Application of Biochips in Cancer Diagnosis

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Abstract. Cancer biomarkers play an important role in understanding tumor location and physiological activity. With the development of interdisciplinary technology, biochips are promising as the main tools for detecting biomarkers. Biochip refers to a microarray hybrid chip with a high-density roof of bioinformation molecules on a solid phase supporting medium, and the principle of microfluidic chip is different from that of ordinary biochips, which is mainly based on the principle of microfluidic mechanics. Biochips have a wide range of applications, playing an important role in the detection of gene expression levels, gene diagnosis, drug screening, personalized medicine, sequencing, bioinformatics research, and other fields. This paper will focus on the mechanism of three main biochips, combined with the principles of different biochips to explain the application and development of biochips in the field of cancer stage and explore the advantages and disadvantages of various biochips.

Keywords: Biochip; cancer diagnosis; microarray; microfluid; biomarker.

1. Introduction

The challenge of cancer treatment is primarily manifested in the following aspects: 1. Heterogeneity-Cancer encompasses numerous types, and even within the same type, there exists a high degree of heterogeneity among individuals, resulting in varying efficacy of treatments; 2. Diagnosis-Early-stage cancers often lack obvious symptoms and are challenging to diagnose; 3. Metastasis-Cancer cells have the ability to separate from the original tumor site and migrate through the blood or lymphatic system to form new tumors in other parts of the body; 4. Recurrence-There is a high risk of cancer recurrence, which poses greater difficulty in treatment. However, by detecting cancer biomarkers, the difficulty of diagnosing cancer can be greatly reduced. Cancer biomarker is a specific attribute that is assessed to indicate the likelihood of developing cancer, the presence of cancer, or the prognosis for patients. These biomarkers are created by tumor cells and can be identified in bodily tissues or fluids, offering valuable information for diagnosing cancer, predicting outcomes, tailoring treatment plans, anticipating drug reactions, and monitoring the progression of cancer. In a broad sense, cancer markers can be DNA, RNA, proteins, peptides, or cellular metabolites, or they can be specific changes in cells, tissues, or biological fluids (such as urine or cerebrospinal fluid).

With more and more tumor markers being identified, the corresponding technologies applied to detect these markers are also developed for cancer diagnosis, among them, biochip was proved to be a promising one. Biochip technology is the result of the cross-fusion of multiple scientific fields, involving the joint application of biology, chemistry, physics, electronics, computer science, engineering, and other disciplines. It can be conducted on cells, proteins, nucleic acids, and other biological molecules. Accurate, fast, and high-throughput detection greatly reduces detection costs. The development of biochips can be traced back to the advances in microelectronics technology in the 1960s and 1970s, which were later applied to the detection and analysis of biomolecules. Until the 1980s, the emergence of microfluidics and microelectromechanical systems (MEMS) technology provided a new platform for the development of biochips.

Professor Patrick O. Brown of Stanford University and his colleagues invented DNA microarrays in the early 1990s, a technology that allowed the expression of thousands of genes to be monitored simultaneously. Stephen P.A. Fodor of Affymetrix and others developed the first commercial biochip product "gene chip", which was also an early example of the biochip concept. The current classification of biochips mainly includes gene chips, protein chips, and lab-on-a-chip (also called

microfluidic chips). With the advancement of biochip technology, there are also cell chips, tissue chips, organ chips, and other types.

This review will focus on the application of biochip technology in cancer diagnosis, in that case, elaborating on the mechanisms of action and the advantages and disadvantages of different types of biochips.

2. Biochips in Cancer diagnose

2.1. Gene chip

As one of the core technologies of biochips, "gene chip," which is also called DNA chip, or DNA microarray, has been developing for several decades. Briefly, based on central dogma, the principle of gene chip is to arrange nucleotides of known sequences on a solid-phase carrier in a specific order at high density as probes to detect complementary sequences in the test product. After that, by detecting the hybridization signal and analyzing the signal using computers and related software, the information and expression level of the target DNA sequence can be seen at a glance. Based on this principle, Researchers can compare the DNA sequences of cancer patients with normal DNA (Fig.1) sequences to find gene fragments that may cause cancer or be related to the development of cancer [1]. Therefore, gene expression data may now be acquired on a scale that was previously unthinkable thanks to DNA microarrays. Its usage can be roughly divided into four steps [2], (1) construction of chip array; (2) sample preparation; (3) hybridization; (4) signal detection and interpretation.

