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# Genomics in Surgery, Trauma, and Critical Care: How Do We Control the Future?

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## Introduction

Deoxyribonucleic acid (DNA) microarray technology evolved from the initial reports published in the 1970s indicating that labeled nucleic acids could be incorporated to monitor the expression of nucleic acid molecules attached to solid state support scaffolds. Over the next 20 years, genomic analysis methods continued to evolve until 1995 when the first article describing the application of DNA microarray technology used for genomic expression analysis was published.

Over the last two decades the manner of application of genome-wide expression analysis at the cellular level has exploded. Microarray technology has made dramatic advancements since its development and gained an increasing popularity among scientist, researchers, biologist, oncologist, statisticians, and physicians. The broad array of technological advancements and biological applications that comprise the field of human genomic

analysis has solidified microarray technology indispensable as a research methodology and robust diagnostic tool.

The prevalence of DNA microarray technology along with its ubiquitous adoption by industry, medicine, and research laboratories has fostered the ability to quickly and accurately perform simultaneous analysis of thousands of genes or proteins. A single human microarray provides the ability to analyze the entire human genome from a single peripheral blood or tissue sample providing extensive and valuable information on gene expression, interaction, and function in a disease-specific manner. In this chapter our aim is to give a brief overview of cDNA microarray technology and how its application has contributed to the evolution of surgical diseases from oncology, to trauma, and critical care.

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## DNA Microarray Technology

DNA microarray technology in its simplest sense is a high-throughput, versatile technology used for prokaryotic and eukaryotic gene expression analysis of thousands of known or unknown genes in a simultaneous fashion. A more precise and accurate definition of DNA microarray technology is an orderly arrangement of thousands of identified sequenced genes printed on an impermeable solid support scaffold, usually glass, silicon chips, or nylon membrane.

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## Basics of Microarray Technology

DNA microarray analysis is an efficient, flexible, and versatile approach to achieve high-throughput explorations of genome structure, expression, and function at the cellular and organism levels. This capability provides researchers and clinicians alike with a powerful tool to discern between, confirm diagnosis, and make predictions in various disease states. DNA microarray analysis is a complex multistep process involving numerous and specific pieces of scientific instrumentation and strong multidisciplinary expertise in various scientific and medical specialties including molecular biology, image analysis, statistical computing, and medical genetics.

Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome. A DNA microarray (also commonly known as DNA chip, biochip, or gene chip) is a collection of microscopic DNA spots attached to a solid surface. Each DNA spot contains a specific DNA sequence, or probes (probe sets, reporters, or oligos). These can vary between a short section of a gene or other DNA-based target elements that are used to hybridize a strand of cDNA (complimentary DNA) synthesized from a purified, tissue-specific mRNA (messenger ribonucleic acid) template in a catalyzed reaction using reverse transcriptase and DNA polymerase reactions. Probe-target hybridization is usually detected and quantified by detection of a fluorochrome, silver, or chemiluminescence, labeled targets to determine relative abundance of nucleic acid sequences in the target.

Once the experimental tissue is processed and hybridized to the gene chip, the gene chip fluorescence is quantified with a specific gene chip scanner that transposes the fluorescence values at each target probe into specific gene or probe set expression levels. Using an ever evolving cadre of powerful statistical analysis packages the genomic expression levels can be compared between groups of patients, tissues, cells, or normal controls to discover genomic differences between control and test groups. A schematic of the methodology involved to process an experimental

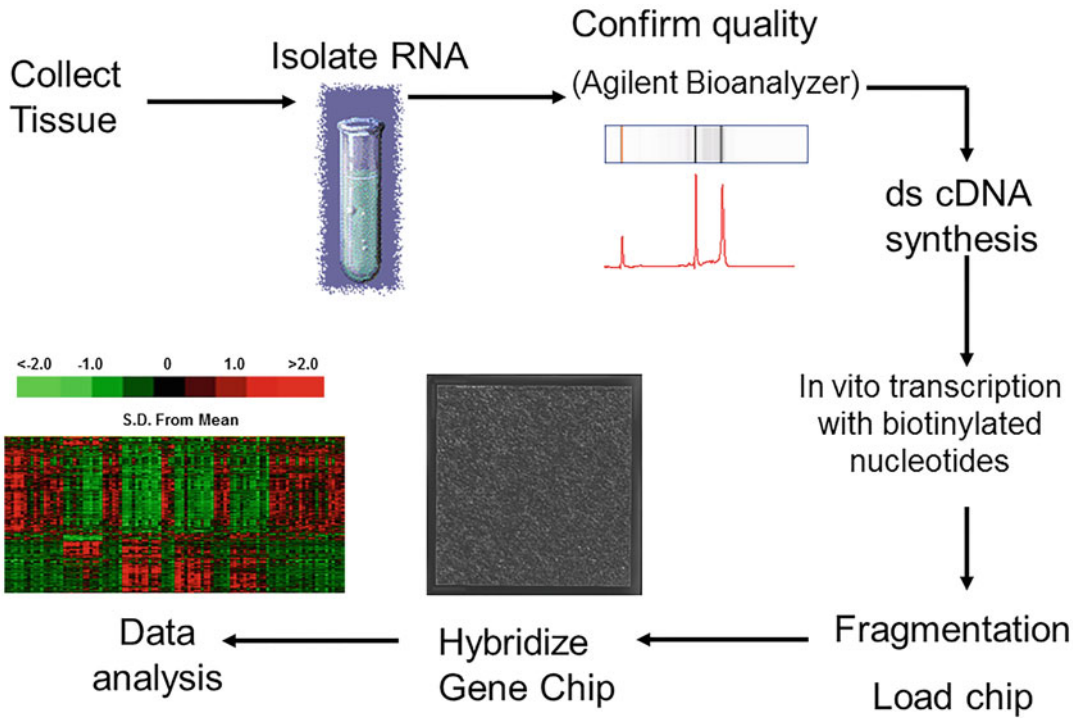
tissue, hybridize the target cDNA to the gene chip, and then analyze the chip for meaningful genomic expression values is summarized in Fig. 3.1.

