MINI REVIEW

Application of electrochemical biosensors in tumor cell detection

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Abstract

Conventional methods for detecting tumors, such as immunological methods and histopathological diagnostic techniques, often request high analytical costs, complex operation, long turnaround time, experienced personnel and high false-positive rates. In addition, these assays are difficult to obtain an early diagnosis and prognosis quickly for malignant tumors. Compared with traditional technology, electrochemical technology has realized the study of interface charge transfer behavior at the atomic and molecular levels, which has become an important analytical and detection tool in contemporary analytical science. Electrochemical technique has the advantages of rapid detection, high sensitivity (single cell) and specificity in the detection of tumor cells, which has not only been successful in differentiating tumor cells from normal cells, but has also achieved targeted detection of localized tumor cells and circulating tumor cells. Electrochemical biosensors provide powerful tools for early diagnosis, staging and prognosis of tumors in clinical medicine. Therefore, this review mainly discusses the development and application of electrochemical biosensors in tumor cell detection in recent years.

Introduction

Tumors, as a nonhereditary genetic disease, can be divided into benign and malignant tumors, the latter can metastasize, grow rapidly, and produce harmful substances, thereby seriously threatening human health. In addition, malignant tumors (also named cancers) have developed a variety of genetic mechanisms to adapt to the stresses of living environment through genetic mutations, thereby escaping growth inhibition signals and immune surveillance systems.[1,2] During the evolution from normal cells to tumor cells, there are specific proteins or small molecules used as markers for tumor diagnosis on the cell surface or in the serum, which brings good gospel for the early diagnosis and treatment of tumors.[3] For a long time, histopathological diagnosis has been the gold standard for cancer diagnosis and the basis for clinical treatment.[4] However, histopathological diagnostic techniques have the disadvantages of high analytical costs, complex operations, long

turnaround time, and high false-positive rates, and it is difficult for them to meet the requirements for early diagnosis and prognosis of malignant tumors. Fluorescence imaging combined with confocal microscopy can directly observe the rich location information of cancer cells.[5–7] However, the technology cannot meet the requirements of high sensitivity measurement. Therefore, the development of new tools is in demand. Recent studies have highlighted an electrochemical technique which has been proven to have ultra-high sensitivity and accuracy in the quantitative detection of breast, prostate, liver and cervical cancer cells.[8–10]

The most classical application of electrochemical biosensors in the early diagnosis of tumors is the detection of tumor cells by biosensors based on cell impedance sensing technology. Cyclic voltammetry (CV), as a commonly used electrochemical research method, can be used to judge the microscopic reaction process on the electrode surface, so as

to detect the change in impedance or microcurrent at the electrode interface caused by the growth of cells on the electrode surface. Differential pulse voltammetry (DPV) is a method based on linear sweep voltammetry and staircase voltammetry which has a lower background current and higher detection sensitivity. In addition, it displays the highly stable and specific capture of cancer cells by producing nontoxic biological modifications on the working electrodes of electrochemical biosensors, such as with covalently linked biotin, monoclonal antibodies, lactoglobulin A and aptamer. Therefore, the detection of tumor cells without lysis and fixation is made possible, which simplifies the analysis process and improves the accuracy of the results. Here, we review the latest developments in electrochemical biosensors for the detection of tumors (Table 1). We highlight four aspects: electrochemical biosensor in tumor cell detection; electrochemical immunosensors in tumor cell detection;

electrochemical nucleic acid biosensors in tumor cell detection and detection of circulating tumor cells (CTCs).

Electrochemical biosensor in tumor cell detection

The electrochemical biosensor consists of an identification system and a transduction system. The function of the identification system is to selectively interact with the analyte and convert the resulting parameters into a certain signal. The function of the transduction system is to receive signals and transmit them to the electronic system in the form of electrochemical signals. The electronic system further amplifies and outputs, realizing the quantification and research of the analyte (Fig 1). Because of the advantages of good selectivity, high sensitivity, simple equipment and low price, the electrochemical biosensor has been widely used in

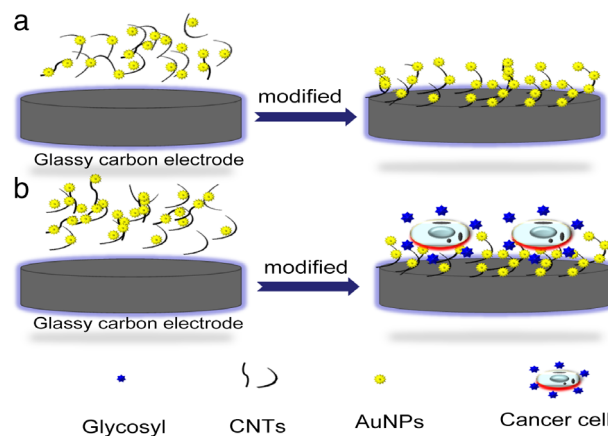**Table 1** Detection of tumor cells using electrochemical biosensors

