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Mapping Molecular Networks Using Proteomics: A Vision for Patient-Tailored Combination Therapy

Emanuel F. Petricoin III, Verena E. Bichsel, Valerie S. Calvert, Virginia Espina, Mary Winters, Lynn Young, Claudio Belluco, Bruce J. Trock, Marc Lippman, David A. Fishman, Dennis C. Sgroi, Peter J. Munson, Laura J. Esserman, and Lance A. Liotta

From the US Food and Drug Administration (FDA) –National Cancer Institute (NCI) Clinical Proteomics Program, Office of Cellular and Gene Therapy, Center for Biologics Evaluation and Research (CBER), FDA; FDA-NCI Clinical Proteomics Program, Laboratory of Pathology, NCI, Center for Cancer Research (CCR)/National Institutes of Health (NIH); Biostatistics Section, Mathematical and Statistical Computing Laboratory, Center for Information Technology, NIH, Bethesda; Johns Hopkins University, Department of Urology, Baltimore, MD; University of Padova, Department of Oncological and Surgical Science, Padova, Italy; University of Michigan, Department of Internal Medicine, Ann Arbor, MI; Department of Obstetrics and Gynecology, New York University School of Medicine, New York, NY; Massachusetts General Hospital Cancer Center and Harvard Medical School, Charlestown, MA; and Departments of Surgery and Radiology, University of California, San Francisco, San Francisco, CA.

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Address reprint requests to Emanuel F. Petricoin III, PhD, Center for Applied Proteomics and Molecular Medicine, George Mason University, 10900 University Boulevard, MS 4E3, Manassas, VA 20110; e-mail: epetrico@gmu.edu.

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ABSTRACT

Mapping tumor cell protein networks in vivo will be critical for realizing the promise of patient-tailored molecular therapy. Cancer can be defined as a dysregulation or hyperactivity in the network of intracellular and extracellular signaling cascades. These protein signaling circuits are the ultimate targets of molecular therapy. Each patient's tumor may be driven by a distinct series of molecular pathogenic defects. Thus, for any single molecular targeted therapy, only a subset of cancer patients may respond. Individualization of therapy, which tailors a therapeutic regimen to a tumor molecular portrait, may be the solution to this dilemma. Until recently, the field lacked the technology for molecular profiling at the genomic and proteomic level. Emerging proteomic technology, used concomitantly with genomic analysis, promises to meet this need and bring to reality the clinical adoption of molecular stratification. The activation state of kinase-driven signal networks contains important information relative to cancer pathogenesis and therapeutic target selection. Proteomic technology offers a means to quantify the state of kinase pathways, and provides post-translational phosphorylation data not obtainable by gene arrays. Case studies using clinical research specimens are provided to show the feasibility of generating the critical information needed to individualize therapy. Such technology can reveal potential new pathway interconnections, including differences between primary and metastatic lesions. We provide a vision for individualized combinatorial therapy based on proteomic mapping of phosphorylation end points in clinical tissue material.

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INTRODUCTION

Although gene microarrays can provide important information about somatic genetic taxonomy, they are unable to provide a full picture of the fluctuating signaling events that occur at the proteomic level.

The discovery and characterization of defective or hyperactive signaling pathways have been a major focus for mechanistic studies of cancer progression, the identification of candidate therapy targets, and the rational selection of patients who are most likely to respond to a therapeutic regimen.[1-19] Gene microarray and transcriptional profiling can provide important insights into coordinate gene expression and transcriptional control mechanisms.[20,21] However, cellular signaling events are driven by protein-protein interactions, post-translational protein modifications, and enzymatic activities that cannot be predicted accurately or described by transcriptional profiling methods alone.[22,23]

Activation of kinases and subsequent protein-protein interactions is an orchestrated event that uses select scaffolding proteins and specific protein phosphorylations and dephosphorylations.[24-26] Protein phosphorylation occurs at tyrosine, serine, and threonine residues, and these modifications, in turn, provide sites in which specific protein-protein interactions drive cellular signaling cascades.[24,25] In the complex tissue microenvironment the interconnections of kinases, phosphatases, their substrates, and the scaffolding proteins that tie them all together are a product of the cellular lineage, the local cellular microecology, and the pathologic state.[27]

Recently, antibodies have been developed to specifically recognize the phosphorylated

isoform of kinase substrates. In theory, it could be possible to evaluate the state of entire portions of a signaling pathway or cascade, even though the cell is lysed, by looking at dozens of kinase substrates at once through multiplexed phospho-specific antibody analysis. Protein microarrays offer the promise to dramatically multiplex, quantify, accelerate, and miniaturize this type of analysis over any existing format.[28,29] In this perspective, we summarize the translational potential of proteomics in the field of clinical oncology and patient-tailored combinatorial therapy.

PROTEIN POST-TRANSLATIONAL MODIFICATIONS: A RECORD OF ONGOING SIGNALING

Molecular networks of signaling cascades are driven by post-translational modifications in proteins (eg, phosphorylation or cleavage) that happen in a small subset of the total pool of proteins that can actively participate in signaling events. Unlike genomics, proteomics lacks a proteomic equivalent of a polymerase chain reaction for protein amplification. Thus the major challenge for translational applications of proteomics to patient-specific cancer network analysis is successfully measuring low-abundance post-translational events.

