Therapeutic Cancer Vaccines and Translating Vaccinomics Science to the Global Health Clinic: Emerging Applications Toward Proof of Concept

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Abstract

As vaccines evolve to be a more common treatment for some cancers, further research is needed to improve the process of developing vaccines and assessing response to treatment. Vaccinomics involves a wide-ranging integration of multiple high throughput technologies including transcriptional, translational, and posttranslational population-based assessments of the human genome, transcriptome, proteome, and immunome. Vaccinomics combines the fields of immunogenetics, immunogenomics, immunoproteomics, and basic immunology to create vaccines that are tailor made to an individual or groups of individuals. This broad range of omics applications to tumor immunology includes antigen discovery, diagnostic biomarkers, cancer vaccine development, predictors of immune response, and clinical response biomarkers. These technologies have aided in the advancement of cancer vaccine development, as illustrated in examples including NY-ESO-1 originally defined by SEREX, and HER2/neu peptides analyzed via high-throughput epitope prediction methods. As technology improves, it presents an opportunity to improve cancer immunotherapy on a global scale, and attention must also be given to utilize these high-throughput methods for the understanding of cancer and immune signatures across populations.

Introduction

VACCINES ARE EMERGING as a standard treatment for some cancers. After a randomized Phase III trial of patients with metastatic prostate cancer receiving a vaccine targeting prostatic acid phosphatase (sipuleucel-T) versus placebo demonstrated a 22% reduction in risk of death, the first therapeutic cancer vaccine was both approved by the Food and Drug Administration for use in the United States and added to the compendium of cancer treatments published by the National Comprehensive Cancer Network (NCCN) as a "category 1" (highest recommendation) treatment for castration-resistant prostate cancer in 2010 (Kantoff et al., 2010; www.nccn.org Retrieved December 1, 2010). A survey of the United States National Institutes of Health Clinical Trials registry Website revealed over 20 Phase III trials actively evaluating therapeutic vaccination for various types of cancer [(www.clinicaltrials.gov Retrieved December 1, 2010, The NCCN Drugs & Biologics Compendium (NCCN Compendium™)].

The development of effective cancer vaccines and methods to assess the efficacy of cancer vaccines are areas of intensive research. Uniting the fields of vaccinology and tumor immunology with high-throughput omics sciences will improve vaccine design across populations as well as the identification

of immune-related biomarkers for diagnosis, prognosis, and assessment of immune response. Vaccinomics involves global population based studies, including the consideration of genetic polymorphisms across groups and their relationship to disease susceptibility and clinical response to immune-based therapies. At present there are no widely used vaccinomics approaches validated in the cancer immunotherapy literature as a means to study the effect of immunity on cancer. However, the literature has demonstrated that in many instances a clinically effective antitumor immune response is associated with acute inflammation, and that a general "immune signature" of tissue rejection may predict for immunity-induced tumor destruction (Disis, 2010). Thus, in addition to an appraisal of what has been studied in cancer, this focused review of cancer vaccinomics extrapolates in some instances, from the autoimmune, infectious disease, and allograft rejection omics literature, given that the mechanism of an acute inflammatory response leading to tissue destruction is a common thread that unites these disciplines (Wang et al., 2008).

Vaccinomics Techniques

A comprehensive systems biology approach to cancer immunotherapy involves the integration of multiple

high-throughput omics technologies from a transcriptional, translational, and posttranslational standpoint. Tools available to achieve such integration include DNA sequencing analysis (genomics) as well as more focused epidemiologic genome-wide association studies (GWAS) to compare how genes vary from individual to individual. GWAS is critical to identify single nucleotide polymorphisms (SNPs) associated with a disease state or response to treatment. Gene expression profiling of mRNA (transcriptomics) can be employed in the analysis of either the tumor or peripheral blood mononuclear cells for prognostic or predictive information. Furthermore, serologic evaluation of proteins and antibodies (proteomics and immunoproteomics) can be useful for diagnostics, vaccine design, and immune response assays. Each technology has specific advantages and disadvantages as well as variations in feasibility (Nambiar et al., 2010). For example, a special consideration for the global translation of omics technologies as clinical biomarkers includes tissue source, with preference toward an easily accessible bodily fluid such as blood. Other considerations involve cost of the approach, ease of reproducibility, and the ability to validate results with these tools. An integrative analysis of high-throughput omics technologies will be discussed below in the context of their potential applications related to therapeutic cancer vaccines.

