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De-Convoluting the “Omics” for Organ Transplantation

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Abstract

Purpose of review—The desire for biomarkers for diagnosis and prognosis of diseases has never been greater. With the availability of genome data and an increased availability of proteome data, the discovery of biomarkers has become increasingly feasible. This article reviews some recent applications of the many evolving “omic” technologies to organ transplantation.

Recent findings—With the advancement of many high throughput “omic” techniques such as genomics, metabolomics, antiomics, peptidomics and proteomics, efforts have been made to understand potential mechanisms of specific graft injuries and develop novel biomarkers for acute rejection, chronic rejection, and operational tolerance.

Summary—The translation of potential biomarkers from the lab bench to the clinical bedside is not an easy task and will require the concerted effort of the immunologists, molecular biologists, transplantation specialists, geneticists, and experts in bioinformatics. Rigorous prospective validation studies will be needed using large sets of independent patient samples. The appropriate and timely exploitation of evolving “omic” technologies will lay the cornerstone for a new age of translational research for organ transplant monitoring.

Keywords

genomics; proteomics; organ transplant; biomarker; translational medicine

Introduction

The field of organ transplantation has generated many fundamental principles of the mechanisms of acute rejection based on the analysis of selected genes, with the characterization of their specific functional roles in the immunological cascade leading to organ rejection [1,2] [3]. Nevertheless, the redundancy of the immune system suggests that numerous other molecular pathways interplay in the effector response and contribute to the heterogeneity of clinical graft outcome. The lack of event and patient therapeutic individualization results in inadequate or over-immunosuppression, resulting in the confounding clinical outcomes varying from the rejection response to malignancy. Additionally, mechanisms of early regulatory responses lead to chronic rejection, at a time when interventions may alter the acute nature of graft injury, remain to be defined.

The progression towards high-throughput data production and analysis represents a global paradigm shift from hypothesis-driven experiments towards large-scale hypothesis-generating data collection. Transplantation is a complex immunologic state involving constant interaction between the graft and the recipient's immune system. Numerous

interrelated immune pathways are responsible for the delicate balance between graft rejection and acceptance, and are further complicated by manipulation of immunosuppressive medications. These precise molecular mechanisms remain poorly understood; specifically, the interplay between co-regulated immune pathways and gene families, that may as yet be “unlinked”, and based on single gene analyses. However, the evolution of the field of “*functional omics*”, including microarray, proteomics, protein-array, metabolomics and antibody-array technologies, have begun to uncover these intricate gene, protein, antibody and metabolite regulation events, thus offering new insights in to the pathogenesis of post-transplant clinical events. These high throughput “omic” profiling technologies provide the link between biological mechanisms and clinical phenotypes by elucidating the underlying molecular processes responsible for the variability in clinical behavior and outcome, such as acute rejection, chronic rejection, drug toxicity, ischemia-reperfusion injury, delayed graft function, infection and tolerance.

Biomarkers in Organ Transplantation: A Critical Need

A *biomarker* is a gene, protein/peptide or metabolite present in a biological system. It is indicative of a physiological or pathological state that can be recognized or monitored. Monitoring the biomarker thus provides a means for monitoring the disease condition and assists in its diagnosis and prognosis, and importantly, the biomarker can correlate with a clinical change in the underlying disease state. Thus, when assessed in biological fluids distant from the actual site of injury, the biomarker can provide a non-invasive means to follow the underlying disease.

A well-known biomarker for kidney function is the serum creatinine, which is a surrogate biomarker for underlying graft injury in renal transplantation. However, the serum creatinine does not meet the criteria for an *ideal biomarker* that would monitor renal transplant patients because it lacks high specificity (serum creatinine is dependent on muscle mass and hydration status, in the absence of graft injury) and sensitivity (serum creatinine can be elevated with multiple causes of intrinsic and extrinsic graft injury). There is an unmet need for non-invasive biomarkers, which are more specific and sensitive to replace the renal transplant biopsy as the gold-standard. Given the inability of our field to generate reliable and non-redundant biomarkers that can track with a specific cause of graft injury and graft survival course, the application of high throughput “omic” techniques, such as genomics [2], proteomics and peptidomics [4], antibiomics [5], and metabolomics [6,7], may uncover novel biomarkers in each of these fields by a process hypothesis generation without *a priori* learning bias from past single pathways experiments.

