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DNA microarrays in infectious diseases

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บทบาทของ DNA microarray ในโรคติดเชื้อ

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DNA microarrays or gene chips are developed based on the currently available information of complete sequences of human and several pathogen genomes and the technology that enables placing of nucleic acid probes on a single array at a microscopic and high-density scale.[1] The working principle behind DNA microarrays is parallel to traditional Southern hybridization, its "macro" array counterpart. The novel fabrication technologies of DNA microarray can immobilize tens of thousands genes (or oligonucleotides) or all genes of an entire genome on a solid substrate or platform in comparison to hundreds of genes on Southern blot.[2-4] Samples for microarrays can either be microbial DNA content or their gene-expression profiles. Hybridization of labeled targets derived from nucleic acids in the test sample to the probes on the array makes probing multiple gene targets possible in a single experiment. Thus, DNA microarray is a high-throughput technology for detection and analysis of genes and gene expression on a "global" or "genome-wide" scale.

Since its emerging in the early 1990s, the number of studies using DNA microarrays has risen markedly during the past decade.[3,5] Early microarray studies focused mainly on human cancers due to the readily available tumor specimens and the social impact of the diseases.[6-8] DNA microarray technology has been gradually becoming a standard tool in regular biological laboratories. This tool is presently applied to other fields of medicine including the infectious diseases.[9-11] The potential of the DNA microarrays in investigation of both sides of the host-pathogen interaction offers a mean to study infectious diseases in a systemic and efficient way. [12-14]

A large number of DNA sequences including antigenic determinants, virulence factors, and multiple different sequence variants of each gene target immobilized on the microarrays allow the detection of a broad range of pathogens with high specificity and discriminatory ability.[15] Therefore, identification of pathogens via DNA microarrays needs not be restricted to a single strain, species, genus, or class of organisms. This advantage is beneficial for identification of causative agents of infectious diseases. Although detection of microbes using the currently available molecular techniques is faster than conventional methods, discrimination of very closely related species or different organisms without prior amplification of target DNA or pre-identification of the pathogen remains difficult. Several studies demonstrated the efficiency of DNA microarrays for rapid and simultaneous identification of closely related

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pathogens and/or distinct etiologic organisms in a single assay.[16-27] In addition, depending on probes designed on the platform DNA microarrays have been previously shown to be a tool with broad identification capacity to identify virulence factors, detect polymorphisms or mutation in the pathogens, study complex microbial populations, determine taxonomic relationships between microbial strains at species to strain level resolution, determine molecular typing, identify multiple antibiotic resistance genes, survey the spread of strain or antibiotic resistance determinants in epidemiological studies, and discover unknown causative agents of infectious diseases.[24, 28-40]

The crucial advantage of microarray-based approach allows the entire biological pathways and coordinated interaction of multiple pathways or genes to be studied without prior bias to a particular gene or pathway. Thus, microarrays have been used for genome-wide analysis of host expression responses to diverse pathogenic stimuli and differential gene expression of microbial virulence factors during infection providing an insight of the host-pathogen interaction, the key to pathogenesis of infectious diseases.[14, 41, 42] To study host immune responses, global expression analyses of both the innate and adaptive immune cells at various stages of differentiation, maturation and activation during microbial invasion is an unbiased approach to comprehend the complex coordination of immune responses to infection.[9] Numerous studies have utilized microarray analysis to follow gene expression alterations of host cells, either target cells or immune cells, in response to interactions with infectious pathogens, and vice versa.[43-55] Distinctive host gene expression patterns observed in patients infected with different etiologies might assist in the differential diagnosis of infectious diseases.[56] The relationship between expression profiles of host inflammatory cells and clinical status may be useful to predict clinical outcome.[57] In addition, susceptibility to infectious diseases and clinical response to antimicrobial drugs may be associated to certain host genetic background.[58, 59] Thus, global analysis of host and microbial interaction contributes to new insights on pathogenesis of infectious diseases leading to novel strategies for therapeutic and prophylactic interventions and prognostic markers of outcome.[11, 60-64]

Microarrays also have limitation or disadvantages. Although the DNA microarray technology is a genome-wide approach, the detection is restricted to DNA immobilized on the platform.[9] As a result, this technique cannot detect sequences or genes that are absent on the array. The genes or oligonucleotides on the array need to be updated according to currently available information. Like other molecular methods, standardization of the system is required before the microarray technique is implemented for clinical practice in infectious diseases.[65] Due to its early application, protocols are not well-defined. In addition, the cost of equipments and software is still high. Personnel require special training and complementary knowledge. Mathematical and statistical knowledge and/or experts are necessary for study design and data analysis due to substantial amount of data derived from this technique. Therefore, most microarray techniques are presently used in research.

In author's opinion, the main advantage of DNA microarray technique is its rapid, sensitive and high-throughput nature. This advantage is particularly useful for urgent diagnosis of or screening for infectious diseases that require immediate treatment and/or infectious control such as diseases caused by highly virulent and contagious agents and infection in critically ill patients or immunocompromised hosts. However, this technique should not replace but may serve as a complement conventional testing in clinical laboratories. The future application of microarrays in routine investigation of the infectious diseases depends on the development of more cost-effective protocols and equipments, more robust and simplified formats, easier or user friendly step of data analysis, and the adequate evaluation of their performance (efficacy) and convenience (efficiency) compared with other molecular methods. Hence, it is necessary to develop more simple assays that could be performed for all diagnostic laboratories.

In conclusion, microarrays will serve as a powerful tool that can be applied in several aspects of infectious diseases including diagnosis, identification of microbial and host factors related to prognosis and response to treatment, screening for antimicrobial resistance, epidemiological investigation, evolution study of microbes, identification of drug targets and development of new antimicrobials, and vaccine design. Thus, the microarray technology will revolutionize infectious disease practices in the future. However, clinicians must learn to use it effectively and appropriately and realize its limitation. The obtained accurate results, it needs to be interpreted in conjunction with other clinical and laboratory data.

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