Characterization of Stress Hormone-Mediated Drug Resistance in Breast Cancer Cells using SILAC combined with High Resolution Mass Spectrometry

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Abstract

Purpose: Study drug-resistance affects of chemotherapeutic agents in breast cancer cells treated with stress hormones using stable isotope labeling with amino acids in cell culture (SILAC™) and Fourier transform (FT) mass spectrometry (MS).

Methods: Breast cancer cells were exposed to paciltaxel and then treated with a physiological concentration of stress hormones. SILAC-based relative quantitation experiments were performed to measure changes in the proteome of the cancer cells as a result of the stress hormone treatment.

Results: Initial results show that SILAC methodology using nanoscale separations coupled online with FTMS is a facile method to study quantitative changes in protein abundance in breast cancer cells and chemotherapy. Identified proteins and their relative abundances were further interrogated using Ingenuity Pathway Analysis™ software to determine potential pathways involved and to provide insight for exploring the biology of drug resistance in cancer.

Introduction

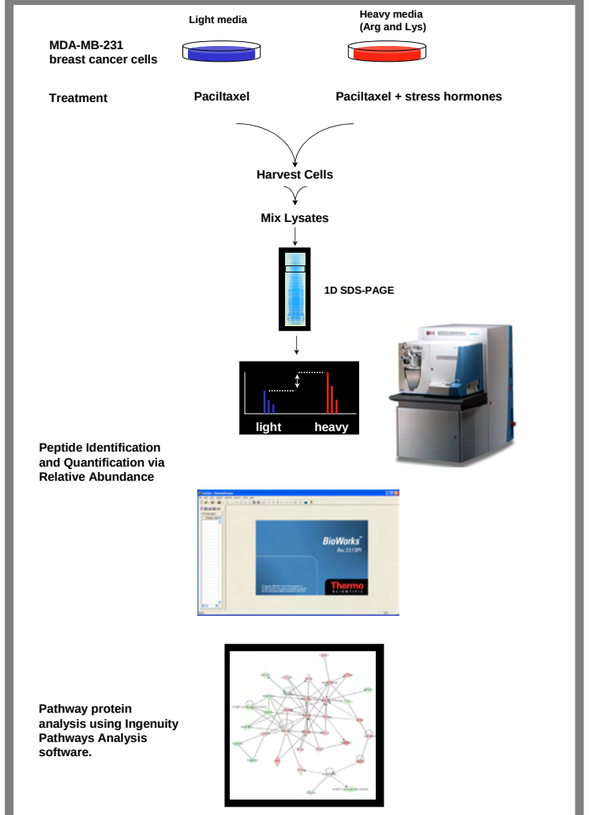
Stable isotope labeling with amino acids in cell culture (SILAC) is a straightforward method for MS-based quantitation of the relative abundance of complex protein samples.[1,2] SILAC involves metabolic incorporation of 'heavy' ^{13}C or ^{15}N-labeled amino acids into proteins of actively growing cell cultures using specially formulated media and dialyzed serum. Typical experiments involve growing two cell populations that are identical except that one of them contains a 'light' and the other a 'heavy' form of a particular amino acid (e.g. $^{12}C_6$ and $^{13}C_6$ Lysine, respectively). Upon alteration of the proteome in one sample through chemical treatment or genetic manipulation, equal amounts of cell lysate from both cell populations are then combined for protein identification and quantitation using high resolution MS (Figure 1).

Breast cancer is one of the most frequently diagnosed cancers among women and chemotherapy treatment has been a successful therapy, however, as many as 50% of patients will not benefit from this care because their breast cancer cells will become resistant to the chemotherapeutic reagents. Great effort is currently focused on understanding what causes drug resistance in breast cancer at the molecular level. To gain a more clear understanding of chemotherapeutic resistance at the level of the proteome, we used SILAC coupled with FTMS to investigate drug resistance in breast cancer cells. Initial results shown by traditional cell biological-based assays indicate that breast cancer cells treated with paciltaxel experience significant cell cytotoxicity. Upon incubation with stress hormones, however, the majority of cells survive paciltaxel treatment. To gain insight into the proteome-level changes commensurate with drug resistance, breast cancer cells were grown in light and heavy SILAC medium, equivalently exposed to paciltaxel, and the heavy cells were incubated with stress hormones. Cells were lysated, mixed in a 1:1 ratio and proteins were resolved by 1D SDS-PAGE. Gel bands were digested with trypsin and the peptides were analyzed using a high resolution FTMS. Initial results show that stress hormones alter the abundance level of several proteins involved in drug resistance. Taken together, we have shown that SILAC combined with nanoscale separations coupled online with FTMS is a facile method to study quantitative changes in protein abundance changes in breast cancer cells and chemotherapy.

Methods and Materials

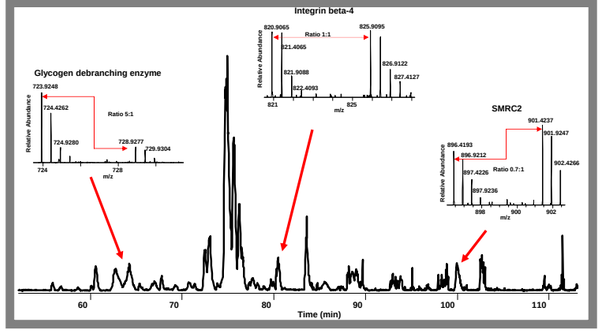
MDA-MB-231 cells were grown in Dubecco's Modified Eagle medium (DMEM) labeled with a mixture of both heavy amino acids $^{13}C_6$-lysine and $^{13}C_6{}^{15}N_4$-arginine using a Thermo Scientific Pierce® SILAC Quantitation Kit. Cells were then treated with paciltaxel to an inhibitor concentration of 50% (IC_{50}). Heavy-labeled cells were treated with a mixture that contained physiological concentrations of stress hormones. Cells were lysed, mixed with control lysates, resolved by 1D SDS-PAGE and digested. Peptides were identified and quantitated using an LTQ Orbitrap™ mass spectrometer (Thermo Scientific). The SILAC method was able to successfully quantify changes in relative protein abundance after chemical or genetic perturbation in a model cancer cell line. Pathway protein analysis was performed using Ingenuity Pathway Analysis software.