An ideal biomarker in the field of organ transplantation would be able to do the following: predict outcomes prior to and after transplantation; predict the onset and the severity of specific events such as acute and chronic graft rejection and infection; and predict the specific injury responses to immunosuppressive medications. The impact of these discoveries would be to finally customize the dose (or load) of immunosuppression for the patient- i.e., finding the individual threshold between rejection and infection so that the individual can avoid allorecognition and retain normal infectious immunity. The examination of patients with spontaneous operational tolerance [8,9] has allowed for identification of a highly regulated gene set in peripheral blood, which can provide a critical supplemental tool for non-invasive monitoring of allograft “acceptance” during the process of immunosuppression customization.

The *hunt for biomarkers* in the field of organ transplantation has been extraordinarily difficult. The primary problem lies with not having a true gold standard for the classification of different etiologies of graft dysfunction. The transplant biopsy faces many dilemmas: (1) it samples a fraction of the kidney while the underlying process of injury is often patchy

(e.g. drug related injury, acute rejection); (2) the fraction of sample processed for biomarker studies is separate and often disparate from the tissue sample processed for histology; (3) the limited amount of tissue received from human transplant studies limits dissection of samples to examine specific cellular compartments or specific infiltrating cells; (4) the heterogeneity of the underlying process may not be recognized by pathology [2]; (5) there is inter-observer variability in pathological diagnosis [10]; (6) chronic graft injury is often pooled together but etiologies for that injury may be highly variable and may be driven by different mechanisms. Thus different biomarkers may be required for graft monitoring. This lack of a true gold standard in transplantation creates the first hurdle in high throughput technology to analyze genome wide differences in genes or proteins. If the input data is flawed, can we expect meaningful output measures with relevant clinical correlations? An additional problem lies in the heterogeneity of the patients, and the source of tissues for the biomarker studies. Biomarkers in transplantation also have to transcend many clinical variables, not just the baseline differences in immunosuppressive drugs, but also other differences such as, variations in recipient and donor gender, age, HLA match, ischemia time for the organ, donor source, recipient hematocrit, recipient white counts, recipient concomitant infection, etc. Since the “omic” technologies are expensive and often time consuming to perform and analyse, application of these strategies in very large patient sample numbers is a daunting task, and often results in a study with a biased validation set, or a relatively small discovery datasets [11], such that results from these studies are difficult to validate in a different validation patient sample set, which may have many recognized or unappreciated differences in clinical or demographic parameters.

An introduction to the Omic Technologies

Genomics refers to the analysis of these genomes and *functional genomics* to the component of this field that uses global approaches to understand the functions of genes and proteins. Microarrays can interrogate gene expression profiles in various systems that range from bacteria to humans. Microarrays are artificially constructed grids of tens of thousands of spots of DNA (oligonucleotide or cDNA targets), such that each element of the grid probes for a specific RNA sequence — that is, each holds a DNA sequence that is a reverse complement to the targeted RNA sequence. Thus, microarrays enable the comprehensive interrogation of a cell's transcriptional activity by simultaneously observing the steady state mRNA levels of thousands of genes, thus making this a crucial platform for interrogating the complexity behind an organism or cell's phenotype. This in reality, results from the expression of multiple gene products that collectively constitute the proteome. Microarray technology is still evolving, and has progressed from early technology using nylon membranes to the current state of the art using glass wafers. As a result, many studies are being done ranging from basic science to clinical applications in cancer, diabetes and acute rejection of allograft in transplant. Rapid biological interpretation of gene lists that result from the analysis of microarray, proteomics, and other high-throughput genomic data can be done by customizable software applications such as Expression Analysis Systematic Explorer (EASE), whereby ‘genes’ can be converted to ‘themes’ [12]. Studies in transplantation defined different molecular sub-groups of acute rejection by tissue microarray analysis, and (Table 1) shows that these categories of rejection were found to have very distinct biological processes and gene functions. Tens of thousands of research articles have been published using the microarray technique since the appearance of the first paper in 1995, which demonstrates its influence and impact on current biological research [13]. Nevertheless, some basic pitfalls face the novice researcher in this field and are outlined in Table 2.