Computational Vaccinology

For the development of cancer vaccines that target specific tumor-associated antigens (TAA), epitopes are valued as the portions of the antigens that interact with MHC complexes to stimulate a T-cell-associated immune response. Immunoinformatics technologies have allowed for a more streamlined approach to vaccine design by identifying epitopes recognized across multiple alleles of major ethnic groups throughout the world (Kessler and Melief, 2007). Multiple Web-based modeling programs are now available to predict both MHC Class I and Class II binding affinity *in silico* [examples include SYFPEITHI (Institute for Cell Biology, Heidelberg, Germany), Propred (Institute of Microbial Technology, Chandigarh, India), Rankpep (Harvard, Boston, MA), and MHCpred (Jenner Institute for Vaccine Research, Compton, Berkshire, UK)] (Guan et al., 2006; Rammensee et al., 1999; Reche et al., 2002; Singh and Raghava, 2001).

MHC Class I epitopes, processed intracellularly and recognized by cytotoxic T lymphocytes (CTL), are smaller than class II epitopes and bind very specifically to HLA alleles. Indeed, investigators have shown that MHC Class I binding affinity is critical, as the extent that epitopes are able to bind determines an epitope's immunogenicity (Sette et al., 1994). Multiple binding algorithms, rather than just a single program, are typically used to predict epitopes, as was shown with the development of Class I peptides from the tumor antigen CEA. Studies demonstrated the use of multiple binding algorithms greatly improved the ability to detect class I peptides which elicited T cells capable of lysing tumor cells expressing HLA-B7 and CEA (Lu and Celis, 2000). It has become clear that intracellular Class I ligand processing is quite complex, and includes TAP processing of peptides into the endoplasmic reticulum as well as proteasomal digestion into short Class I ligands. One of the initial studies that considered proteasomal processing in tumor vaccine design was an analysis of CTL epitopes for the tumor antigen PRAME. The

investigators added *in vitro* proteasome-mediated digestions of polypeptides containing candidate epitopes to complement motif-based Class I binding predictions for the development of peptides that induced CTL responses against PRAME-expressing tumor cell lines (Kessler et al., 2001). More recently, many integrated *in silico* Class I epitope prediction algorithms have been developed to combine predictions of proteasomal cleavage, TAP translocation and HLA class I epitope recognition for improved accuracy (Kessler and Melief, 2007). In addition to Class I considerations, more advanced systems for Class II epitope prediction are also in development.

HLA Class II epitopes, recognized by T helper cells, can also be utilized for vaccine design spanning multiple alleles. CD4⁺ T cells are becoming more recognized as essential in mounting a more efficient CTL response against tumors. Unlike MHC class I, the Class II molecules interact with larger peptides and may be more promiscuous due to the binding groove of Class II being open at both ends to allow peptides of varying size to bind. In a study by Salazar and colleagues (2003), competitive inhibition assays were used to analyze class II HER-2/*neu* peptides for their binding affinity to 14 common HLA-DR alleles. The peptides had been used in a clinical trials and their ability to elicit both peptide and protein specific T cells was established. The peptides that had been shown to be native epitopes of HER-2/*neu* *in vivo* were those that demonstrated high *in vitro* binding affinity to multiple HLA-DR alleles, that is, promiscuous epitopes. Thus, this study was the one of first to show that *in vitro* binding affinity could predict the *in vivo* immunogenicity of class II peptides. This model has been described further more recently for insulin-like growth factor binding protein 2 (IGFBP-2) peptides, in which predicted peptides most likely to be native class II epitopes of self proteins bind with high affinity across multiple HLA-DR alleles (Kalli et al., 2008; Park et al., 2008). Using Web-based modeling, a high-throughput approach has been developed for prediction of epitopes that are presumed high-affinity binders promiscuous to multiple alleles. The methods such as described by Park and colleagues identifies peptide candidates that range from 15–25 amino acids in length, each assigned a score that accounts for the overlaps occurring within and across modeling algorithms. A final binding score is calculated as the sum of scores across the three algorithms multiplied by the number of HLA alleles where each amino acid was predicted to have high affinity binding. For ease of visualizing these “immunogenic hotspots,” a heatmap can be constructed whereby each amino acid is assigned a color shade based on quartiles from lowest to highest binding score. The antigens can also be graphed by amino acid binding score in order to choose peptides and determine presence of extended regions containing high affinity binding epitopes (Fig. 1). By utilizing this approach, epitopes can be designed for use in a therapeutic vaccine that should target HLA Class II alleles for greater than 90% of major ethnic groups. Although MHC Class II-restricted vaccine design has historically been less commonly employed in vaccine formulations, it will likely become more extensively utilized as combinations of Class II epitope prediction algorithms demonstrate improved reliability.