Before analysis of clinical specimens can take place, it is important to select a technology that is sensitive and can recapitulate linked kinase network events. Reverse-phase protein microarrays have the potential to meet this need.[29-34] Example data demonstrating such potential are presented in Figure 1. We analyzed a series of known connected phosphorylation substrates: epidermal growth factor receptor (EGFR; c-erbB1), MEK, and ERK kinases using a colon cancer cell line (CCL250) with overexpressed c-erbB1 receptor levels. On receptor-ligand binding, the EGFR becomes rapidly phosphorylated and downstream substrates (eg, MEK and ERK) of the cascade associate and become phosphorylated. When these events are analyzed by reverse-phase protein microarray, both ERK and MEK phosphorylation show a coordinately linked kinetic profile that is expected given that ERK is a direct substrate of MEK kinase (Fig 2). When the cells were pretreated with a specific MEK kinase inhibitor, as expected, phosphorylation of MEK was not altered. In contrast, phosphorylation of the downstream MEK kinase substrate, ERK, was suppressed. This example supports the utility of analyzing the phosphorylation of the kinase substrate itself as a surrogate for the upstream kinase activity. Phosphorylation of kinase substrates is a transient event, given that phosphatases promptly dephosphorylate the substrate as the cascade continues. Therefore, at any point in time, if two substrates are phosphorylated concurrently, it is likely that they are linked together to some extent in an active pathway, such as that seen with MEK and ERK kinase (Fig 1).

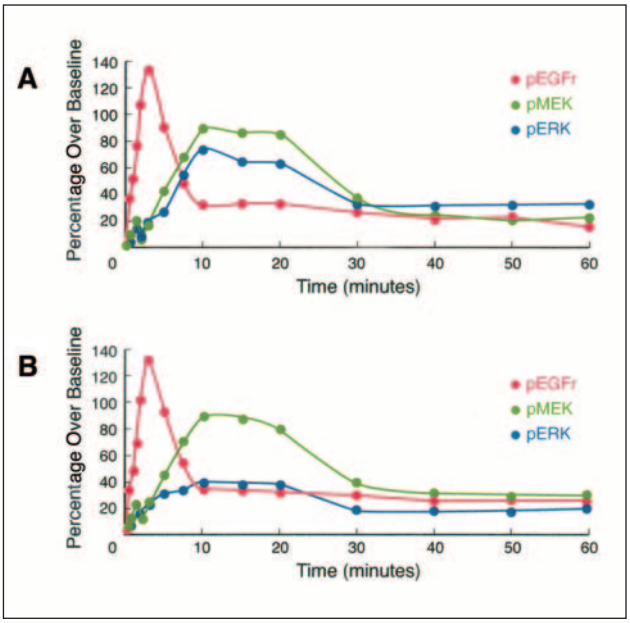

Fig 1. Molecular network analysis of defined pathways can be obtained using reverse-phase protein microarrays. One million CCL250 cells were treated, (A) with and (B) without 10 μmol/L of the MEK kinase inhibitor PD98059 for 60 minutes and then treated with 100 ng/mL of EGF (epidermal growth factor) for the times indicated on the x axis. Arrays were probed with phospho-specific antibodies.

Beyond the challenge of sensitivity, the next hurdles to overcome are the issues associated with the complexity of the tissue microenvironment and the cellular heterogeneity of tissue. In vivo cellular signaling events are an intimate product of the local cellular ecology, cell-cell interactions, and cell-matrix interactions, and include the influence of soluble factors such as cytokines and hormones. Thus, what is happening in the tissue may have little relationship to what is observed or predicted from cultured cells. The merging of microdissection technology with new types of protein microarrays has the potential to meet the challenge of sensitive analyte measurements with the correct tissue and cellular context. We provide example case studies that illustrate the feasibility of this approach.

FEASIBILITY CASE STUDIES

Translational Potential of Protein Microarrays for Routine Use in Clinical Research Specimens

To provide a foundation for the vision presented in this perspective, we conducted feasibility case studies using clinical specimens obtained under clinical trial research approved by the Institutional Review Board.

Laser capture microdissection (LCM) was used to procure an enriched starting population of cells. Microdissection is critical because every cell type may contain differences in its proteomic repertoire.[29] To study kinase

