

Advances in Detection Technologies of Early cancer

Juntao Tan^{1,2}, Yong Hong^{1,2}, Jing Su^{1,2}, Nan Hu^{1,2}, Zhongqiang Lai^{1,2}, Xiaoling Lu^{1,2,*}, Yongxiang Zhao^{1,2,*}

¹ National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Medical University, Nanning, Guangxi 530021, China

² Guangxi Key Laboratory of Biological Targeting Diagnosis and Therapy Research, Guangxi Medical University, Nanning, Guangxi 530021, China

Abstract

This article is dedicated to present a mini-review on the latest progress and future trends in detection methods of cancer cells. The article analyzes the methods that are used to isolate, quantify and detect cancer cells via their distinctively different biological and/or physical characteristics. The advantages and disadvantages of different detection approaches are discussed. The future direction towards cancer early detection was outlined. The importance for developing comprehensive multi-mode detection method was emphasized.

Key words: Cancer biomarker; Early detection; Physical method.

Received: August 10, 2014; **Accepted:** November 20, 2014; **Published:** March 18, 2015

*Corresponding author: National Center for International Research of Biological Targeting Diagnosis and Therapy, Guangxi Key Laboratory of Biological Targeting Diagnosis and Therapy Research, Collaborative Innovation Center for Targeting Tumor Diagnosis and Therapy. Guangxi Medical University, Nanning, Guangxi 530021, China E-mail: yongxiang_zhao@126.com (Yongxiang Zhao), luwuliu@163.com (Xiaoling Lu)

Background

Cancer has become one of the major fatal diseases of serious harm to human health and the global life in recent years. Cancer is generally considered incurable, mainly due to its low cure rate, high mortality and wide distribution [1]. In developed countries, cancer has become leading cause of people's death while the cancer mortality rate is ranked second in all diseases in developing countries.

Up to date, a lot of researchers have devoted their efforts on discovering material resources for cancer prevention, diagnosis and treatment [2]. The direct cost of such malignant disease is topped on fifteen diseases which led to the loss of the world's major economies [3]. Due to the metastasis and invasion, about 90% of cancer patients died of it, however early treatment and intervention will improve the therapeutic effect [4]. Early diagnosis and treatment are vital to improve the survival rate

in cancer patients. In this paper, we review the current advanced early cancer detection technologies for the purpose to provide a reference of the early diagnosis and prevention of cancer.

TNM stage and clinical significance

Cancer is a complex disease. In order to better describe the extent of the development of malignant tumors and allow doctors and patients to be aware of the disease conditions, tumor staging system is employed for classification [5]. The Department of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) have established mature TNM staging system (Table 1). The TNM system determine the phase of development of tumor based on the detection of T, N, and M. The corresponding total installments, namely Phase I, phase II, phase III, etc., in different phase of the development of tumor corresponding to slight differences [6].

Some of the current detection methods which are commonly used, such as X-ray, ultrasound, PET-CT, MRI and other imaging methods in the diagnosis of cancer, are worthy of recognition., However, because of discrepancies between results obtained by using these methods and pathological diagnosis, these methods are considered to be less practical and useful [7]. In addition, multiple tests are not easily afforded, which led to a delay in tumor detection. Some of these methods, such as X-ray, CT, PET, or combinations of different methods, require patients to expose to radiation, which, therefore, are not suitable for frequent tests [8]. Additional histochemical methods which analyze by puncturing to obtain a tissue sample from patients is time-consuming, complicated to operate and

will generate severe damage to the patients [9]. Early tumor detection is desired for applying in a large number of people to find suspected patients and allow implementing frequent detections. Therefore, with this need, the above mentioned methods are not suitable for early detection of cancer, especially among a large number of population. As a result, the development of a low-cost, easy-to-use, portable, low-damaged, high accuracy and high throughput screening method for the early detection of cancer becomes the hotspot of current medical research.

Early tumor detection

Currently, the primary goal of early cancer detection is to reveal the differences between normal cells and tumor cells, as well as normal tissues and tumor tissues. The major differences between tumor cells and normal cells include biological characteristics and physical characteristics.

