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Gene sequencing technologies and their application in cancer research

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Abstract. The continuous development of gene sequencing technology has made the next-generation sequencing technology and the third-generation sequencing technology gradually applied to scientific research, which has greatly improved the efficiency and accuracy of sequencing and reduced the cost. The incidence of tumors in the human population continues to rise, making tumors gradually become one of the most serious diseases that endanger health. In view of the close relationship between gene mutations and tumorigenesis, the progress of gene sequencing technology has a profound impact on clinical research on tumors. In this thesis, literature analysis was used to introduce several gene sequencing technologies and compare them from multiple perspectives. In addition, the causes of tumorigenesis and the role of sequencing technology in the clinical research of human tumors were investigated. This paper found that the third-generation sequencing technology was more efficient than the next-generation sequencing technology, but it was difficult to be widely used due to its lower accuracy. Sequencing technology is of great help to tumor clinical research.

Keywords: Cancer, Gene-sequencing, Application

1. Introduction

Gene sequencing technology is a kind of technology that presents the target gene sequence by computer. Through the unremitting efforts of predecessors, it has been developed into third-generation sequencing technology. However, the next-generation sequencing technology is still widely used, and the defects of the second and third-generation sequencing technology still need to be overcome. The occurrence of tumors is closely related to gene mutations, and gene sequencing technology can play an important role in the treatment process. In this paper, the advantages and disadvantages of various sequencing technologies were integrated through a literature review, and the clinical application of tumor sequencing technology was listed. This study may help people to make better use of this technology for personalized tumor study to achieve better therapeutic effects and realize the treatment and prevention of tumors.

2. Gene sequencing methods

2.1. Next generation sequencing technology

At present, there are three mature next-generation sequencing technologies, namely 454 technology from Roche, Solexa technology from Illumina and Sequencing by Oligo Ligation Detection (SOLiD)

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technology from Applied Biosystems (ABI) [1]. Among them, Solexa is the most widely used. Solexa sequencing technology and 454 sequencing technology both use the chain extension reaction of deoxyribonucleic acid (DNA) polymerase, but 454 technology uses a variety of enzymes such as luciferase and fluorescein to couple the polymerization of deoxyriboside triphosphate (dNTP) along the primer with fluorescence signal, and then uses a charge-coupled device optical system to obtain a detection map, to read the DNA sequence information [2]. In Solexa technology, a 3' terminal protective group was added to the four dNTPs labeled by fluorescent groups to obtain fluorescent signals [3].

2.2. Third generation sequencing technology

TGS emerged shortly after NGS was introduced as the cutting-edge technology. Single-molecule sequencing (SMS) and sequencing in real-time, as two specific features of NGS, enable DNA or ribonucleic acid to be amplified by polymerase chain reaction (RNA) without templates. This quantum leap is of great significance in history, for it induced the bias introduced by PCR when constructing the library [4]. The first mature single-molecule real-time (SMRT) sequencing technology on the basis of a nano sensor was released in 2011, named PacBio RS sequencer [5]. Another sequencing technology introduced by Oxford Nanopore Technologies (ONT) called nanopore sequencing is based on a theory that came up at the end of the 1980s. This technology sequences single strand DNA or RNA by using nanopores on membrane [6].

2.3. Comparison of NGS and TGS

Next-generation gene sequencing technology has greatly improved the level of sequencing, but it also brings a corresponding limitation, that is, the generation of short reads. Short reads are a troublesome drawback, for it must be additionally processed by specialized bioinformatics tools. This step, which cannot be omitted, greatly increases the time to complete sequencing. Third generation sequencing perfectly circumvents this problem by producing longer reads, and it also improves the quality of genome construction. The two most salient features of TGS, single-molecule sequencing and real-time sequencing, bring smaller bias comparing to NGS and increase genome coverage [7]. The advancement of the third-generation sequencing technology is also reflected in its improved sequencing tools, reducing the inconvenient sequencer to the size of the pocket, which greatly facilitates field operation and sequencer storage for researchers.

3. Application of gene sequencing methods in cancer research

3.1. Formation of tumors

In simple terms, tumors arise from the accumulation of cytopathic effects. Tumors were classified as benign or malignant. Benign tumors do not invade adjacent tissues, while malignant tumors not only invade, but also spread throughout the body through metastasis.

The formation of common tumors can be explained by the two-hit hypothesis first proposed by American geneticist Alfred Knudson in 1970. He first proposed the concept of tumor suppressor genes through his studies on retinoblastoma. His theory has driven the discovery of many tumor suppressor genes, and on the basis of it, has explained the cause of many other diseases. Knutson believes that retinoblastoma is not a simple dominant genetic disease in which anyone who carries the disease-causing gene will get affected. He eventually concluded that tumors arise from the proliferation of malignant cells, and that it usually takes two mutations in a cell to cause disease in a carrier, by documenting and analyzing data on the patient's family history, affected eyes, and the condition of the tumor. Patients are divided into two types, the hereditary patients are born with a mutation gene because of inheritance, and the disease occurs after birth. Non-genetic patients require two mutations in the same cell to develop the disease. He concluded that retinoblastoma is controlled by a pair of recessive genes that suppress tumorigenesis, and that the loss of both genes leads to tumorigenesis.

