Early Changes in Protein Expression Detected by Mass Spectrometry Predict Tumor Response to Molecular Therapeutics

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ABSTRACT

Biomarkers that predict therapeutic response are essential for the development of anticancer therapies. We have used matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) to directly analyze protein profiles in mouse mammary tumor virus/HER2 transgenic mouse frozen tumor sections after treatment with the erbB receptor inhibitors OSI-774 and Herceptin. Inhibition of tumor cell proliferation and induction of apoptosis and tumor reduction were predicted by a >80% reduction in thymosin β4 and ubiquitin levels that were detectable after 16 hours of a single drug dose before any evidence of in situ cellular activity. These effects were time- and dose-dependent, and their spatial distribution in the tumor correlated with that of the small-molecule inhibitor OSI-774. In addition, they predicted for therapeutic synergy of OSI-774 and Herceptin as well as for drug resistance. These results suggest that drug-induced early proteomic changes as measured by MALDI-MS can be used to predict the therapeutic response to established and novel therapies.

INTRODUCTION

There is an increasing number of molecule-targeted anticancer therapies, both established and in clinical development. In most cases, the tumors that are dependent on the molecular targets of these therapies and, therefore, potentially sensitive to these drugs, are not easily recognizable. Therefore, it becomes imperative to identify a molecular signature or predictive marker before or during preclinical anticancer drug development to guide this process. Such information would allow the selection of appropriate patients into efficacy studies as well as the exclusion of patients in whom these drugs are unlikely to exhibit any clinical benefit. In some cases, this predictive molecular signature is known. For example, the presence of estrogen and progesterone receptors in breast cancers predicts for response to antitumor (3). Clinical responses to the anti-HER2 (erbB2) humanized IgG1 trastuzumab (Herceptin) are limited to breast tumors with overexpression of the erbB-2 (HER2) receptor RNA and protein have shown limited to no clinical activity (4, 5). Interestingly, two groups recently reported EGFR gene mutations in lung cancers that exhibited robust clinical responses to the EGFR tyrosine kinase inhibitor gefitinib (Iressa; refs. 6, 7), additionally underscoring the presence of molecular signatures or surrogate markers that can predict drug action.

Proteomics approaches, including two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS), have been shown to be powerful tools to discover biomarkers that distinguish normal from diseased tissues. Charand et al. (8) used MALDI-MS to directly analyze mouse colon tissue and showed that protein patterns were significantly different between tumor tissue and normal colon tissue. Several tumor-specific protein markers were identified in this work (8). Yanagisawa et al. (9) recently showed that tumor subsets of non–small-cell lung cancer give rise to unique protein patterns. They reported that MALDI-MS–derived protein profiles from human lung tissue could accurately distinguish normal lung, adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma. They also identified markers that were indicative of patient prognosis (9). In this article, we show that protein changes initiated by treatment with a small molecule inhibitor of the EGFR tyrosine kinase and/or a HER2 blocking antibody predict for cellular and clinical activity in oncogene-expressing mammary tumors. These changes can be observed as early as after 16 hours of treatment and, thus, support the use of proteomic-based approaches to potentially guide individualized anticancer therapies.

MATERIALS AND METHODS

Transplantation and Treatment of Transgenic Tumors. FVB female transgenic mice expressing the human HER2 cDNA under the control of the mouse mammary tumor virus (MMTV) promoter develop mammary tumors with a latency of 8 months (10). Snap-frozen MMTV/HER2 mammary tumors were provided by Sharon Erickson (Genentech, Inc., South San Francisco, CA). Through a small incision in the dorsal flank of syngeneic nontransgenic female mice, an ~1-mm³ tumor pellet was inserted with a sterile probe in the s.c. space near the #1 mammary fat pad just above the shoulder. The incision site was closed with 1 to 2 sterile wound clips under a protocol approved by the Vanderbilt Institutional Animal Care and Use Committee. Within 1 to 2 weeks, tumors measuring >3 mm in diameter were palpable. Tumor size was followed with calipers, and tumor volume was calculated by the formula (volume = width² × length/2). Herceptin was purchased from the Vanderbilt University Hospital Pharmacy and administered in sterile isotonic saline at either 10 mg/kg or 30 mg/kg i.p. twice a week. OSI-774 was provided by Mark Sliwkowski (Genentech, Inc.) and prepared every week in capsitol (from OSI Pharmaceuticals, Boulder, CO) as a 20 mg/mL stock solution and administered daily via orogastric gavage (p.o.) at a dose of 10 to 100 mg/kg in a volume of 100 μL. Where indicated, lungs were examined for surface lung metastases as described previously (11).