Reliability is clearly an important concern in the use of *in silico* epitope prediction programs for both MHC Class I and Class II. Because overlap between programs may be low, the use of multiple programs remains strongly advisable

FIG. 1. *In silico* assessment of MHC binding peptide sequences. Graph shows epitope binding scores for 15–25 amino acid sequences along a protein sequence for a tumor antigen. The epitopes receiving the highest binding scores are considered “immunologic hotspots.”

(Kessler and Melief, 2007). Furthermore, after the prediction phase is completed, formal *in vitro* immunogenicity studies are needed to validate use of specific epitopes for cancer vaccine design. In summary, immunoinformatics tools such as epitope prediction algorithms are a valuable complement to wet-lab studies for simpler and more efficient development of immunotherapies, in particular, cancer vaccines.

Intratumoral Immune Signatures

Assessment of gene expression profiles in tumors or tumor immune infiltrates has been performed both in evaluation of prognosis and as a predictor of response to immunotherapy. These types of assessments work best when serial tumor biopsies are feasible and available for evaluation. The bulk of the data in humans so far has been focused on prognostics. Galon and colleagues (2006) reported that gene expression profiling of tumor infiltrating lymphocytes in colorectal cancer was a better predictor of patient survival than standard histopathological methods, with upregulated genes associated with Type I (Th1) immune response strongly associated with lower recurrence rates ($p < 0.05$). Gene expression arrays of cancer cells have similarly demonstrated a correlation between an “immunologic signature” of immune response-associated genes and improved prognosis, as demonstrated extensively in the literature for breast cancer, lung cancer, and melanoma (Bogunovic et al., 2009; Mandruzzato et al., 2006; Reyat et al., 2008; Roepman et al., 2009; Staaf et al., 2010; Teschendorff et al., 2007). To date, these immunologic signatures have not been validated outside of clinical protocols.

Conversely, less is known regarding validated-specific intratumoral immunologic signatures that could be predictive of immune response to immunotherapies such as cancer vaccines. Rates of response to cancer immunotherapy vary widely. Given similarities in the process of acute inflammation leading to tissue destruction, one could extrapolate from the acute allograft rejection literature. One study revealed a clustering of upregulated inflammatory genes associated with interferon-gamma (IFN- γ) and granzyme molecules linked with biopsy samples of patients undergoing acute renal allograft rejection compared to tissues derived from the same patients during periods of stable graft function (Sarwal et al., 2003). Preclinical studies in mouse cancer models have provided an ease of access to tissue for the evaluation of immune rejection signatures in tumors. Investigators compared transcriptional patterns within the tumor microenvironment of