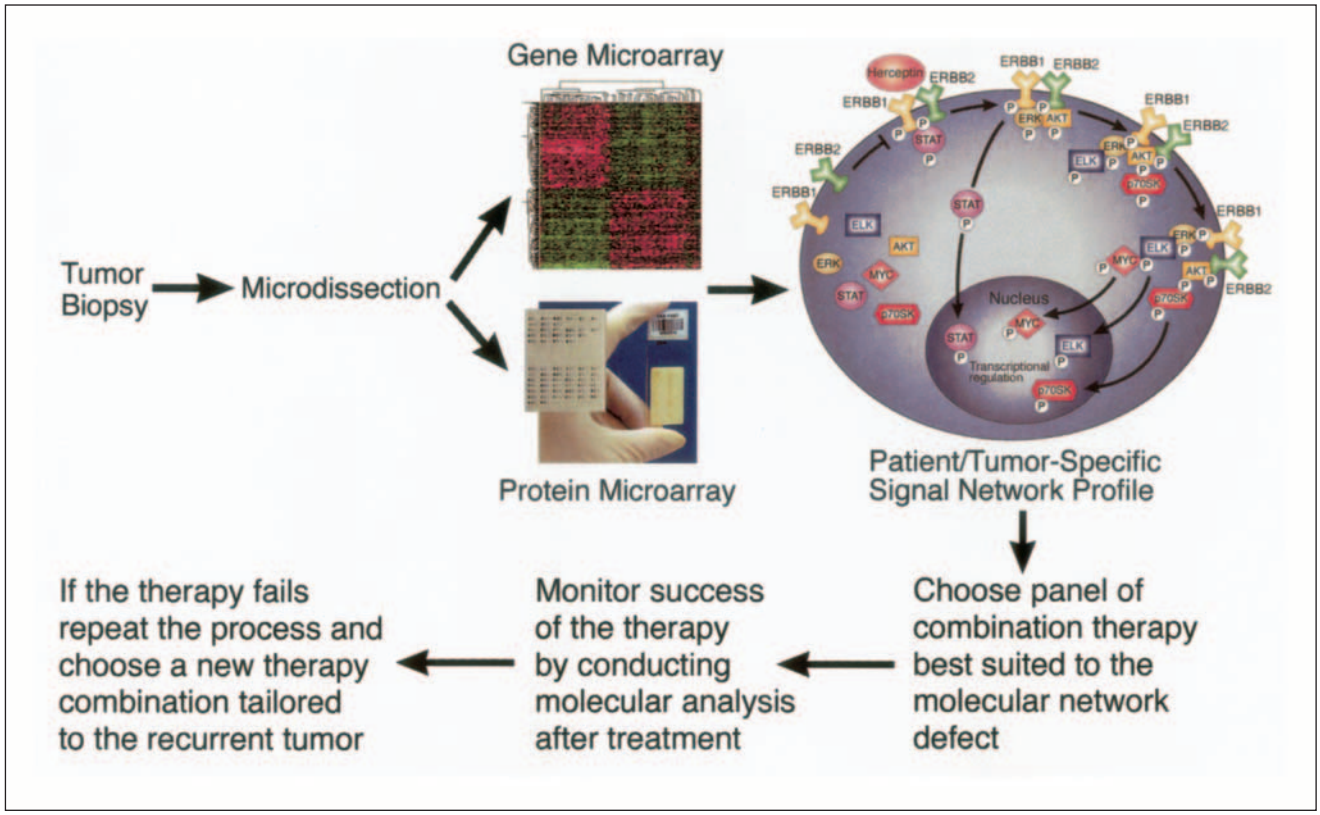

Fig 2. Individualized cancer therapy paradigm. After biopsy, molecular network analysis is conducted. A tumor-specific network portrait becomes the basis for combinatorial therapy selection. A panel of molecular target inhibitors is selected and administered. After therapy, the molecular network is re-evaluated for efficacy, and a new therapeutic regimen is considered depending on target assessment.

substrate and signal pathway analysis, multiple reverse-phase protein arrays containing immobilized cell lysates were constructed. Protein phosphorylation was detected using a set of antibodies that was validated for specificity and sensitivity using a subset of the LCM breast tissue. Before use for array-based investigation, all antibodies underwent extensive validation by Western blot analysis. These antibodies recognize the protein only when it is phosphorylated on a specific tyrosine and/or threonine residue substrate, effectively providing a readout for a specific upstream kinase activity, and recognize the protein regardless of phosphorylation state. A full list of the antibodies validated by our laboratory can be found at http://home.ccr.cancer.gov/ncifdaproteomics. The phosphorylation pattern attained from different discreet LCM cell populations in the same tissue specimen was reported to be reproducible using reverse-phase protein microarray technology.[29,32] In these studies, linearity, sensitivity, and inter- and intra-assay variability were assessed. Analysis was performed by measuring the relative intensity of each phosphorylation end point within multiple independent LCM samples procured from different regions within the same tissue sample. As a result of these previous studies, we determined that a pooled lysate consisting of about 25,000 epithelial cells procured from different regions of the tissue specimen gave reproducible and linear results for phosphorylation outcomes with a coefficient of variation of less than 10%.[29,32-34] The sensitivity was fully adequate to detect the phosphorylated subpopulations of low-abundance signaling proteins such as phosphorylated AKT.[32]

Network analysis of expected kinase substrate cascades was performed on a set of patients with high and low levels of AKT phosphorylation. Glycogen synthase kinase 3 (GSK3) and the Forkhead protein family (FKHRL/FKRH) are well-known AKT kinase substrates involved in glucose mobilization and energy metabolism as well as transcriptional regulation of pro-survival pathways.[35] Concordance was observed between phosphorylation of AKT and the downstream kinase substrates GSK3 and FKHRL/FKHR, but not with other substrates such as ER and STAT1 (Fig 3). The working hypothesis demonstrated by both cell line–based studies (Fig 1) and microdissected tissue cells (Fig 3) is that closely linked events will show tight correlation of phosphorylation (activation), whereas those phosphorylation events that are not coordinate are likely not to be directly linked in a network.