In addition to high-throughput genomic analysis, another complimentary tool for the analysis of proteins and other physiologic biomarkers is based on multiplex technologies intended to detect low levels of multiple protein types from a single tissue, blood, or urine sample. Multiplex technology is bead-based multiplexing, where beads are internally dyed with fluorescent dyes to produce a specific spectral or fluorescence address. Biomolecules, such as antibodies, can be conjugated to the surface of inert beads to capture physiologic molecules of interest. This technology uses flow cytometric or other imaging technologies for characterization of the beads as well as detection of the light emission due to target molecule presence. Luminex™ (Austin, TX) technology enables up to 500 proteins to be detected in each well of a 96-well plate, using very small starting sample volumes. Common applications include cytokines, chemokines, metabolic markers, autocrines, and phosphorylated proteins. A schematic representation of the tissue processing through data readout using a multiplex approach to cytokine analysis is depicted in Fig. 3.2.

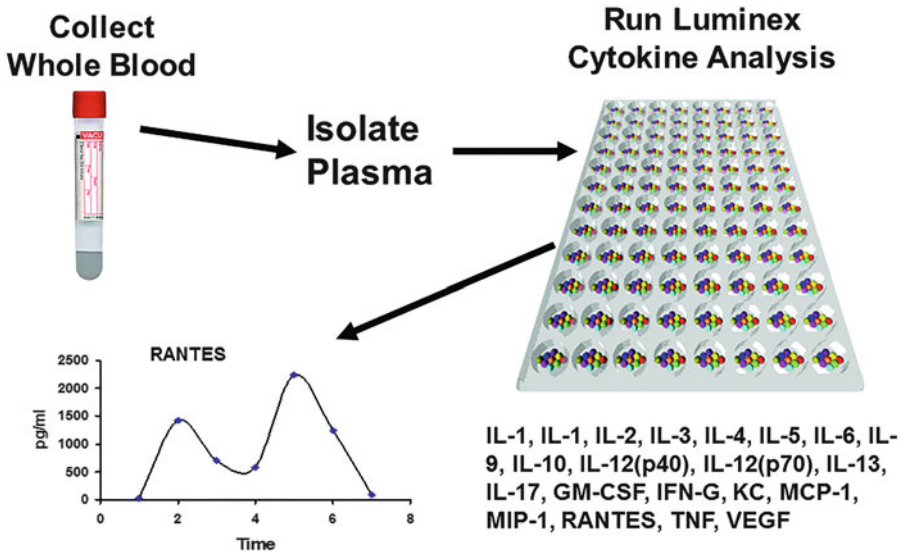
## Affymetrix GeneChip® Arrays (Santa Clara, CA)

Affymetrix makes quartz chips for genome-wide analysis called GeneChips. Affymetrix's GeneChips enable researchers to quickly scan for the presence of particular genes in a variety of biological samples. Moreover, Affymetrix is uniquely focused on oligonucleotide microarray technology. These specific microarrays are incorporated to determine which genes exist in a biologic sample by detecting precise pieces of mRNA.

Each gene on an Affymetrix microarray Gene Chip is typically represented by a probe set consisting of 11 different pairs of 25-bp oligos covering features of the transcribed region of that gene.



**Fig. 3.1** Schematic of sample processing to gene chip analysis. A schematic of the methodology involved to process an experimental tissue, hybridize the target cDNA to the gene chip, and then analyze the chip for meaningful genomic expression values



**Fig. 3.2** Multiplex cytokine analysis. A schematic representation of the tissue processing through data readout required using a multiplex approach to cytokine analysis



**Fig. 3.3** Affymetrix U133 2.0. This is an actual Affymetrix U133 2.0 human GeneChip<sup>®</sup>. A single gene chip packed with fragments from each of the 35,000 known genes that make up the human genome. (Courtesy of Affymetrix, Inc., Santa Clara, CA, USA)

Each pair consists of a perfect match (PM) and a mismatch (MM) oligonucleotide. The PM probe exactly matches the sequence of a particular standard genotype, often one parent of a cross, while the MM differs in a single substitution in the central, 13th base. The MM probe is designed to distinguish noise caused by nonspecific hybridization from the specific hybridization signal. Affymetrix GeneChip microarrays are the most popular high density oligonucleotide gene expression arrays and have become an invaluable tool in genomics studies worldwide. Figure 3.3 represents an actual Affymetrix Human Gene Chip U133 plus 2.0.

## Microarray Technology Impacts Surgical Care

Each human cell contains approximately three billion DNA base pairs that are estimated to encode approximately 30,000 genes that are responsible for maintaining the structural and functional integrity of a cell. These genes encode the RNA and proteins that produce the cell phenotype. A neoplastic phenotype develops out of an aberration or alteration in the normal expression of genes. A variety of specific mechanisms have been implicated in the transformation process including chromosomal rearrangement, deletions, amplification, methylation, and mutations of genes. Elucidating the fundamental molecular mechanisms that are involved in the stepwise progression from normal tissues to malignant tumors, organ-specific inflammation or systemic inflammation is essential in our knowledge of the development of cancers. This would ultimately lead to improved methods of detection, treatment, and cures for cancers. Comprehensive analyses of cancer genomes promise to better inform prognoses and improve precise cancer treatments improving patient-centered and specific cancer care and chemotherapeutics [1].

## Melanoma

Malignant melanoma is a highly aggressive disease accounting for a majority of the deaths from skin malignancies with patient survival dependent on early detection and diagnosis. Although some promising new therapies have recently emerged, a better understanding of the molecular alterations involved in melanoma progression, particularly from localized tumors to metastasis, such as genomic and epigenetic aberrations, will aid in early detection and development of biomarkers and future targeted treatment strategies.

Melanoma, like other solid tumors, is thought to arise from a series of genetic and epigenetic events. Genetic aberrations have been identified

in the past decade and have potential utility as biomarkers. Multiple studies have revealed that epigenetic events, such as genomic promoter region methylation of CpG islands, histone modification, and microRNA (miRNA) expression, have been shown to be important regulators of melanoma progression, and that these epigenetic changes can potentially serve as molecular biomarkers in tumor tissues and in blood as circulating DNA, for diagnosing disease and predicting disease outcome and progression [2].

