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REVIEW ARTICLE

SELDI ProteinChip® Array Technology: Protein-Based Predictive Medicine and Drug Discovery Applications

Guru Reddy and Enrique A. Dalmasso*

Ciphergen Biosystems, Inc, 6611 Dumbarton Circle, Fremont, CA 94555, USA

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Predictive medicine, utilizing the ProteinChip® Array technology, will develop through the implementation of novel biomarkers and multimarker patterns for detecting disease, determining patient prognosis, monitoring drug effects such as efficacy or toxicity, and for defining treatment options. These biomarkers may also serve as novel protein drug candidates or protein drug targets. In addition, the technology can be used for discovering small molecule drugs or for defining their mode of action utilizing protein-based assays. In this review, we describe the following applications of the ProteinChip Array technology: (1) discovery and identification of novel inhibitors of HIV-1 replication, (2) serum and tissue proteome analysis for the discovery and development of novel multimarker clinical assays for prostate, breast, ovarian, and other cancers, and (3) biomarker and drug discovery applications for neurological disorders.

INTRODUCTION

The ProteinChip Array technology is used for the discovery, validation, identification, and characterization of disease-associated proteins from biological samples. The versatile nature of this technology is enabling for a wide variety of applications in both research and clinical proteomics and will be reviewed in three application areas. A recent publication presents the use of the flexibility and power of the ProteinChip Array platform to elucidate the nature of novel protein inhibitors of HIV-1 replication, the molecules previously known as the $CD8^+$ antiviral factors. Numerous cancer-related publications have demonstrated the discovery and development of biomarkers and multimarker patterns for protein-based predictive medicine. Finally, we discuss a variety of drug discovery applications using Alzheimer's disease as the model system.

SELDI PROTEINCHIP® ARRAY TECHNOLOGY

The ProteinChip Array technology is based on surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) [1]. The key components of this technology are the ProteinChip Arrays, the ProteinChip Reader, and the associated software. ProteinChip Arrays use various chromatography surfaces or biological surfaces to capture proteins from complex biological mixtures according to their physicochemical properties. Chromatographic surfaces are composed of hydrophobic, hydrophilic, ion exchange, immobilized metal, or other chemistries. These surfaces are often used for profiling proteins from biological mixtures, for biomarker discovery, and for assay implementation. This is considered a *de novo* protein discovery approach in that no prior knowledge of the specific proteins is required as would be the case using antibody-based arrays. The activated surfaces are used to covalently immobilize specific bait molecules such as antibodies, receptors, or oligonucleotides often used for biomolecular interaction studies such as protein-protein and protein-DNA interactions.

Biological samples such as cell lysates, tissue extracts, or biological fluids are added to a spot on a ProteinChip Array and proteins are allowed to bind to the surface based on the general chromatographic or specifically designed biological affinity properties. The unbound proteins and mass spectrometric interfering compounds are washed away and the proteins that are retained on the array surface are analyzed and detected by SELDI-TOF-MS using a ProteinChip Reader (Figure 1). The MS profiles from the various sets of samples are then compared in a technique described as differential protein expression mapping, whereby relative expression levels of proteins at specific molecular weights are compared by a variety of statistical techniques and bioinformatic software systems [2].

DISCOVERY AND IDENTIFICATION OF HIV REPLICATION INHIBITORS

HIV has so far infected 40 million people and 20 millions have died of the AIDS disease. It is expected that

