

Application of different analysis methods to detect telomerase activity in cancer cells

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Abstract. Telomerase plays an important role in abnormal cell proliferation, metastasis, stem cell maintenance, and immortality in different types of cancer. As a result, the design of drugs targeting telomerase is of great significance for the treatment of cancer. In recent years, treatment strategies for telomerase have attracted extensive attention. However, finding an effective treatment is still more difficult. Understanding the structure, function and biological process of telomerase and their related proteins is of great help to drug development and clinical treatment. As a result, this research mainly introduces different biological detection methods of telomerase, including PCR, western blot, CCK-8 assay, sphere formation assay, immunocytochemistry and nanobiotic detection technology. By understanding the detection range, different aspects of detection, and advantages and disadvantages of several detection methods, the development of telomerase detection technology can be well understood. And it can better understand the activity and expression of telomerase, and hope to provide reference for future drug development targeting telomerase, telomerase and related proteins.

Keywords: cancer, telomerase, biological detection, analysis method

1. Introduction

Telomerase is a eukaryotic ribonucleic protein (RNP) complex that helps stabilize the telomere length of human stem cells, germ cells, and cancer cells by adding TTAGGG repeats to the telomere, using its inherent RNA as a template for reverse transcription. Therefore, it has been hypothesized that maintaining telomere stability is necessary for long-term tumor proliferation [1].

Cancer is one of the leading causes of death in the world over ten million people die of cancer each year. Cancer both disrupts and exploits mechanisms of multicellular organization. Cancer treatment and control is still a major problem in the medical field. One of the reasons that makes cancer difficult to treat and control is that cancer cells can multiply indefinitely and can spread or metastasize to other parts of the body with body fluids such as blood or lymph. Not only the original site of the disease has cancer cells, but also other parts of the body will be invaded by cancer cells and affect health, so the simple removal of lesions cannot control the development of the disease.

Unlike normal cells, cancer cells replicate and multiply in an uncontrolled manner, disrupting normal cell networks, infiltrating tissues, and metastasizing to distant organs, eventually causing the body to die. This pattern of activity in cancer cells is controlled by telomerase cells [2]. In normal cells, telomere length shrinks with each round of cell division to control cell proliferation and malignant transformation. However, the length of telomerase in tumor cells does not shorten with cell division, which is an important characteristic of human malignant cells, and is also a necessary factor for the infinite proliferation of cancer cells and other cancer properties [3]. Therefore, inhibiting the catalytic function of telomerase, thus stopping the maintenance of telomere length, will help to inhibit the infinite proliferation of tumor cells. [4]. In addition, because of telomerase as an important cancer target, anti-cancer drugs developed on the basis of inhibiting telomerase activity can achieve higher medical effects with minimal side effects.

So far, there are many different methods to detect telomerase activity, such as PCR-ELISA kit. In addition, a variety of different biosensing techniques are used to detect telomerase activity in cancer cells and normal cells [5]. The qPCR/Western blot was used to detect the expression of stem cell-related genes/proteins and human telomerase reverse transcriptase (hTERT) genes/proteins in tumor

cells and normal cells. Immunocytochemistry method was used to detect some special substances in labelled reserved cells (LRCs). The proliferation, toxicity and tumorigenesis of cancer cells can be analyzed and compared by CCK-8 assay, spherogenesis assay and *in vivo* assay. The expression of stem cell-related protein and hTERT protein in cancer cells was significantly higher than that in normal cells, and telomerase activity was significantly higher than that in normal cells. At present, these biological detection techniques can effectively detect telomerase activity and expression in different cells, and show high selectivity and sensitivity to a certain extent. The reliability of telomerase determination depends on the accurate evaluation of telomerase activity. In order to maintain the accuracy of the results and minimize the variability of the analysis, accurate assessment of telomerase activity may also need to rely on some emerging technologies. In addition, significant challenges in telomerase inhibitor development and clinical trials, such as limitations in clinical models and the lack of high-resolution human telomerase structures, have hindered the development of successful clinical therapies [6].