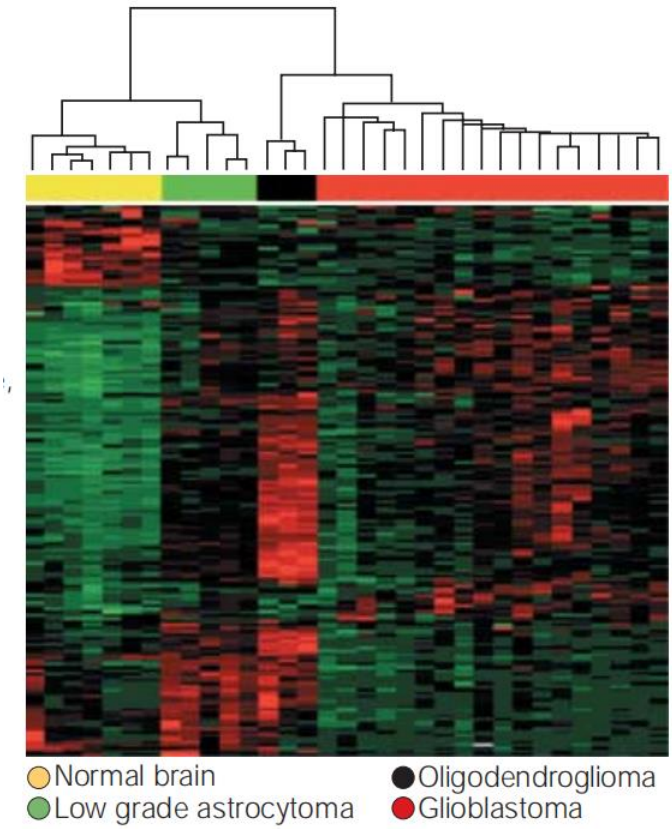

Fig 1. Normal brain compared with brain cancer by DNA microarray [3].

As mentioned earlier, biomarkers can be protein markers (such as enzymes, receptors, ligands, tumor antigens), nucleic acid markers (DNA, mRNA, non-coding RNA), metabolite markers (special metabolites), immune Markers (antibodies, cytokines), cell markers (proteins or other molecules on the cell surface) [4]. Moreover, biomarkers can be classified according to their different functions, such as Screening, identifying recurrence, treatment monitoring, and disease diagnosis. Here are some biomarkers used in various cancer detection (Table 1).

Table 1. Common cancer biomarker (the information of this table was from [5]).

Type of Cancer/Infected Location	Biomarker
Liver	AFP, CT antigens,CEA (Carcinoembryonic antigen)
Breast	CA 15-3,CA27.29,ER & PgR (Estrogen, progesterone receptors) ,BCL2,HER2
Prostate gland	PSA,CT antigens,PCA3
lung	CT antigens,HER2,Nse,EGFR,KRAS, ALK,ROS1
bladder	CT antigens,NMP 22,FGFR2 & FGFR3,Fibrin-fibrinogen
skin	CT antigens, NY-eSO-1
Stomach	RCAS1
Pancreas	CA 19-9
colon	CA 19-9, CEA
Thyroid gland	Calcitonin,Thyroglobulin
Ovary	CA 125, HCG-β, OVA1
Blood	Tdt,CD30,BCL2,BCR-ABL fusion gene,CD20,CD22,CD25