Several commercial arrays are currently available for researchers. Among these widely used platforms are: Affymetrix geneChips® (Affymetrix Inc, Santa Clara, CA), Agilent

oligonucleotide microarrays (Agilent technologies, Palo Alto, CA), Illumina (Illumina Inc., San Diego, CA), and Nimblegen (Nutley, NJ). It is now widely accepted that each of these platforms have their own strengths and weaknesses. The researcher must choose the appropriate platform based on his/her priorities. We have seen the array technology platform along with several other genomic techniques evolve in recent years. For example, single nucleotide polymorphism (SNP) arrays detect SNPs of human genome [14], aberrations in methylation patterns [15], alterations in gene copy-number [16] alternative RNA splicing [17] are now available. Details on these issues on the most recently published microarray studies, which are relevant to human organ transplantation and experimental models in transplantation, are summarized in recent reviews [18-21].

The field of **proteomics** involves identification and characterization of the proteins encoded by the genome. Since proteins are involved in different cellular processes, an understanding of proteins inside the cell provides an insight into the cellular events. The proteomics approach provides an unbiased high-throughput approach to identify differentially expressed proteins in the healthy and disease states. For this very reason, proteomic analysis for biomarker discovery has been extensively applied to many fields of biomedical research, including oncology, diabetes, renal and urine related diseases, and solid organ transplantation [42-47].

Analysis of tissue samples has led to the patterns of tissue-specific proteomes, or the set of proteins expressed in the cells of a particular organ system. Body fluids, such as cerebrospinal fluid (CSF), synovial fluid, and nipple fluid aspirates, have been used to identify protein signatures that differentiate samples with disease from healthy controls, and provide candidate proteins as surrogate biomarkers of disease. Blood and urine become more appealing candidates for their potential application in the clinical setting [42,45,48,49].

Protein analysis methods traditionally used *western blot* and *immunohistochemistry* (IHC) and relied on the availability of respective antibodies, and required a relatively large protein quantity. Recent proteomic analysis methods include *2D gel electrophoresis* (2 DE) [50] and *two dimensional difference gel electrophoresis* (2D DIGE) in which as many as three fluorescently labeled protein samples can be analyzed in a single gel, thereby reducing gel-to-gel variation and increasing reproducibility [51]. Surface enhanced laser desorption/ionization-time of flight mass spectrometry (*SELDI-TOF*) [18] [43] generates patterns of peaks representing proteins and protein fragments, which then need to be identified using other technologies. *Electrospray ionization* (ESI) and *Matrix-assisted laser desorption/ionization* (MALDI) [52] [53] and *liquid chromatography coupled with mass spectrometry* (LC-MS), have become increasingly popular in efforts to discover biomarker molecules [54] [55]. *Multidimensional protein identification technology* (*mudPIT*) analyzes small proteolytically digested peptides from complex biological samples and has become a popular “bottom-up” approach [56]. Application of depletion of abundant proteins as confounders in samples [55], enrichment or fractionation has facilitated analysis of a complex protein mixture, which has been popularly known as “*Shotgun Proteomics*” [57]. The availability of highly sensitive mass spectrometers such as triple quadrupole MS instruments, and newer 2D linear ion trap instruments such as *LTQ FT* and *LTQ-orbitrap* and *Fourier-transform ion cyclotron resonance mass spectrometry* (FT ICR) [58], have facilitated peptide identification of thousands of proteins. Unlike microarrays, where gene identification is easily known due to the completion of the human genome project, accurate quantification of proteins or peptides is not easy. Some of the methods employed for quantification include tagging of samples with some stable isotope such as labeling by amino acids in cell culture (*SILAC*) [59]; Isotope Coded Affinity Tags (*ICAT*) [60]; *iTRAQ*™ [61] and $^{18}\text{O}/^{16}\text{O}$ labeling methods [62].