Biological characteristics of tumor cells

The biological characteristics of human tumor cells which differ from normal cells are that allows tumor to invade and transfer to other parts of the human body. Normal cells through procedural regulation will ultimately become apoptosis, and, due to genetic changes, cancer cells growth is not restricted, thus will be able to skip the stage of apoptosis and continue to proliferate.

Gene markers

The cancer cells can change the genetic material of the cell. All human beings carry oncogene; however the expression of oncogene of cancer patients is abnormal and is

different from the one of healthy people. Tumor suppressor genes are lost or inactive in patients with cancers [10]. For example, the development of colon cancer is due to the loss or mutation of normal epithelial cells of the APC gene, DNA methylation abnormalities, Ras mutations and DCC, p53 gene loss and other genetic changes and the formation of tumors [11].

Protein biomarkers

Proteins are the product of the genes. When the normal cells become cancerous, the expression of gene will become abnormal and the corresponding encoded protein will change. Numerous studies have shown that the level of expression of some proteins in tumor tissue is higher than that of normal tissue. Therefore, the biomarkers of these proteins have become an important tool for the diagnosis of neoplastic disease. The basic principle of the detection of tumor-specific antigens is mainly relying on the corresponding specific antibodies (The basic principle of the detection of tumor-specific antigens is mainly based on measuring the responses of interactions to the corresponding specific antibodies). In addition, through a combination of white matter genomics and other techniques,

including gel electrophoresis, liquid chromatography, mass spectrometry and nuclear magnetic resonance, are commonly used for detection. Moreover, categories and amount of biomarker expression in tumor cells are different. For example, the biomarker of prostate cancer is prostate specific antigen (PSA) and the serum PSA in patients with prostate cancer will increase. The CA125 is the biomarker of ovarian cancer and AFP is the biomarker for liver cancer, etc. Table 2 shows the some well-known markers for cancer diagnosis and prognosis [12].

Physical properties of tumor cells

Being influenced by the genetic factors or environmental factors, the genetic and cellular structure of tumor cells changes. Such structure changes will cause the physical properties which is different from normal cells. Because of such changes, the methods of detecting specific physical properties of tumor cells can be applied. For instances, density can be detected by using the density gradient separation method; the size and deformability of cells can be detected by using the method of separation by filtration. Figure 1 summarizes the changes in the physical properties of the tumor.

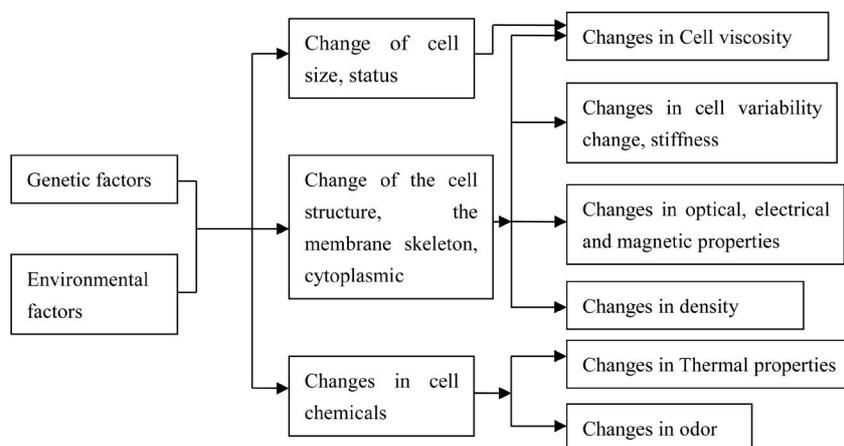


Figure. 1 Changes in physical properties of cancer cells.

