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Understanding microbial infections using microarray technology

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Abstract

Human microbial infections are symbiotic processes between pathogens and humans that often lead to human disease and death. Microbial infections involve the attachment, growth, and survival of microorganisms on human skin, inside the body, or inside specific cells. Microbial infections can be localized to one body region or migrate to secondary body locations utilizing various transport mechanisms. An understanding of host-pathogen interactions related to the expression of essential genes during and after infection can lead to valuable information for biologists and clinicians. Microarray technologies allow researchers to perform genomic characterization experiments rapidly and efficiently. Microarray experiments support the resolution of underlying molecular events that play a role in normal and aberrant physiologic activities in living systems. Microarray technology, coupled with bioinformatics analysis, generates comprehensive insights into relevant genes, proteins, and protein-protein interactions. This review article explores recent microarray research studies from select protozoan and bacterial pathogens to illustrate how researchers utilize microarray technology to examine aspects of microbial infection. Microarray studies of pathogen and host genomes at different stages of the infection process will generate a more precise understanding of pathogenic life cycles and pathogen survival strategies. Detailed knowledge of the genes involved in the microbial infection process will lead to the discovery of disease biomarkers and potent therapeutic solutions.

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Introduction

The completion of the human genome project paved the way for the development of high throughput technologies in functional genomics such as DNA microarrays and next generation RNA sequencing. DNA microarray protocols are widely useful methods for investigating genome-level transcription events in cells and tissues in a separate experiment (Dufva, 2009). DNA microarrays are solid surfaces that contain microscopic copies of complementary DNA or oligoncleotides of varying lengths (e.g., short or long) arranged in spots (Petersen *et al*., 2005). Common microarray platforms such as Affymetrix, Agilent, and Illumina are more ubiquitous in today's market and can even be customized to address specific research questions. In the past, the costs to conduct microarray experiments were not cost-effective for most life science laboratories. Today, however, costs associated with microarray experiments are somewhat manageable for laboratories with relatively small budgets.

Nucleic acid microarray experiments involve the selection of an appropriate biological process to examine. Next, mRNA is isolated and converted to complementary DNA (cDNA). The cDNA is then labeled with a fluorescent molecule and applied to a microarray surface containing oligonucleotides. Fluorescence intensity comparisons of experimental treatments and baseline samples, following hybridization of nucleic acid sequences provide evidence regarding up-regulated genes, down-regulated genes and non-active genes (Dufva, 2009). Comparing differential gene expression in normal cells vs. abnormal cells or healthy cells vs. diseased cells is a routine strategy to elucidate the relevance of underlying genetic mechanisms that participate in biological processes. In addition to the identification of particular activated genes and gene expression levels, analysis of microarray data sets can provide information about biological processes, transcription factors, signal transduction pathways, biomarkers, and diseases associated with gene expression clusters.

DNA microarrays are now being used to rapidly diagnose microbial pathogens in patients, food samples, and water samples (Hou *et al*., 2018; Kostić, Stessl, Wagner, Sessitsch, & Bodrossy, 2010; Sakai, Kohzaki, Watanabe, Tsuneoka, & Shimadzu, 2012; Thissen *et al*., 2014). Microarrays are demonstrating application in resolving antimicrobial resistance in clinically-relevant microbes (Charnock, Samuelsen, Nordlie, & Hjeltnes, 2018; Uddin *et al*., 2018) and in biomarker elucidation (Chen *et al*., 2019; Tiwary, Kumar & Sundar, 2018). Microbial-based microarrays are cost-effective when compared to other microbe detection techniques and offer the added benefit of providing valid identification of test samples in less time. Using the approach under discussion, clinical samples such as nasopharyngeal, respiratory, blood, buccal, fecal, urine, spinal fluid, and saliva samples can be used to detect the presence of specific microbes or a spectrum of microbes known to cause disease. As referenced above, this technique has been used to detect the difference between antibiotic susceptible and antibiotic resistant bacteria. Recognition of antibiotic resistance genes in clinical samples can impact therapeutic strategies and potentiate patient recovery and survival.

Microbial Infections

A microbial infection occurs when a microorganism (e.g., protozoa, bacteria, fungi) invades the human body through a specific portal of entry. Humans can be exposed to microbes in a number of ways including ingestion, inhalation, fecal contamination, physical contact with fomites, and body fluid transfer. Moreover, the existence of co-infections in which more than one type of microbe (e.g., bacterial and viral) participates in a primary and secondary infection is possible and represents a dangerous situation (Abelenda-Alonso *et al*., 2020; Jia *et al*., 2017). Fig. 1 summarizes the general stages of many

microbial infections. Fig. 1 does not account for all known microbial infections. The first major step involved in the infection process is the attachment phase. Microbes possess attachment factors that mediate attachment to the skin or mucous membranes. Specific receptor molecules on the surface of the skin and mucous membranes are complementary to microbial attachment factors. During this phase some microbes remain attached to the skin or membranes, others penetrate these outer layers and become positioned in the internal environment. Some pathogenic microbes are ingested via contaminated food and water.