Immunoprecipitation, Western Blotting, and Immunohistochemistry. Tumors were harvested and either fixed in 10% formalin (VWR Scientific, West Chester, PA) or homogenized as described previously (12). Tumor lysates were precipitated with a COOH-terminus erbB2 (HER2) polyclonal antibody (NeoMarkers, Freemont, CA) and protein A-Sepharose (Sigma, St. Louis, MO) followed by Western blot procedures with HER2 (NeoMarkers) and phosphorytrosine (Upstate Biotechnology, Lake Placid, NY) antibodies.
(13). Tumor cell proliferation was measured as described previously (14). In brief, paraffin-embedded tumors were sectioned (5-μm) and then subjected to immunohistochemistry with a proliferation cell nuclear antigen (PCNA) antibody (NeoMarkers). Tumor cell apoptosis was assessed by terminal deoxyribonuclease transferase (Tdt)-mediated nick end labeling (TUNEL) analysis with the Apoptag Detection Kit (Serologicals Corp., Norcross, GA) according to the manufacturer’s instructions.

**Tissue Preparation and MALDI-MS Analysis.** Tissues from animals were snap-frozen in liquid N₂ and stored at −80°C until additional analysis. Frozen tissues were cut at −15°C into 12-μm thick sections on a cryostat (Leica Microsystems Inc., Bannockburn, IL); sections were transferred and thawed onto gold-coated stainless-steel MALDI target plates. The sections were desiccated for at least 1 hour before matrix application. For regional analysis, 0.25 μL of the matrix solution (sinapinic acid, 20 mg/mL in 50:50 acetonitrile:0.2% trifluoroacetic acid) was deposited on the tissue, dried, and an additional 0.25 μL was deposited directly on the first spot. For imaging applications, sinapinic acid matrix was coated over the tissue until there was a homogeneous layer of matrix crystals over the surface (15).

MALDI time-of-flight mass spectra were acquired on a Voyager DE-STR mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a nitrogen laser (337 nm) run at 2 Hz. Linear mode was used in conjunction with delayed extraction. Data were obtained with an accelerating voltage of 25 kV, 95% grid voltage, 0.05% ion guide wire voltage, a delay time of 150 nanoseconds, and a flight path length of 2.0 m. A QStar Pulsar i (Applied Biosystems) hybrid QqTOF mass spectrometer equipped with a MALDI source and a nitrogen laser (337 nm) was used to acquire MALDI-tandem mass spectra (MS/MS) of the drug OSI-774. The laser was run at 20 Hz. OSI-774 was effectively analyzed by selected reaction monitoring as described previ-ously (18). Briefly, the protonated molecular ion at m/z 394.2 was dissociated, and the predominant fragment ion at m/z 278.1 was analyzed. Sensitivity was increased by allowing a wide mass range into the collision quadrupole (~5 Da) and by detecting only a small mass range surrounding the fragment of interest (m/z 240 to 320) in the time-of-flight region.

**Imaging.** The Voyager DE-STR MALDI time-of-flight instrument is equipped with imaging software described previously (17). The software consists of a graphical user interface that allows the user to specify the area to be imaged, the distance between laser shots (spatial resolution), the instrument acquisition method to be used, the number of laser shots to be averaged in a spectrum, and the mass ranges of interest to monitor. As the image is acquired, the sample stage is moved from spot to spot, creating a raster of desorbed areas over the tissue surface. A spectrum is acquired at each spot, and the intensity of each signal from all of the mass ranges specified is plotted as a function of the location on the tissue surface. Thus, ion density maps are obtained showing the localization of compounds of interest within the tissue. The QStar Pulsar i instrument is equipped with similar software (16).