The emerging field of **metabolomics** examines a metabolite or a panel of these molecules, as they track with specific cellular processes left behind in disease states. The term metabolome refers to the complete set of small-molecule metabolites (such as metabolic intermediates, hormones and other signalling molecules, and secondary metabolites) to be found within a biological sample, usually with a molecular mass up to 2000 Dalton. Researchers have attempted to catalogue almost 2500 metabolites in the human body [74], the most referenced being Citric Acid, D-Glucose, Lactic Acid, L-Alanine and L-Valine. Important steps during metabolite analysis include extraction and chromatographic separation of metabolites, and detection, quantification and identification of the analytes, using mass spectrometry.

Antibiomics is another newly emerging field which allows for the rapid analysis of novel antibody specificities to a host of target antigens or proteins gridded on an array [5]. The Protoarray by Invitrogen is one platform like this that can allow for rapid analysis for thousands of antibody specificities and may be an exciting new approach to assay the humoral response in transplantation.

Integrative Omics- An answer to the challenge of intrinsic heterogeneity and biomarker discovery

An approach that combines data from individual research groups conducted on similar disease conditions and then applies is for robust cross-validation in a large dataset, may be one way to remedy this issue. In a recent report Kong *et al* [75] applied a non-parametric meta-analysis approach for combining independent microarray datasets pertaining to chronic allograft nephropathy (CAN). The work used non-parametric meta-analysis approach based on two CAN studies, and identified 309 distinct genes that expressed differently in CAN. With the help of Fisher's exact test, the study found 6 KEGG pathways to be over-represented among the identified genes [75]. Another approach that utilized a unified framework for finding differentially expressed genes (DEG) has been shown to be better than other gene selection methods [76]. A framework for this kind of approach can use the following modules: (i) gene ranking –using two gene selection algorithms, namely, a) two-way clustering and b) combined adaptive ranking to rank the genes, ii) significance analysis of genes – converting the gene ranks into p-values (iii) validation – using - three fold validations of the obtained DEGs selected by false discovery rates (FDR) analysis [76].

Another powerful approach of integrative genomics, antibiomics (where multiple antibody specificities are simultaneously measured by the use of sera samples hybridized to high density protein microarrays) and bioinformatics [5] has uncovered a novel means to define the differential immunogenicity of the human kidney. This study utilized genomic data sets generated from 7 different microdissected kidney regions [77], mapped the genes and proteins between a cDNA, oligonucleotide and protein array platform, and then determined the specificity of de novo post-transplant antibody responses after renal transplantation, to specific regions of the kidney. We have utilized large published datasets from GEO and performed integrated bioinformatics analysis of archived renal transplant genomic, antibiomic and proteomic datasets in the Sarwal Lab, together with publically available genomic data pertaining to different types of organ transplants (kidney, heart, lung, liver, pancreas, intestine) performed on multiple array platforms, with a view to identify shared critical pathways and validate common non-invasive etiology-specific bio-markers for transplant injury across different solid organs. An approach to develop novel antibody alloimmune markers after kidney transplantation is shown in (Figure 1). This approach utilizes the integration of antibody, cDNA microarray and oligonucleotide microarray measurements to find novel, de novo alloimmune responses against non-HLA targets after kidney transplantation [5].

Avoiding noise in omic datasets

Given the heterogeneity of the patient population and complexity of any subset of genome or proteome being interrogated, the task of biomarker search is daunting. To add to the complication, as reported by Mueller et al in a recent report, that the transcriptomic footprint could be continuous instead of dichotomous [78]. The presence of thousands of proteins and their concentration can span a dynamic range of at least 10 orders of magnitude. This only adds to the already complex proteomic sample [54]. Having a low signal to noise ratio is a well-recognized problem in these experiments. Sophistication in automated data analysis software has resulted in minimizing the problem of background noise from hybridization and wash variables, but biological noise still remains a problem; specifically, in samples where the signal level for the disease specific signatures can be relatively low. This was recently shown in a genomics study geared towards the discovery of peripheral blood biomarkers for graft rejection using whole blood analysis from PAXgene tubes [20]. The study reports how globin genes may interfere with biomarker discovery efforts for allograft rejection in peripheral blood samples.