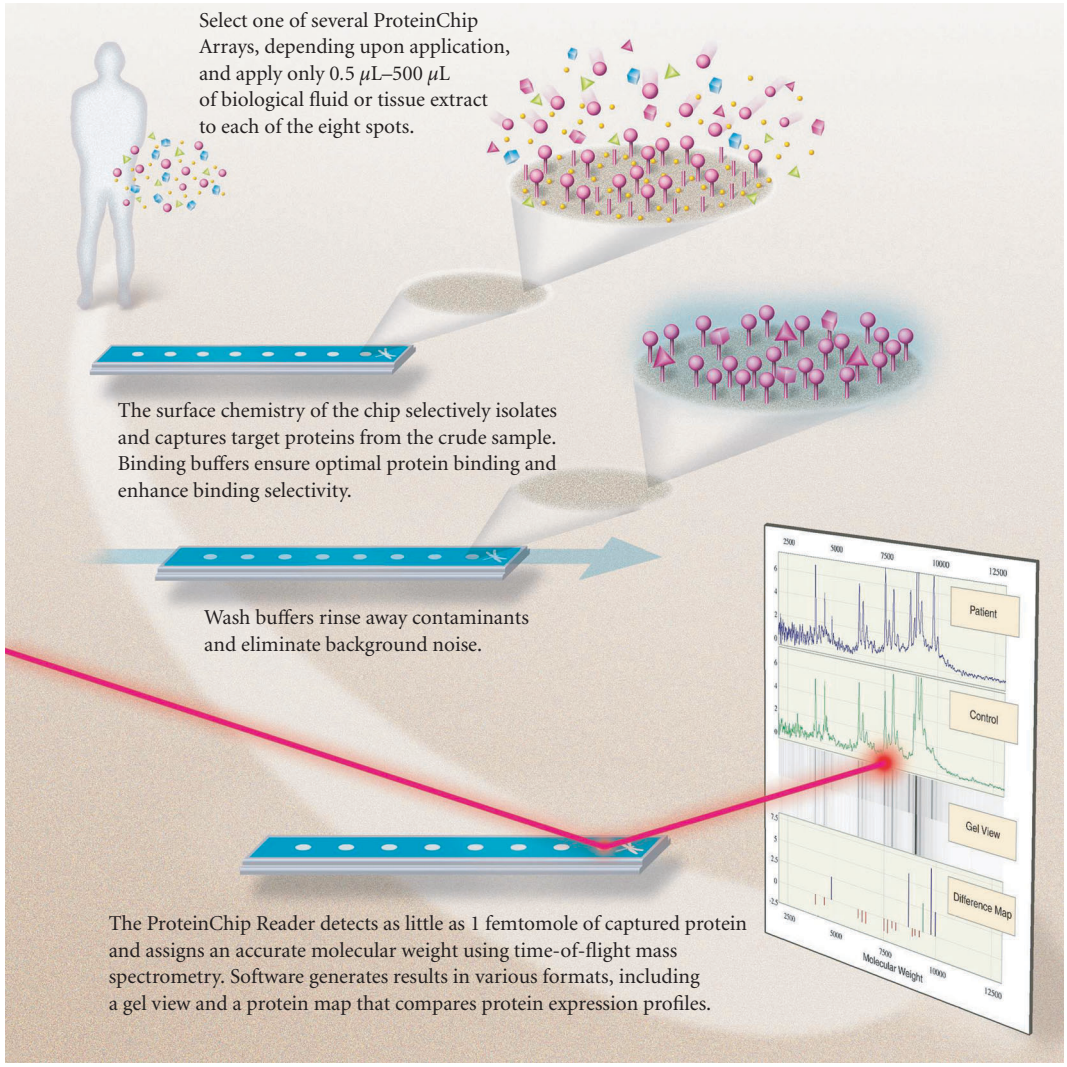

FIGURE 1. Protein profiling protocol. Procedure for preparing the ProteinChip Arrays with biological samples and for analyzing the retained proteins by SELDI-TOF-MS using a ProteinChip Reader.

around 3 millions more deaths will occur over the next 12 months, equating to over 9,000 deaths per day. Every day, 15,000 more people are infected. Certain individuals infected with HIV-1 virus remain in clinically stable condition for many years after the infection and are classified as long-term nonprogressors (LTNP). It has been known for some time that $CD8^+$ T lymphocytes from these individuals secrete a soluble factor, $CD8^+$ antiviral factor (CAF), that suppresses HIV-1 replication, irrespective of viral phenotype [3]. CAF is found in greater abundance in supernatants from LTNP $CD8^+$ cells and not usually detected from patients that progress to develop AIDS.

Despite enormous efforts by numerous laboratories over the past 16 years, the identity of CAF has remained elusive. Traditional approaches, including protein expression based on mRNA levels and several proteomic technologies, had failed to define the molecules responsible for the full extent of this activity. Several studies have shown that CAF lacks full identity to known chemokines. Interleukin 16 (IL-16) was suggested as the identity of CAF by Kruth and colleagues [4]; however, it is not present in all CAF-containing fluids and it only reduces HIV replication in an acute infection assay at high concentrations. Hence, it does not account for the CAF activity. It was postulated by Gallo and colleagues that beta-chemokines such as RANTES, MIP-1-alpha, and MIP-1-beta were responsible for the antiviral activity of $CD8^+$ cells [5]. But later it was shown that beta-chemokines can competitively block the R5 viruses that use CCR5 as a coreceptor but not the X4 viruses that use CXCR4 as a coreceptor. Since CAF can inhibit both types of viruses, beta-chemokines do not fully account for CAF activity.

Drs David Ho and Linqi Zhang and their colleagues from the Aaron Diamond AIDS Research Center were supported by the Ciphergen Biomarker Discovery Center® team in their discovery of the identity of CAF.

