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REVIEW ARTICLE

A high-performance gene chip platform for detecting genetic markers from circulating tumor cells

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Abstract Diagnosis of and therapy for early-stage tumors has the potential to decrease morbidity and mortality among patients with such malignancies. Although promising advances in imaging technology and other diagnostic modalities have been achieved, evidence that primary cancers begin shedding neoplastic cells into the circulation at an early stage is accumulating. Specifically, approximately 10^6 cells are shed daily per gram of tumor. Thus, circulating tumor cells (CTCs) are a potential source for the noninvasive and early diagnosis of cancers. Several studies have indicated that a preoperative detection of micrometastases may reflect the transient shedding of tumor cells, metastatic potential, or residual disease, but that postoperative micrometastases are likely to indicate minimal residual disease. Such neoplastic cells may be present in the bloodstream in very low numbers and would be difficult to detect by conventional methods. In this article, we introduce a highly sensitive gene chip analysis method, developed from a colorimetric membrane array into a weighted enzymatic chip array, to detect the gene clusters of CTCs. This gene chip method provides a fast and accurate analysis of gene clusters, with performance that is substantially better than that of traditional biochemical examinations. The development of this gene chip solves the problem of costs and artifacts that hampers traditional detection methods, and thus marks an important milestone in the field of clinical diagnosis.

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Introduction

Circulating tumor cells

Circulating tumor cells (CTCs) are cells that are present in the circulating blood of cancer patients and have the antigenicity and genetic characteristics of the primary tumor.[1] Many studies have reported that, even in the early stages of a cancer or when the tumor is very small, such cancer cells may shed into the bloodstream or lymphatic system. A study by Folkman et al found that active angiogenesis to provide nutrients to a tumor is observable when the tumor grows to a size of 2 mm in diameter.[2] At that point, tumor cells can enter the circulation, leading to micrometastases. A study by Chang et al reported that about 10^6 cancer cells per gram of tumor are shed into the circulating blood every day.[3] Because blood samples are easy to collect, methods using peripheral blood samples as specimens to detect whether CTCs are present in patients are the most sensitive, accurate, and relatively noninvasive auxiliary methods to assist in the early clinical diagnosis of cancer.

Currently, there are many biotechnologies that can detect CTCs in the bloodstream. These techniques include immunohistochemical staining,[4] flow cytometry,[5] the reverse transcriptase polymerase chain reaction (RT-PCR),[6] and the CellSearch system (Veridex LLC, Raritan, NJ, USA).[7] However, these methods have their shortcomings and limitations. For example, immunohistochemical staining methods cannot accurately determine the number of circulating cancer cells in the blood of cancer patients. Flow cytometry cannot describe the cell morphology of the CTCs and requires further immunohistochemical staining for confirmation,[8–10] causing an increase in time and expense for cancer detection that cannot meet the clinical requirements of effectiveness. CellSearch system, a US Food and Drug Administration-approved CTC test for metastatic breast, prostate, and colorectal cancer, is based on a combination of immunomagnetic labeling and automated digital microscopy. Its detection limit is one CTC per 7.5 mL blood. Moreover, the anti-EpCAM antibody-based enrichment strategy does not confer tissue-type specificity, and the analysis requires specific equipment to perform it.[11]

Cancer genetic markers

With advances in molecular biology techniques, not only have the genetic changes and molecular mechanisms involved in various carcinogenic and transfer mechanisms been elucidated, but clinically early diagnosis and prognostic evaluation have also become simpler and easier by detecting specific genetic markers of the cancer tissues. Therefore, this has become an important direction of development in the early diagnosis of cancer by using cancer-specific target genes and changes in gene expression, for example in DNA sequence variants and mRNA expression, as biomarkers for the differential diagnosis of cancer.[12] In addition, scientific evidence argues for the existence of CTCs in patients with early cancer. Therefore, this has become an important trend in cancer diagnosis and treatment in terms of developing the detection of special genetic markers of CTCs to assist in the early diagnosis of cancer.[13]

Genetic testing

Two well-known methods, RT-PCR and real-time quantitative PCR, are highly sensitive methods that can detect genetic markers of CTCs.[14–16] However, these techniques can detect only one type of genetic molecule at one time, which is the greatest limitation of these techniques in clinical applications.[17] Due to the heterogeneity and characteristics of simultaneous variation in multiple genetic molecules in cancer cells, methods using multiple genetic markers can improve the sensitivity and specificity of cancer detection compared to methods using a single marker.[18,19]

Gene chip operation platform

Gene chips, when implanted with a probe for multiple genetic markers, can simultaneously detect several, dozens, or even hundreds of genetic markers in one test. Through analysis by specific software, an evaluation of the overall experimental results for all markers can be provided to greatly improve test accuracy in clinical application.[20–22] Gene chips have the advantages of being fast and accurate, and can be used to analyze large numbers of tests; therefore, this approach is very convenient in investigating the association between diseases and genomic changes.

Wang et al used a method combining suppression subtractive hybridization and cDNA microarray chips to investigate changes in all genes involved in the carcinogenic pathway from colorectal hyperplastic polyps to colorectal cancer.[23] They successfully identified 71 genes specific for colorectal cancer as diagnostic markers and obtained patents in Taiwan (No. I278519), the USA (No. US 7575928), and the European Union (No. 04 003 301.1). In order to implement the clinical application of specific gene groups, it is necessary to develop and establish a gene chip detection platform that has high sensitivity, high performance, and low cost so that it can be efficiently used in clinical detection.

Colorimetric membrane array

Traditionally, microarrays use glass carriers and fluorescent labeling. Although this method has excellent sensitivity, it is not always convenient in terms of operation. In addition, its biggest drawbacks are expensive reagents and costly equipment necessary for the related analysis.[24,25] In order to detect multiple genetic markers with a simple, convenient, fast and low-cost method, Lin et al, in 2005, first used a nylon membrane carrier to replace the glass carrier, and digoxigenin cDNA labeling to replace the fluorescent labeling.[26] They successfully established a low-cost and easy-to-operate colorimetric membrane array (CLMA) that was patented in Taiwan (No. I287581; Fig. 1). There are dozens of publications reporting clinical experimental results pertaining to the related technology platform, including for gastric cancer, colorectal cancer, breast cancer, and lung cancer.[26–30]