FIGURE 1. Diagram representing the SILAC workflow used to quantify differentially expressed proteins in breast cancer cells exposed to paciltaxel and paciltaxel plus stress hormones. Proteins with significant differences in abundance were identified, and Ingenuity Pathway Analysis software was used to demonstrate the biochemical processes which are affected by the treatment.



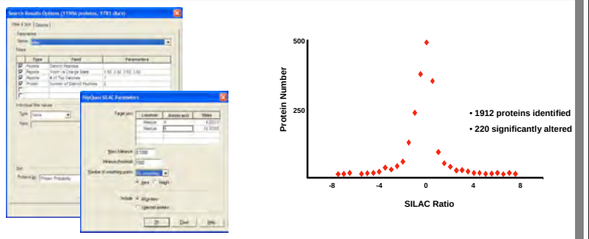
Results

FIGURE 2. Representative LC-MS base peak chromatogram of a gel band.



Upper panels are representative mass spectra of light and heavy peptides of glycogen debranching enzyme (VAPGYYTLTADQDAR, upper right panel), integrin beta-4 (LTAELNQVLYR, upper middle panel) and SMRC2 (YYEAADTVTQFDNVR, upper right panel). Mass spectra of heavy peptides containing $^{13}C_6$ L-lysine or $^{13}C_6{}^{15}N_4$ L-arginine are increased in mass by 6 and 10 amu, respectively for singly-charged ions. Integrin beta 4, a laminin receptor, upregulated in tumor cells, was approximately equal in both conditions and plays a key role in the signaling networks that drive angiogenesis and tumor progression. Glycogen debranching enzyme was significantly decreased by hormones and SMRC2 was increased by stress hormones. SMRC2 is a protein complex involved in the activity of multiple transcription factors and signaling pathways, and is thought to be associated with cancer.

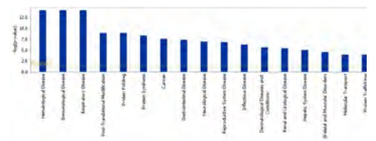
FIGURE 3. Shown below are the BioWorks™ search filters and the PepQuan™ parameters used for relative quantitation. A plot of SILAC ratio vs number of proteins shows very even distribution of both positive and negative changes in abundance as a result of the treatment. Based on the plot, a 95 % confidence error estimate was determined resulting in a significant alteration of 220 proteins out of the total 1912 proteins identified.



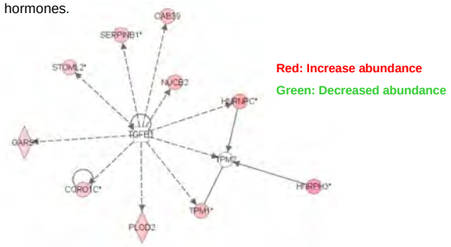
SILAC ratios were calculated using BioWorks 3.3.1 SP1 PepQuan SILAC function using the following settings: target ions = residue = K, Mass = 6.02013, residue = R, Mass = 10.03355, mass tolerance = 0.1, minimum threshold = 1000, no smoothing, and perform calculation based on area. Identified proteins were plotted on semi-log graphs sorted by average SILAC ratios to determine most up- and down-regulated proteins.

FIGURE 4: Proteins involved in cell cycle, proliferation and cancer. A protein of interest, Tropomyosin alpha-1 (TPM1), is highlighted in the pathway and the RIC are shown for a peptide from this protein for both the light and heavy labeled form.

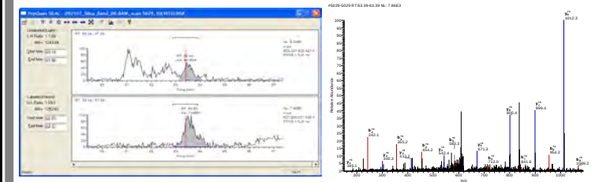
Canonical Pathways Influenced by Hormone-mediated Chemotherapeutic Resistance



Strong activation of the TGFβ pathway may play a role in paclitaxel-induced cell cycle arrest by stress hormones.



Tropomyosin alpha-1 (TPM1) Peptide IQLVEELDR#, where '#' denotes heavy Arg.



Conclusions

- The SILAC method resulted in the successful quantification of over 1900 proteins from breast cancer cells, where 220 showed significant altered abundances resulting from stress hormone exposure.
- Key pathways identified in these experiments will be further analyzed to determine the mechanisms by which the hormones interfere with the treatment of the cancer cells with paciltaxel.

References

1. **Everly, P.A., et al. (2004). Quantitative cancer proteomics: Stable isotope labeling with amino acids (SILAC) as a tool for prostate cancer research.** ***Mol & Cell Proteomics*****. 3.7: 729-735.**
2. **Mann, M. (2006). Functional and quantitative proteomics using SILAC.** ***Nature Reviews*****. 7:952-959.**

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