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Review

Is this the real time for genomics?

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ABSTRACT

In the last decades, molecular biology has moved from gene-by-gene analysis to more complex studies using a genome-wide scale. Thanks to high-throughput genomic technologies, such as microarrays and next-generation sequencing, a huge amount of information has been generated, expanding our knowledge on the genetic basis of various diseases. Although some of this information could be transferred to clinical diagnostics, the technologies available are not suitable for this purpose. In this review, we will discuss the drawbacks associated with the use of traditional DNA microarrays in diagnostics, pointing out emerging platforms that could overcome these obstacles and offer a more reproducible, qualitative and quantitative multigenic analysis. New miniaturized and automated devices, called Lab-on-Chip, begin to integrate PCR and microarray on the same platform, offering integrated sample-to-result systems. The introduction of this kind of innovative devices may facilitate the transition of genome-based tests into clinical routine.

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1. Introduction

The genomic era started with the completion of the Human Genome Project in 2001, opening new interesting challenges from biological research to medicine applications. During this period, we have witnessed the astonishingly fast development of high-throughput technologies, including hybridization and sequence-based ones, which allowed the transition from studies involving single genes to those employing a more extended genomic approach. This is generating a plethora of

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data that are collected in public databases such as GenBank for DNA/RNA and protein sequences, and OMIM (Online Mendelian Inheritance in Man) for gene and genetic phenotypes. These public resources, daily updated, currently contain 46,608 human gene sequences and 4023 phenotypes with a known molecular basis [1–5]. Some of this information could be already transferred to clinical diagnostics, but the technologies available are not adequate for this purpose. The actual challenge is the use of genome-based technologies in clinical practice.

In the next paragraph, we will briefly discuss the two most important technologies used in genomic screening analysis.

2. High-throughput technologies in genomics: DNA microarrays and next-generation sequencing

DNA microarrays and next-generation sequencing (NGS) are the two most important technologies for high-throughput genomic analysis [6]. During the past 20 years, DNA microarray technology has been developed and consolidated as a routine tool in research laboratories and is now transitioning to the clinic. While we are witnessing this transition, NGS is catching up [7,8]. Over the past 8 years, a number of NGS technologies have emerged that enable the sequencing of large amounts of DNA in parallel and at significantly lower costs than conventional methods. NGS technologies are suitable to different applications, such as whole or targeted genome sequencing, and RNA sequencing (RNA-seq). Due to cost reduction, the latter application may soon replace DNA microarrays in transcriptome profiling analysis [9]. However, the transition of NGS into clinical practice is slowed up by non-automated experimental procedures and lack of efficient and user-friendly methods to store, process and analyze the large amount of data produced [10].

Despite the great potential of traditional DNA microarrays and NGS, a number of issues need to be tackled to implement these technologies in clinical diagnostics. In this review we will analyze emerging DNA microarray platforms that may offer immediate opportunities to implement genomic tests in clinical medicine. Thanks to several characteristics, these sample-to-result systems are available for real-time detection and offer a more reproducible, qualitative and quantitative multigenic analysis.

3. DNA microarray technology: state of the art

DNA microarrays technologies are based on the ability of DNA to find and spontaneously bind its complementary sequence in a highly specific, rapid and reversible manner [11]. Over the years, this technology has been applied to genome analysis in distinct medical fields allowing the association of polygenic alterations to specific pathologies [12–22]. Obtained DNA microarray data are collected in public repositories, such as Gene Expression Omnibus (GEO), which contains also NGS and other forms of high-throughput functional genomic data [23]. Although the collected information could be relevant from a medical perspective, they are not easily accessible from a clinical practice standpoint and only few of them have already been transferred to the bed-side.

To date, a small number of microarray-based tests have been cleared for diagnostics. The main reason is related to the complexity of this technology, which is suitable to research laboratories but not to diagnostic ones. Below we will briefly describe the first diagnostic, prognostic or pharmacogenetic tests based on DNA microarray technology.

The *MammaPrint* test by Agendia, the first in vitro diagnostic multivariate index assay (IVDMIA) to be cleared by the US Food and Drug Administration (FDA) in 2007, is an individualized metastasis risk assessment test with prognostic value for breast cancer patients with stage 1 or 2. It analyzes the expression of 70 genes and stratifies patients into two distinct groups: low risk or high risk of distant recurrence [19,24–26].

The *Pathwork Tissue of Origin* Test by Pathwork Diagnostics is a microarray-based gene expression assay for improving classification of clinicopathologically ambiguous tumors. It can detect the expression level of 1550 genes in each tumor sample of unknown or poorly differentiated origin (primary or metastatic) and to determine the similarity to 15 tissue types belonging to known tumors [25,27,28]. This test gained FDA clearance in 2008 for frozen samples and in 2010 for formalin-fixed and paraffin-embedded samples.

The *AmpliChip CYP450* by Roche, cleared by the FDA in 2004, is a pharmacogenetic test based on Affymetrix microarray technology that analyzes allelic variations in two highly polymorphic cytochrome P450 genes (CYP2D6 and CYP2C19), whose encoded enzymes regulate the metabolism of drugs from a variety of classes. By predicting altered drug metabolism, it is possible to prevent harmful drug interactions and to ensure the optimal use of drugs [25,29–31].

Despite the undeniable advantages of these tests, their applications in clinical settings are still limited by the use of traditional microarray technology at few highly specialized laboratories. In the next section, we will analyze in detail the advantages and limits of traditional microarray technology.

4. DNA microarray technology: advantages and limits

The main benefits of DNA microarray technology are *high-throughput analysis*, *miniaturization* and *safety* [32]. The *high-throughput analysis* allows parallelism through a direct comparison between thousands of probes spotted on the microarray and their complementary targets. This advantage has been reached thanks to *miniaturization* of the array surface, leading also to a significant improvement in terms of decrease of reaction volumes, increase of sample concentration and acceleration of hybridization kinetic. *Safety* derives from the use of fluorochrome labeling methods, which avoid handling by the operator of radioactive or toxic compounds during the experimental process.

Despite the undisputed advantages of DNA microarray technology, there are many limiting factors that hinder their routine use in diagnostics. Some of these, such as the lack of *accuracy* and *reproducibility* [33–37], depend on the experimental phases. Although a detailed description of these limiting factors goes beyond the focus of this review, we will briefly describe them below.

- *Sample preparation and labeling*. In this phase, the sample quality and quantity have a crucial role since its partial or total degradation can affect the entire experimental outcome.
- *Hybridization and post-hybridization washing*. The specific binding between probes and their complementary targets mainly depends on the stringency of washing buffers and temperature. Small changes in these phases, which are not fully automated, may produce non-specific interactions.
- *Image acquisition*. Image acquisition can be influenced by scanning parameters, such as intensity and signal resolution, which are used to increase the sensitivity and reduce the noise. The resulting image has to be further submitted to quality assessment and pre-processing data analysis steps (such as grid overlay) that could greatly affect the final results.

Another important aspect affecting accuracy and reproducibility of DNA microarrays is the *low dynamic range* of detection. Part of the signals detected by traditional DNA microarrays fall in a window with a linear dynamic range, whereas those near to background or saturating levels are not.

The complexity of traditional DNA microarray resides not only in its experimental procedures and image acquisition, but also in analysis and interpretation of obtained data that require dedicated software and bioinformatics staff. Almost all experimental and analytical phases depend on the operator, and the *lack of automation* further reduces accuracy and reproducibility. Additional limits of traditional DNA microarray technology include the *requirement of highly skilled personnel*, *high costs*, and *prolonged procedures* (generally 48 h).