A meaningful network analysis, of course, should begin with data representing the status of a large number of

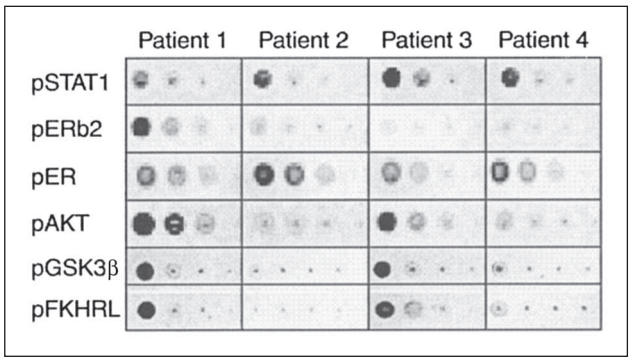

Fig 3. Kinase substrate analysis demonstrates concordance of AKT-mediated signaling using reverse-phase protein microarrays. Four patient samples, two with high and two with low levels of phosphorylated AKT (ser 473) were chosen and analyzed for phosphorylation of STAT1 (Tyr 701), c-erbB2 (Tyr 1248), AKT, GSK3β (Ser 9), and FKHRL/FKHR (Thr 32/Thr 24). STAT1, signal transducer and activator of transcription 1; ER, estrogen receptor; Erb2, erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) GSK3β, glycogen synthase kinase 3; FKHRL, forkhead protein family.

signaling phosphorylation events measured concomitantly. Bioinformatic analysis methods, such as those applied routinely to gene arrays, can be applied to this type of data to highlight phosphorylation events that appear simultaneously activated or suppressed, and therefore are potentially linked. Figure 4 shows the clustering results obtained using an unsupervised Bayesian clustering methodology for each of the 54 patients in the feasibility set (45 patients with cancer and nine normal patients) as a heat map on which relative protein levels are measured and colorized representing higher (red) or lower (green) relative expression. Clustering analysis was performed as described previously.[29]

In this feasibility set we noted a striking degree of heterogeneous signaling (Fig 3). Each patient's microdissected sample reflected a unique constellation of kinase-driven signaling events. This observation concurs with recent gene microarray analysis[36] and indicates that although some other cancers such as chronic myelogenous leukemia and stromal tumors are underpinned by a common pathway defect (eg, c-kit family signaling activation), sporadic human breast cancer and other epithelial tumors are controlled by a multiplex of protein circuitry derangements. Many of the normal epithelial signaling portraits are grouped together in large families that are distinct from the tumor fingerprint subsets (Fig 4). However, within the context of the underlying tumor heterogeneity, the clustering reveals larger subsets of tumors that have common pathway activation, some of which are expected and some of which are unexpected (Fig 4A). For example, the 10 patients at the top of the map are dominated by estrogen receptor overexpression and phosphorylation. Principal component analysis (Fig 4B) of the data reinforce the observation that a significant number of tumor and histologically normal epithelium can be distinguished from each other using the STAT1 and PKCα kinase substrates (43 of 45 cancers [95% CI, 85% to 95%] and 15 of 16 histologically normal epithelium [95% CI, 70% to 100%]). In keeping with this preliminary observation, in a neoadjuvant setting, PKCa phosphorylation was found to be dramatically decreased, or below the limits of detection in seven of eight patients (95% CI, 47% to 100%) with more than four positive lymph nodes compared with a relatively elevated level in eight of nine patients (95% CI, 52% to 100%) with fewer than four positive nodes.

Translational protein network signaling analysis is not restricted to the primary tumor site. Too often the presenting pathology is metastatic disease. Because the tissue microenvironment of the metastatic cancer cell is completely different from the primary tumor, there is no reason to expect that the phosphorylation events of the metastatic tumor cells will be identical to those of the primary tumor cells. Indeed, the state of signaling for the metastatic lesion may be the most appropriate basis for the selection of targeted therapy. An example of how the kinase substrate profile of the metastatic tumor is different from its primary tumor lesion is shown in Figure 5, which presents a case study of laser capture, microdissected, patient-matched primary colorectal tumor tissue and liver metastasis obtained simultaneously at surgery. In this small study set of three patient-matched primary and metastatic tumor specimens, 36 separate protein end points were measured. Thirty-two of these end points measured the phosphorylated form of specific kinase substrates. This preliminary result, using a small number of samples, could reflect important pathway changes influenced by the organ microenvironment.

Translational Applications of Proteomic Network Analysis: Patient-Tailored Combination Therapy

Evidence is emerging from gene microarray data to support the concept that each patient's cancer may have a unique complement of pathogenic molecular derangements. In our feasibility case studies, we found the same type of patient heterogeneity at the cell signaling level in human breast cancer, and colorectal cancer primary lesions compared with liver metastasis. In the face of such heterogeneity, we would expect no single targeted therapy to be equally successful for all patients. A given class of therapy may be effective for only a subset of patients who harbor tumors with susceptible and specific protein network defects. Such defects may have a direct or indirect basis in somatic genetic mutations within the tumor genome. At a functional level, the protein network alterations within the tumor presumably have been selected out because they provide a survival value for the individual tumor within its specialized tissue microenvironment.

