Abstract. Background: Selection of the human drug sensitive and invasive cell line (MDA-MB-435S-F) with the chemotherapeutic agent paclitaxel, resulted in the development of drug resistant cell lines displaying enhanced invasion-related characteristics. Materials and Methods: Serum-free conditioned media from the human cancer drug-sensitive and invasive cell line (MDA-MB-435S-F) and its paclitaxel-resistant superinvasive variant (MDA-MB-435S-F/Taxol10p4pSI) were analyzed using Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS). Results: A differentially expressed protein was observed at 7.6 kDa, which was 4-fold up-regulated in MDA-MB-435S-F/Taxol10p4pSI. The differentially expressed protein was identified using matrix-assisted laser desorption ionization tandem time-of-flight mass spectrometry (MALDI-TOF/TOF MS), as a fragment of bovine transferrin. The transferrin receptor was also found to be overexpressed in the superinvasive cell line. Conclusion: Cleavage of serum proteins such as transferrin could provide a valuable source of markers for malignant tumours and could also play a role in aspects of cancer pathogenesis, such as tumour cachexia.

The development of invasive and metastatic behaviour during cancer progression requires the concerted action of multiple genes and cellular functions, including cytoskeleton remodeling, acquisition of migratory phenotypes, ability to invade the tumour surrounding tissues, and survival of disseminated tumour cells. There is evidence in the literature that invasion and metastasis may sometimes be related to the multidrug resistance (MDR) phenotype (1). In some cases, invasive/metastatic cells develop drug resistance more readily than non-invasive/metastatic cells (2, 3). Some tumour cell lines selected for resistance to chemotherapeutic drugs are more invasive/metastatic relative to non-resistant parental cells (4-8). However the possible relationship between drug resistance and invasion/metastasis and how prevalent this phenotype is remains poorly understood, especially from a proteomics perspective.

Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) is a relatively new proteomics technology for the analysis of serum, plasma, intestinal fluid, urine, cell lysates and cellular secretions focusing on the discovery and identification of potential biomarkers for various diseases. SELDI-TOF MS utilizes the different chromatographic properties of ProteinChip® arrays from Ciphergen to bind different subsets of peptides and proteins for subsequent analysis (9). Recent reports employing SELDI-TOF MS-based approach have demonstrated that protein profiles are useful for the early detection of breast, prostate and colon cancers (10-12). Distinct protein profiles of many carcinoma cell lines and associated variants are now established using this technology (13, 14). More recently, SELDI-TOF MS-based serum profiling has been subject to criticism, with results published by some groups not being reproduced by others (15-18). However, the same level of criticism may not apply to cell culture based SELDI-TOF MS analysis since multiple replicate samples from the same cell line are readily obtainable.

Paclitaxel (MDA-MB-435S-F/Taxol10p4pSI)-selected variants of an in vitro invasive clonal population of the human breast cancer cell line, MDA-MB-435S-F, were established by pulse selection, and exhibited a novel ‘superinvasive’ phenotype (19). This phenotype is characterized by an ability to relocate to another surface following invasion through matrigel and membrane pores. Decreased adhesion to extracellular matrix proteins and increased motility are also typical of this phenotype. This
may represent an in vitro model of a step in the metastatic process occurring subsequent to invasion.

The secretome of cells and tissues may reflect a broad variety of pathological conditions and thus represents a rich source of information. In order to investigate changes in the secretome that might be associated with mechanisms involved in drug resistance and invasion/metastasis, or for establishing biomarkers associated with this phenotype, conditioned media from the parental cell line, MDA-MB-435S-F and the drug-selected superinvasive population, MDA-MB-435S-F/Taxol-10p4pSI were subject to biochemical and proteomic analysis.

Materials and Methods

Chemicals. All media and serum used in the maintenance of the cell lines were obtained from Sigma Aldrich (Dublin, Ireland). Chemicals were obtained from Sigma (Dublin, Ireland) and IMAC30 ProteinChip Arrays, IMAC Hypercel spin columns and sinapinic acid from (Ciphergen Biosystems, CA, USA).