Table. 1 TNM classification and clinical significance

Staging symbol	Clinical Significance	Staging symbol	Clinical Significance	Staging symbol	Clinical Significance
T	Primary tumor	N	Regional lymph nodes	M	Distant metastasis
Tx	primary tumor cannot be assessed	Nx	Regional lymph nodes cannot be assessed	Mx	Distant metastasis cannot be assessed
T0	None of the primary tumor evidence	N0	No regional lymph node metastasis	M0	No distant metastasis
Tis	Carcinoma in situ	N1-3	Increase With the extent of lymph node involvement and range	M1	Distant metastasis
T1-4	increase with the primary tumor size and scope				

Table. 2 Tumor markers and related tumors

Tumor markers	Tumor types	Tumor markers	Tumor types
CEA	Stomach cancer, colon cancer, liver cancer	NES	Lung Cancer
AFP	liver cancer	CYFRA21-1	Lung Cancer
CA125	Ovarian cancer	Ferritsn	Breast cancer
CA153	Breast cancer	HCH	Lung Cancer
CA19-9	Colorectal cancer	SCCA	Cervical Cancer
CA72-4	Gastric cancer	BAT	Bladder Cancer
CA242	Colorectal cancer	TPS	Breast cancer
CA50	Pancreatic Cancer	S100	Melanoma
PSA	Prostate Cancer	TPS	Breast cancer

The method of tumor detection

Typical approaches

Typical detection approaches include history taking, physical examination, blood tests and imaging. Detection of tumor markers and tumor imaging have made a great contribution to the early diagnosis of cancer. Commonly used techniques that can detect markers are ELISA, 2D-PAGE, multidimensional protein

identification technology, proteomic pattern recognition and protein microarrays [13]. However, these detection methods are time-consuming and with high-standard operation, and need large auxiliary equipment for operations. Tumor imaging techniques include ultrasound imaging (ultrasonography), optical imaging, X-ray computed tomography (CT), magnetic resonance imaging (MRI), single photon emission tomography (SPECT) and positron emission tomography (PET) techno-

logy. In recent years, much progress of a variety of diagnostic imaging techniques has been made. However, there are still many deficiencies in such methods.

Objective of tumor detection

Tumor detection can be divided to be *in vivo* and *in vitro* detection. Most imaging techniques aim to detection with non-destructive test, but, generally, only big-scaled hospitals possess such expensive equipment. *In vitro* tests include detection of biopsy tissue, body fluids, excretions and secretions detection. Studies have shown that a variety of epithelial tumor cells migrate via peripheral blood circulation system (Circulating tumor cells, CTC), such as breast cancer, bladder cancer, prostate cancer, colorectal cancer, cervical cancer, and pancreatic cancer [14]. The detection of CTC can provide valuable clinical information, including early detection of tumor recurrence, and monitoring the effectiveness of adjuvant therapy as an independent prognostic factor. However, only a few CTCs exist in the blood which limits the detection applications [15].

Density gradient centrifugation

The density gradient centrifugation is commonly used to detect monocytes. Since the buoyant density of tumor cells is different from other cells in human blood, hence tumor cells can be separated via density gradient centrifugation [16]. Biomarkers of tumor cells can be stained via immunochemical staining method so that the tumor cells can be identified under the fluorescence microscope [17]. By employing density gradient centrifu-

gation method, cells with low density, including epithelial cells, tumor cells and cells are separated from cells with high density and located at upper layer, while most of the lymphocytes and red blood cells are located at the lower layer. However, some residual tumor cells in the plasma layer and red blood cells may reduce the accuracy of the detection. Therefore, such method can only be used as the primary tumor cells detection and isolation.

Tumor cells isolated according to the size, stiffness

The size and stiffness of tumor cells are different from other cells in blood [18, 19] hence tumor cells in the blood can be isolated according to their different physical properties, which is considered to be a more effective approach to separate tumor cells and other cells in blood. In 1964, Seal first proposed that tumor cells can be isolated from the peripheral blood by using a filtration system [20]. Typical dimensions of blood cells, i.e. erythrocytes, are ranging 5-9 μm in diameter. Since the red blood cells are deformable, when the filter pore diameter is larger than 3.3 μm , all of the normal human red blood cell can pass through the filter [21]. For Granulocytes, the diameter of the filter pore can be 10-18mm for lymphocytes and 12-20mm for monocytes [22]. The diameters of the most tumor cells are larger than cells in blood. For examples, the diameter of MCF-7 is about 22.5mm, diameter of NCI-H358 is about 18.1mm, diameter of AGS is about 14.9mm, and the diameter of LNCaP cell line is $17 \pm 1.5\text{mm}$ [23]. Depending on the cell diameter and rigidity, the filter can be designed to possess the pores with certain