Epigenetics refers to heritable changes in gene expression that are not caused by changes in the genomic DNA sequence. DNA methylation is one of the hallmark epigenetic events most studied in cancers. DNA methylation involves the addition of a methyl group to the 5' carbon of a cytosine ring located 5' to a guanosine base in a CpG dinucleotide and is catalyzed by DNA methyltransferases. Methylation events of promoter regions have been strongly implicated in cutaneous melanoma progression. Many of these genes are involved in cell cycle control, cell signaling, migration and invasion, apoptosis, angiogenesis, and metastasis. Comparing the methylation status of melanoma primary tumor and metastasis, an increase in hypermethylation of WIF1, TFPI2, RASSF1A, and SOCS1 was seen with increasing clinical tumor stage. Despite advances in this field and development of multiple platforms for studying genomic methylation, uniformity and standardization remain significant issues in evaluating and comparing results.

miRNAs are evolutionarily conserved, endogenous, noncoding RNA transcripts of 22 nucleotides in length that serve to temporally and spatially regulate biological function. miRNAs are considered an epigenomic mechanism that can have normal regulatory function but also can have negative influence when dysregulated, particularly in cancer progression as in melanoma. They are derived from noncoding intergenic or intronic regions of DNA that, once in their mature form, interfere with protein translation from mRNA transcripts. miRNA can preferentially bind with mRNA transcripts to inhibit translation or degrade the mRNA transcript before translation

can take place. miRNA can modulate biological functions such as cell cycle, proliferation, apoptosis, and angiogenesis, which, if aberrantly regulated, can lead to malignancy. As an example miR-221/222 was found to interfere with c-KIT and p27, causing dysregulation of the cell cycle during the progression of melanoma. miRNA regulation may influence the microenvironment and can contribute to tumor cell invasion, migration, and metastasis. miRNA regulation of protein coding genes is, therefore, an essential regulatory element in biological development and function [2]. Lastly, deregulated miRNA expression may serve as diagnostic or prognostic biomarker in cutaneous melanoma.

By collectively assimilating the ever evolving cadre of miRNA, biomarkers, epigenetic phenomena, and small molecule expression markers, genomic expression finger prints can be developed that serve to stratify malignant melanoma metastatic risk, responsiveness to therapy, and even long-term survival odds prospectively and prior to therapeutic intervention. No more will the responsiveness to therapy be the clinicians best guess and experience but rather based on individualized genetic and biomarker-specific expression matrix gathered from each patient.

## Prostate Cancer

Prostate cancer is the most common type of cancer in men and the second leading cause of cancer death in men in the United States. The recent surge of high-throughput sequencing of cancer genomes has supported an expanding molecular classification of prostate cancer. Translation of these basic science studies into clinically valuable biomarkers for diagnosis, prognosis, and biomarkers that are predictive for therapy is critical to the development of precision medicine in prostate cancer. Many recent genomic applications are aimed at improving screening specificity in prostate cancer by differentiating aggressive versus indolent prostate cancers. Over the past decade a host of candidate gene biomarkers have been centered on three distinct groups involving

*ETS* gene rearrangements, *PTEN* inactivation, and androgen receptor signaling. These and other putative biomarkers may provide a rationale for matching patients with molecularly targeted therapies in clinical trials [3].

One of the major barriers prohibiting scientist, oncologist, and clinicians from better understanding comprehensive analyses of prostate cancer genomes is the inaccessibility of metastatic tissue. A potential solution is to characterize circulating prostate tumor cells (CTCs). In the past this has required overcoming the challenges of isolating rare and metastatic prostate cancer cells and sequencing low-input material. However, recently investigators have reported an integrated process to isolate, qualify, and sequence whole exomes of prostate CTCs with high fidelity using a census-based sequencing strategy. The authors' data support the notion that CTC sequencing can reveal early mutations in tumor evolution and those that could be shared among metastatic sites. The genomic technology involved provides a minimally invasive window into the mutational landscape of metastatic prostate cancer and will provide investigators and clinicians with more robust tools to better tailor prostate cancer chemotherapeutics in cases of metastatic disease [4].

## Breast Cancer

Cancer genomics has already revolutionized our knowledge of breast cancer molecular pathology, fostering the development of new and more effective clinical protocols. The most recent advances in the field of cancer genomics and epigenomics include DNA alterations and driver gene mutations, gene fusions, DNA methylation, and miRNA expression [5].

Genome-wide association studies have allowed the discovery of only a portion of genomic alterations associated with hereditary breast cancer risk. The genes *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *STK11*, and *CDH1* with high-penetrance mutations account for only 20–25 % of all cases of hereditary breast cancer. It has been speculated that the majority of breast cancer may be

explained by the accumulation of low-penetrance and acquired genetic alterations, interacting together in a polygenic model, associated with a modification of the epigenetic component. The exact definition of the patient-specific occurrence and interaction of genomic alteration is expected to be the greatest contribution that next generation sequencing will provide to breast cancer management. Until the identification of these low-penetrance genomic influences is completed, treatment of breast cancer will continue to depend on the level of expression of receptors for estrogen, progesterone, and epidermal growth factor type 2 receptor (*HER2/neu*) [5]. However as the genomic expression profiles evolve new chemotherapeutics and chemotherapeutic combinations will be tailored to treat individual patients with breast cancer.

## Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) affects over 2.5 million people of European ancestry, with a rising prevalence in other countries. There is overwhelming evidence for the role of genetics in IBD as evidenced by initial reports of familial clustering. The last 15 years have seen a tremendous degree of progress regarding the identification of genetic loci involved in IBD. This has happened in part because of technological advances and growth in the genetic approaches used to identify these genes. Although other variations do exist, Crohn's disease and ulcerative colitis are the two most common forms of inflammatory disease and continue to confound investigators and clinicians [6].