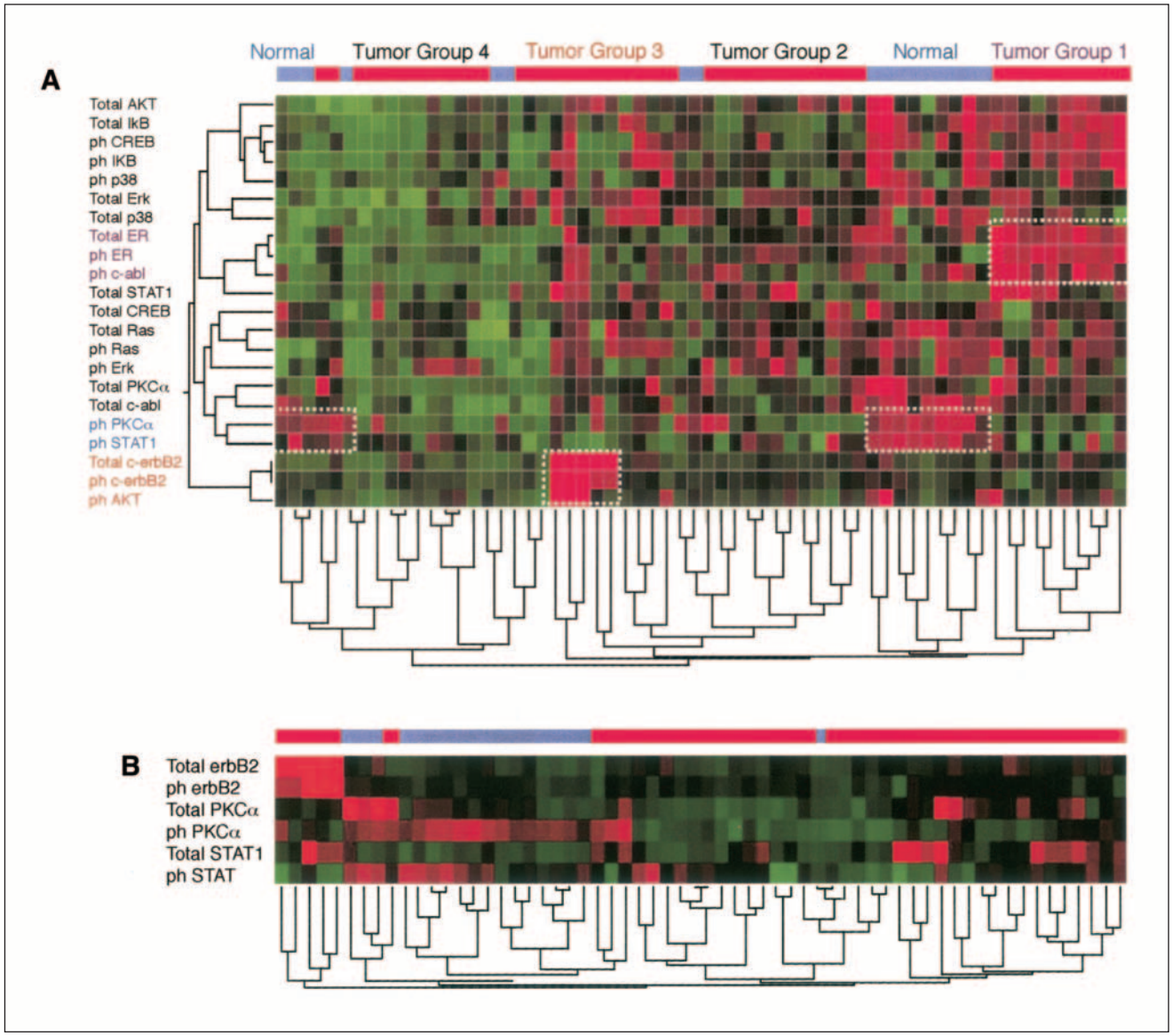

Fig 4. (A) Molecular network analysis of human breast cancer and normal breast epithelium. Twenty-two end points (11 matched phospho-specific and total protein-specific end points) of the 54-patient study set (normal = blue; tumor = red) were used. Example molecular networks and activated signaling pathways that separate patients into different groups (eg, tumor group 1) are identified by dotted boxes. (B) Subsequent principal component analysis of (A); normal, blue; tumor, red.

This conclusion is in keeping with the existing body of published clinical experience for molecular targeted cancer therapy. The recent literature has many examples of targeted therapies proving effective for only subsets of patient populations. EGF mutations in lung cancer correlate with the clinical response to gefitinib, an EGFR kinase inhibitor.[18,19] Mutational analysis of specific kinases (eg, c-kit, PDGFr) in gastrointestinal stromal tumors,[1-3] B-Raf in colorectal cancer,[6] PI3K in a variety of cancers,[7] or global mutational analysis of the so-called kinome,[8] all highlight the fact that patients will stratify into degrees of response to a selected targeted therapy. These studies emphasize the clinical need to understand more than the quantitative measurement of the expression of a protein analyte such as c-erbB1 and c-erbB2 when considering EGF-related therapeutic targeting. In fact, it would seem more important to elucidate the degree of activation of the pathway, the engagement with downstream network components, and the phosphorylation state of the molecules.[37]

