resistance is not yet known.

7. Redox proteins

The cellular redox system is essential for living cells to monitor, control and maintain the intracellular redox balances ^{53,54}. The proteins involved in the cellular redox system have been frequently detected in drug resistant cells as expected due to their anti-stress functions. However, the detailed mechanisms on how these enzymes of redox system cause drug resistance are not yet clear. Their involvement in the removal of oxidant, which causes DNA damage and apoptosis and in DNA repair, may explain how their altered expressions render cancer cells resistant to DNA-damaging radiation and drugs.

Peroxiredoxins (Prx) are antioxidant enzymes that reduce H₂O₂ and other reactive oxygen species using thioredoxin as the immediate electron donor and their peroxidase activity to eliminate reactive oxygen species. Six Prx isoforms have been identified in the past. Except for Prx 2, most other Prx isoforms (1, 3, 4 and 6) are increased in various drug resistant cancer cell lines (see Table 3). These drug resistant cancer cells include vincristine-resistant stomach cancer cell line SGC7901/VCR ³³, Adriamycin-resistant breast cancer cell line MCF7/Adr and MCF7/AdVp3000 ^{8,17}, carboplatin-resistant ovarian cancer cell line SKOV3/CBP and cisplatin-resistant ovarian cancer cell lines A2780/CDDP ⁷, tamoxifen resistant breast cancer xenograft MaCa3366/TAM ⁴⁹, intrinsically vincristine-resistant leukemia xenograft ⁵⁵, and

radiation resistant breast cancer cell line MCF7+FIR3⁵⁶. It has recently been shown that ectopic over-expression of Prx 3 in WEHI7.2 thymoma cells causes resistance to H₂O₂ and the anticancer drug imexon⁵⁷ and that transgenic mice with over-expression of Prx 6 are resistant to hyperoxia-induced lung injury⁵⁸. These observations are consistent with the predicted role of Prx in resistance to damages.

It has been shown previously that the increased expression of Prx 2 causes resistance to DNA-damage-induced cell death^{59,60}. Silencing Prx 2 expression in the radiation-resistant MCF7 +FIR3 cells using siRNA partially reversed the resistance of these cells to ionizing radiation⁵⁶. It has also been shown that down-regulating Prx 2 expression causes sensitization to cisplatin-induced cell death⁶⁰. These observations suggest that the over-expression of Prx 2 may be involved in causing the resistance phenotype to radiation- and chemically-induced DNA damages and cell death. Likely, over-expression of other Prx isoforms in the drug resistant cancer cell lines (Table 3) contributes to resistance to various stresses induced by anticancer drugs. However, the expression of Prx 2 was decreased in Adriamycin-resistant breast cancer cell lines^{8,17} and vincristine-resistant leukemia xenograft⁵⁵. Thus, it is not clear if the down-regulated expression of Prx 2 as revealed by proteomic analyses contributes to resistance observed in these cases, or these observations are simply artifacts of proteomic approaches. Interestingly, Prx 2 expression was decreased in B-cell chronic lymphocytic leukemia patients with shorter survival compared with patients with longer survival³⁹, suggesting that the decreased Prx 2 expression may contribute to the aggressiveness and resistance to therapy in B-cell chronic lymphocytic leukemia. Clearly, further studies are needed to verify how Prx 2 expression affects responses of cancer cells to anticancer drugs.

8. Signal transduction

In the category of proteins involved in signal transduction, kinases, and phosphatases, many candidates were found to have altered expression in the drug resistant cancer cell lines by proteomic analyses. One of these proteins is the Ras-related nuclear protein (RAN), which was increased in expression in three different cell lines (deoxycholate resistant HCT-116 cells⁶¹, cisplatin resistant A431/Pt⁶², and Adriamycin resistant MCF7/Adr³¹). RAN has been shown to play important roles in the nuclear transport of proteins and RNA, cell cycle progression, and nuclear structure in mitotic regulation, as well as RNA and DNA synthesis⁶³. Recently, it was shown that RAN is important for the survival of activated K-ras-transformed cells⁶⁴. However, it is not yet known if RAN over-expression causes resistance to anticancer drugs. Nevertheless, the finding that RAN expression is increased in three different drug resistant cell lines suggests that its over-expression may cause drug resistance possibly by enhancing the survival of cells under drug attack⁶⁴.

9. Transmembrane proteins

Transmembrane proteins such as ABC transporters were rarely identified in the comparative proteomic studies despite the fact that many cancer cell lines listed in Table 2 over-express them. This is likely due to the hydrophobic nature, low abundance, and heterogeneity in glycosylation of the transmembrane proteins which interfere with solubilization and isoelectrofocusing during 2-DE separation. Recently, Rahbar and Fenselau⁶⁵ used SDS-PAGE to separate a plasma membrane protein mixture (at 1:1 ratio) isolated from MCF7 cells metabolically labeled with [¹³C]Lysine and [¹³C]Arginine and its mitoxantrone resistant subline, which was not labeled. Gel slices were then subjected to LC-MS analyses and the abundance ratios of proteins (unlabeled/[¹³C]labeled) were determined. With this approach, the authors were able to find transmembrane proteins that have differential levels between the two cell lines. The transmembrane proteins with >2 fold changes are shown in Table 3. One of the over-expressed proteins, ABCG2, is a known ABC transporter which causes multidrug

resistance by actively effluxing anticancer drugs^{66,67, 68}. Another interesting membrane protein, metabotropic glutamate receptor 4 (mGluR4), was increased in expression in 5-FU resistant human colon cancer cell line SNU-769A/5-FU⁶⁹. Subsequently, it was shown that the 5-FU resistance in SNU-769A/5-FU was significantly increased by mGluR4 agonist L-AP4 but decreased by antagonist MAP 4, validating the potential role of mGluR4 in 5-FU resistance⁶⁹.