Gel based proteomic techniques have inherent issues of reproducibility and their high detection limit impedes the effort of detecting the low abundance protein biomarkers. The problem caused by masking of low abundance potential protein biomarkers in blood by high abundance plasma proteins was realized which led to the design and implementation of commercially generated depletion columns, such as the Multiple Affinity Removal System column (<http://www.chem.agilent.com>).

One of the strengths of the omic tools is that they have tremendous power to generate a large volume of data dealing with multiple samples at the same time very effectively. This strength has been very useful in a broad spectrum, ranging from the research trying to elucidate basic cellular events to translational research aimed at discovering biomarkers for diseases. Publicly available data repositories are being used to ask questions pertaining to different health conditions ranging from obesity to organ transplant tolerance [81,8]. Since many of the omic techniques are being developed and studied in small datasets, there is often no existing preliminary data that can be utilized for sample size prediction, or power calculation purposes. Some of the informatics tools applied towards OMIC analyses are discussed in Table 3.

Data Warehousing and Reporting- essential requisites for collaborative data analysis

Currently much of the data generated by an individual or group is still unavailable to the scientific community or in a format that is unusable by other laboratories. In addition, the field of transplant immunology is relatively new and information on the mechanisms of acute rejection, chronic rejection, and tolerance or the effects of immunosuppressive medications is in its infancy. Making data sets publicly available will foster scientific collaborations that can result in better patient care through the prediction of disease processes or individualization of treatment. Standard formats for archiving expression data are available for a number of repositories such as the Stanford Microarray Database (SMD), ExpressDB, The Gene Expression Database (GXD) and Gene Expression Omnibus (GEO).

Translating Omic Discoveries to Clinical Practice

Ongoing and future multi-center collaborative studies, as well as meta-analyses of existing data, will enable the validation of important correlative biomarkers emerging from omic technologies. In addition, incorporation of these *omic* profiling approaches into large-scale prospective clinical trials will allow for better correlation with clinical data (i.e. immunosuppression effects), improve prognostic capabilities, and enable researchers to delineate the variables involved in multifactorial processes such as chronic graft injury.

In clinical settings, typical *omic* signature-based classifiers and predictors are composed of very few markers (typically less than 20 genes or proteins or patterns)[62,63,78,82], which can then be tested using more simplified analysis, and at lower cost. Examples are the translation or validation of microarray data by PCR and of antibody data by ELISA assays. These tests, compared to their *omic* counterparts, can be performed rapidly, and in most facilities, have less “noise”. Hence analysis is statistically simpler and swifter.

One of the criticisms of the *omic* efforts in biomarker discovery is that very few biomarker molecules have been successfully applied in a clinical setting, due to some of the following reasons: (1) *Lack of a gold standard* (the biopsy) in the classification of the disease condition and the *lack of a proper normalization method* to categorize the samples accurately. (2) There are different techniques and platforms for generation of data and the methodology for *normalizing the data across platforms* without losing its biological relevance is not as developed [83,84]. (3) Validation and verification steps usually require a *large set of samples*- which can be a roadblock for development of the biomarker for subsequent clinical use. (4) Because of the sensitivity of the methods being used and the method of sample preparation process (extraction, amplification, hybridization etc) there is *no consensus on the threshold of significance*. Usually, methods being employed for discovery use expensive and sophisticated instruments not suitable for a clinical setting, and they are relatively expensive and require skilled personnel for their operation. A threshold set for such a technique may not hold true when the biomarker reaches the clinical testing method using a different technique.