sizes so that the cells in blood and tumor cells can be separated. However, white blood cells have similar diameters with tumor cells. In order to avoid omitting such cells from normal cells in blood, the methods combined with experimental fluorescence staining or electroporation are used to distinguish these two cells [24].

Antigen - antibody that specifically binds for cell separation.

Currently, the majority of tumor cells separation methods are closely related to antigen-antibody immunity. Cell Search technology is the first implementation of CTC detection and counting techniques can be used to monitor the condition of patients with metastasis of breast cancer, colorectal cancer and prostate cancer. The principle is the use of magnetic beads connected EpCAM antibody that specifically binds to tumor cells by immunomagnetic separation. EpCAM is overexpressed at most epithelial tumor cell surfaces. CK is expression by 0%-20% at normal cells [25]. However, due to tumor heterogeneity, the research of Cell Search technology shows variable sensitivity and capabilities, which makes its low value in clinical use, and a further study for improvement is needed.

Electrical method

Tumor cells are variation of the normal cells, the electrical properties of the cells will vary. The impedance of lymphoma cells and myeloma cells have been measured to evaluate the dielectric properties of cells

through the experimental investigation. The findings of these studies show that, if the traveling wave dielectrophoresis frequencies is above 1 MHz, the separation of these two cells can be achieved, meanwhile activities of these two types of cells retain [26]. Changing the electric field by dielectrophoretic excitation of microelectrode is to attract or repel cells with different shapes, sizes and then cells are mediated by electrostatic force in the separation process [27]. Using the dielectrophoretic method, some researchers have successfully separated breast cancer cells and other cells in the blood [28, 29].

Optical detection method

Optical methods are commonly used for biological detection, including fluorescence, interference, thermal lens, surface plasmon resonance and chemiluminescence. Fluorescence and automatic digital microscope (ADM) can be used as a reliable means of cell detection. The detection rate can reach 800 cells per second [30, 31]. However, for a large number of cells in blood such speed is not high enough. Krivacic chose a fiber optic array scanning technology (FAST) whose detection rate is 500 times high of the ADM method. The high sensitivity and specificity of FAST method have facilitated the development of fluid technology for optical detection facilitated [32].

Nanotechnology in tumor detection

Nanotechnology has been rapidly and successfully developed and has been applied to many fields, including medicine, pharmacy, chemistry and manufacturing [33]. Nanoparti-

cles have unique optical, electrical, chemical, mechanical and physical properties, which play an important role in solving the medical problems. [34]. Different nanoparticles have diverse characteristics, hence the applications of nanoparticles also vary.

Gold nanoparticles, possessing unique biological optical properties, can be connected to tumor cells as an optical marker in the process of detection [35]. For example, a gold nanoparticles membrane electrode also play an important role in the detection of tumor biomarkers. The gold nanoparticles can be used to achieve signal amplification [36] (any examples). The breath of patients are affected by lung cancer. Peng et al designed 9 array of chemical impedance sensor cross-reaction. Each sensor can be detected by a variety of gas species lung respiratory monitoring method. This chemical impedance sensor use different organic-modified gold nanoparticles of 5 nm [37]. Some researchers use a carbon nanotube, carbon nanotube construction "forest" in the chip, thus improving the detection efficiency of trace substances can be improved [38].

Summary

Researchers have devoted great efforts on the studies of early cancer cell detection. In this paper, a review of the latest development in the field is provided. Obviously, multiple methods are involved in most of the testing process. Methods which are currently used in clinical tumor cell separation are not mature enough, which means that further studies and improvement are needed. The ultimate goal in this area is to achieve early tumor discovery

and give timely treatment, thus to relieve and even solve this major problem which human beings are facing.

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