Concluding remarks

Taken together, it is clear that comparative proteomic approaches are powerful tools to investigate drug resistant mechanisms in cancer cells. As shown in Table 2, any single cancer cell line selected for resistance to a particular anticancer drug likely has multiple mechanisms of resistance, in addition to their known resistance mechanisms such as over-expression of ABC transporters which are not discussed here. Thus, targeting any single mechanism to sensitize drug resistant cancers may not be effective. Combination therapies targeting multiple mechanisms to sensitize drug resistance may be necessary to help eradicate human cancers.

As shown in Tables 1 and 2, any specific mechanism of resistance may be selected in multiple lines of drug resistant cancer cells regardless of origin and drugs used. Analysis of the listed proteins in Table 3 showed that the most striking and commonly selected mechanisms of resistance are the up-regulated expressions of HSP27, 14-3-3 σ , and peroxiredoxins. Particularly, the role of HSP27 and 14-3-3 σ in drug resistance has been subsequently validated and their potential roles in causing poor prognosis have also been observed clinically. Thus, these proteins may represent a common target to sensitize drug resistant human cancers. However, the mechanism of action of these proteins in drug resistance has not yet been fully elucidated.

Despite successful comparative proteomic studies in the past, the practical value of this approach is to give a global overview of cellular processes, which might set the foundation for targeted confirmational studies using RNAi technology, western blot analysis, and immunocytochemistry. Furthermore, the proteomic approach has inherent technical problems. One of the main concerns is that most of the time only the abundant proteins were detected by the conventional proteomic approach. The second major concern is the limitations in separation and identification of hydrophobic membrane proteins and the proteins with extreme mass or pI. For example, ABC transporters that are known mechanisms of resistance in many cell lines listed in Table 2 were not found in these studies simply due to the limitations of these techniques mentioned above. New developments in technologies and methodologies of proteomic profiling will certainly help overcome these limitations. Fractionation of cell lysates may also help enrich proteins with low abundance. Furthermore, most of the proteins identified in the proteomic analyses of drug resistant cancer cells have not been validated for their expression changes using another approach such as western blot and for their role in drug resistance using functional assays such as MTT and colony formation. In future studies, the role of these proteins in drug resistance needs to be validated so that they may be used as targets for sensitizing drug resistance in cancer chemotherapy. Mechanism of resistance should also be investigated to help understand and design chemo-sensitizing agents.

Nevertheless, the use of the comparative proteomic approaches has contributed significantly to our understanding of various biological processes including the mechanism of MDR. Without the advancement of this approach, the newly discovered drug resistance mechanisms, such as the 14-3-3 σ -mediated drug resistance in the Adriamycin resistant MCF7/AdVp3000 cells, would not have been found. As discussed above, the use of comparative proteomic profiling also has helped us elucidate that any given drug resistant cancer cell lines may have

multiple mechanisms of resistance. This latter finding alone demonstrates the power and the strength of the comparative proteomic approach.

Perspectives

It is clear from the various proteomic profiles that multiple mechanisms of drug resistance exist in model cancer cell lines. This observation, if also true in clinics, imposes a serious challenge for successful cancer chemotherapy. Alteration in expression of any one of these genes could generate enough resistance to drugs and cause chemotherapy failure. Future proteomic studies of drug resistant cancer cells using advanced technologies and approaches such as CE-MS, LTQ-MS, and Protein-AQUA [for in-depth review of these technologies see ^{70,71}]; and more detailed collective analyses of the proteomic data will likely help identify predictive factors for prognosis and help design individualized therapies. Furthermore, these proteins may be used as targets for developing chemo-sensitizing therapeutics that can be used to enhance the chemo-sensitivity of cancers to currently available anticancer drugs in combination therapy.

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Table 1

Proteomic approaches used in the reviewed studies

Approaches	References
2-DE, MALDI-TOF	6,7,14,17,30,32,33,41,47,50,55,62,69,72,73
2-DE, ESI-MS/MS	33,49
2-DE, LC-MS/MS	12,56,74
ICAT-MS/MS	5
1-DE, metabolic labeling, LC-MS/MS	65
Q-TOF/Metabolic labeling	8,9,31
2-DE, unspecific MS	4,18,44
PowerBlot™ Western Array	61