With the advent of DNA microarray, many early works on cancer diagnosis using DNA chips were carried out. Using cDNA microarray technology, L. Paskins reported an experiment aimed at identifying putative diagnostic markers for prostatic intraepithelial neoplasia and prostate cancer. The microarray, which consisted of 1877 cDNA clones representing a range of stages and grades of prostate cancer, precursor lesions, and normal tissue, was assembled and used, and a few genes were found to be overexpressed in PIN [6]. In 2007 Tetsuo Ito et al. reported a "3D DNA chip" for diagnosing esophageal cancer, which can accurately identify characteristic genes with an accuracy of up to 95% [7]. Employing a small square chip covered in carbon that resembles a diamond to increase the signal-to-background ratio, Tsunedomi's group has invented a unique DNA microarray which is the potential to be used in vitro diagnosis. In other words, this DNA microarray could be useful in personalized treatment. The Invader UGT1A1 Molecular Assay system was utilized to construct the targeted DNA microarray on a 3 mm^2 chip, which resulted in a nearly two-fold reduction in cancer diagnosis time. By using specific probes for each polymorphism, the assay uses fluorescent labeling of sample DNA to detect polymorphisms. The newly created DNA microarray test produced genotyping findings for UGT1A1*28 and UGT1A1*6, and it was demonstrated that these results were highly compatible with those from previously existing methods [8]. The accuracy of DNA microarray depends on not only the specificity of detecting samples but also the precision of data analysis, thus, the strategy used to process the large amounts of genetic information draws researchers' attention as well. In 2020, Hamed Tabesh and others used deep learning methods combined with DNA microarray to analyze and diagnose Acute Myeloid Leukemia [9].

Except for DNA, currently, microRNA (miRNA) as a type of biomarker for detection, is also applied to gene chips [10]. As small noncoding RNAs, miRNAs participate in a variety of important physiological activities, such as apoptosis, differentiation, and proliferation [11]. Furthermore, miRNAs are expressed in all bodily fluids, including blood, semen, and saliva. That means it would be possible to be used in cancer detection in an in vitro environment, simply by extracting the patient's body fluids and analyzing their genetic profile via miRNA. To demonstrate the accuracy of DNA microarray [12], a parameter called MIAME standard was proposed, which aimed to provide a foundation for reporting microarray results, ensuring that the data can be easily interpreted and independently verified.

2.2. protein chip

Protein chip (protein microarray) is an extension of DNA chip technology. Therefore, it has many in common with gene chip, both protein chips and gene chips work in the form of microarrays, which helps them perform high-throughput analysis. The difference between them is that the probes of

protein chips are proteins (such as enzymes, antigens, antibodies, receptors, ligands, and cytokines) instead of DNA. Protein chips, also known as phenotypic fingerprints, can concurrently identify, in contrast to gene chips, the content of every protein in biological samples that may be connected to sickness or damage brought on by fluctuating circumstances. Put differently, it may show protein interactions, functions, and activities directly, which improves the clarity and ease of analysis of the results. For example, Ciphelxen Biosystems found six putative prostate cancer biomarkers in three days by using protein chips to identify serum samples from patients and healthy individuals. Additionally, Pisarchick et al. employed the substrate to spot antibodies and utilize them to identify variations in protein expression between malignant and normal tissue. They discovered that the production of a few proteins, including the gelatinase protein and the prostate tissue-specific antigen, is crucial to the growth of tumors [13]. The above are early examples of exploring protein microarrays for cancer detection.

Protein microarrays are mainly divided into two categories: Forward protein microarrays, in this type of microarray, the elements on the array are capture molecules, which include antibodies, proteins, nucleotides, or aptamers. Among them, antibody array is the most common form, which uses the specific interaction between antibodies and proteins to identify and analyze proteins; The other one is reverse microarray, unlike forward protein microarray, the array itself of reverse protein microarray are composed of proteins on which subsequent analyses can be performed [14].

By detecting biomarkers, protein chip is also used in diagnosing cancer. And it may be a more effective way to diagnose cancer through protein microarray detection of markers because proteins often directly reflect the physiological processes of tumor cells. SUN and others used a combination of multiple biomarkers to perform protein microarray detection and successfully discovered 16 cancer patients among 15,867 individuals. In tests on 793 healthy individuals, the specificity of this protein chip reached 97 % [15]. Furthermore, Wang et al. used protein microarray to identify novel autoantibodies against tumor-associated antigens (TAAs) and to create diagnostic models that could help distinguish between pulmonary nodules and lung cancer [16]. The advantages of protein chip is that it can be used directly with crude biological samples and can quickly find multiple biomarkers. Its disadvantages are also obvious. The stability of proteins is extremely poor, and the experimental conditions must be strictly controlled in the laboratory, which has caused obstacles to the development of protein chip.