Cell lines. The human breast cancer cell line MDA-MB-435S was obtained from the American Type Culture Collection (ATCC) and was maintained in culture at 37°C using RPMI 1640 media supplemented with 2 mM L-glutamine (Gibco BRL, Grand Island, NY, USA) and 10% foetal calf serum.

MDA-MB-435S has a spindle-shaped morphology which evolved from the parental line MDA-MB-435 isolated by Cailleau et al. (20), and was originally derived from the pleural effusion of a 31-year-old female with metastatic, ductal adenocarcinoma of the breast. Recently, the tissue origin of MDA-MB-435 cell line has been the subject of considerable debate. In 2002, results were published suggesting that MDA-MB-435 cells was of melanoma origin (21). It was recently reported that the MDA-MB-435 cells used widely have identical origins to those that exhibit a melanoma-like gene expression signature, but exhibit a small degree of genotypic and phenotypic drift (22), but the possibility that some breast tumours may have gene expression profiles similar to melanoma cannot be ruled out.

Cell culture. MDA-MB-435S-F was obtained by clonal dilution from MDA-MB-435S in this laboratory. The MDA-MB-435S-F paclitaxel-resistant variant was derived by pulse selection of the parental cells with the IC_{90} value of doxorubicin or paclitaxel. Cells were exposed to the IC_{90} value of 15 ng/ml paclitaxel for 4 h, once a week for 10 weeks (MDA-MB-435S-F/Taxol-10p). These cells were then exposed to 125 ng/ml paclitaxel for 4 h, once a week for 4 weeks (MDA-MB-435S-F/Taxol-10p4p). Antibiotics were not used in the growth media. All cell lines were free from mycoplasma.

Invasion assays. Invasion assays were performed by a modification of the method described by Albini et al. (23). Matrigel was diluted to 1 mg/ml in serum-free RPMI medium then 100 μl of 1 mg/ml Matrigel (Sigma, Dublin, Ireland) was placed into each insert (Falcon, Franklin Lakes, NJ, USA) (8.0 μm pore size), which stood in wells of a 24-well plate (Bio-Rad Labs., Hemel Hempstead, Herts., UK). The inserts and the plate were incubated overnight at 4°C. The following day, cells were harvested and suspended in RPMI-1640 containing 10% FCS at a concentration of 1x10^6 cells/ml. The inserts were washed with serum-free RPMI-1640 then 100 μl of the cell suspension was added to each insert and 250 μl of RPMI-1640 containing 10% FCS was added to the insert well. Cells were incubated at 37°C for 48 h. After this time period, the inner side of the insert was wiped with a wet swab to remove the cells, while the outer side of the insert was gently rinsed with PBS and stained with 0.25% crystal violet for 10 min, rinsed again and then allowed to dry. The surface of the 24-well plate was rinsed gently twice with PBS and stained with 0.25% crystal violet for 10 min, rinsed again and then allowed to dry. Cells were quantified by counting with the aid of a light microscope.

Isolation of MDA-MB-435S-F/Taxol-10p4pSI cell populations. The superinvasive populations, (SI’s) were isolated by allowing cells to grow which had invaded, detached from the bottom of the insert and re-attached to the bottom of the 24-well plate, until they reached suitable confluency levels for further subculturing.

Preparation of serum-free conditioned media. Cells were grown to 60-70% confluency in RPMI-1640 media supplemented with 2 mM L-glutamine (Gibco BRL, Grand Island, NY, USA) and 10% foetal calf serum and then washed three times in serum-free medium. Cells were subsequently incubated in serum-free medium for 1 h, washed two more times in serum-free medium, then incubated in serum free media for 72 h. Conditioned medium was collected, centrifuged at 1000 rpm for 15 min to remove cellular debris, decanted into clean tubes and stored at –80°C.

Surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS). Conditioned medium was concentrated using 3000 MW cut-off centricon (Millipore, Billerica, MA, USA). Samples were then diluted with 250 mM sodium chloride. An IMAC30 ProteinChip Array, 8-spot (Ciphergen Biosystems, CA, USA) was selected for this study. IMAC30 chips were coated with 5 μL of 100 mM CuSO4 for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland). The copper ions were charged by applying 50 μl of 100 mM sodium acetate for a total of 30 min (2x15 min applications) and then rinsed with high-performance liquid chromatography grade water (Sigma, Dublin, Ireland).
the spots of the array. The array was placed on a shaker and gently agitated for 90 min to allow for interaction with the array surface. After removing the sample, the array was washed twice with 300 μl of 250 mM sodium chloride for 5 min, followed by a brief high performance liquid chromatography grade water wash. After removing the array from the bioprocessor, a 0.8 μl aliquot of saturated sinapinic acid (SPA; dissolved in 50% acetonitrile containing 0.5% trifluoroacetic acid) was added to the spots.

Figure 2. (A) Chromatographic view of the conditioned media from the human cancer drug-sensitive and invasive phenotype MDA-MB-435S-F and its paclitaxel-resistant superinvasive variant MDA-MB-435S-F/Taxol10p4pSI run in triplicate on IMAC30-Cu2+ ProteinChip Arrays. The arrow indicates the position of a differentially expressed species at approximately 7.6 kDa. (B) 1D-Gel view of the corresponding chromatographs. The 7.6 kDa species was found to be fourfold up-regulated in MDA-MB-435S-F/Taxol10p4pSI cells.
allowed to dry and the application repeated. The IMAC30-Cu²⁺ ProteinChip Arrays were then analyzed in a Ciphergen Series PBS-IIC ProteinChip® System (SELDI mass spectrometer) and TOF data generated by averaging a total of 220 laser shots collected at a laser intensity of 200, a detector sensitivity of 8 and molecular mass range from 0-20000 Da.

**Analysis of SELDI spectra.** Data were analyzed using Biomarker Wizards Software (Ciphergen Biosystems). After automatic baseline noise correction, the spectra were normalized by the “total ion content” method as described by the manufacturer. The peaks with an m/z value <2 kDa were excluded, as these peaks were mainly ion noise from the matrix (sinapinic acid). Peak clusters were generated by automatically detecting qualified mass peaks (S/N>5) then completed with second-pass peak selection (S/N>3, with a 0.3% mass window).

**Protein purification.** To fully characterize the 7.6-kDa protein, conditioned media from the MDA-MB-435S-F/Taxol 10p4pSI population was fractionated first using BioSepra IMAC-Cu²⁺ HyperCel spin columns (BioSepra, Cergy, France). Each of the fractions eluted from the spin columns using 50 to 500 mmol/l imidazole in binding buffer containing 250 mM sodium chloride was analyzed further on a hydrophilic NP20 ProteinChip Array (1 ìl per spot) to monitor the elution and recovery of the protein of interest. The eluted fractions in which the marker was the most abundant were concentrated to 30 ìl using 3000 MW cut-off centricons (Millipore, Billerica, MA, USA) and were loaded onto 4-12% NuPAGE Bis-Tris Gels (Invitrogen, NY, USA) and run using a MES/SDS buffer. The samples were run according to the manufacturer’s instructions and were stained using Invitrogen SilverQuest protocols.

**MALDI TOF/TOF MS and protein identification.** In-gel digestion of proteins was carried out following a modified protocol from Schevchenko et al. (24). Milli-Q water (200 ìl) was added to the excised gel piece and placed on a shaker for 15 min. Acetonitrile (200 ìl) was then added and incubated with shaking for a further 15 min. The solution was then removed and replaced with 200 ìl 100 mM NH₄HCO₃ and left to shake for 15 min after which the solution was replaced with 200 ìl 100 mM NH₄HCO₃ in 50% acetonitrile and left for a further 15 min with shaking. This solution was removed and 100 ìl of acetonitrile was added; following incubation on a shaker for 10 min, the solution was then removed and the gel piece was dried in a SpeedVac concentrator (Thermo Fisher Scientific, MA, USA) for 10 min. Fifty ìl of 10 mM dithiothreitol in 20 mM NH₄HCO₃ was added to gel piece which was then placed at 56ºC for 1 h after which the liquid was removed and replaced with 50 ìl of 50 mM iodoacetamide in 20 mM NH₄HCO₃. This was left at room temperature, in the dark with shaking for 30 min. Gel pieces were then washed again with subsequent solutions of 100 mM NH₄HCO₃, 100 mM NH₄HCO₃ in 50% acetonitrile and 100 ìl acetonitrile, as previously, and the gel piece was dried in a SpeedVac concentrator for 10 min.