Until recently, the characterization of the structure and composition of human telomerase holoenzyme was unclear, which hindered drug design and mechanism analysis [7]. A team of US scientists used cryo-electron microscopy to determine the structure of telomerase with the highest resolution to date. However, until now, scientists have not been able to fully determine the true causes of aging and cancer, and although electron microscopes used to be able to show details of the microscopic world, high-resolution information on biological samples has been difficult to obtain because biological samples cannot withstand radiation damage from electron beams. Therefore, improved structural resolution should facilitate the design of more effective human telomerase small molecule inhibitors.

Overall, there are many challenges to using telomerase as an effective target for cancer therapy. Detection of telomerase is an urgent need for basic biochemical research and clinical diagnosis. Therefore, the use of highly sensitive and selective telomerase detection technology plays an important role in the understanding of telomerase activity and the treatment of cancer. As a result, this research will outline a diverse of different detection methods for the analysis of telomerase activity, including PCR-TRAP, western blot, CCK-8 assay, sphere formation assay, immunocytochemistry and nanobiotic detection technology.

2. Application of Methods for detection of telomerase activity

2.1. PCR-TRAP

Kim et al. developed the telomeric repeat amplification protocol (TRAP) assay to elongate the telomeric repeats by coupled polymerase chain reaction (PCR) [8]. Taking advantage of telomerase's ability to add 6 bases to the end of an appropriate oligonucleotide chain *in vitro* using the region of its own RNA as a template, PCR was used to amplify this repeat to a detectable number. Then, TRAP method was used to display the results on gel electrophoresis to show that the ladder bands indicated the size of telomerase activity. Although TRAP is easy to operate and has high stability and sensitivity, its use is greatly affected by sample purity and quality. And impurity contamination is inevitable in PCR amplification experiments, so it is important to improve the selectivity of TRAP. Therefore, in the subsequent improved experiment, the primers modified with gold nanoparticles (AuNPs) significantly improved the sensitivity and specificity of traditional TRAP detection, which may be an effective method for direct PCR of concentrated cell lysates [9], as shown in Figure 1. At the same time, various other modifications have been proposed, particularly to simplify the laborious steps and to improve quantification. A TeloTAGGG telomerase PCR-ELISA kit was developed based on this. This kit uses Amplifluor™ primers to detect telomerase amplification products and fluorescein labelled internal PCR controls, allowing quantification while measuring telomerase activity [10]. The entire assay is performed in a closed PCR container, which greatly reduces the risk of contamination and simplifies the experimental procedures. To sum up, because of the many advantages of the PCR-TRAP assay, this method is generally our first choice for detecting telomerase activity.

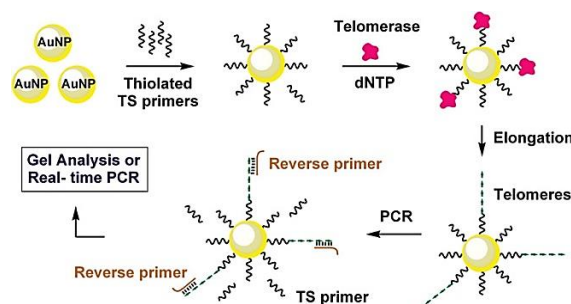


Figure 1 Scheme of TRAP assay [9].

2.2. Western blot

Western blot is commonly used in immunogenetics, which provides qualitative and semi-quantitative analysis of proteins. Compared with PCR, if the changes in protein expression are at the protein level but not at the RNA or DNA level, such as protein modification, PCR cannot detect them, and western blot can be used in this case. In addition, most of the functions are meaningful at the protein level, and western blot detection is relatively more accurate. In the telomerase assay, western blot was performed to analyze cellular apoptosis and signalling pathways [12]. It is intended as an efficient method for detecting protein targets in complex samples. In addition, the technology continues to evolve to provide ultra-sensitive imaging systems that provide a wide range of dynamic detection and can accurately and accurately quantify signals from the same imprinting of low and high expression proteins [13]. However, different from DNA-based detection methods, there are significant differences in protein expression, stability, conformation and activity under different buffers and experimental conditions. This affects the accuracy of Western blot detection and its results to some extent. For example, in studying the atypical function of reverse transcriptase of telomerase, different antigens were added to the extract for quantitative western blot analysis of proteins [14]. Western blot showed the results of overexpression of TERT. The results show that our method works within the linear range, so the quantitative methods of hTR level and hTERT are accurate. As a result, the western blot has been widely used to analysis the telomerase activity [15].