Genome-wide association studies and meta-analyses of patients with these two diseases have implicated a sundry of previously unsuspected cellular mechanisms, such as autophagy, in their pathogenesis. Furthermore these findings have shown that some IBD loci are shared with other inflammatory diseases like ankylosing spondylitis. Meta-analysis of Crohn's disease and ulcerative colitis combined a total of more than 75,000 cases and controls in genome-wide association scans. The investigators found 71 new genomic

associations for a total of 163 IBD loci that met genome-wide significance thresholds. The authors found that most of the discovered loci contribute to both IBD phenotypes. In addition many of the IBD-associated loci were also implicated in other immune-mediated disorders such as ankylosing spondylitis and psoriasis. An additional and unexpected finding was that there is considerable overlap between susceptibility loci for IBD and mycobacterial infection. Gene co-expression canonical pathway and network analysis emphasize this relationship, with pathways shared between host responses to mycobacteria and those predisposing to the development of IBD [7]. The findings suggest that either mycobacteria directly or the immune processes involved in mycobacteria eradication cause immune dysregulation which potentiates IBD development.

As a consequence of the ability to simultaneously scan and catalog thousands of genomic expression patterns for a disease state, biomarker panels with a high level of diagnostic sensitivity and specificity have been developed to more quickly and accurately identify patients with IBD. One such panel used in the diagnosis of Crohn's disease is the PROMETHEUS® Crohn's Prognostic test (Prometheus Labs, San Diego, CA). This technology combines proprietary serologic and genetic biomarkers in a logistic regression model to provide individual patients with probabilities for developing disease complications after diagnosis with Crohn's disease. The test enables physicians to stratify patients according to their risks of developing complications and develop patient-centered treatment plans rather than a blanket therapeutic approach.

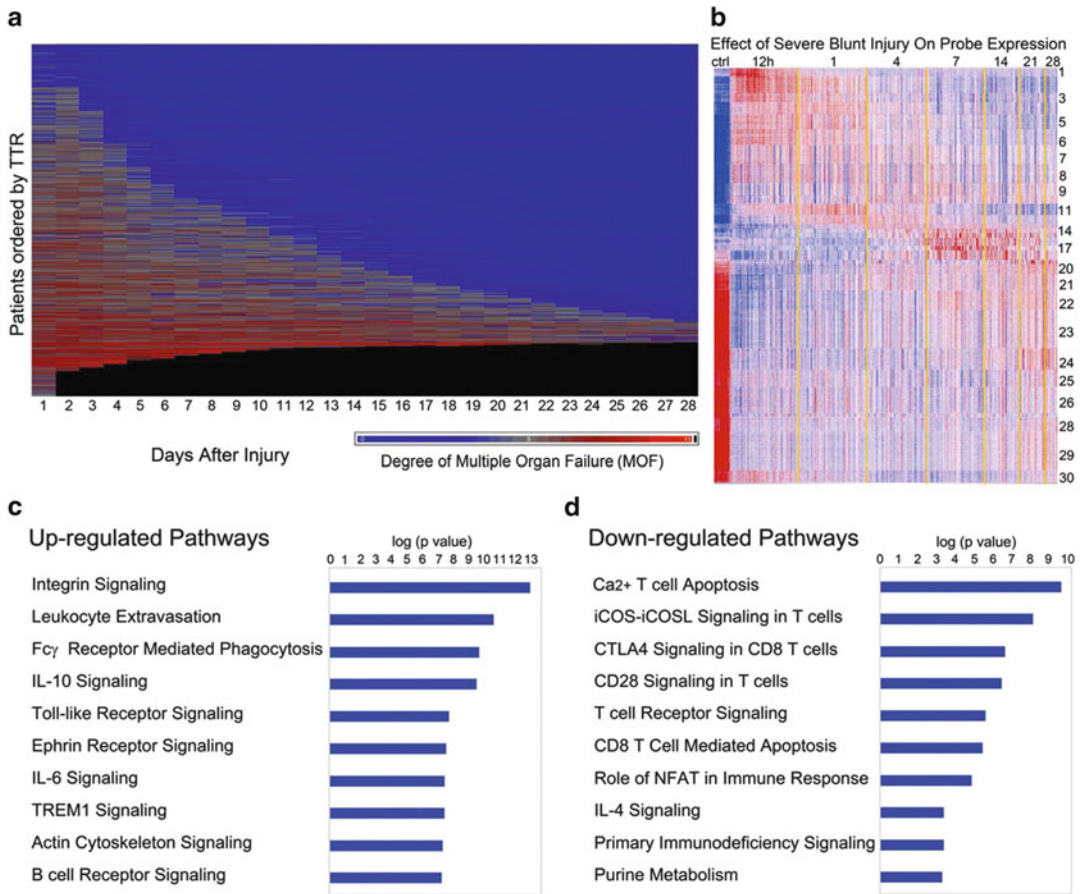
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### Microarray Technology in Trauma and Critical Care

The *Inflammation and the Host Response to Injury* collaboration is a large-scale research program centered on the immune-inflammatory response in trauma and sepsis. The mission of the multi-institutional collaborative effort funded by

the National Institute of Health is to understand key regulatory elements that drive the host's response to traumatic injury and its accompanying systemic inflammation response. The participating investigators used a discovery-driven approach to acquire large amounts of new human genomic data to try and identify genomic expression patterns that may serve as new targets for therapeutic development. In an effort to streamline the clinical sample processing and analysis, the investigators were able to develop novel genome-wide microarray technologies and introduce high-throughput proteomics to clinical medicine. These efforts have culminated in the identification of novel genomic and proteomic markers that predict patient outcomes and serve as new therapeutic targets for basic and clinical research and pharmaceutical development.