mouse mammary carcinoma cells that underwent rejection with those that resisted rejection due to mechanisms of tumor evasion. Gene profiles confirmed that immune rejection of tumor cells, in this model, is mediated through Type I immunity, whereas lack of immune responsiveness is associated with increased expression of immunosuppressive cytokines such as IL-10, that is, Type II (Th2) immunity (Lu et al., 2010; Worschech et al., 2008). This “rejection signature” has now been reported in preliminary studies of human tumors for prediction of immune response. In an innovative prospective analysis of gene expression profiles by DNA microarray in humans, serial fine needle aspiration (FNA) biopsies were obtained from tumors of patients with metastatic melanoma before and after various immunotherapy regimens were administered in order to identify candidate predictors of immune responsiveness and further understand pathways of immune rejection (Wang et al., 2002). Although the study did not identify genes associated with clinical outcome, approximately 30 genes differentially expressed in pretreatment samples were found to correlate with clinical response ($p < 0.001$); interestingly, about half of the genes preferentially expressed in the responding melanoma lesions were associated with T-cell regulation, suggesting that it may be possible to identify tumor microenvironment immune response genes or “rejection signatures” in cancer that could predict which patients should receive immunotherapy or are responding to immunotherapy. Researchers from the same group also performed similar analysis to evaluate early transcriptional pathways associated with immune rejection in basal cell carcinoma punch biopsies before and after topical immunotherapy with the toll-like receptor-7 agonist imiquimod compared with placebo (Panelli et al., 2007). The study documented an association between immune rejection and induction of genes associated with cellular and innate immune effector functions, including IFN- γ and genes associated with IFN- γ expression, natural killer cells, and cytotoxic T cells. Notably, these omics studies in cancer patients took advantage of evaluating tumor types that were readily accessible by FNA or punch biopsy, and may be more useful in driving tumor immunology hypotheses rather than translating to globally utilized biomarker assays.

Peripheral Blood Signatures

One important consideration in the development of high-throughput techniques related to cancer immunotherapy is

tissue source, with preference toward easily accessible products such as blood rather than the need for serial tumor biopsy, which is not as feasible in some tumor types. Blood-based technologies can focus on interrogation of antibody-rich serum (immunomics) or gene expression signatures from peripheral blood mononuclear cells (PBMC). Serologic screening can identify biomarkers not only for early detection of cancer but also potential targets for vaccine development and even serologic signatures of immune response predictive of improved outcome. Although PBMC signatures have not been well validated in tumor immunology, they have again been described in the autoimmune, infectious disease, and solid tumor allograft rejection literature in the characterization of acute inflammatory response that can be extrapolated to tumor immune rejection. An overview of peripheral blood-based omics technologies that could be applied to tumor immunology are assessed below.

Cancer immunomics

Antibodies detected in the serum can aid in the identification of tumor-associated antigens as potential biomarkers for the early diagnosis of cancer or as identification of therapeutic vaccines targets. Multiple tools described below are available for serologic analysis (Fig. 2). *SEREX*. One powerful technique

for evaluation of humoral immunity to tumor antigens is Serological Screening of cDNA Expression Library (*SEREX*) (Sahin et al., 1995). This approach uses a tumor cDNA library in lambda-phage vectors expressed in *Escherichia coli* that are transferred to nitrocellulose membrane and incubated with sera from cancer patients versus control donors. The clones reactive to cancer patient sera are then identified and sequenced. So far, thousands of TAAs have been identified and published using *SEREX* (Lu et al., 2008). A notable *SEREX*-defined tumor antigen is NY-ESO-1, which was discovered from an esophageal cancer cDNA library and has since been tested in over 30 clinical trials of therapeutic vaccination for a variety of tumor types (Chen et al., 1997; Old, 2008). *SEREX* is also being utilized in transgenic mouse models to predict human TAAs, as has been demonstrated with multiple mouse tumor antigens with human homologues identified from the neu-transgenic mouse (Lu et al., 2006). The successful incorporation of animal models in cancer immunomics research not only allows for prioritization of potential immunologic targets but also provides opportunities to evaluate humoral immunity at multiple timepoints along the disease continuum of oncogenesis. *SEREX* databases also provide the opportunity for public access of immunomics data as well as the ability to contribute data. There is now a publicly available Cancer Immunome Database through the Academy of Cancer

FIG. 2. Methodology for identification of TAAs using *SEREX*, *SERPA*, or *MAPPING*, and protein array. (A) *SEREX* involves the use of tumor cDNA made into a lambda phage library, infection of bacteria, transfer of expressed antigen to membrane to screen with sera, and identification of positive clones. (B) Tumor proteins evaluated by 2D immunoblot for *SERPA* and 2D immunoaffinity chromatography for *MAPPING* can be further identified using mass spectrometry. (C) Protein microarray chips can be prepared with spots of fractionated tumor proteins probed with patient sera and TAAs identified by mass spectrometry.