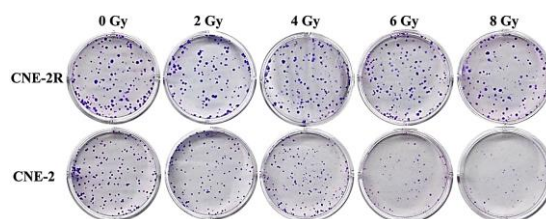


Figure 2 The proliferation of cells cultured in different treatment solutions was detected by CCK-8 assay [19].

2.3. CCK-8 assay

The CCK8 is a commonly used biological assay to detect cell proliferation and toxicity, as shown in Figure 2. In some studies, the number of living cells is dynamically measured by CCK-8 method to determine the optimal concentration of drug action on cells, which plays an important role in drug screening and development [16]. Moreover, the results of CCK-8 assay could indicate the degree of inhibition of different compounds on cell proliferation, migration and invasion [17]. For example, when testing the antitumor effect of a compound on cancer cells, the CCK-8 detection technology can be used to treat different concentrations of compound solutions and cancer cells and drugs to see whether the compound inhibits the proliferation of tumor cells. To determine the effect of different compounds against cancer cells. And because CCK-8 solution can be directly added into cells without premixing with other components and is easy to operate and accurate to detect, so it is widely used tumor-related experiments and drug development. In the study of telomerase resistance of tumor cells to chemotherapy drugs, CCK-8 detection method was used to evaluate the sensitivity of

chemotherapy drugs [18]. The count of negative cells can reveal the resistance and sensitivity of cancer cells to different drugs.

2.4. Sphere formation assay

Pelletizing ability is an important method to identify tumor stem cells *in vitro*. It measures the capacity of a single cell to self-renew in a suitable conditioned medium, generally expressed as cell pellet formation efficiency. The pelletized cells showed enhanced proliferation, self-renewal and tumorigenesis. The sphere formation assay (SFA) is an important criterion for evaluating the self-renewal ability of stem cells, as shown in Figure 3. The SFA technology has also been used to detect the effects of drugs, chemical components or microenvironmental components on the self-renewal ability of stem cells [20]. The *in vitro* SFA was used to verify self-renewal of melanoma CSC and to investigate the influence of tumor microenvironmental factors on it. The pellet formation efficiency of melanoma CSCs in stem cell culture medium was proportional to the number of them in culture medium, thus the number of CSC in culture medium could be directly quantified. This relatively simple approach helps to investigate the ability of any external factors such as growth factors. Since tumor stem cells are involved in resistance therapy, the cancer treatment could be affected [21]. Therefore, studying the pellet-forming ability of cells is very important in inhibiting cell proliferation and reducing DNA damage repair ability, and is also of great help to the treatment of tumors.

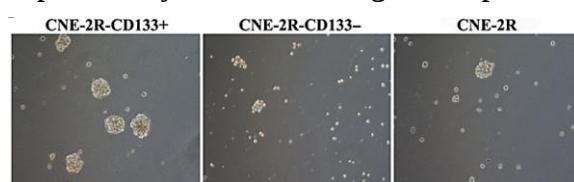


Figure 3 Sphere formation of three groups of cells [19].

2.5. Immunocytochemistry

Immunocytochemistry is widely used to examine specific substances in cells or tissues. Immunohistochemistry is based on the ability that antibodies specifically bind to cell antigens. In immunochemistry experiments, the expression of hTERT protein can be detected in a single cell by immunohistochemistry. Different from TRAP method, the TRAP method can estimate telomerase activity, but cannot measure telomerase activity of individual cells. This method allows specific antigens to be displayed with minimal background staining while preserving the structural integrity of the cell/tissue. Results of immunostaining can be displayed as the proportion of positively stained cells, the distribution of the staining and the intensity of staining [22]. In tumor detection, antigen-antibody complexes are isolated in magnetic fields using antibodies against tumor-associated antigen-specific targeted binding [23]. The antibody can then be used for immunocytology to obtain tumor information, as shown in Figure 4.