Although cancer therapy has been directed at a single molecular target, in the future we can imagine targeting an entire set of interconnected kinase-driven events all along a deranged signaling pathway.[38] Interconnecting points within a signaling cascade are interdependent. This

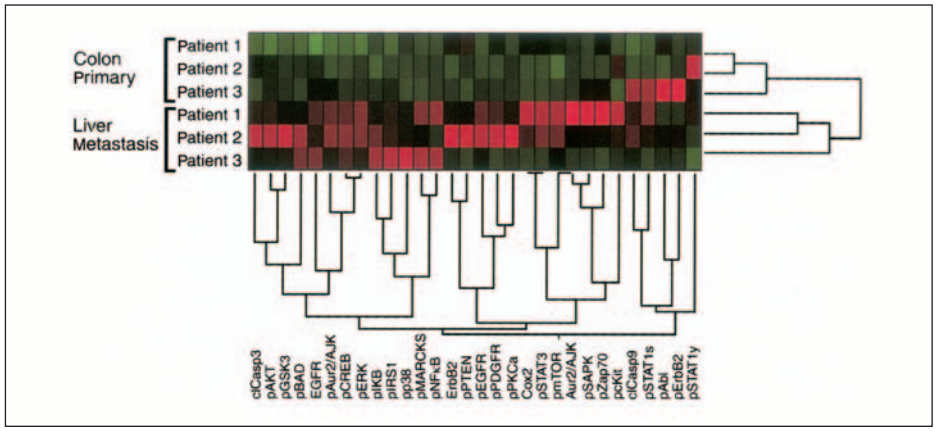

Fig 5. Signaling networks dramatically change after metastasis. Unsupervised clustering of a multiplexed kinase substrate heat map obtained by reverse-phase array analysis of laser capture microdissection procured cellular lysates. The study set consisted of three sets of primary colorectal cancer tissue with patient-matched hepatic metastasis obtained concomitantly at surgery.

is because a downstream phosphorylation event is driven by upstream events leading to the activation of the kinase acting on the target substrate. For example, as shown in Figure 2, phosphorylation of ERK is dependent on the activity of MEK, which is in turn driven by an entire series of upstream events. Consequently, in the context of this active pathway, a potential combinatorial therapeutic strategy would use an MEK inhibitor (the readout being ERK phosphorylation) and a Raf farnesyltransferase inhibitor (the readout being MEK phosphorylation). Given that the molecular network signaling pathways share an interacting and interdependent linkage, strategically selected combinatorial therapies could be given at a potentially lower dose, which could result in reduced toxicity compared with that seen using either agent alone. The potential reduction in toxicity combined with increased therapeutic efficacy is a promising hallmark of the potential for combinatorial therapeutics. Moreover, the likelihood of a tumor developing resistance to a cocktail of inhibitors that target an entire pathway could be significantly less probable.

Of course the biologic complexity of the cellular protein networks are not as simple as the box and arrow diagrams that are often drawn in research publications and vendor catalogs. The interconnections and cross talk are undoubtedly vast, and constantly assembling and dissolving. It has been theorized that cellular molecular networks can be modeled as scale-free communication linkages resembling power grids and the worldwide Web.[39-48] Efficient targeting of such a complex system will require the gathering of information about the following questions. In the neoplastic state of an individual tumor, what segments of the network are hyperactive, abnormally suppressed, or otherwise deranged? How does the network rewiring provide a survival advantage for the tumor cell? What are the weak points in the deranged network that can be used as a rational basis for targeting? What are the feedback loops and alternate pathway connections that influence the recovery of a tumor cell population after unsuccessful therapy? What are the interconnections within the relevant regions of the network that provide a rational selection for combinations of targeted therapy? Most (if not all) of this information is embodied or reflected by the protein post-translational modifications and protein-protein interactions. Existing and emerging proteomic technology is being developed for the purpose of gathering the necessary information to answer these critical questions. Although efforts are underway to begin developing an information repository for molecular networks, the elucidation of molecular networks is an evolving process with many gaps in our current knowledge base. The understanding of proteomic networks in actual human tissue specimens is even more limited, and how these networks truly interact (eg, regular, random, scale-free) and are perturbed in the disease microenvironment, will be critical components of effective therapeutic intervention strategies[38,46] and clinical trial design.[49-52]

In the past, a critical bottleneck for translational application of proteomics has been the paucity of validated antibodies with high specificity to the activated signaling molecules. It is encouraging that within the last few years, a large compendium of well-characterized and carefully validated phospho-specific antibodies have become commercially available. These antibodies can be used to discover new pathway interconnections and directly monitor therapeutic targets; however, they cannot be used as a de novo discovery tool. That is, these antibodies can only measure an occurrence that one knows beforehand may be important to measure. Identification of unknown phosphorylation events and new phosphorylation sites are an important component of ongoing proteomic research. A number of new and emerging proteomic technologies that use affinity capture reagents that enrich for phosphorylated proteins followed by mass spectrometry–based sequencing are being used for phosphoproteomic discovery.[53-59] These ongoing discoveries will result in an ever-growing list of phospho-specific end points with their respective antibodies. The two major applications of proteomic technology—discovery

and profiling—will be necessary driving forces for the advancement of translational clinical applications.