Using Ciphergen's ProteinChip System in their laboratory, Ho and colleagues discovered a cluster of low molecular weight proteins (3.3 to 3.5 kd) that were present in stimulated $CD8^+$ cells from normal individuals and LTNP, but not from patients progressing to AIDS. These unique proteins were enriched by ion exchange followed by reverse-phase chromatography. "This enrichment and purification process was monitored by SELDI-TOF-MS." Following enrichment, these proteins were then definitively identified as human alpha-defensin-1, -2, and -3 using Ciphergen's PCI-1000 ProteinChip Interface on a tandem MS system [6].

These findings were confirmed by in vitro inhibition of viral replication for many HIV-1 isolates. Antibodies specific for human alpha-defensins completely eliminated the CAF activity, while the specific antibody-based depletion of the molecules was demonstrated by an on-chip SELDI-TOF-MS assay. Furthermore, the authors demonstrated that commercially synthesized alpha-defensins also inhibit the in vitro replication of HIV-1. Taken together, all of these results indicated that alpha-defensin-1, -2, and -3 collectively account for most of the anti-HIV activity of CAF. Detailed mechanism and in vivo studies will define the modes of action and biological utility of the alpha-defensins. This discovery will have a profound impact on the development of novel AIDS therapeutic and diagnostic approaches. This is a powerful example demonstrating that in addition to the *de novo* discovery of biomarker and multimarker patterns, SELDI ProteinChip Array technology can identify novel protein therapeutic drug candidates and assign novel biological function to known proteins.

MULTIMARKER CLINICAL ASSAYS FOR CANCER

Cancer is the second leading cause of death in the Untied States, exceeded only by heart disease. In the USA, one out of every four deaths is from cancer. This year 555,000 Americans are expected to die of cancer, more than 1500 people a day. Discovery and development of better diagnostics and therapies are urgently needed to fight this deadly disease. Most current diagnostic tests detect cancer in advanced stages when treatment is often difficult and prognosis is poor. Numerous studies have shown that early detection of cancer increases treatment options and improves survival rates. Novel biomarkers can be discovered by comparing the differences in protein expression profiles between serum or tissue extract samples from cancer patients and normal individuals. SELDI ProteinChip Array technology has been used extensively to profile proteins and discover biomarkers in different types of cancers [7, 8, 9].

The laboratories of Drs George Wright, Jr, Daniel Chan, Lance Liotta, Emanuel Petricoin, and many others are currently using the SELDI ProteinChip Array technology for serum proteome analysis. These laboratories are focused on the discovery of markers and biomarker patterns for the early detection of prostate, breast, ovarian, and other cancers. The main objectives of these studies are to find signature proteomic patterns, or multimarker clinical assays, in serum that differentiate normal individuals from cancer patients and have clinical performance better than current single markers, thereby enabling more accurate diagnoses by accounting for the heterogeneity of cancer. In these studies, protein profiling data is generated by SELDI ProteinChip Array technology followed by analysis utilizing numerous types of multivariate software algorithms.

Research from these laboratories utilizing hundreds of serum samples per study has led to the discovery of multiple biomarkers or biomarker patterns that, when used in combination, have higher clinical sensitivity and specificity relative to the best available single-marker assays. For example, Wright and colleagues [7] discovered that a SELDI multimarker profile combining nine different proteins generates an assay with better sensitivity (83%) and specificity (97%) for diagnosis of prostate cancer than the prostate specific antigen (PSA) test. Chan and colleagues [8] demonstrated that when three newly discovered biomarkers for breast cancer are used in combination, the SELDI assay has a significantly higher sensitivity (93%) and specificity (91%) relative to CA15.3, the best available protein marker. For ovarian cancer, Liotta and Petricoin et al [9] demonstrated that a SELDI multimarker profile has sensitivity (100%) and specificity (95%), compared to the poor performance of the CA125 test.

While studying serum protein profiles is helpful in the early diagnosis of cancer, the final confirmation and staging of the disease comes from examining the tumor tissue itself. Since tumor tissue is heterogeneous in nature, new molecular techniques are needed to study the biomarkers that are present. Laser capture microdissection (LCM) developed by Liotta and colleagues at the National Institutes of Health has enabled researchers to selectively procure pure population of cells from a stained tissue section under direct microscopic visualization. The comprehensive analysis of several cancer samples using SELDI-TOF-MS to generate tissue-specific profiles of LCM-procured samples has been reported [10, 11]. Wright and colleagues [11] used ProteinChip Array technology to discover several protein biomarkers, in cells procured by LCM, that were specifically distinguished prostate cancer cells from surrounding normal prostate cells from the same patient. Pawaletz et al [10] analyzed protein profiles from patient-matched normal, premalignant, malignant, and metastatic microdissected cell populations from human esophageal, prostate, breast, ovary, colon, and hepatic tissue sections by SELDI-TOF-MS. They obtained reproducible and discriminatory protein biomarker profiles that differentiated normal cells from tumor cells and discriminated different tumor types. Coupling LCM for specific cell procurement with SELDI ProteinChip Array technology has a tremendous potential for the discovery

of specific biomarkers that are associated with each stage of tumor development.