Table 2

Drug-resistant cancer cell lines with altered processes and pathways

Cell Lines	Processes/Pathways									Ref
	Ca ⁺⁺ Binding	Cell Cycle	Chaperone	Cytoskeleton	Metabolism	Protein Synthesis	Redox	Signal Trans.	Transcription	
A2780/CDDP	✓			✓	✓		✓	✓	✓	7
A2780/CBP	✓		✓	✓	✓		✓			7
A431/Pt	✓		✓	✓	✓			✓		62
ALL17 xenograft				✓		✓				55
CEM/VCR R		✓	✓	✓			✓			41
CEM/VLB100			✓	✓						41
EPF86-079RNOV							✓			44
EPP85-181RDB				✓						18
EPP85-181RNOV		✓		✓	✓					18
HCT15/A204197	✓	✓		✓	✓			✓		4
HCT-116				✓	✓				✓	61
H460/T800	✓									4
H69AR	✓									10
HRT-18	✓		✓	✓	✓		✓			12
HT-29RNOV				✓	✓					44
HT-29RDB				✓	✓					44
IGROV1-R10	✓			✓	✓					6
IGROV-1/CP	✓	✓		✓	✓	✓	✓	✓	✓	5
MaCa3366/TAM	✓		✓	✓	✓	✓	✓	✓		49
MCF7/Adr	✓	✓	✓	✓	✓	✓	✓	✓		8,9,31
MCF7/AdrVp		✓	✓	✓		✓	✓			8,
MCF7/AdVp3000		✓	✓	✓	✓		✓			50
MCF7/Mel	✓	✓		✓				✓		17
MCF7/MX			✓	✓		✓		✓		72
MCF7/MXR				✓				✓	✓	50
MCF7/NP			✓	✓		✓		✓		65
MCF7+FIR3									✓	50
MeWo/Eto	✓	✓	✓		✓	✓	✓	✓		56
							✓			14,
MeWo/Fote	✓		✓		✓		✓	✓		30
										14,
MeWo/Vin	✓	✓	✓		✓	✓	✓	✓		30
										14,
MeWo/Cis	✓		✓		✓	✓	✓	✓		30
										14,
SGC7901/VCR	✓		✓	✓	✓	✓	✓	✓		33
SKOV3/CDDP	✓		✓	✓	✓	✓	✓	✓	✓	7
SKOV3/CBP	✓		✓	✓	✓	✓	✓	✓		7
SH-SY5Y	✓		✓	✓	✓		✓		✓	32
SNU-C4R					✓					73
SNU-638/CP					✓					47
SNU-769A/5-FU					✓					69

Cell Lines	Processes/Pathways									
	Ca ⁺⁺ Binding	Cell Cycle	Chaperone	Cytoskeleton	Metabolism	Protein Synthesis	Redox	Signal Trans.	Transcription	Ref

The changes in the level of proteins in the indicated categories in drug resistant cells are indicated by √. Note that some of these cell lines also have other known mechanisms of drug resistance such as over-expression of ABC transporters which are not listed here since they are not found using proteomic profiling due to the limitations of these methods. These cell lines include but are not limited to MCF7/Adr, MCF7/AdrVp, MCF7/AdVp3000^{66,75}, MCF7/NP⁷⁶, and MCF7/MX⁷⁷

Table 3
Summary of proteins identified to have altered expression in drug resistant cancer cell lines

Proteins ^a	Accession # ^c	Changes ^d	Drug	Cell Lines	References
Ca⁺⁺-binding and control proteins Annexin I	P04083	U	Adriamycin	MCF7/Adr	8
		U	Adriamycin	MCF7/Adr	9
		D	Carboplatin	SKOV3/CBP	7
	P07355 P12429	U	Adriamycin	H69AR	10
		U	Adriamycin	MCF7/Adr	9
		U	Cisplatin	SKOV3/CDDP	7
		U	Cisplatin	A2780/CDDP	7
	P09525	U	Carboplatin	SKOV3/CBP	7
		U	Carboplatin	A2780/CBP	7
		D	C225	HRT-18	12
Annexin IV	P09525	U	A204197	HCT115/A204197	4
		U	Paclitaxel	H460/T800	4
		D	Cisplatin	IGROV-1/CP	5
	P08758	D	Cisplatin	SKOV3/CDDP	7
		D	Cisplatin	IGROV1-R10	6
		U	Adriamycin	MCF7/Adr	8
		U	Adriamycin	MCF7/Adr	9
	P06703 P62158 P31949 P27797	U	Cisplatin	MCF7/Adr	9
		D	Adriamycin	A431/Pt	62
		U	Melphalan	MCF7/Mel	72
Caleyclin Calmodulin Calgizzarin Calreticulin Calumenin Sorcin Translationally controlled tumor protein 1	P08758	U	Cisplatin	A431/Pt	62
		D	Adriamycin	MCF7/Adr	9
		U	Adriamycin	MCF7/Adr	9
	P06703 P62158 P31949 P27797	U	Cisplatin	A431/Pt	62
		D	Adriamycin	MCF7/Mel	72
		U	Cisplatin	A431/Pt	62
		U	Vincristine	SGC7901/VCR	33
	P30626 P13693	U	Etoposide	MeWo/Eto	14
		U	Fotemustine	MeWo/Fote	14
		U	Cisplatin	MeWo/Cis	14
Cell cycle and checkpoint 14-3-3 τ 14-3-3 σ	P27348 P31947	U	Vindesin	MeWo/Vin	14
		D	Tamoxifen	MaCa 3366/TAM	49
		D	Vincristine	CEM/VCR R	41
	P61981	U	Adriamycin	MCF7/Advp3000	17
		U	Adriamycin	MCF7/Adr	8
		U	Adriamycin	MCF7/Adr	9
		U	Mitoxantrone	EPP85-181RNOV	18
	P25205	U	Etoposide	MeWo/Eto	14
		U	Vindesin	MeWo/Vin	14
		D	Deoxycholate	HCT-116	61
Chaperones Cyclin A (unknown as A1 or A2) ^b DNA replication licensing factor MCM3 hRad9 (unknown as A or B) ^b Mitotic checkpoint protein BUB3 BCL2-associated athanogene 5 Cyclophilin A	P61981	U	Cisplatin	IGROV-1/CP	5
		U	Deoxycholate	HCT-116	61
		U	Adriamycin	MCF7/Advp	50
	O43684	D	Vincristine	SGC7901/VCR	33
		D	Vincristine	SGC7901/VCR	33
		D	Tamoxifen	MaCa 3366/TAM	49
		D	Tamoxifen	MaCa 3366/TAM	49
	Q9UL15 P62937	D	Vincristine	SGC7901/VCR	33
		D	Vincristine	SGC7901/VCR	33
		D	Tamoxifen	MaCa 3366/TAM	49