Immunology and the Ludwig Institute for Cancer Research, which describes gene products against which there is a document immune response in cancer patients as defined by SEREX and more recently other immunomics technique (<http://ludwig-sun5.unil.ch/CancerImmunomeDB>). SEREX remains a useful immunomics tool for working with a large cDNA library to identify multiple TAAs, and it serves as a platform for global data sharing related to antigen discovery. Limitations of SEREX include the use of *E. coli* as the expression system rather than mammalian-based systems that take into account posttranslational modifications; thus, other proteomics-based sciences have also been employed in the evaluation of autoantibodies.

SERPA/MAPing. Other immunomic modalities for autoantibody biomarker identification include either two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) isolation or two-dimensional immunoaffinity chromatography followed by proteolysis and mass spectrometry identification of the resulting peptides to interrogate with sera of cancer patients versus controls, called serological proteome analysis (SERPA) and multiple affinity protein profiling (MAPing), respectively (Hardouin et al., 2007; Klade et al., 2001). These tools are both very robust; SERPA remain relatively labor intensive with limitations in sample capacity while MAPing may be more amenable to automation in the future.

Protein arrays. Similar proteomics approaches with more high-throughput capabilities than SERPA or MAPing include automated protein microarrays on which serum antibodies from cancer patients versus controls can be evaluated against thousands of proteins at a time, typically derived from cDNA libraries or peptide-phage display libraries, as demonstrated for serologic evaluation of prostate, lung, breast, and ovarian cancer (Anderson et al., 2008; Chatterjee et al., 2006b; Qiu et al., 2004; Sioud and Hansen, 2001; Wang et al., 2005; Zhong et al., 2005). Protein arrays can either be biased with a known panel of proteins probed, or they can be unbiased, where tumor protein is fractionated and separated before being printed on a chip (Gunawardana and Diamandis, 2007). Unbiased approaches are more useful for TAA discovery because they include unknown proteins. However, in a successful application of biased protein arrays, Fong and colleagues (2009) assessed the induction of antibody responses to TAAs in the study of combined immunotherapy treatment using CTLA4 blockade (ipilimumab) plus GM-CSF in patients with metastatic castration-resistant prostate cancer. Array-based screening with phage-spotted immunoblot was used to study antibody responses to 30 known cancer-testis antigens (CTA) in patient sera before and after treatment. The development of antibodies to NY-ESO-1 posttreatment was identified in one clinical responder and one nonresponder, suggesting that immunotherapy can induce immune responses to other known TAAs. Although libraries are one source of protein for immunomic arrays, other sources include recombinant proteins or fractionated tumor lysates. Recombinant proteins for the arrays are more pure but also more costly and may not account for posttranslational modifications, whereas tumor cell proteins may retain their natural conformation but present more challenges related to fractionation and reproducibility. In order to further

take into account the effects of posttranslational modification on epitope recognition with respect to aberrant glycosylation of tumor proteins, a very recent advance includes a high-throughput O-glycopeptide discovery platform for seromic profiling (Blixt et al., 2010). Investigators have developed methods in which O-glycopeptides can be synthesized and immobilized on protein arrays to be analyzed with patient sera in order to detect autoantibodies directed to aberrant O-glycopeptide epitopes. The O-glycopeptide platform is very high-throughput but still in its early discovery stage. Valid concerns with protein arrays in general, as with most techniques, include reproducibility and proper analysis of the data. So far, none of these immunomic techniques have been validated on a large scale, but should present opportunities for broader antigen screening in the future as the technology improves.