Gel slices were rehydrated with trypsin digestion buffer with enough liquid to cover each gel piece (12.5 µg/ml modified bovine trypsin (Roche, NJ, USA), 0.1% n-octyl glucoside (Calbiochem, San Diego, CA, USA) and 20 mM NH₄HCO₃). After 16 h incubation on a shaker at 30ºC, an equal volume of acetonitrile was added and the peptides were allowed to extract for 10 min with shaking. One tenth of the peptide solution from the digest was added to a 192 spot MALDI sample plate (Applied Biosystems, Warrington, UK) and run using a MES/SDS buffer. The samples were run according to the manufacturer’s instructions and were stained using Invitrogen SilverQuest protocols.

MALDI TOF/TOF proteomics analysis was performed using a MALDI-TOF/TOF Proteomics Analyzer instrument (Applied Biosystems). Prior to analysis, the instrument was externally calibrated using the 4700 Proteomics Analyzer Calibration Mixture (4700 Cal Mix; Applied Biosystems). All MS and MS/MS experiments were carried out in positive reflectron mode. Five precursor ions for MS/MS were selected.
automatically on the basis of intensity from the MS spectra. The MS and MS/MS data were combined and searched against a number of databases using GPS Explorer software (Applied Biosystems) and a local MASCOT (Matrix Science, MA, USA) search engine for protein identification. A mass window of 20 ppm was set for database searching on all precursors. All mass spectrometry and protein identification was performed with "FingerPrints", Wellcome Trust Biocentre, University of Dundee, Scotland.

**Immunoblot analysis.** Electrophoretic transfer of proteins to Hybond-ECL nitrocellulose membranes (Amersham, Bucks., UK) was carried out using a Bio-Rad Transblot SD cell (Bio-Rad Labs., Hemel Hempstead, Herts., UK). Proteins were transferred for 80 min at 0.34 mA. Efficiency of transfer was evaluated using Ponceau-S-Red staining of nitrocellulose membranes, followed by destaining in phosphate buffered saline (PBS; 50 mM sodium phosphate, 0.9% (w/v) NaCl, pH 7.4). Membranes were blocked for 1 h in 5% (w/v) fat-free milk powder in PBS containing 0.5% Tween-20. Membranes were then incubated overnight at 4°C with the primary antibody, anti-transferrin receptor (Abcam, Cambridge, UK; ab1086) diluted to 1:1000 with blocking buffer. Nitrocellulose replicas were subsequently twice washed for 10 min in blocking solution and then incubated with a 1:1000 dilution of the corresponding peroxidase-conjugated secondary antibody for 1 h at room temperature (DakoCytomation, Glostrup, Denmark). Nitrocellulose membranes were washed twice for 10 min in blocking solution and twice rinsed for 10 min in PBS. Visualization of immuno-decorated 1D bands was carried out using an enhanced

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**Figure 4. IMAC-Cu2+ Hypercel fractionation of MDA-MB-435S-F/Taxol10p4pSI using increasing concentrations of Imidazole.** All seven fractions collected from unbound peptides/proteins through to 500 mM Imidazole were analysed on NP20 ProteinChip Arrays. From the chromatographs it is clear that the greatest concentration of the 7.6 kDa species elutes in the 250 mM fraction with the majority of the contaminating proteins removed in earlier fractions.
chemiluminescence kit (Amersham). The immunoblots were subsequently scanned and densitometric analysis performed using Labworks 302 software (Labworks, CA, USA).

Results

Invasion assay. MDA-MB-435S-F was highly invasive, but selection with paclitaxel resulted in a more aggressive invasive phenotype characterized by a proportion of the invading cells detaching from the bottom of the insert and reattaching to the bottom of the 24-well plate (superinvasion), which was negligible in MDA-MB-435S-F as shown in Figure 1.