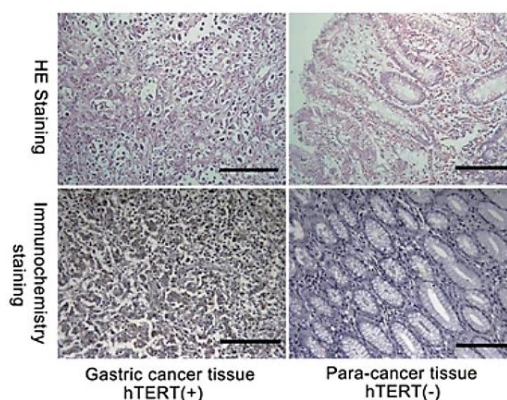


Figure 4 Immunohistochemistry of hTERT-positive gastric cancer tissues and hTERT-negative corresponding adjacent tissues [24].

2.6. Nanobiotic detection technology

Although the immunoassay and fluorescence detection methods mentioned above are indispensable tools for molecular diagnosis, these analysis methods still have some limitations. Enzyme linked immunosorbent assay (ELISA), for example, relies on fluorescent labelling of samples, a time-consuming and expensive process. Nanotechnology, by contrast, can provide label-free detection and is being used to overcome some limitations. Current nanotechnology can be manipulated and positioned at a scale of 300 nm. The use of nanotechnology in the laboratory can detect extremely small amounts of compounds and viruses with good sensitivity and selectivity. Some nanoparticles, such as gold nanoparticles, can serve as particularly useful labels for microbiological detection. They can be detected by light absorption, fluorescence, Raman scattering and conductivity, and to some extent replace PCR and fluorescence labelling techniques currently used [25].

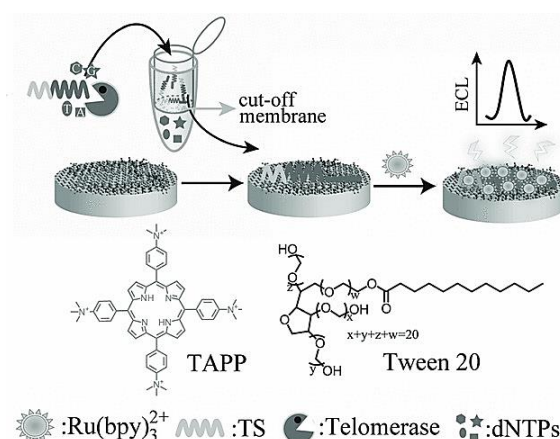


Figure 5 Scheme of the used developed electrochemical method for telomerase activity detection [27].

The detection system based on nanomaterials relies on chips embedded in DNA. If the target disease is present in the sample, its DNA binds to complementary strands of DNA on both the chip and the gold particle. At the same time, the chip is coated with a solution that enhances the signal and can detect extremely small amounts of DNA under a photoscanning detector. In addition, nanodetection can be combined with cyclic tumor cell (CTCs) detection to directly target CTCs *in vivo* [26]. Antibodies can be attached to the surface of the nanodetector to remove immunocytochemical or PCR analysis of CTCs and directly obtain the number of CTCs in circulating human blood, as shown in Figure 5. The most important feature of nanoprobe technology is its biocompatibility and no side effects in any application.

3. Conclusion

The above, this research introduces several kinds of detecting methods for analysis of telomerase activity. The elaboration of the advantages and disadvantages of different methods such as detection sensitivity and selectivity are outlined, to understand the nature of the telomerase and expression in the cancer cells. Although great efforts have been made over the past 20 years to create telomerase tests, using telomerase as a reliable clinical biomarker for cancer detection still remains a challenge. Detection of telomerase activity is an urgent need for basic biochemical research and clinical diagnosis. There is no doubt that highly interdisciplinary collaboration will enable faster progress for developing new detection method.

Authors' Contributions

Runjia Yu completed the work design and article writing.

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