Proteins ^d	Accession # ^e	Changes ^d	Drug	Cell Lines	References
Cyclophilin B	P23284	D	Adriamycin	MCF7/Adr	9
		U	Adriamycin	MCF7/Adr	31
		U	Adriamycin	MCF7/AdrVp	50
		U	VP-16	MCF7/VP	50
DJ-1 protein FK506 binding protein 1A HSC20 HSP27	Q99497 P62942 Q8IWL3 P04792	U	Mitoxantrone	MCF7/MX	50
		D	Tamoxifen	MaCa 3366/TAM	49
		U	Adriamycin	MCF7/Adr	31
		D	Carboplatin	A2780/CBP	7
		U (1.6 fold)	Adriamycin	MCF7/AdVp3000	17
		U	Vincristine	SGC7901/VCR	33
		U	Etoposide	SH-SY5Y	32
		U	Adriamycin	MCF7/Adr	31
		U	Etoposide	MeWo/Eto	30
		U	Fotemustine	MeWo/Fote	30
HSP27 phosphorylated HSP cognate protein	P11142	U	Vindesin	MeWo/Vin	30
		U	Cisplatin	MeWo/Cis	30
		U	Cisplatin	SKOV3/CDDP	7
		U	C225	HRT-18	12
		D	Carboplatin	SKOV3/CBP	7
		D	Tamoxifen	MaCa 3366/TAM	49
		D	Melphalan	MCF7/Mel	72
		U	Cisplatin	A431/Pt	62
		U	Vinblastine	CEM/VLB100	41
		D	Vincristine	SGC7901/VCR	33
HSP60	P10809	U	Etoposide	MeWo/Eto	30
		U	Fotemustine	MeWo/Fote	30
		U	Vindesin	MeWo/Vin	30
		U	Cisplatin	MeWo/Cis	30
HSP70 variant	Q53FC7	U	Etoposide	MeWo/Eto	30
		U	Fotemustine	MeWo/Fote	30
		U	Vindesin	MeWo/Vin	30
		U	Cisplatin	MeWo/Cis	30
HSP70.1	P08107	U	Cisplatin	SKOV3/CDDP	7
		U	Carboplatin	SKOV3/CBP	7
HSP90 α	P07900	D	Vincristine	CEM/VCR R	41
		D	Fotemustine	MeWo/Fote	30
HSP90	P08238	D	Cisplatin	MeWo/Cis	30
		D	Etoposide	MeWo/Eto	30
Nucleophosmin	P06748	D	Fotemustine	MeWo/Fote	30
		D	Vindesin	MeWo/Vin	30
Protein disulfide isomerase precursor Protein disulfide isomerase A3 T-complex protein 1, β T-complex protein 1, ϵ T-complex protein 1, γ Telomerase-binding protein Tetratricopeptide protein	P07237 P30101 P78371 P48643 P49368 Q15185 Q99614	D	Adriamycin	MCF7/AdVp3000	17
		U	Tamoxifen	MaCa 3366/TAM	49
		U	Cisplatin	A431/Pt	62
		D	Vincristine	SGC7901/VCR	33
		U	Cisplatin	A2780/CDDP	7
		U	Adriamycin	MCF7/Adr	31
		U	Etoposide	MeWo/Eto	14
		U	Cisplatin	MeWo/Cis	14
		U	Vindesin	MeWo/Vin	14