Both serum and tissue proteome analysis by SELDI ProteinChip Array technology will have great utility in protein-based predictive medicine as demonstrated in these studies. Detailed molecular analysis of tissue has great potential to assist with improved definition of tumor aggressiveness and patient prognosis, and to assist with selection of appropriate treatment option. Also, SELDI ProteinChip Array multimarker serum protein patterns appear to perform significantly better in diagnosing different types of cancers than currently used single-marker assays. If these multimarker SELDI-TOF-MS protein profiles are validated in larger and more clinically diverse study sets, this approach can have immediate and substantial benefit for the early detection of many kinds of cancers. As these studies expand, applications for protein-based predictive medicine will further develop by establishing multimarker serum assays that address the more defined clinical questions described above but with a more easily acquired biological sample, possibly when the solid tumor itself cannot be located.

BIOMARKER AND DRUG DISCOVERY APPLICATIONS IN NEUROLOGICAL DISORDERS

The SELDI ProteinChip technology has been used by several researchers to discover novel biomarkers and multimarker panels that are relevant to the diagnosis and patient stratification for Alzheimer's disease, Parkinson's disease, multiple sclerosis, schizophrenia, HIV-induced dementia, and so forth. In this section, we focus on drug discovery applications of SELDI ProteinChip technology in Alzheimer's disease (AD). As the most prevalent form of neurodegenerative disorders, AD affects about 4 million people in the USA, generally people over the age of 65. Clinical features of AD include beta-amyloid deposits in brain, neuritic plaques, and degeneration of synapses [12]. In the familial forms of AD, mutation in the amyloid precursor protein (APP) or in a presenilin gene (PS1 or PS2) leads to increased amounts of the 40- and 42-amino acid beta-amyloid peptides that are primary components of plaques. The peptides originate from the proteolysis of APP by two proteases known as beta-secretase and gamma-secretase [13]. The advantage of using ProteinChip technology for the analysis is that they can be detected directly from a wide range of samples including cell culture supernatant, CSF, and brain/nerve lysates. Moreover, very low amounts of sample are required.

All of the beta-amyloid peptides share a common N-terminal sequence, so an antibody that is specifically raised against this N-terminal sequence can be immobilized on the ProteinChip Array and used to capture beta-amyloid peptides from complex samples. The SELDI-TOF-MS analysis of such a capture can be used to monitor the relative amounts of peptides of various lengths, including many from beta-amyloid 1–15 to 1–42, some of which correlate more strongly with the development of AD than others. This assay has been used successfully to profile peptide variants secreted into the media of cultured human neuronal cells that express the APP [14]. In addition, this assay was used to establish that BACE-1 is the beta-secretase that is involved in the generation of beta-amyloid peptides by neurons [15] and to discover candidate biomarkers for AD from human CSF (unpublished results, 2002). Secretases are thought to be potential drug targets and this assay has been widely used to discover novel secretase inhibitors [16], to study the function of secretase inhibitors [17], and to monitor the changes of beta-amyloid in serum and brain mediated by beta-amyloid vaccination [18]. The drug discovery capabilities of SELDI ProteinChip technology have been best demonstrated in the various uses of this on-chip assay for monitoring relative changes in various lengths of the beta-amyloid peptides.

DISCUSSION

In this review, we have described a number of recent publications that demonstrate the power of the SELDI ProteinChip Array technology when utilized directly by the translational medicine clinical researcher. The technology enables rapid testing of a clinical hypothesis, which greatly enhances and accelerates discovery potential and will enable protein-based predictive medicine. In the first example, demonstration of alpha-defensin-1, -2, and -3 as novel inhibitors of HIV-1 replication, the technology was instrumental in discovering and identifying a set of small proteins that had eluded researchers for 16 years. In the second set of examples, we summarized results from several publications presenting serum and tissue proteome analyses. These studies describe the discovery and development of multimarker clinical assays for prostate, breast, ovarian, and other cancers. These assays hold great promise for the early detection of cancer and for development of protein-based predictive medicine. Finally, in the third set of examples, we described publications that show the utility of the technology as demonstrated by drug discovery applications for AD. In addition to novel applications for protein-based predictive medicine, the technology is powerful in its ability to discover and characterize novel protein drug candidates, protein-protein interactions, and signal transduction pathways in the tumor cells which would in turn be used to customize therapy specific for each individual.

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* Corresponding author.
E-mail: edalmasso@ciphergen.com
Tel: +1 510 505 2245