Human survival from traumatic injury whether penetrating or blunt requires an appropriate inflammatory and immune systemic response. Until recently, the immune cell genomic expression patterns underlying the systemic inflammatory response to traumatic injury were unknown. Investigators from the *Inflammation and the Host Response to Injury* have described the circulating leukocyte transcriptome after severe trauma and burn injury and have shown that severe injury produces a global reprioritization affecting greater than 80 % of the cellular functions and pathways in a manner they termed a "genomic storm." Early after severe traumatic injury, the investigators found that the leukocyte genomic response is consistent with a simultaneously increased expression of genes involved in the systemic inflammation, innate immunity, and the compensatory anti-inflammatory response with concomitant suppression of genes involved in adaptive immunity; see Fig. 3.4a–d. Moreover, complications such as nosocomial pneumonia and organ failure were not associated with any genomic evidence of a physiologic second hit phenomenon that has been historically postulated in the literature and clinical reports. The similarities in gene expression patterns between the various injury models investigated reveal a fundamental human genomic signature to



**Fig. 3.4 (a–d)** Immune cell gene expression in trauma. Organ injury and genomic changes associated with severe blunt trauma. **(a)** Whole blood was taken from severe blunt trauma patients, leukocytes were isolated, and total cellular RNA was extracted and hybridized onto an HU133 Plus 2.0 GeneChip<sup>®</sup> (Affymetrix, Santa Clara, CA). The continuum of clinical responses to severe blunt trauma in the 1,637 total patients from which the 167 sampling trauma patients were drawn is shown graphically. Each row represents an individual patient ordered by time to recovery (TTR), and the  $x$  axis represents time from injury in days. Patients are sorted from least to most severe organ injury and mortality. The presence and severity of organ injury is represented by colors from *blue* (least severe) to

*red* (most severe). *Black* indicates death. **(b)** K-means clustering of the genes into 30 clusters based on patterns of expression over time. *Red* indicates increased and *blue* indicates decreased expression relative to the mean (*white*). Five thousand one hundred and thirty six genes were differentially expressed between patients and controls (ctrl; FDR <0.001 and at least twofold change). **(c, d)** Summary of the canonical pathways that are most affected by trauma. The graph shows the  $\log_{10}(p)$  value of the enrichment of the pathway. **(a–d):** Copyright © Xiao et al. 2011. Originally published in Xiao W, Mindrinos MN, Seok J, Cuschieri J, Cuenca AG, Gao H, et al. A genomic storm in critically injured humans. The Journal of Experimental Medicine. 2011;208(13):2581–90

inflammatory stress that are far more common than different [8]. This notion explains why simplistic models of inflammation based on one or several overexpressed biomarkers have simplistically failed over the past 40 years. Moreover, the

data suggest a complex cellular system-based approach toward therapeutic intervention targeted at optimizing the patient's individualized inflammatory response may be more beneficial than the current single-agent interventions.



## **Class Prediction and Outcome of Sepsis in Trauma**

Despite the advances in medical sciences, the morbidity and mortality due to sepsis in severe trauma patients remains high and thus there is a need for early and accurate identification of those patients that are genetically predisposed to develop severe sepsis or other complications that predispose the patient to poorer outcomes. Over the past three decades a vast amount of resources have been dedicated to determining a prediction model sensitive and specific enough to reasonably prognosticate which patients will develop severe sepsis and which will not. Using a high-throughput genomic strategy several recent reports show promise in being able to differentiate the groups of patients that will develop complications based on their genomic signature.

## **Complicated and Uncomplicated Outcomes in Trauma**

Many patients have complicated recoveries following severe traumatic injury due to the development of end organ injury. Physiological biomarkers and prognosticators to date have had limited success in predicting patient clinical trajectories. Recently investigators from the *Inflammation and the Host Response to Injury* collaboration developed a retrospective validation of a simple genomic composite score that shows promise to rapidly predict clinical outcomes. Microarray-derived genomic data obtained from 167 severely traumatized patients over 28 days were assessed for differences in messenger RNA abundance among individuals with various clinical trajectories.

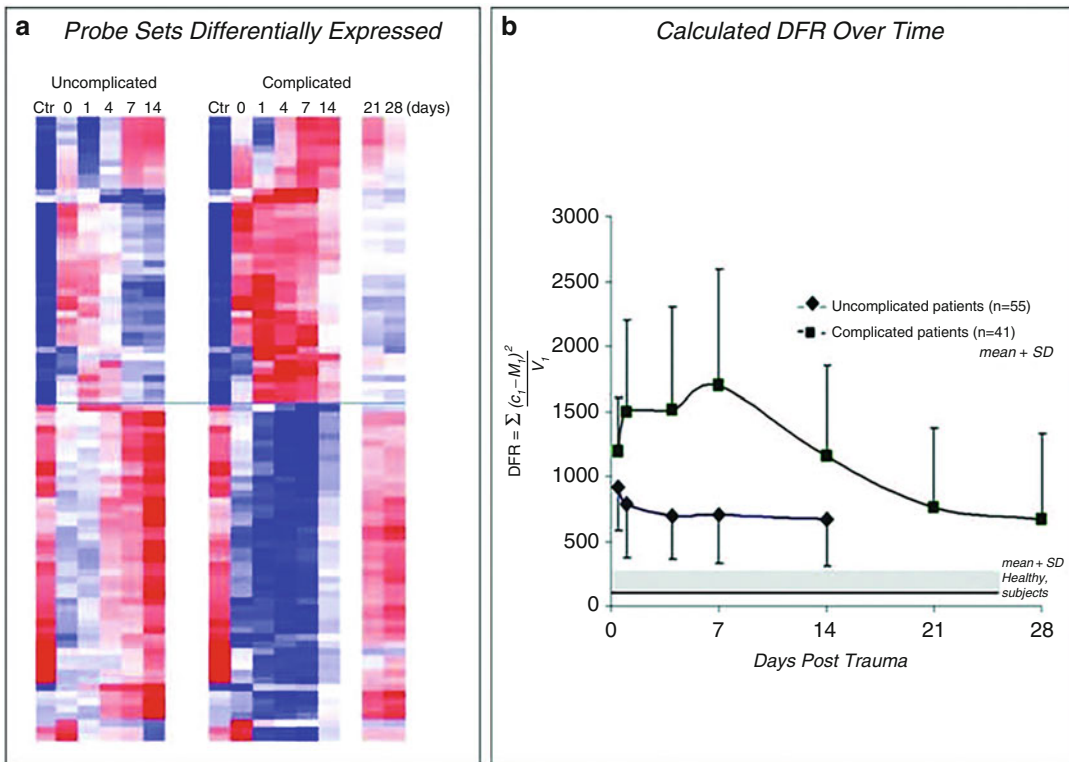
Once a set of genes was identified based on differences in expression over the entire study period, messenger RNA abundance from these subjects obtained in the first 24 h was analyzed using a rapid multiplex platform, and the genomic expression data reduced to a single metric. The researchers identified development of a genomic metric that can be rapidly used to predict clinical outcome in severely injured trauma patients; see Fig. 3.5a, b. The group then developed a composite score that showed good discriminatory

capacity to distinguish patients with a complicated outcome which was significantly better than the predictive power of either Acute Physiology and Chronic Health Evaluation II or new injury severity score scoring systems. The excitement surrounding the study stems from the fact that clinicians can now obtain a rapid genomic composite score after trauma that can identify trauma patients who are likely to develop complicated clinical trajectories rather than the wait and see approach that is now used. The ability to accurately predict patient complication patterns would enable clinicians to develop new strategies to prevent rather than treat complications [9].