Translational Potential of Proteomics in Oncology: Concluding Vision

In the future, we can visualize a time when cancer patient management is an exercise in patient-specific network targeting (Fig 2). A named pathologic diagnosis, such as infiltrating ductal carcinoma, will be supplemented or replaced by a molecular profile map, which may even be unique to the individual patient. The information within the profile can then be used to select an optimal panel of therapies, best suited to the individual's tumor. After therapy administration, the molecular profile can be monitored by repeat biopsy or molecular imaging to judge the success of the therapy. Finally, if the therapy fails, the molecular profiling process can be repeated, and will become the basis for a revised therapy strategy that is, once again, best suited to the individual patient's tumor portrait.

Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. Other Remuneration: Emanuel F. Petricoin III, US Government; Lance A. Liotta, US Government. For a detailed description of these categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration form and the Disclosures of Potential Conflicts of Interest section of Information for Contributors found in the front of every issue.

REFERENCES

1. Heinrich MC, Corless CL, Demetri GD, et al: Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 21:4342-4349, 2003
2. Duensing A, Medeiros F, McConarty B, et al: Mechanisms of oncogenic KIT signal transduction in primary gastrointestinal stromal tumors (GISTs). Oncogene 23:3999-4006, 2004
3. Heinrich MC, Corless CL, Duensing A, et al: PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299:708-710, 2003
4. Lim YP, Diong LS, Qi R, et al: Phosphoproteomic fingerprinting of epidermal growth factor signaling and anticancer drug action in human tumor cells. Mol Cancer Ther 2:1369-1377, 2003
5. Druker BJ, Sawyers CL, Kantarjian H, et al: Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. N Engl J Med 344:1038-1042, 2001
6. Davies H, Bignell GR, Cox C, et al: Mutations of the BRAF gene in human cancer. Nature 417:949-954, 2002
7. Samuels Y, Wang Z, Bardelli A, et al: High frequency of mutations of the PIK3CA gene in human cancers. Science 304:554, 2004
8. Bardelli A, Parsons DW, Silliman N, et al: Mutational analysis of the tyrosine kinome in colorectal cancers. Science 300:949, 2003
9. Giaccone G, Herbst RS, Manegold C, et al: Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: A phase III trial—INTACT 1. J Clin Oncol 22:777-784, 2004
10. Slamon DJ, Leyland-Jones B, Shak S, et al: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344: 783-792, 2001
11. Wakeling AE, Guy SP, Woodburn JR, et al: ZD1839 (Iressa): An orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. Cancer Res 62:5749-5754, 2002
12. Arteaga CL: ErbB-targeted therapeutic approaches in human cancer. Exp Cell Res 284: 122-130, 2003
13. Baselga J, Rischin D, Ranson M, et al: Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. J Clin Oncol 20:4292-4302, 2002
14. Kris MG, Natale RB, Herbst RS, et al: Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: A randomized trial. JAMA 290:2149-2158, 2003
15. Herbst RS, Giaccone G, Schiller JH, et al: Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: A phase III trial—INTACT 2. J Clin Oncol 22:785-794, 2004
16. Pegram MD, Konecny G, Slamon DJ: The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of Herceptin (trastuzumab) therapy for breast cancer. Cancer Treat Res 103:57-75, 2000
17. Li B, Chang CM, Yuan M, et al: Resistance to small molecule inhibitors of epidermal growth factor receptor in malignant gliomas. Cancer Res 63:7443-7450, 2003
18. Paez JG, Janne PA, Lee JC, et al: EGFR mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science 304:1497-1500, 2004
19. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 350:2129-2139, 2004
20. Slonim DK: From patterns to pathways: Gene expression data analysis comes of age. Nat Genet 32:502-508, 2002
21. Ferrando AA, Neuberg DS, Staunton J, et al: Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 1:75-87, 2002
22. Ge H, Walhout AJ, Vidal M: Integrating 'omic' information: A bridge between genomics and systems biology. Trends Genet 19:551-560, 2003
23. Petricoin EF, Liotta LA: Molecular profiling of human cancer. Nat Rev Genet 1:48-56, 2000
24. Manning G, Whyte DB, Martinez R, et al: The protein kinase complement of the human genome. Science 298:1912-1934, 2002
25. Pawson T: Regulation and targets of receptor tyrosine kinases. Eur J Cancer 5:S3-S10, 2002 (suppl)
26. Spirin V, Mirny LA: Protein complexes and functional modules in molecular networks. Proc Natl Acad Sci U S A 100:12123-12128, 2003
27. Liotta LA, Kohn EC: The microenvironment of the tumour-host interface. Nature 411: 375-379, 2001
28. Nielsen UB, Cardone MH, Sinskey AJ, et al: Profiling receptor tyrosine kinase activation by using Ab microarrays. Proc Natl Acad Sci U S A 100:9330-9335, 2003
29. Liotta LA, Espina V, Mehta AI, et al: Protein microarrays: Meeting analytical challenges for clinical applications. Cancer Cell 3:317-325, 2003
30. Grubb RL, Calvert VS, Wulkuhle JD, et al: Signal pathway profiling of prostate cancer using reverse phase protein arrays. Proteomics 3:2142-2146, 2003
31. Wulfkuhle JD, Aquino JA, Calvert VS, et al: Signal pathway profiling of ovarian cancer from human tissue specimens using reverse-phase protein microarrays. Proteomics 3:2085-2090, 2003
32. Paweletz CP, Charboneau L, Bichsel VE, et al: Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. Oncogene 20:1981-1989, 2001
33. Nishizuka S, Chen ST, Gwadry FG, et al: Diagnostic markers that distinguish colon and ovarian adenocarcinomas: Identification by genomic, proteomic, and tissue array profiling. Cancer Res 63:5243-5250, 2003
34. Nishizuka S, Charboneau L, Young L, et al: Proteomic profiling of the NCI60 cancer cell lines using new high-density 'reverse-phase' lysate microarrays. Proc Natl Acad Sci U S A 100: 14229-14234, 2003