Proteins ^d	Accession # ^c	Changes ^d	Drug	Cell Lines	References
Cytoskeleton and associated proteins					
Actin, β	P60709	U	Tamoxifen	MaCa 3366/TAM	49
Actin, γ	P63261	D	Vinblastine	CEM/VLB100	41
Actin-regulatory protein CAP-G	P40121	D	Vincristine	ALL17 xenograft	55
Calponin	Q15417	U	Cisplatin	A431/Pt	62
Cofilin	P23528	D	Vincristine	SGC7901/VCR	33
		D	Cisplatin	SKOV3/CDDP	7
		D	Carboplatin	SKOV3/CBP	7
		U	Mitoxantrone	EPP85-181RNOV	18
		U	Daunorubicin	EPP85-181RDB	18
		U	Cisplatin	A2780/CDDP	7
		U	Carboplatin	A2780/CBP	7
		U	Cisplatin	SKOV3/CDDP	7
		U	Carboplatin	SKOV3/CBP	7
		U	Cisplatin	A2780/CDDP	7
		U	Carboplatin	A2780/CBP	7
Destrin	P60981	U	Tamoxifen	MaCa 3366/TAM	49
		U	Vincristine	ALL17 xenograft	55
Filamin A	P21333	U	Deoxycholate	HCT-116	61
Gelsolin	P06396	U	Deoxycholate	HCT-116	61
Kinesin F1A	Q12756	D	Adriamycin	MCF7/AdrVp	50
Keratin 8	P05787	D	VP-16	MCF7/VP	50
		D	Mitoxantrone	MCF7/MX	50
		U	Cisplatin	IGROV1-R10	6
Keratin 18	P05783	U	Cisplatin	IGROV1-R10	6
Keratin 19	P08727	D	VP-16	MCF7/VP	50
		D	Adriamycin	MCF7/AdrVp	50
		U	Adriamycin	MCF7/AdrVp3000	17
L1	P32004	D	Deoxycholate	HCT-116	61
Lamin B1	P20700	U	Vinblastine	CEM/VLB100	41
Mena	Q8N8S7	D	Deoxycholate	HCT-116	61
Microtubule associated protein RP/EB1	Q15691	D	Cisplatin	A431/Pt	62
		D	Deoxycholate	HCT-116	61
		D	C225	HRT-18	12
Myosin light chain alkali isoform 3	P06741	U	Cisplatin	IGROV-1/CP	5
Myosin regulatory light chain 2	P19105	D	Vincristine	CEM/VCR R	41
		U	Vinblastine	CEM/VLB100	41
Nestin	P48681	D	Deoxycholate	HCT-116	61
Profilin I	P07737	U	C225	HRT-18	12
Septin 2	Q15019	D	Adriamycin	MCF7/AdrVp	50
		D	VP-16	MCF7/VP	50
		D	Mitoxantrone	MCF7/MX	50
Septin 7	Q16181	D	Adriamycin	MCF7/AdrVp	50
SPFH domain family member 2 precursor	O94905	U	Tamoxifen	MaCa 3366/TAM	49
Stathmin	P16949	D	Cisplatin	A431/Pt	62
		D	Adriamycin	MCF7/Adr	8
		D	Carboplatin	A2780/CBP	7

Proteins ^d	Accession # ^c	Changes ^d	Drug	Cell Lines	References
Stomatin-like protein 2	Q9UJZ1	U	Adriamycin	MCF7/Adr	31
γ -synuclein	O76070	U	Carboplatin	SKOV3/CBP	7
Thymosin- β -10	P63313	D	Cisplatin	SKOV3/CDDP	7
Tropomodulin-3	Q9NYL9	U	Carboplatin	SKOV3/CBP	7
Tropomyosin α	P09493	U	Mitoxantrone	HT-29RNOV	44
Tubulin, β (class I)	P07437	U	Danunorubicin	HT-29RDB	44
Tubulin, β (class II)	P68371	U	Adriamycin	MCF7/Adr	31
Tubulin, α	P68366	D	Vincristine	CEM/VCR R	41
Villin 2	P15311	D	Vinblastine	CEM/VLB100	41
Vinculin	P18206	D	Vincristine	CEM/VCR R	41
Vimentin	P08670	D	Vinblastine	CEM/VLB100	41
Metabolism					
Adenine phosphoribosyl transferase	P07741	D	Vincristine	CEM/VCR R	41
Aldehyde dehydrogenase 1	P00352	U	Mitoxantrone	HT-29RNOV	44
Aldehyde reductase	AA892369	U	Danunorubicin	HT-29RDB	44
Aldose reductase	Q51031	U	Carboplatin	A2780/CBP	7
Aldose reductase-related protein 2	O08782	U	Cisplatin	IGROV1-R10	6
Argininosuccinate synthetase	Q16217	U	Cisplatin	A2780/CDDP	7
ATP synthase α	P25705	U	Cisplatin	A2780/CDDP	7
ATP synthase β	P06576	U	H ₂ O ₂	OC14	74
ATP synthase D	O75947	U	Deoxycholate	HCT-116	61
dUTP pyrophosphatase	NP_001939	D	5-FU	SNU-C4R	73
Catechol- <i>O</i> -methyltransferase	P21964	D	Adriamycin	MCF7/Advp3000	17
ECH1 protein	Q13011	U	Cisplatin	SKOV3/CDDP	7
Fatty acid-binding protein (epidermal)	Q01469	D	Etoposide	SH-SY5Y	32
Fructose- biphosphate dehydrogenase ^b		D	Cisplatin	A2780/CDDP	7
Glucose-6-phosphate dehydrogenase	P11413	D	Carboplatin	A2780/CBP	7
		D	Carboplatin	SGC7901/VCR	9
		D	Carboplatin	A2780/CBP	33
		U	Carboplatin	A2780/CBP	7
		U	Mitoxantrone	SKOV3/CBP	7
		U	C225	EP85-181RNOV	18
		D	Carboplatin	HRT-18	12
		D	Adriamycin	SKOV3/CBP	7
		D	Adriamycin	MCF7/Adr	8
		D	Adriamycin	MCF7/Adr	8
		D	Adriamycin	MCF7/Adr	9