**Protein Identification and Tandem MS.** Tumor aliquots of ~300-mg were suspended in homogenization buffer [0.25 mol/L sucrose, 10 mmol/L Tris HCl buffer (0.1 mol/L), agitated with a motor-driven pestle for 1 minute, and centrifuged at 680 x g for 10 minutes at 4°C, and the pellet was labeled “crude nuclear fraction.” The supernatant was transferred to an Eppendorf tube and labeled “cytosolic fraction.” To determine which tumor fraction contained the proteins of interest, dilutions of antibody to the ectodomain of the HER2 receptor (20). To inhibit tumor growth, we used the following: (1) OSI-774 (erlotinib, Tarceva), an ATP-competitive small molecule (MW 393) that inhibits the EGFR and the HER2 tyrosine kinases with an in vitro IC₅₀ of 0.02 and 0.35 μmol/L, respectively (18, 19); and (2) the humanized IgG1 trastuzumab (Herceptin), which binds the ectodomain of the HER2 receptor (20). Treatment with either OSI-774 or Herceptin eliminated established MMTV/HER2 tumors of ≥100 mm³ in volume (Fig. 1A). Within 48 hours, OSI-774--treated tumors exhibited loss of HER2 phosphorylation (Fig. 1B). We next compared global protein expression patterns between treated and untreated tumor transplants via MALDI mass spectrometry.

MMTV/HER2 tumors from untreated mice were first analyzed to obtain a baseline protein profile. An example of such a profile, representing an average of three mass spectra, is shown in Fig. 1C. There are >200 signals present over the mass range of 2,000 to 30,000 Da. These signals are thought to arise from abundant, soluble proteins present in the tissue section. Several abundant proteins are labeled, including thymosin β4 (Tβ4), ubiquitin, acyl-CoA-binding protein (ACBP), histone H4, and the α and β chains of hemoglobin (α⁺ and β⁺, singly charged, and α⁺² and β⁺², doubly charged).

**Therapy-Induced Changes in the Tumor Proteome Are Dose- and Time-Dependent.** To examine if the effects of OSI-774 are dose-dependent, mice bearing two established (≥250 mm³) contrat-eral tumor transplants each were treated with different daily drug concentrations. Twenty hours later, the right transplant was harvested. To control for an antitumor effect, treatment was continued for 9 days, and volume of the contralateral transplant was measured serially. Only tumors treated with the higher dose (100 mg/kg) of OSI-774 exhibited evidence of apoptosis at 20 hours as well as growth arrest after 9 days of treatment (Figs. 2, A and B).

**RESULTS**

Drug-Mediated Inhibition of HER2 Induces Rapid Changes in the Proteome. We first determined the efficacy of signaling inhibitors against tumors arising in MMTV/HER2 transgenic mice (10). These mice develop metastatic mammary tumors with a median latency of 8 months. These transgenic tumors can be serially transplanted in FVB wild-type mice where they retain HER2 overexpression. Unless otherwise specified, all of the experiments were done with a tumor from an F2 mouse (F2–1282). To inhibit tumor growth, we used the following: (1) OSI-774 (erlotinib, Tarceva), an ATP-competitive small molecule (MW 393) that inhibits the EGFR and the HER2 tyrosine kinases with an in vitro IC₅₀ of 0.02 and 0.35 μmol/L, respectively (18, 19); and (2) the humanized IgG1 trastuzumab (Herceptin), which binds the ectodomain of the HER2 receptor (20). Treatment with either OSI-774 or Herceptin eliminated established MMTV/HER2 tumors of ≥100 mm³ in volume (Fig. 1A).

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Mass spectra were acquired and averaged from a total of 3 tumors per dose harvested 20 hours after the first dose of OSI-774. Each average spectrum was compared with a control mass spectrum acquired from 9 spectra collected from 3 animals. Selected mass ranges of the four averaged traces are shown in Fig. 2C. The top spectrum...
shows the protein signals in the range of 4,700 to 5,100 Da. There is a signal at m/z 4,913 that is of similar intensity in all four groups. The signal at m/z 4,965, identified as Tq4 (21), is of similar intensity in the control group and the groups treated with 10 mg/kg and 30 mg/kg, but is significantly decreased (~80%) in the 100 mg/kg group. Tq4 is an actin-sequestering protein and has been recently implicated in having a role in tumor metastasis by promoting cell migration and angiogenesis (22). In contrast, there was no signal at m/z 4,794 in the untreated or low dose groups, which was prominent in the tumors treated with 100 mg/kg (Fig. 2C). This signal was sequenced by tandem MS and identified as a fragment of an E-cadherin binding protein (SwissProt accession number Q8C7WS). Electrospray ionization-tandem MS (LTQ, ThermoFinnigan) on the triply charged ion at m/z 1599.95 returned the following sequence via Sequest searching: KAPQGDEGFD YNEEQYRDCK GGEFGNQRR FPGHILFWDFK, with a predicted molecular weight of M, 4,783. This sequence corresponds to residues 53-92 of the protein. E-cadherin is a transmembrane protein that mediates cell-cell adhesion. Inactivating mutations or inhibition of E-cadherin function have been associated with tumor cell motility and progression (23–25). Recently, a novel E-cadherin binding protein called Hakai was identified (26), which acts as an ubiquitin-ligase (E3) involved in ubiquitination of E-cadherin after tyrosine phosphorylation. The sequence of the protein fragment observed from the treated tumors differs from the analogous sequence of Hakai by the loss of a single glutamic acid residue after Glu59. A possible role of this protein on OSI-774-induced tumor growth inhibition remains to be studied.