Concluding Remarks

The use of *omic* technologies will be undoubtedly beneficial in providing rapid and global views of the gene, protein, peptide, antibody and metabolite profiles of different disease states, thereby allowing improved understanding of the molecular mechanisms of the diseases and identify attractive and potentially important diagnostic, prognostic and therapeutic markers. As the cost of many of these technology platforms is high and the analysis of the data is fairly complex, these platforms will never be used as general monitoring tools in the clinic. Nevertheless, a combination of genomics and the emerging proteomics, antibiomics and metabolomics technologies provide excellent screening tools and stringent attention to sample selection, processing, confounding variables, and data analysis will help to fill in our knowledge-gaps in mechanisms, molecular pathways and monitoring in transplantation medicine.

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Figure 1.
Integrated Informatics Approach to combine analysis of cDNA, oligonucleotide and protein array measurements. Detailed methods described in Li et al PNAS 2009 [5]

Table 1

The Major Groups of Acute Renal Transplant Rejection identified by Microarray Analysis of 63 Renal Transplant Biopsies [2], are shown as AR1, AR2 and AR3, and can be seen to have different biological processes and gene functions in acute graft rejection. Expression Analysis Systematic Explorer=EASE

<i>AR1 Functional Category</i>	Genes	Genes in Category	EASE Score	Fisher Exact	Example Genes
Defense/immune response	47	747	1.36E-30	1.73E-31	IL2RB, IL6R, IGLL1, LTB, LTF
MHC class II receptor activity	10	18	2.09E-15	1.44E-17	HLA-C, -DM, -DP, -DQa, CD74
Antigen processing/presentation	11	28	1.1E-14	1.54E-16	MHC class I and II genes
Signal transduction	34	1971	1.46E-06	6.29E-07	ITGA2, ITGB2, STAT1, -3, -6
Humoral immune response	9	144	2.56E-05	3.26E-06	CD53, CSF1R, UBD
Complement activation	4	29	0.00177	9.71E-05	DAF, BF, C1s, SERPING1
MHC class I receptor activity	3	16	0.0062	0.000219	HLA-C, HLA-E, B2M
Innate immune response	6	175	0.0155	0.00362	CXCL9, LYZ, SPP1, NMI
Cell adhesion	10	549	0.0394	0.0172	CD9, CD69, TSPAN-1, VCAM1
Chemokine activity	3	43	0.041	0.00416	CCL5, CXCL9, CXCL10
<i>AR2 Functional Category</i>	Genes	Genes in Category	EASE Score	Fisher Exact	Example Genes
Mitochondrion	20	476	1.59E-05	4.89E-06	ACAT1, ATP5B, DLD, GATM
Regulation of coagulation	3	9	0.00653	0.000215	ANXA5, ANXA7, KLKB1
Energy pathways	8	160	0.00704	0.00185	ATP5B, ATP5G3, ME2, OXCT1
Regulation of translation	5	60	0.00969	0.0015	EEF1A1, EIF4A2, HRSP12
Actin polymerization	3	13	0.0136	0.000702	CXCL12, DSTN, PFN2
Membrane fusion	3	23	0.0404	0.00392	CALM, CDH17, CLTB, GCA
Calcium ion binding	12	499	0.0608	0.0309	CDH17, RGN, EFHD1, CLTB
Cell motility	8	253	0.0639	0.0263	ATP1A1, CD9, C4A, TM4SF8
Negative regulation of cell cycle	4	71	0.0767	0.0176	RAP1A, RBL1, RBL2, CDK2AP1
<i>AR3 Functional Category</i>	Genes	Genes in Category	EASE Score	Fisher Exact	Example Genes
Defense/immune response	20	747	1.74E-06	4.83E-07	CCL13, CD6, CD79, APOH
Cell proliferation	17	899	0.000899	0.000351	CDC20, CCNA2, -B1, -B2, IL1B
Regulation of cell cycle	10	337	0.000926	0.000228	CKS1B, CKS2, CDC20
Response to wounding	8	250	0.00271	0.000616	GAGE8, IL1B, IL9, LBP