Treatment planning. More individualized or strategically targeted tumor vaccines could also be developed using high-throughput technology. In tumor vaccine development, much work is focused on the identification of specific biologically relevant proteins that are overexpressed in patients' cancers and can become the basis of a vaccine if those targets are proven to be antigens. In the future, an individual patient's patterns of autoantibody binding may even be used for development of personalized immunotherapy such as a cancer vaccine specifically targeting a panel of individually identified tumor antigens (Chatterjee et al., 2006a). Although the design of therapeutic cancer vaccines targeting specific TAA epitopes in an individual's tumor is an appealing strategy, it has not been widely developed due to various challenges including acquisition of tumor specimens and the laborious nature of the analysis. Other strategic immune-based approaches for treatment planning have been reported using combinations of omics sciences. A unique study of donor lymphocyte infusions (DLI), a type of immunotherapy used in patients with residual disease after hematopoietic stem cell transplantation for chronic myelogenous leukemia (CML), utilized immunoscreening with both SEREX and protein microarray to identify effective therapeutic targets (Biernacki et al., 2010). By evaluating pre- and post-DLI treatment sera from patients with clinically effective graft-versus-leukemia effect by immunoscreening followed by the evaluation of identified antigens in gene expression microarray analysis, it was concluded that the CD34⁺ malignant CML progenitor cells seemed to be the target of DLI; furthermore, three antigens expressed in the majority of patients after treatment have been identified as potential CML cancer vaccine targets. It is expected that the use of high throughput tools will continue to serve in identification of vaccine targets and other treatment planning.

Immune response monitoring. Predictive biomarkers are also needed to link immunity with improved outcome for patients receiving cancer vaccines (Disis 2011). A clinically effective antitumor response is associated with the generation of Type I immunity, which is associated with enhanced crosspriming of antigen-presenting cells and associated epitope spreading, and is measured by development of immunity to tumor-associated antigens not present in the vaccine formulation. Epitope spreading has been linked to clinical response in some in patients with metastatic melanoma and

renal cell carcinoma treated with immunotherapy (Butterfield et al., 2003; Wierecky et al., 2006). Additionally, clinical trials using tumor vaccines targeting HER-2/*neu* in patients with advanced-stage breast cancer demonstrated that immunization of these often heavily pretreated patients produced significant levels of T helper 1 (Th1) tumor antigen-specific T cells as well as corresponding evidence of epitope spreading (Disis et al., 2004, 2009). Moreover, when vaccinated patients were followed out over a decade, epitope spreading was identified as an independent predictor of improved overall survival (Salazar et al., 2009). Epitope spreading is typically evaluated by enzyme-linked immunosorbent spot (ELISPOT) assay, a sensitive but laborious technique to evaluate antigen-specific T cells. More recently, peptide-MHC microarray and other multimer technologies have been developed as a higher throughput technique for evaluation of antigen-specific T cells (Casalegno-Garduno et al., 2010; Soen et al., 2003). Although these microarrays do not appear to currently be as sensitive as ELISPOT, improvements in array technology over time may improve the performance of the assay as a means to more simply measure epitope spreading as a potential clinical correlate of immune response and prognosis.

PBMC arrays

Peripheral blood mononuclear cell expression arrays may represent a useful tool in the identification of immune signatures associated with cancer immunotherapy. Because little is known about PBMC gene expression immune signatures with cancer vaccines and immune rejection, evidence can be extrapolated from the autoimmune, infectious disease, and allograft rejection literature. Several studies in the autoimmune literature have demonstrated utility of PBMC expression arrays to help identify predictors of response to therapy and prognosis. These include the development of an eight gene expression profile associated with T-cell regulation, which predicted the response to infliximab in patients with rheumatoid arthritis (Julia et al., 2009). Gene expression profiles have also been associated with acute inflammation linked to pathogenesis and worse prognosis in many other autoimmune diseases. In patients with systemic lupus erythematosus and ANCA-associated vasculitis, purified CD8⁺ T cells were transcriptionally profiled to avoid other confounders, and genes associated with interleukin-7 receptor, T-cell receptor signaling, and memory T cells were identified as prognostic biomarkers correlating with disease exacerbation (McKinney et al., 2010). Likewise, global gene expression patterns from the blood cells of patients with Sjogren's syndrome identified a prominent pattern of overexpressed genes that are inducible by interferons when compared to non-diseased controls, aiding to further understand disease pathogenesis (Emamian et al., 2009). Furthermore, in the first study linking immune response and microarray technology to prophylactic HPV virus-like particle vaccines, PBMC expression arrays were performed in patients before and after prophylactic HPV vaccination in order to identify a recall response gene profile after vaccination. Important innate and adaptive response-related genes including IFN- γ -related genes were induced by the vaccine, although more studies associating immunogenicity and long-term protection with immune signatures are still needed (Garcia-Pineros et al., 2009). The renal transplant literature has also demonstrated