## **Gram-Negative Bacteremia and Outcomes**

Trauma patients that develop bacteremia during their hospitalization have a higher risk of death than their non-bacteremic counterparts. However, the role that trauma altered immunity plays in the development of bacteremia was unknown until a recent report further elucidated the subject. Total leukocyte gene expression was used to compare trauma subjects in whom Gram-negative bacteremia developed and those in whom it did not develop. The results showed that Gram-negative bacteremia was found to be an independent risk factor for death.

The investigators went further to show that as hospitalization time progressed to 96 h after injury, 102 probes of genes were differentially expressed. Twenty probe sets represented fifteen innate or adaptive immunity genes. Some of the noteworthy genes discovered were a downregulation of *IL1B* and an upregulation of *IL1R2* which reflect suppression of innate immunity in patients developing Gram-negative bacteremia. In addition downregulation of adaptive immune genes in Gram-negative bacteremia subjects was also observed. The authors concluded that by 96 h after injury, there are differences in leukocyte gene expression associated with the subsequent development of Gram-negative bacteremia, reflecting suppression of both innate and adaptive immunity; see Fig. 3.6a, b. Importantly, Gram-negative bacteremia after trauma is, in part, the consequence of host immunity failure and



**Fig. 3.5** (a, b) Heatmap and calculated difference from reference (DFR). These are the 63 genes that were found to distinguish clinical trajectory. Using a false discovery adjusted probability of less than 0.001 and a twofold difference in expression, the temporal pattern of expression of 63 genes that differed between patients with the two clinical outcomes is presented. (a) Cluster analysis of the two cohorts. (b) Summary of the difference from reference score calculated for each patient in the uncomplicated and complicated cohorts at each time point.

Statistical analysis at each time point (0, 1, 4, 7, and 14 days) revealed significant differences in difference from reference (DFR) between complicated and uncomplicated patients ( $p < 0.05$ , Mann-Whitney rank analysis). (a, b): Used with permission from Cuenca AG, Gentile LF, Lopez MC, Ungaro R, Liu H, Xiao W, et al. Development of a genomic metric that can be rapidly used to predict clinical outcome in severely injured trauma patients. *Critical Care Medicine*. 2013;41(5):1175–85

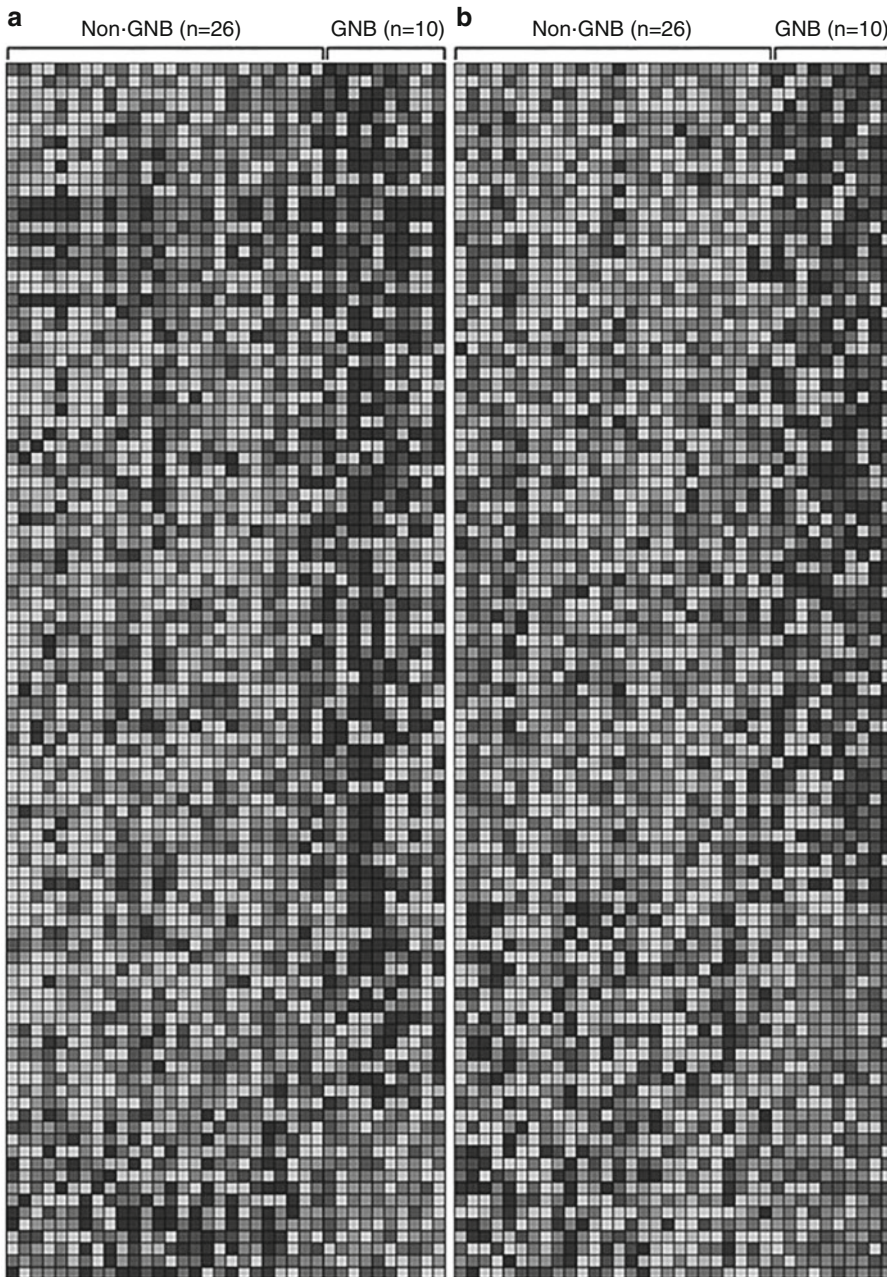
may not be completely preventable by standard infection-control techniques.