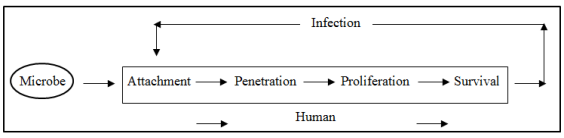

Fig. 1. Basic microbial infection strategy.

Some microbes also have the ability to invade specific cells, tissues, and organ systems using complex host-pathogen mechanisms in which microbial growth, differentiation, proliferation, and survival are the main biologic objectives. In terms of microbial infections, examination of gene expression data can reveal how microbes activate human and microbial genes in order to complete the fundamental infection strategy shown in Fig. 1. The next section of this review discusses research utilizing DNA microarrays to detect specific microbes and examine microbial gene expression profiles.

Protozoa and Microarray

Protozoa are unicellular eukaryotes that cause a variety of human diseases such as Toxoplasmosis, Giardiasis, Malaria, and Trypanosomiasis. There are now a pleothora of useful DNA microarray platforms to explore pathogenic protozoa (Kafsack, Painter, & Llinás, 2012; Moon, Xuan, & Kong, 2014; Wang, Orlandi, & Stenger, 2005). Chen *et al*. (2016) developed a DNA microarray system to concurrently detect 18 different species of human bloodborne protozoa from 5 of the most common genera of protozoa found in mammalian blood (*Plasmodium*, *Leishmania*, *Trypanosoma*, *Toxoplasma gondii*, and *Babesia*). A diagnostic test with a relatively low limit of detection was designed to detect waterborne pathogenic protozoa such as *Cryptosporidium parvum* (Lee, Seto, & Korczak, 2010). The microarray was deemed effective and clinically relevant based on comparative verification tests.

The investigators utilized protozoan small ribosomal RNA (rRNA) nucleotide sequence probes on the microarray platform. Ribosomal RNA subunit gene sequences serve as excellent sources of microbial probes for diagnostic microarrays for a number of reasons. First, it is well known that both the small and large subunits of prokaryotic and eukaryotic ribosomes are highly conserved among species. Additionally, rRNA gene sequences undergo fewer nucleotide modifications when compared to their macromolecular counterparts.

Classification of gene profiles during the various stages of infection could be used to create molecular countermeasure approaches that suppress microbial infection capacity and kill the microorganism or activate host immune mechanisms. Some protozoal parasites, such as amoeboid microbes have the ability to transition into multiple biological and structural forms (e.g., trophozoite, cyst) depending on the type of host they inhabit. The trophozoite stage is the vegetative phase or feeding and amplification stage of the parasite and is typically found in the human host, while the cyst stage is the protective form of the microbe and is found in the environment after being passed in the feces of mammals.

The cyst stage is a resistant form of the parasite that can survive harsh stimuli (e.g., climatic changes, chemicals). The cyst form is converted back to the trophozoite stage inside the intestines of the human host following human consumption

of the cyst via contaminated water or food. Understanding the genetic changes that occur during trophozoite-cyst conversion is paramount to control efforts.

Moon, Xuan, Chung, Hong, and Kong (2011) performed a microarray study to map the key genes responsible for *Acanthamoeba castellanii* encystation. Gene expression profiles of cysts were compared to *Acanthamoeba* trophozoites following microarray and bioinformatics procedures. There were 701 upregulated genes and 859 downregulated genes in the cyst stage compared to the trophozoite stage. Not surprisingly, a portion of the differentially expressed genes were associated with metabolic functions according to KOG analysis. Since *Acanthamoeba* encystation within the host further exacerbates immunological eradication efforts, understanding the essential genes involved in the encystation process may be beneficial.

Recently, a group of molecular parasitologists set out to identify biomarkers associated with miltefosine-resistance in visceral leishmaniasis. Following treatment with miltefosine, patients were evaluated. *Leishmania* parasites were then extracted from patients demonstrating visceral leishmaniasis relapse. Comparing differential gene expression characteristics of parasites from relapsed and cured patients, Tiwary, Kumar, and Sundar (2018) demonstrated that a cysteine protease-like protein was highly upregulated in the parasites from relapsed patients, suggesting that the cysteine protease-like protein could serve as a biomarker to monitor patient relapse.

Bacteria and Microarray

Bacteria are unicellular prokaryotes that account for a large number of human microbial diseases. Some of the well-known bacterial diseases are leprosy, diphtheria, plague, tuberculosis, and cholera. Ranjbar, Behzadi, Najafi, and Roudi (2017) recently designed a DNA microarray platform that contained distinct oligonucleotide sequences that were specific to ten different medically relevant bacteria (*Escherichia coli Shigella boydii*, *Sh.dysenteriae*, *Sh.flexneri*, *Sh.sonnei*, *Salmonella typhi*, *S. typhimurium*, *Brucella sp.*, *Legionella pneumophila*, and *Vibrio cholera*). Following the experiment, the microarray successfully detected all ten bacteria at the same time. Moreover, researchers recently used microarray technology (FDA-ECID DNA Microarray) to identify and characterize virulence gene composition of non-O157 *E. coli* serovars (Shridhar *et al*., 2019). Virulence gene identification in clinical isolates is equally as important as microbial identification and can provide a greater depth of understanding regarding the nature of infection and genetic factors influencing patient-related pathop hysiological outcomes.