Expression difference mapping analysis. A total of 98 peaks were detected and 5 peaks were found to be differentially expressed with statistical significant $p$-value of $<0.05$. The most prominent differentially expressed peak at m/z 7.6 was found to be fourfold greater in the superinvasive phenotype with a statistically significant $p$-value of $<0.04$. Figure 2(A) shows comparative chromatographs of mass signals and Figure 2(B) shows the one-dimensional gel view. Figure 3 shows the Biomarker Wizard plot from conditioned media analyzed on IMAC30-Cu$^{2+}$ ProteinChip arrays. Conditioned media from MDA-MB-435S-F and MDA-MB-435S-F/Taxol10p4pSI were analyzed in triplicate using IMAC30-Cu$^{2+}$ chip surface. Results were cross-checked for their reproducibility by using different biological sample and replicates on different ProteinChip batches.

Purification of the 7.6-kDa marker candidate. The IMAC HyperCel sorbent has greater capacity than an IMAC30 ProteinChip Array and is used where enrichment of the target protein is desired. The conditioned media from MDA-MB-435S-F/Taxol-10p4pSI cells was fractionated using BioSepra IMAC-Cu$^{2+}$ HyperCel spin columns, with the 250 mM Imidazole fraction showing the highest concentration of the 7.6-kDa protein along with the least number of contaminating peaks (Figure 4). The 250 mM fraction was run on a 4-12% NuPAGE Bis-Tris 1D gels using a MES/SDS running buffer and antioxidant under reducing conditions. The MES/SDS running buffer is specially formulated for greater resolution in the lower molecular weight range. Silver staining of the gel allowed a band at 7.6 kDa to be excised.

Identification of the 7.6-kDa marker candidate. In-gel digestion of proteins was carried out using a modified version of the method used by Schevchenko et al. (24). Tryptic digests of the two proteins were subject to MALDI TOF/TOF analysis. All MS and MS/MS experiments were carried out in positive reflectron mode. Five precursor ions for MS/MS were selected automatically on the basis of intensity from the MS spectra. The MS and MS/MS data were combined and searched against a number of databases and identified as a 7.6-kDa protein fragment of bovine transferrin.

Immunoblot analysis of MDA-MB-435S-F and MDA-MB-435S-F/Taxol10p4pSI cells. Immunoblot analysis of whole cell lysates from both MDA-MB-435S-F and MDA-MB-435S-F/Taxol10p4pSI phenotypes using an antibody against the transferrin receptor showed a marked increase in the abundance levels of the receptor in MDA-MB-435S-F/Taxol10p4pSI phenotype compared to the parent (Figures 5 and 6). This 3.5-fold increase in the receptor corresponds to a fourfold increase in the transferrin fragment identified using SELDI analysis found in the MDA-MB-435S-F/Taxol10p4pSI conditioned media (Figure 6).

Discussion

To identify patterns of differential expression that may correlate with the drug-selected superinvasive phenotype exhibited by MDA-MB-435S-F/Taxol-10p4pSI, conditioned media was subject to SELDI-TOF MS analysis. Initial
analysis carried out using the IMAC30-Cu\textsuperscript{2+} ProteinChip arrays demonstrated that the expression level of the 7.6 kDa protein in MDA-MB-435S-F/Taxol-10p4pSI cells was increased when compared with MDA-MB-435S-F cells. The 7.6-kDa protein was found to be 4-fold up-regulated in the superinvasive taxol-resistant cells with a statistically significant \( p \)-value of <0.04.

Purification and identification of the 7.6 kDa was performed using conditioned media from the MDA-MB-435S-F/Taxol-10p4pSI population and using MALDI TOF/TOF analysis was identified as a 7.6-kDa protein fragment of bovine transferrin.

Plasma transferrin is an 80-kDa glycoprotein with homologous N-terminal and C-terminal iron-binding domains (25). The primary role of transferrin is to transport iron safely around the body to supply growing cells and plays a central role in DNA replication with one of the key enzymes, ribonucleotide reductase, requiring iron as a co-factor. Each transferrin molecule can carry two Fe\textsuperscript{3+} ions and contains an array of amino acids that are perfectly arranged to form four bonds to the iron ion, which locks it in place (26). Transferrin is synthesized predominantly by hepatocytes (27). Other tissues expressing transferrin include metastatic melanoma cell lines (28), and human breast cancer cell lines (29).