Malignant tumors include cancer and sarcoma, among which cancer is the most common. The formation of cancer is mainly caused by the transformation of proto-oncogenes into oncogenes and

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tumor suppressor gene mutations. Tumor suppressor genes, such as the TP53 gene, act as guardians of the cell cycle. When a cell is damaged, the TP53 gene shuts down the cell cycle until the cell is repaired. When the TP53 gene is mutated, the cell cycle is no longer arrested because of cell damage, so that the damaged cells continue to proliferate normally, which may lead to cancer. Note that cancer requires more than one mutation, which is called the multi-hit concept of carcinogenesis.

In normal cells, the expression of telomerase at the end of chromosomes decreases with time, and telomere repeats are also shortened to control cell life span and inhibit cell proliferation. And cancer cells will reactivate the telomerase to ensure the normal length of the telomere, so as to achieve eternal life and unlimited proliferation.

3.2. Application of single-cell sequencing technologies in pancreatic cancer

Pancreatic cancer is the third leading cause of cancer-related death in the world. Due to the heterogeneity of cancer cells caused by various factors, the research and treatment of pancreatic cancer are difficult to implement. In the background that traditional techniques cannot meet the research needs, single-cell sequencing is tried to be applied (see Table 1).

Single-cell sequencing is divided into two steps: single-cell separation and single-cell analysis, and these two steps are closely related, including laser capture microdissection (LCM) and so on. With these steps and techniques, single-cell sequencing technology can detect some specific genetic modifications and tumor cells.

First, the investigators performed single-cell transcriptome analysis of samples obtained by puncture from six patients to generate further differentiated cells. The researchers found that one cell matched the basal cells, suggesting that aggressive basal cells are widespread in the body. Since tumor cells are generally highly metastatic, scientists have become curious about the relationship between initial tumors and those that develop after metastasis. This is where a transcriptomic analysis including multiple single-cell sequencing techniques was applied. The sequencing results of pancreatic cancer samples showed that 224 samples were primary tumors, while only 95 samples were metastatic tumors. Since then, single-cell RNA sequencing has been used to identify therapeutic targets, and next-generation sequencing has been used to analyze circulating tumor cells. The former enhances the inhibitory effect of the drug on surviving, allowing researchers to better control tumor cell growth.

Sequencing techniques have also been used to determine tumor subtypes. Bailey et al. used whole-genome sequencing of hundreds of pancreatic cancer tumors and classified them into several subtypes, including squamous and so on.

The update and maturation of single-cell sequencing has become an important method to reduce the cost of sequencing. For example, researchers are conducting experiments to combine single-cell sequencing with other technologies, making single-cell sequencing technology capable of functional analysis of heterogeneous cell populations with increased sensitivity and efficiency. Single-cell mapping is expected to be improved under the development of this new technology, and further contribute to the clinical research and diagnosis of tumors.

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Table 1. Single cell sequencing techniques and their functions

Single cell sequencing technique	Function	Abstract
Single-cell combinational marker sequencing technique (SCI-seq)	Detects somatic cell variations and constructs thousands of single cell libraries	SCI-seq analysis done to generate thousands of single-cell libraries for variant detection of somatic copy number within in PC. The libraries constructed from 16,698 single cells taken from primate frontal cortex tissue and 2 human adenocarcinomas
Single-cell whole genome amplification method (WGA)	Can efficiently detect mutations in multiple diseases	KRAS mutations in CTCs were detected with a rate of about 27.7% from samples 11 of 12 PC patients. Moreover, KRAS mutations were found in WBC sequenced cells
Topographic single-cell sequencing (TSCS)	Describes spatial characteristics invasion and metastasis of tumor cells	KRAS mutations in CTCs were detected with a rate of about 27.7% from samples 11 of 12 PC patients. Moreover, KRAS mutations were found in WBC sequenced cells
Single-cell multiple sequencing technique (scCOOL-seq)	Analysis of single-cell chromatin state, DNA methylation	Measures genomic number profile of a single tumor cell while preserving the spatial context in tissue sections taken from both ductal adenocarcinoma in situ and invasive ductal carcinoma of 10 synchronous patients. Additionally, a direct lineage was determined in between invasive and in situ tumor cells that shows aberrations evolved and mutations present within the ducts prior to invasion

4. Conclusion

Gene sequencing technology, from the original Sanger sequencing to the current next generation sequencing and the third-generation sequencing technology, has gradually overcome many technical problems and plays an important role in the clinical research, diagnosis, and treatment of diseases. Among them, the next generation sequencing technology represented by SOLiD is the most widely used sequencing technology. The third-generation sequencing technology overcomes the problems of error rate and high cost of the next generation sequencing technology, but it also brings the problem of short reads, which still needs to be improved and matured. Malignancy in tumors, especially cancer, remains an important cause of death worldwide. Cell-level causes of tumors are intracellular mutations, such as double gene mutations and tumor suppressor gene mutations in the hit theory. Gene sequencing technology, such as single cell sequencing, has greatly brought convenience and accuracy to the study of tumors. Whole genome sequencing and other work have been carried out, and the effect of detecting the heterogeneity within tumors is unprecedented. It is believed that with the continuous refinement and improvement of gene sequencing technology, the clinical research of tumors will achieve more breakthrough progress.

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