The above report highlights the unique ability offered to scientist and clinician alike to more precisely identify patients that may be predisposed to developing hospital acquired infections while recovering from their primary injury. Not only is the genomic identification of subsets of immune compromised patients valuable in its own regard but also gives us genetic insight into how subtle variations in human genomic expression can predispose patients to develop life-threatening infections. As further genomic signatures are acquired and linked to human

diseases and patient outcomes, improved, more proactive solutions and treatments will evolve to better serve select subgroups of patients of high risk within a disease group [10].

### Ventilator-Associated Pneumonia and Outcomes

Ventilator-associated pneumonia is a prevalent complication in the critical care setting and predisposes many patients recovering from traumatic injury to unnecessary death. A microarray study of ventilator-associated pneumonia (VAP) patients suggested that 85 leukocyte genes can be used to diagnose VAP. Furthermore a validation



**Fig. 3.6** (a, b) Trauma patients may be predisposed to develop bacteremia. (a) Heatmap of top 100 ranked differentially expressed probe sets from within 12 h after injury. (b) Heatmap of top 100 ranked differentially expressed probe sets at 96 h after injury. The lighter gray colors represent decreased expression, and the darker black colors represent increased expression with the darker

colors indicating a greater degree difference. GNB=subjects in whom Gram-negative bacteremia developed, non-GNB=subjects in whom Gram-negative bacteremia did not develop. (a, b): Used with permission from Thompson CM, Park CH, Maier RV, O’Keefe GE. Traumatic Injury, Early Gene Expression, and Gram-Negative Bacteremia. *Critical Care Medicine*. 2014 Jun;42(6):1397–405

of this gene set to detect VAP was tested using data from an independent patient cohort. Circulating leukocyte GeneChip U133 plus 2.0 expression values were measured over a 28 days time period after injury. Leukocyte transcriptional profiles were analyzed using repeated measures logistic regression. A compound covariate model based on leukocyte gene transcriptional profiles in a training subset of patients was tested to determine predictive accuracy for VAP up to 4 days prior to clinical diagnosis in the test subset. Using gene expression values measured on each study day 27 of the 85 genes were associated with the diagnosis of VAP 1–4 days before diagnosis. However, the compound covariate model based on these 85 genes did not predict VAP in the test cohort better than chance ( $P=0.27$ ). In contrast, a compound covariate model based upon de novo transcriptional analysis predicted VAP better than chance 4 days before diagnosis with a sensitivity of 57 % and a specificity of 69 % [11].

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### **The Future of Microarray Technology in Trauma, Sepsis, and Critical Care**

Although high-throughput genomics and proteomics have revolutionized the ability of clinicians to diagnose disease prognosticate patients outcome, the science is hampered by genetic variation within a disease group and with the technicalities of tissue analysis which require large amounts of patient-derived RNA. Affymetrix, in association with the *Inflammation and the Host Response to Injury* collaboration or “Glue Grant,” has attempted to solve the problem by the development of a new gene chip technology minimizing the amount of initial patient RNA used while maximizing the amount of genomic information recovered.

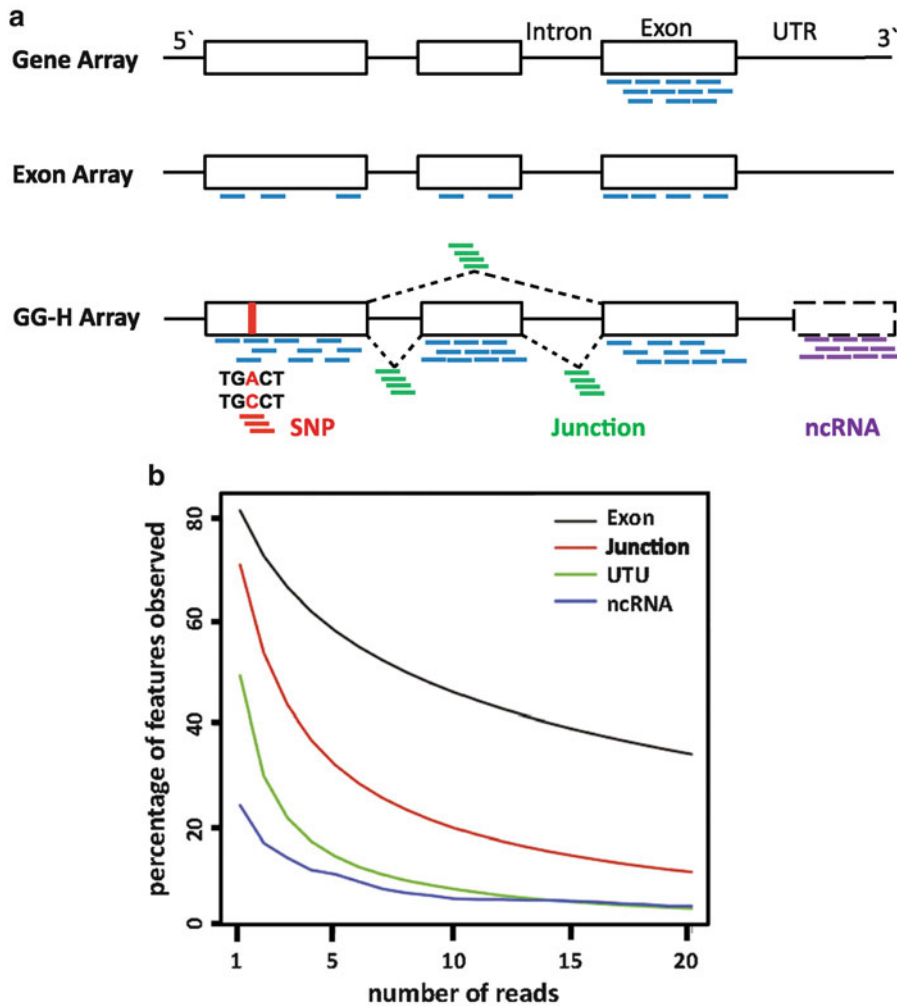
The new gene chip is a 6.9 million-feature oligonucleotide array of the human transcriptome [Glue Grant human transcriptome (GG-H array)] that has been developed for high-throughput and cost-effective analyses in clinical studies. This array allows comprehensive examination of gene