The transferrin receptor is expressed on the surface of human cells that require iron and is responsible for internalisation of transferrin-bound iron and its intracellular release. Non-dividing cells can have extremely low levels of transferrin receptor expression, whereas rapidly proliferating cells, for example carcinoma cell lines, can express up to 100000 transferrin receptors per cell (29). In this study, the abundance levels of the transferrin receptor were found to be approximately 3.5-fold increased in the MDA-MB-435S-F/Taxol-10p4pSI cells compared to the parent.

Transferrin is implicated in the growth, differentiation and cytoprotection of many cell types. Bovine transferrin is very abundant in serum used in the culturing of MDA-MB-435S-F, MDA-MB-435S-F/Taxol-10p4pSI cells. The positive growth-promoting effect of transferrin on human colon tumour cell lines, for example, has been reported (30). Transferrin was also found to ensure the survival of ovarian carcinoma cells when apoptosis was induced by TNF\textalpha or FasL (31), and previous publications have also implicated transferrin in enhancing human colon tumour cell growth by differentiation class specific mechanisms (32).

Increased expression levels of transferrin receptors could also explain the increased abundance of the 7.6 kDa fragment detected in MDA-MB-435S-F/Taxol-10p4pSI cells. In this study, the fourfold increase in the detectable levels of the 7.6 kDa bovine transferrin fragment correspond to the 3.5-fold increase in the expression levels of the transferrin receptor found in the MDA-MB-435S-F/Taxol-10p4pSI population compared to MDA-MB-435S-F.

Transferrin receptor levels are elevated in various types of cancer cells and correlate with the aggressive or proliferative ability of tumour cells (33-35). A report also showed that transferrin differentially stimulated the growth of highly metastatic variant lines of murine melanoma and that these highly metastatic cells also had greater numbers of transferrin receptors on their cell surfaces (28). More results suggest that neoplastic cells displaying various metastatic properties may express differing numbers of...
transferrin receptors and respond differently to growth factors such as transferrin (29). Of recent interest was the finding that weak or absent transferrin receptor 1 expression in primary tumours was related to the presence of nodal or distant metastasis in patients with colorectal cancer (36).

Previous results demonstrated that selection of the human breast cell line MDA-MB-435S-F with paclitaxel resulted in a more aggressively invasive phenotype (19). Results presented here have associated this more aggressively invasive phenotype with an increase in expression levels of a 7.6-kDa protein fragment of transferrin. A recent report indicates that drug resistance in melanoma cells appears to be associated with a more aggressive behaviour, with resistant M14 ADR cells displaying significantly higher motility and invasion than parental sensitive M14 WT cells. P-glycoprotein and CD44 might cooperate to confer this more invasive phenotype (8).

The results shown here suggest that SELDI-TOF MS-based proteomic profiling may be useful in monitoring the relationship between drug resistance and invasion/metastasis. SELDI-TOF-MS is a recently established technology using ProteinChip arrays to purify and detect a selection of peptides/proteins in samples that have specific affinity to the chromatographic surface used. SELDI-TOF-MS is especially well suited to analyze a variety of body fluids such as serum and also conditioned media from cell culture. Only small sample amounts are needed for SELDI-TOF-MS to generate data giving rapid analysis and protein expression profiles between samples. We have demonstrated that ProteinChip technology is a powerful proteomics technology for elucidating differentially expressed proteins involved in the molecular and biological processes that are characteristic of important biological profiles, taking as examples the drug-resistant superinvasive cell lines MDA-MB-435S-F/Taxol-10p4pSI. The fragmentation pattern of major serum protein generated by proteinases/enzymes unique to specific tumours may represent a rich source of potential biomarkers in the future. It is also possible that cleavage of serum proteins with important roles in cell metabolism, such as transferrin, could play a part in aspects of cancer pathology such as tumour cachexia.

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