35. Mills GB, Kohn E, Lu Y, et al: Linking molecular diagnostics to molecular therapeutics: Targeting the PI3K pathway in breast cancer. Semin Oncol 30:93-104, 2003

36. Ma XJ, Salunga R, Tuggle JT, et al: Gene expression profiles of human breast cancer progression. Proc Natl Acad Sci U S A 100:5974-5979, 2003

37. Thor AD, Liu S, Edgerton S, et al: Activation (tyrosine phosphorylation) of ErbB-2 (HER-2/neu): A study of incidence and correlation with outcome in breast cancer. J Clin Oncol 18:3230-3239, 2000

38. Petricoin EF, Zoon KC, Kohn EC, et al: Clinical proteomics: Translating benchside promise into bedside reality. Nat Rev Drug Discov 1:683-695, 2002

39. Carter SL, Brechbuhler CM, Griffin M, et al: Gene co-expression network topology provides a framework for molecular characterization of cellular state. Bioinformatics 20:2242-2250, 2004

40. Jenster G: A visualisation concept of dynamic signaling networks. Mol Cell Endocrinol 15:218:1-6, 2004

41. Toroczkai Z, Bassler KE: Network dynamics: Jamming is limited in scale-free systems. Nature 428:716, 2004

42. Kunin V, Pereira-Leal JB, Ouzounis CA: Functional evolution of the yeast protein interaction network. Mol Biol Evol 21:1171-1176, 2004

43. Bar-Yam Y, Epstein IR: Response of complex networks to stimuli. Proc Natl Acad Sci U S A 101:4341-4345, 2004

44. Yook SH, Oltvai ZN, Barabasi AL: Functional and topological characterization of protein interaction networks. Proteomics 4:928-942, 2004

45. Hasty J, McMillen D, Isaacs F, et al: Computational studies of gene regulatory networks: In numero molecular biology, Nat Rev Genet 2:268-279, 2001

46. Bray D: Molecular networks: The top-down view. Science 301:1864-1865, 2003

47. Jansen R, Yu H, Greenbaum D, et al: A Bayesian networks approach for predicting protein-protein interactions from genomic data. Science 302:449-453, 2003

48. Bader GD, Hogue CW: An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics 4:2, 2003

49. Arteaga CL: EGF receptor as a therapeutic target: Patient selection and mechanisms of resistance to receptor-targeted drugs. J Clin Oncol 21:289S-291S, 2003 (23 suppl)

50. Arteaga CL, Baselga J: Clinical trial design and end points for epidermal growth factor receptor-targeted therapies: Implications for drug development and practice. Clin Cancer Res 9:1579-1589, 2003

51. Shawver LK, Slamon D, Ullrich A: Smart drugs: Tyrosine kinase inhibitors in cancer therapy. Cancer Cell 1:117-123, 2002

52. Gschwind A, Fischer OM, Ullrich A: The discovery of receptor tyrosine kinases: Targets for cancer therapy. Nat Rev Cancer 4:361-370, 2004

53. Metodiev MV, Timanova A, Stone DE: Differential phosphoproteome profiling by affinity capture and tandem matrix-assisted laser desorption/ionization mass spectrometry. Proteomics 4:1433-1438, 2004

54. He T, Alving K, Feild B, et al: Quantitation of phosphopeptides using affinity chromatography and stable isotope labeling. J Am Soc Mass Spectrom 15:363-373, 2004

55. Barati MT, Powell DW, McLeish KR: Proteomic approach to identification of novel kinase substrates in mesangial cells. Contrib Nephrol 141:231-244, 2004

56. Ficarro SB, McCleland ML, Stukenberg PT, et al: Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae*. Nat Biotechnol 20:301-305, 2002

57. Zhou H, Watts JD, Aebersold R: A systematic approach to the analysis of protein phosphorylation. Nat Biotechnol 19:375-378, 2001

58. Stancato LF, Petricoin EF: Fingerprinting of signal transduction pathways using a combination of anti-phosphotyrosine immunoprecipitations and two-dimensional polyacrylamide gel electrophoresis. Electrophoresis 22:2120-2124, 2001

59. Oda Y, Nagasu T, Chait BY: Enrichment analysis of phosphorylated proteins as a tool for probing the phosphoproteome. Nat Biotechnol 19:379-382, 2001