expression and genome-wide identification of alternative splicing as well as detection of coding SNPs and noncoding transcripts. The authors tested the performance of the array with mRNA sequencing results over multiple independent replicates of human tissue samples; see Fig. 3.7a, b. The GG-H array is highly reproducible in estimating gene and exon abundance while requiring minimal amounts of patient tissue samples. Affymetrix, in conjunction with the *Inflammation and the Host Response to Injury* collaboration, has implemented the GGH gene chip in a multicenter clinical program and has generated high-quality reproducible data. Considering the clinical trial requirements of cost, sample availability, and throughput, the GG-H array has a wide range of applications [12].

This technology will offer a robust platform from which to analyze all the variations of human disease genetic targets with very little starting material. Now not only can disease diagnosis be made rapidly and early, but also the known genetic variability within a disease process explored and treated. These microarrays will be the future of multicenter disease-focused collaborative efforts that are aimed at genetic prognostication for diseases in general and select patients in specific.

### **Microfluidics and Point of Care Genomic Prognostication**

Francis Collins, one of the fathers of the Human Genome Project, clarified his vision for the future of “personalized medicine,” where the fruits of the Human Genome Project would ultimately influence not only human biology and medicine, but human society as well. The goals of individualized gene-based medicine are, for the most part, still beyond the capabilities of most health-care centers because of several remaining technical challenges, only some of which have been addressed by the Human Genome Project. Technologies have recently become available to cost-effectively conduct genomic analyses such that previously unobtainable questions have become technically possible. However, several



**Fig. 3.7** (a, b) Affymetrix glue grant human transcriptome (GG-H array) (Santa Clara, CA). Design of the GG-H human transcriptome array for comprehensive examination of gene and exon expression, alternative splicing, and additional contents of the human transcriptome. (a) On a 3' gene array, such as the Affymetrix HU-133 Array, 11 probes were designed for the 3'-end exon(s) of each gene. On an exon array, such as the Affymetrix Human Exon 1.0 ST Array, there are two to four probes for each exon of the gene. In contrast, the GG-H array uses, on average, ten probes for each exon/PSR and four probes for each exon-exon junction; in addition, six pairs of probes were

designed for each coding SNP, and ten probes were designed for each noncoding RNA transcript. (b) Comparison of the GG-H array contents with mRNA sequencing data on multiple tissues. The percentages of exons, junctions, UTUs, and ncRNAs (y axis) supported by at least a specified number of sequencing reads (x axis) are shown. (a, b: Copyright © National Academy of Sciences. Originally published in Xu W, Seok J, Mindrinos MN, Schweitzer AC, Jiang H, Wilhelmy J, et al. Human transcriptome array for high-throughput clinical studies. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;108(9):3707–12)

technical challenges still remain that prevent widespread use of these technologies in the clinical setting, including rapid sample accrual, large sample volumes, lengthy processing times, and complex data analysis.

Gene expression analysis can be a powerful tool in predicting patient outcomes and identifying patients who may benefit from targeted therapies. However, isolating human blood polymorphonuclear cells (PMNs) as well as other

human immune cells for genomic analysis has been challenging. Several investigators have thrust their efforts into providing a platform from which human neutrophils can be rapidly isolated from peripheral blood. Using a novel microfluidic technique that isolates PMNs by capturing CD66b<sup>+</sup> cells, the authors use bronchoalveolar lavage (BAL) samples of patients with acute respiratory distress syndrome (ARDS) to evaluate PMN genomic alterations secondary to pulmonary sequestration. Blood and BAL fluid PMNs were also isolated using microfluidics from seven hospitalized patients with ARDS. Gene expression was inferred from extracted RNA using Affymetrix U133 Plus 2.0 GeneChips. Patterns of gene expression from blood and BAL PMNs differed significantly from each other in the patients with ARDS. The authors further demonstrate the cost savings, alacrity, and ease that can be gained by using a microfluidics approach to point of care cell separation and testing rather than using traditional isolation and analysis methods [13, 14].

The ability to rapidly isolate circulating blood PMNs for diagnostic and laboratory evaluation arms physicians with a powerful tool to more efficiently and accurately predict which patients are at risk for the development of ARDS. Early stratification of those at risk for the development of ARDS will allow for the development of better ARDS prevention strategies.

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## Final Conclusions

As we better understand the genomic etiologies of more diseases states, our focus will inevitably shift from reaction to prevention. Patient treatment plans will be based on the sets of complications that may hinder their recovery progress. Rather than focus on generalized disease prevention, we will focus on patient-centered disease identification before the symptomatology and sequel are apparent. By applying genomics and other high-throughput technologies, clinicians and researchers will have a robust platform for using the human transcriptome as a diagnostic tool in the clinical setting.

The technical, statistical, and methodological challenges of handling human genomic information and translating it into reliable tools applicable in clinical decision-making will overcome one major hurdle preventing the widespread use of genomics in clinical medicine, and that is translating basic science discoveries into new and better treatments. Over the past 30 years a cadre of clinicians and researchers alike has described the genomic and proteomic response to inflammation and severe injury in leukocyte populations from humans. Now is the time to take advantage of the large scientific database of information and use it to implement a diagnostic test for predicting outcome and appropriateness for therapy in the critically ill trauma patient, cancer patient, or acute care surgery patient.

Advances in the treatment of critically ill trauma, cancer, and acute care surgery patients have stagnated over the past decade. The enthusiasm in the 1990s for new therapeutics to treat surgically oriented diseased patients has been stymied by an inability to identify those patients who would benefit from such therapies. Current stratification and diagnostics to identify patients who will have an adverse clinical trajectory or outcome are imprecise. The future of improved outcomes is rooted in new genomic diagnostics that can accurately identify patients who may benefit from therapies aimed at disease prevention and not reaction.

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