Proteins ^d	Accession # ^e	Changes ^d	Drug	Cell Lines	References
Glyceraldehyde 3-phosphate dehydrogenase	P04406	U (1.5 fold)	Adriamycin	MCF7/Adr	8
GMP synthase	P49915	U	Adriamycin	MCF7/Adr	31
3-hydroxyacyl-CoA dehydrogenase 2	Q99714	D	Tamoxifen	MaCa 3366/TAM	49
Inorganic pyrophosphatase	Q15181	U	Vincristine	SGC7901/VCR	33
		D (1.9 fold)	Adriamycin	MCF7/Advp3000	17
		D	Tamoxifen	MaCa 3366/TAM	49
		U	Vincristine	SGC7901/VCR	33
	P50213	U	C225	HRT-18	12
		D	Vincristine	SGC7901/VCR	33
Isocitrate dehydrogenase, mitochondria		D	Cisplatin	SKOV3/CDDP	7
Isocitrate dehydrogenase, cytoplasm	O75874	D	Carboplatin	SKOV3/CBP	7
		D	Cisplatin	A2780/CDDP	7
		D	Carboplatin	A2780/CBP	7
		D	Carboplatin	SKOV3/CBP	7
		D	Cisplatin	A2780/CDDP	7
		D	Carboplatin	A2780/CBP	7
L-lactate dehydrogenase B chain	P07195	U	Adriamycin	MCF7/Adr	9
		U	Cisplatin	A2780/CDDP	7
Lactamase, β 2	Q9Y392	D	Carboplatin	SKOV3/CBP	7
Lanosterol synthase	P48449	D	Cisplatin	IGROV-1/CP	5
5-lipoxygenase	P09917	U	Deoxycholate	HCT-116	61
Lysophospholipase 1	O75608	U	Tamoxifen	MaCa 3366/TAM	49
Microsomal epoxide hydrolase X	P07099	U	Deoxycholate	HCT-116	61
Mitochondrial ATPase inhibitor	Q9UII2	U	Cisplatin	A431/Pt	62
NADP-dependent isocitrate dehydrogenase	O75874	U	Vincristine	SGC7901/VCR	33
Nicotinamide N-methyltransferase	P40261	U	Etoposide	MeWo/Eto	30
		U	Vindesin	MeWo/Vin	30
		U	Adriamycin	MCF7/Adr	9
	Q15274	D	C225	HRT-18	12
Nicotinate-nucleotide pyrophosphorylase	Q29459	D	Vincristine	SGC7901/VCR	33
Platelet-activating factor acetylhydrolase 1b	P09874	D	VP-16	MCF7/VP	50
PARP-1		D	Mitoxantrone	MCF7/MX	50
	P00558	U	Cisplatin	A431/Pt	62
Phosphoglycerate kinase 1	P18669	D	Etoposide	MeWo/Eto	30
Phosphoglycerate mutase 1		D	Fotemustine	MeWo/Fote	30
		D	Vindesin	MeWo/Vin	30
		D	Cisplatin	MeWo/Cis	30
Phosphoserine aminotransferase	Q9Y617	U	Vincristine	SGC7901/VCR	33
		U	C225	HRT-18	12
		U	Carboplatin	SKOV3/CBP	7
Purine nucleoside phosphorylase	P00491	U	Cisplatin	A2780/CDDP	7
Pyruvate kinase, M2	P14618	D (1.9 fold)	Cisplatin	IGROV-1/CP	5
		D	Vincristine	SGC7901/VCR	33
		D	Cisplatin	SNU-638/CP	47
Triosephosphate isomerase	P60174	D	Cisplatin	A2780/CDDP	7

Proteins ^d	Accession # ^e	Changes ^d	Drug	Cell Lines	References
Ubiquinol-cytochrome c reductase	P47985	U	Tamoxifen	MaCa 3366/TAM	49
UMP-CMP kinase	P30085	U	Carboplatin	A2780/CBP	7
Uracil DNA glycosylase	P13051	D	Vincristine	SGC7901/VCR	33
Valyl-tRNA synthetase 2	P26640	D	Cisplatin	IGROV-1/CP	5
Protein synthesis, processing, modification, and degradation					
Coatomer α	P53621	D	Cisplatin	IGROV-1/CP	5
ERp29	P30040	D	Vincristine	ALL17 xenograft	55
40S ribosomal protein SA	P08865	D	Vincristine	SGC7901/VCR	33
40S ribosomal protein S4	P62701	U	Tamoxifen	MaCa 3366/TAM	49
40S ribosomal protein S28	P62857	U	VP-16	MCF7/VP	50
60S acidic ribosomal protein P0	P05388	U	Mitoxantrone	MCF7/MX	50
Proteasome subunit, α type, 1	Q53YE8	D	Adriamycin	MCF7/Adr	31
Proteasome subunit, α type, 7	O14818	U	Adriamycin	MCF7/Adr	31
Proteasome subunit, β type	AAB31085	D	Tamoxifen	MaCa 3366/TAM	49
Signal recognition particle 9 kDa protein	P49458	D	Vincristine	SGC7901/VCR	33
Translation elongation factor 1 β	P24534	U	Cisplatin	SKOV3/CDDP	7
Translation elongation factor 1 δ	P29692	U	C225	HRT-18	12
Ubiquitin	P62988	U	Carboplatin	SKOV3/CBP	7
Ubiquitin carboxyl hydrolase-L1	P09936	U	Cisplatin	IGROV-1/CP	5
Ubiquitin-conjugating enzyme H6	P51965	D	Adriamycin	MCF7/AdrVp	50
Ubiquitin-conjugating enzyme H7	P68036	D	Mitoxantrone	MCF7/MX	50
Ubiquitin-like protein NEDD8	Q15843	U	Etoposide	MeWo/Eto	14
Redox					
Glutathione S-transferase μ 3	P21266	U	Fotemustine	MeWo/Fote	14
Glutathione S-transferase π	P09211	U	Cisplatin	MeWo/Cis	14
Glutathione S-transferase ω 1	P78417	D	Vindesin	MeWo/Vin	14
Glyoxalase	Q04760	U	Adriamycin	MCF7/Adr	31
Peroxioredoxin 1	Q06830	U	Adriamycin	MCF7/Adr	8
		U	C225	MCF7/Adr	9
		D	HRT-18	MCF7/Adr	12
		U	Cisplatin	SKOV3/CDDP	7
		U	Cisplatin	A2780/CDDP	7
		U	Carboplatin	A2780/CBP	7
		D	Vincristine	SGC7901/VCR	33
		U	Tamoxifen	MaCa 3366/TAM	49
		U	Vincristine	SGC7901/VCR	33
		U	Adriamycin	MCF7/AdrVp	8
		U	Tamoxifen	MaCa 3366/TAM	49
		U	Etoposide	SH-SY5Y	32
		U	Etoposide	MeWo/Eto	30