Nosocomial infections are contracted during a stay at a healthcare facility and were not present before the patient was admitted. Keum *et al*. (2006) developed a DNA microarray-based detection system to identify nosocomial pathogenic *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in clinical isolates. The microarray technology demonstrated a sensitivity of 84.6% and 96.2% for *A. baumannii* and *P. aeruginosa*, respectively. Both nosocomial pathogens displayed a positive predictive value of 100%. Purulent meningitis is characterized by acute inflammation of the membranes associated with the central nervous system and is particularly devastating in neonatal populations (He, Li, & Jiang, 2016). Purulent meningitis is primarily caused by bacterial and viral infections. Purulent meningitis leads to a variety of unpleasant symptoms which in part depends on the microbial agent and can lead to death if untreated. Hou *et al*. (2018) designed a DNA-based microarray to enhance diagnostic efforts of the bacterial agents that cause purulent meningitis. A significant number of positive test results (87.5%) were generated using the microarray detection approach compared to only 58.3% using the traditional cerebrospinal fluid

culture detection method. The application of a rapid identification and detection procedure significantly reduces diagnostic deliberations. Antibiotic resistant bacteria are the scourge of healthcare facilities across the world. Antibiotic resistant bacteria lead to unimaginable loss of life and account for millions in medical treatment costs.

Antibiotic resistant bacteria are a global health threat that could render today's powerful antimicrobial options essentially useless. Moreover, as many have pointed out, antibiotic resistance may be further complicated by severe acute respiratory syndrome coronavirus 2 infections and COVID-19 (Rawson *et al*., 2020). The use of rapid molecular recognition technology that allows multiple bacteria to be tested for genetic signatures that confirm antibiotic resistant genes is extraordinarily beneficial to physicians and patients. Carbapenemase and extended-spectrum β-lactamases (ESBLs) are particularly worrisome enzymes produced by some bacteria because they confer antibiotic resistance to bacteria. Carbapenemase- and ESBL-producing bacteria have generated a significant number of hospital-acquired infections (HAI) worldwide. Uddin *et al*. (2018) created a microarray platform designed to detect *Acinetobacter baumannii* carbapenemase and ESBL genes in patient specimens. Researchers demonstrated that their microarray-based method (CT 103XL Check-MDR) of antibiotic resistance genes detection is equally or more effective than other methods.

Conclusions

Less than 1% of the microbes on earth actually lead to human disease. However, the impact that microbial infections have on the economy, human health, and other societal factors elicit enormous responses from the medical and research communities. Techniques such as DNA microarrays and now next generation RNA sequencing or RNA Seq are becoming increasingly more prevalent in life science laboratories because of their sensitive nature, high throughput capacity and application. Moreover, these techniques are more advantageous for microbial detection and identification compared to traditional sequencing and PCR arrays. Microbial detection microarrays are constructed by adding oligonucleotides (probes) from specific microbes to a solid matrix. From a microbial perspective, nucleic acid hybridization techniques are largely applied to clinical diagnostic assays. However, a growing segment of the literature points to a shift in the use of DNA microarrays to study underlying genetic mechanisms of fundamental biological processes. The determination of the molecular constituency, biomolecular interactions, and canonical signaling pathways associated with microorganisms can provide a wealth of beneficial biologic and clinical information.

A review of the DNA microarray investigations in this article highlight previous uses of this technology to examine the biology of microorganisms. This review also focuses on the use of nucleic acid technology to accurately and rapidly detect unique microbial species from clinical, food, and water samples.

In clinical and hospital environments, tests that have the ability to rapidly detect and identify microbes is paramount. A delay in the diagnosis of an infectious entity may provide the microbial agent more time to proliferate and potentially expand to other ectopic sites in the human body thereby causing more damage to tissues and vital organs. Elaborate time course studies could be developed that allow microbiologists an opportunity to map the global gene expression profiles of bacteria and protozoa at different phases of the infection process (e.g., attachment, penetration, proliferation, etc.).

It is hypothesized that unique genes or gene families are involved in discrete stages of the microbial infection process. Further, microbial genes can be identified that likely play a role in

host symptoms and clinical outcomes. With such detailed molecular characterizations, it would be possible to not only identify biomarkers but to link specific microbial gene changes with distinct phases of infection (i.e., pathogenesis markers). Moreover, connecting gene expression profiles with microbial responses to drugs and other treatments is also possible using DNA microarrays.

Performing microarray procedures to analyze host cell gene expression profiles during a microbial infection can also have tremendous scientific and therapeutic value. New microarray platforms are needed to further assist microbiologists, clinicians, and other healthcare workers. The authors future microbiological investigations will explore the use of microarray technology to understand host responses following viral and bacterial infections at the molecular level. The advent of new, quality control, normalization, and bioinformatics software is certain to have a constructive impact on microarray data usability and diversity of data visualizations. Findings generated from DNA microarray studies will open up new possibilities to treat and prevent microbial diseases.

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