The bottom mass spectrum of Fig. 2C highlights the range of 8,300 to 8,800 Da, where the same dose dependence observed for Tq4 is observed for ubiquitin (m/z 8,565). Many proteins are targeted for proteolysis by ubiquitination (27). Therefore, a marked decrease in free ubiquitin suggests the possibility that more ubiquitin is covalently bound to proteins targeted for degradation or that there is a decrease in the production of ubiquitin. Whereas the actual fate of ubiquitin in these tumors remains unknown, the reduction of ubiquitin correlates with tumor reduction.
presence of histone H4 in these areas seems to be reduced relative to the rest of the tumor.

The spatial localization of OSI-774 was analyzed on a serial tumor section via MALDI QqTOF tandem mass spectrometry as described previously (16). Fig. 3 shows an optical image of the tumor section mounted on the MALDI plate before matrix application. The ion density map obtained for OSI-774 via selected reaction monitoring of the transition m/z 394.2 → 278.1 is also shown in Fig. 3. Spectra were obtained every 200 μm in the x-direction and 400 μm in the y-direction. Forty laser shots were averaged at each spot. As shown, OSI-774 is distributed throughout the tumor section 16 hours after drug administration predominantly in the more vascularized peripheral sections of the tumor.

Drug-Induced Changes in the Proteome Correlate with Therapeutic Synergy. The EGFR tyrosine kinase inhibitor gefitinib has been shown to synergize with Herceptin against HER2-overexpressing breast cancer xenografts (29). Therefore, we next tested if the combination of OSI-774 and Herceptin would be synergistic against MMTV/HER2 tumors and whether this synergy can be predicted by early proteomic changes in situ. Tumors measuring about 250 mm³ were used for these studies. Tumors were randomized to no treatment, OSI-774, a less than therapeutic dose of Herceptin, or the combination (n = 13/condition). At 72 hours, 3 tumors per condition were analyzed by MALDI-MS, with 5 to 10 spectra acquired per tumor. The resulting spectra were baseline-subtracted and normalized with a computer program written in-house, and spectra from each treatment group were averaged together (spectra in which signals for hemoglobin were dominant were not included). Fig. 4 shows mass spectra in the ranges of m/z 4,700 to 5,100 and m/z 8,300 to 8,800. In tumors treated with OSI-774 or the combination, the spectrum from each time point is the average of 4 spectra from one animal, whereas the control spectrum is the average of 54 spectra from 9 animals.
combination, a decrease in Tβ4 and ubiquitin was observed. The signal at m/z 4,913 is the same among all four groups, as previously observed (see Fig. 2C). Interestingly, the largest changes observed for Tβ4 and for the E-cadherin binding protein fragment are from the group treated with both drugs.

The changes observed for the OSI-774-treated group are of a smaller magnitude than the changes observed in Fig. 2, C and D. There are several possible reasons for this difference. First, the tumors used in the drug synergy study were much larger (≈3 to 4×) than the tumors examined in Fig. 2. This results in slower-growing tumors and potentially slower turnover of proteins. This would potentially result in lesser alterations in protein content in response to any stimulus. Consistent with this change, however, the response of the larger tumors to OSI-774 alone was not as large as the response observed in smaller tumors. Thus, the lesser magnitude in protein alteration may reflect the lower level of tumor reduction.