Proteins ^d	Accession # ^e	Changes ^d	Drug	Cell Lines	References
Peroxioredoxin 2	P32119	U	Fotemustine Vindesin Cisplatin Carboplatin Adriamycin Adriamycin Vincristine Radiation	MeWo/Fote MeWo/Vin MeWo/Cis SKOV3/CBP MCF7/Advp3000 MCF7/Adr MCF7/Adr ALL17 xenograft MCF7/FIR3	30 30 30 7 17 8 9 55 56
Peroxioredoxin 3	P30048	U	Vincristine Etoposide Fotemustine Vindesin Cisplatin Tamoxifen Adriamycin Cisplatin Adriamycin Adriamycin Adriamycin	SGC7901/VCR MeWo/Eto MeWo/Fote MeWo/Vin MeWo/Cis MaCa 3366/TAM MCF7/Advp3000 A2780/CDDP MCF7/Adr MCF7/Adr MCF7/Adr	33 30 30 30 30 49 17 7 31 8 31
Peroxioredoxin 4	Q13162	U	Deoxycholate	HCT-116	61
Peroxioredoxin 6	P30041	U	Deoxycholate	HCT-116	61
Superoxide dismutase	P00441	U	Deoxycholate	HCT-116	61
Thioredoxin	P10599	U	Deoxycholate	HCT-116	61
Signal transduction, kinases, phosphatases, proteases, and modifications of					
Acetylcholine receptor α	P02708	U	Deoxycholate	HCT-116	61
Bid	P55957	D	Deoxycholate	HCT-116	61
CaM kinase II (unknown as α , β , δ)		D	Deoxycholate	HCT-116	61
Cathepsin D	ILYWB	U	Adriamycin	MCF7/Advp3000	17
Guanine nucleotide-binding protein, α -13	Q14344	U	Deoxycholate	HCT-116	61
Guanine nucleotide-binding protein G, α	P63092	D	Mitoxantrone	MCF7/MXR	65
G protein β subunit	P62879	U	Mitoxantrone	MCF7/MXR	65
β galactoside soluble lectin	P09382	D	Vincristine	SGC7901/VCR	33
Heat stable protein phosphatase 2A inhibitor	P39687	U	Etoposide	MeWo/Eto	30
Interleukin-18 precursor	Q14116	U	Fotemustine	MeWo/Fote	30
I κ B kinase γ	Q9Y6K9	U	Vindesin	MeWo/Vin	30
MAP kinase kinase 2 (MEK2)	P36507	U	Cisplatin	MeWo/Cis	30
MAP kinase kinase kinase 6	O95382	U	Etoposide	SH-SY5Y	32
P36/MAT1	P51948	D	Cisplatin	A431/Pt	62
Phosphatidylethanolamine binding protein	P30086	D	Adriamycin	MCF7/Adr	9
Prenylcysteine lyase	Q9UHG3	U	Deoxycholate	HCT-116	61
Prohibitin	P35232	U	Mitoxantrone	HCT-116	61
		U	Cisplatin	IGROV-1/CP	5
		D	Deoxycholate	HCT-116	61
		D	Adriamycin	MCF7/Adr	9
		U	Deoxycholate	HCT-116	61
		U	Mitoxantrone	MCF7/MX	50
		U	Cisplatin	SKOV3/CDDP	7