<i>ARI Functional Category</i>	<i>Genes</i>	<i>Genes in Category</i>	<i>EASE Score</i>	<i>Fisher Exact</i>	<i>Example Genes</i>
Nuclear division	6	137	0.00369	0.000609	CDC20, NEK2, PTTG1
Acute-phase response	3	20	0.00978	0.000447	LBP, ORM2, SERPINA3
Chemokine binding	3	24	0.0102	0.000485	CCL13, CCR5, CX3CR1
Humoral immune response	5	144	0.0233	0.00483	ADA, BATF, IL1B, PAX5

Table 2

Some Pitfalls and their Solutions for Microarray Studies

Pitfalls in microarray analysis	Solutions
Data variability, particularly for genes with low expression levels.	Use replicate arrays to reduce false positives.
Small sample amounts which limits replication.	Use of amplified RNA (aRNA).
Expression bias due to amplification.	Use improved protocols with single-round amplification.
Difficult to accurately control input RNA amounts.	Use a normalization standard and 2-color labeling strategy to minimize.
Spot quality may vary.	User stringent data filtering criteria assessing signal/noise and spot signal consistency.
Lot-to-lot variation in PCR yields on cDNA arrays.	Use data filtering methods such as SVD to reduce batch biases.
Hybridization efficiency varies with different probes.	Use of long-oligonucleotide arrays minimizes selected hybridization artifacts.
Unequal labeling efficiency of Cy3 and Cy5 dyes.	Use reciprocal labeling to confirm observations.
Small numbers of samples and very large number of genes analyzed may contribute to false discovery.	Confirm mRNA measurements using independent test method such as PCR and independent samples.
Heterogeneity within study groups may contribute to false discovery.	Use statistical modeling such as logistic regression to combine multiple genes. Test on independent samples.
Protein expression levels and function not measured.	Confirm with protein expression methods (e.g. immunohistochemistry, protein arrays).

Table 3

Some of the available informatic tools that can be applied towards OMIC analyses

Commercial microarray analysis tools	Academic microarray analysis tools
Affymetrix Microarray Analysis Suite	AMADA (Hong Kong University)
AIDA Array Compare (Raytest)	BASE (Lund University)
ArrayInformatics (PerkinElmer)	BClust (Yale School of Medicine)
ArrayScout (Lion)	BRB ArrayTools (Simon Rich, NCI)
ArrayStat (Imaging Research)	Cleaver (Stanford)
BioMine (Gene Network Sciences)	Click (Tel Aviv University)
BioXM (BioMax)	Clusflavor (Baylor)
Clententine (SPSS)	Cluster Identification Tool (Van Andel Research Institute)
Expressionist (GeneData)	Cluster Treeview (Michael Eisen, Stanford)
GeneLinker (Molecular Mining)	CyberT (University of California, Irvine)
GeneMaths (Applied Maths)	dChip (Wing Wong, Harvard School of Public Health)
GenePlus (Enodan)	Expression Profiler (Alvis Brazma, EBI)
GeneSight (BioDiscovery)	Gene Cluster (Whitehead)
GeneSpring (Silicon Genetics)	Genesis (Alexander Sturn, University of Graz, Austria)
GeneTraffic (Iobion)	GMEP (Michael Eisen, LBNL)
GeneViz (Contentsoft)	MAExplorer (NCI)
J-Express (MolMine)	MA ANOVA (Gary Churchill, Jackson Lab)
MicroHelper (Chang Bioscience)	MatArray Toolbox (Gary Churchill, Jackson Lab)
Rosetta Resolver (Rosetta Biosoftware)	PAM: Prediction Analysis for Microarrays (Tibshirani, Stanford)
SAS Microarray (SAS)	Plaid (Stanford)
Visual Gene (Visipoint)	TIGR Multiple Experiment Viewer (Inst. Genomic Research)
X-Miner (X-Mine)	R: Statistics for Microarray Analysis (Terry Speed, UC Berkeley)
Xpression NTI (InforMax)	SAM: Significance Analysis of Microarrays (Tibshirani, Stanford)
	SNOMAD (Jonathan Pevsner, Johns Hopkins)
	TreeView (Michael Eisen, LBNL)
	XCluster (Gavin Sherlock, Stanford)