Two additional observations can be made from these spectra. First, there is no change in the ubiquitin signal between the control and Herceptin-treated groups. In addition, there is no difference between the ubiquitin signal in the OSI-774 group and the combination group, but the ubiquitin signal from both OSI-774–treated groups is notably reduced compared with the signal in control and Herceptin-treated groups. Thus, it seems that the reduction of ubiquitin originates solely from administration of the small molecule inhibitor OSI-774. The second observation concerns the signal at m/z 8,719, subsequently identified by peptide mapping as a calmodulin fragment, which is considerably increased only in the combination group. In fact, many signals over the entire mass range (2,000 to 40,000 Da) showed larger changes in the group treated with the combination with intermediate levels in the single drug groups, possibly reflecting the enhanced therapeutic effect of the combined therapy (Fig. 4D; ANOVA, P < 0.05). In addition to Tβ4 and ubiquitin, these included several histones (H3 and both dimethylated, diacetylated H4 at m/z 11,344 and dimethylated, monoacetylated H4 at m/z 11,307), acyl-CoA binding protein, and calgizzarin, as well as a number of as yet unidentified signals. Finally, the E-cadherin binding protein fragment and a signal at m/z 6,081 were also up-regulated in the tumors treated with the combination.

### Table 1

<table>
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<tr>
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<th>Control</th>
<th>Herceptin</th>
<th>OSI-774</th>
<th>Herceptin + OSI-774</th>
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<tr>
<td>Complete responses</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>6/10</td>
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<tr>
<td>Deaths</td>
<td>4/10</td>
<td>2/10</td>
<td>1/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Lung surface metastases</td>
<td>4.5 ± 1.3</td>
<td>3.1 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Lung weight (mg)</td>
<td>179.3 ± 10.5</td>
<td>168.8 ± 12.5</td>
<td>148.6 ± 12.8</td>
<td>138.0 ± 3.0</td>
</tr>
</tbody>
</table>

Fig. 3. Localization of OSI-774 in tumors correlates with mass spectrometric evidence of cellular response. A. Wild-type FVB mice bearing MMTV/HER2 tumors measuring ≈200 mm³ were treated with 100 mg/kg OSI-774 p.o. Sixteen hours later tumors were harvested. A mass spectrum is shown for the analysis of OSI-774 in spot #3 of one tumor section. The drug is analyzed via selected reaction monitoring of the transition m/z 394.2 → 278.1. B. One section of untreated tumor and two serial sections of treated tumor were analyzed via imaging MS. Two selected protein images are shown, one for histone H4 (dimethylated, diacetylated, m/z 11,344) in yellow and one for ubiquitin (m/z 8,565) in green. Whereas histone is fairly homogenously distributed in both untreated and treated tumor sections, ubiquitin has been markedly down-regulated in treated compared with the untreated tumor. A mass spectral image of the localization of the drug itself, OSI-774, was done on a serial section of the treated tumor. OSI-774 is distributed throughout the tumor section but is less evident in the necrotic center.
Therapy-Induced Changes in the Proteome Predict for Drug Resistance. We finally studied if MALDI-MS can be predictive for drug resistance by comparing the Herceptin-induced protein profile in F2–1282 tumors versus a second tumor from the same transgenic founder line (Fo5), which acquired spontaneous resistance to Herceptin. Fo5 tumor cells bind Herceptin and exhibit equal levels of HER2 protein as the F2–1282 tumors. Within 10 days of continuous administration of a therapeutic dose of Herceptin (30 mg/kg), all F2–1282 tumors were eliminated, whereas Fo5 tumors continued to grow (Fig. 5A). Forty-eight hours after the second dose of antibody, F2–1282 tumors exhibited a 10-fold increase in TUNEL+ cells compared with controls. Consistent with the lack of response, this was not seen in Fo5 tumors (Fig. 5B).