peripheral blood mononuclear cell biomarker signatures including a "tolerance footprint" associated with allograft tolerance versus a footprint of early allograft rejection, both of which seem to be linked to the degree of acute inflammatory versus immune regulatory gene expression (Brouard et al., 2007; Gunther et al., 2009). Although peripheral blood mononuclear cell signatures have been well described in autoimmune disease, infection, and solid organ transplant, PBMC-based biomarkers of immune response in patients receiving cancer immunotherapy need to be further explored as means to guide therapy and prognosis.

Population-Based Vaccinomics

Population-based studies such as GWAS have demonstrated multiple genetic susceptibility variants associated with complex human diseases across ethnic groups. Genetic polymorphisms within the population have been shown to impact therapeutic efficacy of cancer treatments on a global scale, ultimately impacting clinical outcome. Indeed, there is a genetic component to therapeutic vaccine immune response, and polymorphisms of immune response genes may have a profound impact on the development of immunity and tumor rejection. As cancer becomes a growing global health problem, vaccinomics can include the use of polymorphisms as biomarkers to predict outcome to cancer therapy including immunotherapy. Detailed studies of polymorphisms involved in the immune response have been performed by infectious disease and autoimmune disease researchers, the majority of which are believed to be related to HLA genes, cytokines/chemokines and their receptors, costimulatory molecules and their ligands, and toll-like receptors (Poland et al., 2008; Yang and Roden, 2003; Zenewicz et al., 2010). For example, a recent study evaluated humoral response and associated SNPs in children receiving measles mumps and rubella (MMR) vaccination since up to 50% of antibody response to vaccine is believed to be genetic. A panel of immune response SNPs were identified from non-HLA genes as candidates for relationship to humoral response to rubella, and rubella antibody levels were shown to correlate in a multigenic assessment with SNPs associated with cytokines/cytokine receptors as well as other genes involved in Th1/Th2 immunity balance (Pankratz et al., 2010). Furthermore, in an infectious disease GWAS study of polymorphisms affecting therapeutic response to IFN- γ in hepatitis C virus (HCV)-infected African-Americans compared to European-Americans, researchers hypothesized that race-related genes would explain the relative poor response to treatment in the African American population; however, it was actually identified that associated SNPs were not linked with racial differences but instead with host immune responsiveness genes to HCV (Pos et al., 2010). Researchers continue to study the relationship between the genome and immune responsiveness related to inflammatory conditions spanning continents and various fields of medicine.

Limited studies have been performed connecting genetic polymorphisms in cancer patients to immunotherapy treatment response and outcome. One study identified a SNP in the interleukin-6 gene promoter as being associated with improved outcome in patient with high risk breast cancer, but this did not involve any evaluation of response to immunotherapy treatments (DeMichele et al., 2003). However, in patients with follicular lymphoma receiving treatment, IgG

FIG. 3. Schematic of potential opportunities for use of high throughput technologies applied to cancer immunotherapy research.

fragment C (FC) receptor polymorphisms did predict response to rituximab, thought to be attributable to differences in effectiveness of rituximab-associated antibody-dependent cell-mediated cytotoxicity (ADCC). This study aided in the theory that ADCC-mediated tumor destruction is a major mechanism of action for rituximab and suggested the possible need for SNP testing in prediction of response to ADCC-associated immunotherapy (Weng and Levy, 2003). Moreover, a deletion-associated polymorphism in the chemokine receptor 5 gene (CCR5Delta32) in a large cohort of almost 800 patients with melanoma receiving immunotherapy predicted for poor survival, suggesting that the decreased immune response associated with treatment may help guide who might benefit from these types of therapies in the future (Ugurel et al., 2008).