2.3. lab-on-a-chip

A chemical or biological laboratory on a chip measuring several square centimeters is referred to as a "lab-on-a-chip." It includes sample preparation, reaction, separation, detection, and cell culture in the domains of chemistry and biology. It is also known as a microfluidic chip or a micro-total analysis system (μTAS). The system's controlled fluid is distributed throughout a network of microchannels formed by the integration of fundamental operating units like selection and lysis. The four fundamental parts of a lab-on-a-chip are microfluidics, living cells or tissue, stimulation, and a sensor component [17]. Unlike microarray chips, in order to more accurately replicate the microenvironment of tumor tissue in vivo, lab-on-a-chip manipulates fluids at the microscopic level to regulate parameters relevant to cell culture. Because a series of operations such as sample collection, extraction of specific biomarkers, signal conversion, and data processing can be performed on a carrier of only a few square centimeters. Lab-on-a-chip could revolutionize cancer screening by taking advantage of low-cost, rapid, specific detection of biomarkers [18].

Circulating in bodily fluids, exosomes are released from stem cells, including tumor cells. serving as messengers between various cells, sending and receiving a range of cargoes, including proteins, mRNA, and ncRNA [19]. Exosomes are currently being considered as a potential biomarker for the diagnosis of cancer. They can be isolated from bodily fluids including blood, urine, pleural effusion, ascites, saliva, and so on [20]. Yoon-Tae Kang and his team developed a microfluidic chip device that can effectively isolate exosomes, a new Exo-Chip. The alternating narrow and wide ripple-like architecture of this annexin V immobilized microfluidic enhances the binding interaction between

particular exosomes and PS-targeting molecules. In that case, both capture efficiency and purity are elevated. Furthermore, the article also showed, by using this kind of microfluidic device, that patients with lung adenocarcinoma had more exosomes detected in their blood than did healthy controls. More precisely, it was found from initial research employing biocompatible elastomer polydimethylsiloxane (PDMS) blocks and small chamber devices (Supporting Information S5) that the annexin V immobilized microfluidic chip captured more exosomes than the immuno-affinity method based on anti-CD63 [21]. Not only have that, lab-on-a-chip can performed three-dimensional (3D) cell culture, which allows establishment preclinical tumor cell models. By observing organ models grown from cancer patient cells, researchers can study the mechanisms of cancer cell development and transport. For example, Hao and his team developed a miniaturized bone-on-a-chip (BC) based on the principle of simultaneous growth dialysis. Furthermore, the co-cultures of metastatic human breast cancer cells and osteoblastic tissue grown in the BC exhibited numerous important markers indicating breast cancer spreading to the bone [22]. The development of microfluidic chips has resulted in many types, but many aspects of microfluidics still have broad room for improvement, such as how to avoid contamination when extracting samples, developing more advanced data analysis equipment, etc.

3. Summary

Biochips have undoubtedly brought a ray of light to the diagnosis of cancer. Using different types of biochips to detect different cancer biomarkers can effectively obtain information of cancer progression, which provides great convenience for the classification of cancer and the prognosis of cancer. For example, using gene chip to detect cancer signature genes, the advantage is that gene chip can determine the site of cancer gene mutation, but the accuracy of gene chip data still needs to be improved. The use of protein chip to detect cancer autoantibodies can intuitively reflect the physiological activities of tumor cells. However, due to the instability of proteins and the complexity of their structure, the application of protein chip is limited. Using lab-on-a-chip to detect exosomes is non-invasive and has better patient compliance, but it still has great potential in data analysis and other aspects. These methods have high sensitivity and specificity. When compared to alternative detection technologies, biochip significantly lowers the cost and difficulty of detection. It is worth mentioning that microarray chip technology is relatively mature and widely used in many fields, but its cost is higher than that of lab-on-a-chip. Lab-on-a-chip has attracted great attention due to its high integration and three-dimensional cell culture characteristics and has become a research hotspot in recent years. At the same time, organ-on-a-chip technology has also been derived. In summary, biochips are emerging as a powerful tool for cancer diagnosis, enabling accurate and efficient detection of cancer biomarkers. As they continue to be developed and optimized, they will become an indispensable aid in advancing precision medicine, leading to better outcomes and brighter futures for cancer patients.

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