Proteins ^a	Accession # ^c	Changes ^d	Drug	Cell Lines	References
Protein kinase C inhibitor protein-1	P31946	D	Cisplatin	A2780/CDDP	7
Protein kinase R	P19525	D	C225	HRT-18	12
PTPID/SHP2	Q06124	U	Deoxycholate	HCT-116	61
Ras-related nuclear protein (RAN)	P62826	U	Deoxycholate	HCT-116	61
		U	Deoxycholate	HCT-116	61
		U	Cisplatin	A431/Pt	62
		U	Adriamycin	MCF7/Adr	8
Rho GDP dissociation inhibitor 1	P52565	D	Tamoxifen	MaCa 3366/TAM	49
Rho GDP dissociation inhibitor 2	P52566	U	Mitoxantrone	EPF86-079RNOV	44
Transcription, RNA processing, and stability					
Activated RNA Pol II cofactor 4	P53999	U	Cisplatin	SKOV3/CDDP	7
Four and a half LIM domains 2	Q6P792	D	Cisplatin	IGROV-1/CP	5
isoform 1					
Heterogeneous nuclear ribonucleoprotein K	P61978	U	Etoposide	SH-SY5Y	32
HMG-1	P09429	U	VP-16	MCF7/VP	50
		U	Mitoxantrone	MCF7/MX	50
		U	Deoxycholate	HCT-116	61
Late SV40 transcription factor ^b					
NHP2-like protein 1	P55769	U	Cisplatin	IGROV-1/CP	5
Nucleolin	P19338	U	VP-16	MCF7/VP	50
		U	Mitoxantrone	MCF7/MX	50
Splicing factor, Arg/Ser-rich 3	P84103	U	Cisplatin	A2780/CDDP	7
TAFII 135	O00268	D	Deoxycholate	HCT-116	61
TAT-SF1	Q99730	D	Deoxycholate	HCT-116	61
Transmembrane Proteins					
ABCG2	Q9UNQ0	U	Mitoxantrone	MCF7/MXR	65
Clathrin heavy chain 1	Q00610	U	Mitoxantrone	MCF7/MXR	65
Nuclear chloride ion channel	O00299	D	Vincristine	ALL17 Xenograft	55
		D	C225	HRT-18	12
Dihydropyridine receptor $\alpha 2$	Q9UTU0	U	Mitoxantrone	MCF7/MXR	65
Ephrin type-B receptor 4	P54760	U	Mitoxantrone	MCF7/MXR	65
Erythrocyte band 7	P27105	U	Mitoxantrone	MCF7/MXR	65
4F2 light chain	Q01650	U	Mitoxantrone	MCF7/MXR	65
4F2 heavy chain	P08195	U	Mitoxantrone	MCF7/MXR	65
Facilitated glucose transporter, member 1	P11166	D	Deoxycholate	HCT-116	61
Integrin α -2	P17301	D	Mitoxantrone	MCF7/MXR	65
Integrin α -3	P26006	D	Mitoxantrone	MCF7/MXR	65
Liver X receptor (unknown as α or β) ^b		U	Deoxycholate	HCT-116	61
Metabotropic glutamate receptor 4	Q14833	U	5-FU	SNU-769A/5-FU	69
TM9sf protein member 3 precursor	Q9HD45	D	Cisplatin	IGROV-1/CP	5
Transferrin receptor protein 1	P02786	U	Mitoxantrone	MCF7/MXR	65
Tumor associated Ca^{++} signal transducer 2	Q7Z7Q4	D	Mitoxantrone	MCF7/MXR	65
Voltage dependent anion selective channel 1	P21796	U	Tamoxifen	MaCa 3366/TAM	49
Voltage dependent anion selective channel 2	P45880	U	Vincristine	SGC7901/VCR	33
Others					

Proteins ^a	Accession # ^c	Changes ^d	Drug	Cell Lines	References
16 kDa protein ^b		U	Cisplatin	IGROV-1/CP	5
78-kDa glucose regulated protein	P11021	U	Vinblastine	CEM/VLB100	41
		U	Mitoxantrone	MCF7/MX	50
ALG-2-interacting protein 1	Q8WUM4	M	Tamoxifen	MaCa 3366/TAM	49
Galectin-3	P17913	D	C225	HRT-18	12
Hepatocellular carcinoma autoantigen	Q9Y6M1	D	Cisplatin	IGROV-1/CP	5
Macrophage migration inhibitory factor	P14174	U	Melphalan	MCF7/Mel	72
Metallothionein-1X	P80297	U	Adriamycin	MCF7/Adr	31
Prothymosin α	P06454	U	Adriamycin	MCF7/Adr	31
Retinoic acid binding protein II	P29373	U	Melphalan	MCF7/Mel	72
Testin splice isoform 1	Q9UGI8	U	Cisplatin	IGROV-1/CP	5
Unnamed protein product	BAA91719	D	Carboplatin	SKOV3/CBP	7
Hypothetical protein FLJ34068	Q8NB89	U	Cisplatin	IGROV-1/CP	5
		D	Mitoxantrone	MCF7/MXR	65
Hypothetical protein DKFZp686D0452		U	Etoposide	MeWo/Eto	30
		U	Fotemustine	MeWo/Fote	30
Hypothetical protein DKFZp566J2046		U	Vindesin	MeWo/Vin	30
		U	Cisplatin	MeWo/Cis	30

^a Only proteins with ≥ 2 fold changes (except the ones with indicated changes) are collected in this table. For the study by Stewart et al. ⁵, only proteins with consistent changes at the mRNA level were collected here. For the study by Bernstein et al. ⁶¹, only the proteins which have consistent changes in all three cell lines were chosen.

^b The accession numbers of these proteins were not given in the original publication and they cannot be defined in the protein database due to existence of more than one isoform or simply cannot be found in the database.

^c Websites to access the data of the listed proteins: <http://ca.expasy.org/sprot/> and <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=search&DB=protein>

^d D and U represent down- and up-regulated in the drug resistant cells compared with sensitive parental cells. M represents posttranslational modification of the protein in drug resistant cells.