Fig. 5C shows mass spectra in the mass range of 8,880 to 9,400 Da obtained for both sensitive (1282) and resistant (Fo5) tumor lines. An increase in $m/z$ 9,212 is observed in Herceptin-treated 1282 tumors. In contrast, the same mass range for the resistant tumors shows no difference in the signal at $m/z$ 9,212 between the untreated and treated groups, suggesting that $m/z$ 9,212 is a potential biomarker of tumor response to the HER2 antibody. Several other signals seemed different between the sensitive and resistant tumors after Herceptin treatment, and they are presented in Fig. 5D. The signals are plotted as the absolute intensity difference from untreated tumors (either sensitive or resistant). The down-regulation of five signals ($m/z$ 6,252, 17,837, 17,877, 15,334, and 11,307, corresponding to an unidentified protein, peptidyl-prolyl cis-trans isomerase A, acetylated peptidyl-prolyl cis-trans isomerase A, histone H3, and dimethylated, monoacetylated histone H4, respectively) was shown to be statistically significant (*, ANOVA, $P < 0.05$) for the combination therapy compared with controls. Peptidyl-prolyl cis-trans isomerase A was detected in unmodified (*) and acetylated ($) forms. Histone H4 was detected in dimethylated, diacetylated (†) and dimethylated, monoacetylated ($) forms.
induced “cellular activity” in situ is assessed after a short therapy interval. For example, in patients with breast cancer, evidence of inhibition of proliferation and/or apoptosis in tumor cells as measured by Ki67 immunohistochemistry or TUNEL, respectively, in a second biopsy done as early as 10 days after antiestrogens or chemotherapy has been shown to predict for clinical response to therapy (30). In these studies, antiestrogen-induced inhibition of proliferation was limited to estrogen receptor-positive cancers, suggesting that this approach could have potentially identified estrogen receptor expression as the molecular signature predictive of good odds of response to antiestrogens. Chang et al. (31) treated locally advanced HER2-overexpressing breast cancers with weekly Herceptin followed by the addition of docetaxel after week 3. Significant tumor regressions at 3 weeks correlated with a 2-fold increase in tumor cell apoptosis, as measured by cleaved caspase 3 in a biopsy obtained on day 8 after start of therapy. Additionally, the IMPACT trial randomized women with estrogen receptor-positive breast cancers to 12 weeks of the aromatase inhibitor anastrozole, the selective estrogen receptor modulator tamoxifen, or the combination. In a core biopsy obtained after 2 weeks of therapy, a statistically superior inhibition in proliferation, as measured by Ki67 immunohistochemistry, was observed in tumors treated with anastrozole compared with the other two arms. This result predicted for improved clinical outcome as measured by anastrozole increasing the odds of breast-conserving surgery (32). Taken together, these data suggest that drug-induced cellular activity in situ can predict clinical outcome. However, the assays used in these studies are time consuming, cumbersome, expensive, and require a few days of treatment to allow sensitive tumor cells to exhibit evidence of drug action.

Because drug-induced protein changes precede inhibition of proliferation or cell death, we proposed the use of MALDI-MS to detect therapy-induced early changes in the tumor proteome that would predict for clinical efficacy. Using MMTV/HER2 transgenic tumors, we show herein that early changes in tumor protein profiles predict for dose- and time-dependent effects of signaling inhibitors. Drug-induced changes in the proteome also predicted for therapeutic synergy and drug resistance. Finally, in drug-sensitive tumors, the spatial distribution of the erbB tyrosine kinase inhibitor OSI-774 mapped with biomarkers of antitumor activity.

These changes were detected in frozen material that did not require fixation or any additional preparation and occurred as early as 16 hours after a single drug dose. Some of the proteins that changed as
a function of response may well be causally related to this response. Tp4 was decreased in tumors treated with the therapeutic dose of OSI-774 as well as by the combination of OSI-774 and Herceptin. Interestingly, Tp4 is an actin-sequestering protein involved in cell migration, angiogenesis, β-catenin-mediated transcription, and metastatic progression (22). The association of this change with the inhibitory drug intervention suggests that MALDI-MS may be able to identify molecular mechanisms associated with the antitumor response. Taken together, these data also illustrate the ability of this technique to correlate changes in protein expression with drug bio-distribution and, therefore, potentially evaluate the effect of molecularly targeted therapeutics on the microenvironment of the tumor. These features in combination with clinical endpoints and more traditional assays (for apoptosis, cell proliferation, immunohistochemistry, and others) should allow for a more complete understanding of the biochemical effects of novel therapeutics and thus allow for well-designed clinical trials and optimal drug development.

In summary, imaging MS can be applied to detect the molecular fingerprints predictive of the antitumor action of molecular therapies. This methodology is fast with analysis times of ~5 minutes per frozen unprocessed single tumor section. In addition, it is unbiased, reproducible, and amenable to high throughput screening. The amount of information that MALDI-MS can provide from a single frozen tumor section should improve the predictive value of current, more cumbersome antibody-based assays.

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