As the differences between genetic and environmental variants in human disease including cancer are further elucidated, population-based genomics studies are strengthened by inclusion of multiple populations and ancestries throughout the world. In the future, more population-based studies are likely to guide immunotherapy treatment decisions in cancer patients, spanning from the development of vaccines using epitope prediction methods that target the majority of ethnic groups to the identification of SNPs that predict immune response. Ultimately, much is left to be learned at the

population level, as differences in immune response to cancer vaccines are likely related to a complex combination of pathways influencing predisposition to immunity, spanning multiple inherited genomic polymorphisms, as well as regulatory pathways influencing transcription and translation.

Global Health

Cancer represents a major disease burden worldwide. Noncommunicable diseases such as cancer are on the rise in developing countries, with a projected 75% increase in cancer incidence in the developing world by 2020 (Parkin et al., 2001). Therefore, biotechnologic advancements such as vaccinomics sciences represent a priority not only to the developed world but also to low and middle income countries. Movements have been made to tackle illnesses such as cancer with collective action rather than individually by nation (Pang et al., 2010). Moreover, to encourage the use of genomics to improve global health, a panel of international scientific experts have used the Delphi Method to prioritize the top 10 biotechnologies for improving health in developing countries (Daar et al., 2002). Notably, the group emphasized the need for technologies to improve vaccines and drug delivery systems, as well as the use of recombinant technologies to make therapeutic products more affordable. One potential

TABLE 1. EXAMPLES OF VACCINOMICS APPLICATIONS AS PROOF-OF-CONCEPT IN THE DEVELOPMENT OF CANCER IMMUNOTHERAPIES (REFERENCES CITED IN TEXT)

Technology	Target	Tumor types	Study
Epitope prediction modeling	HER2/ <i>neu</i>	Breast, ovarian	High affinity binding epitopes predicted for immunogenic peptides in Phase I/II cancer vaccine trials
PBMC expression array	HPV-16 L1	Prevention of HPV-associated neoplasia	Identification of response-related genes in vaccinated patients from Phase II trial
Protein array	Anti-CTLA4 + GM-CSF	Prostate cancer	Development of antibodies to TAAs by phage-spotted immunoblot array after treatment
SEREX	NY-ESO-1	Bladder, esophageal, melanoma, NSCLC, ovarian, prostate, sarcoma	Discovery of antigen by SEREX lead to vaccine development and Phase I, II, III cancer vaccine trials

application within the realm of cancer vaccinomics is the movement toward use of omics technologies for the development of plasmid DNA-based targeted vaccines (Liu, 2011). The potency, ease of manufacturing, and stability of the DNA-based cancer vaccine platform could lend itself to improved transportability to the developing world. Focusing on biotechnologies with global health applications may have the greatest impact in the fight against cancer.

Conclusions

Cancer remains one of the most substantial global health threats of our time. Recent advances in the omics sciences can allow for tumor immunologists to further explore the complex nature of the human immune system and its relationship to cancer. As more sophisticated high throughput technologies become available, their utility will emerge in cancer immunotherapy research, both in the preclinical realm and for use in clinical trials. Studies cited above present potential opportunities for omics technologies to be more directly applied to cancer immunotherapy research (Fig. 3). Although the existing data is limited, some "proof-of-concept" applications of vaccinomics sciences have led to the development of cancer immunotherapies being tested in humans and even improvements in immune monitoring of patients after treatment (Table 1). In the future, vaccinomics will be further utilized in directed cancer vaccine development strategies, with options spanning from simpler and more stable transportable vaccines for global health needs to complex individualized vaccines for the advancement of personalized medicine. Furthermore, simpler and more consistent tools are needed to diagnose cancer, streamline treatment strategies, predict treatment responses, and understand immune signatures across populations. Data generated from biomarker studies for early cancer detection, prognostics, and correlates of antitumor immune response will universally facilitate the potential use of cancer vaccines and ultimately improve cancer treatment. As massive amounts of biological and immunological data are generated with the use of these tools, a remarkable opportunity is presented to improve cancer immunotherapy on a global scale. Although barriers remain, this technological era represents a great opportunity for further development and application of omics in the design and clinical evaluation of vaccines to treat cancer worldwide.

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Author Disclosure Statement

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