Analyte	Detection technique	Nanomaterials	Performance	Reference
MCF-7	Electrochemical impedance	Au nanoparticles (AuNPs)	LOD: 10 cells/mL	Wang et al.[11]
Hela	Electrochemical impedance	Multiwall carbon nanotubes (MWCNTs)	Linear range: 2.1×10^2–2.1×10^7 cells/mL LOD: 70 cells/mL	Liu et al.[12]
HL-60	Cyclic voltammetry (CV) Electrochemical impedance Differential pulse voltammetry (DPV)	Multiwall carbon nanotubes (MWCNTs)	Linear range: 2.7×10^2–2.7×10^7 cells/mL LOD: 90 cells/mL	Xu et al.[13]
K562	Cyclic voltammetry (CV) Electrochemical immunosensors	Au nanoparticles (AuNPs)	Linear range: 1.0×10^2–1.0×10^7 cells/mL	Ding et al.[14]
MCF-7	Electrochemical nucleic acid biosensors	DNA-AgNC	LOD: 3 cells/mL	Cao et al.[15]
MCF-7	Electrochemical nucleic acid biosensors	Multiwall carbon nanotubes (MWCNTs)	Linear range: 1.0×10^2–1.0×10^7 cells/mL LOD: 25 cells/mL	Yazdanparast et al.[16]
CTCs	Cyclic voltammetry (CV) Electrochemical impedance	Pt@Ag nanoflowers AuNPs/Acetylene black	Linear range: 20–10^6 cells/mL LOD: 3 cells/mL	Tang et al.[17]
CTCs	Cyclic voltammetry (CV) Differential pulse voltammetry (DPV) Electrochemical impedance	Magnetic Fe3O4 nanospheres (MNs) Cu2O nanoparticles (Cu2O NPs)	Linear range: 3.0–3000 cells/mL LOD: 1 cells/mL	Luo et al.[18]
CTCs (MCF-7)	Cyclic voltammetry (CV) Electrochemical impedance	Ni micropillars/ PLGA electrospun nanofbers	Linear range: 10–10^5 cells/mL LOD: 8 cells/mL	Wu et al.[19]
K562	Differential pulse voltammetry (DPV)	Graphene oxide/ quantum dots (QDs)	LOD: 60 cells/mL	Zheng et al.[20]
CTCs	Cyclic voltammetry (CV) Electrochemical impedance		Linear range: 1.0×10^2–1.0×10^5 cells/mL LOD: 25 cells/mL	Wang et al.[21]
HepG2	Electrochemical impedance	Carbon nanotubes (CNTs)	Linear range: 10–10^5 cells/mL	Liu et al.[22]
CTCs	Cyclic voltammetry (CV) Electrochemical impedance		Linear range: 30–10^6 cells/mL LOD: 10 cells/mL	Shen et al.[23]
HT 29 FR-positive cancer cells	Electrochemical	Functionalized fibrous Nanosilica (KCC-1)	Linear range: 50–$1 \times 1.2 \times 10^4$ cells/mL LOD: 50 cells/mL	Soleymani et al.[24]
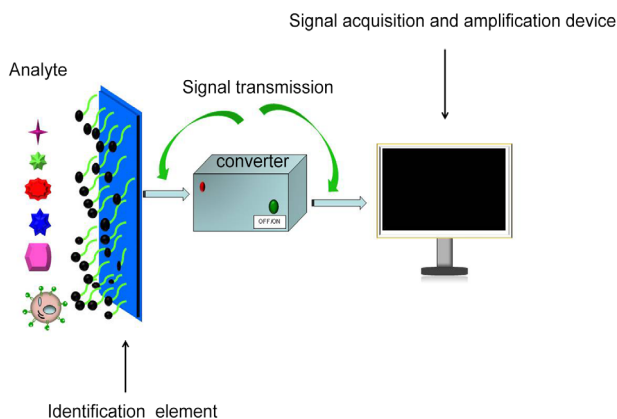
Figure 1 The recognition system selectively interacts with the analyte and converts the resulting chemical parameters into a certain signal. The transduction system receives the signal and transmits it to the electronic system in the form of an electrochemical signal, and the electronic system further amplifies the output.

Figure 2 Cells that grow on the surface of microelectrodes can change the impedance at the interface of the electrode, thus obtaining biological information related to the physiological functions of the cells. (**a**) Glassy carbon electrodes are modified by composite materials composed of carbon nanotubes (CNTs) and gold nanoparticles (AuNPs) to improve their sensitivity and detection range (control). (**b**) Cancer cell is adhered to a composite modified glassy carbon electrode, and the change in cell layer resistance is detected (experiment).

many fields, including food testing, environmental monitoring, clinical medicine, animal disease detection and drug screening.[25–30] One of the most classical techniques in electrochemical biosensor technology is cell impedance sensing. It is based on the principle that cells growing on the surface of a microelectrode can change the impedance at the interface of the adherent electrodes, where biological information related to the physiological functions of cells can be obtained. Therefore, an electrochemical biosensor based on cell impedance sensing technology can measure changes in cell layer resistance caused by cell morphology, cell movement, or cell contact, which can monitor cell dynamic behavior in real time quantitatively without damage (Fig 2). Based on the above principles, researchers have constructed various electrochemical impedance sensors with modified working electrodes based on the innovative technique of capturing tumor cells, and the detection of the following human tumor cells has been demonstrated: HeLa, MCF-7 (breast cancer), HL-60 (human promyeloacute leukemia cell line), HCT-116 (human colorectal cancer cells) and HepG2 (hepatocellular carcinomas).[11–13,22,31–33]

The most important step in building the cell electrochemical sensor is to fix the cells onto the surface of the working electrode to produce electrochemical signals. The traditional fixing method has some disadvantages, such as low survival rate, poor stability, additional increase in diffusion resistance and so on.[34,35] As a kind of biosensor-fixed matrix with good biocompatibility, no toxicity, superior protein adsorption and gel-forming ability, gelatin is widely used in the construction of living cell-fixed and bionic interface. For example, Zhu *et al.* obtained c-SWCNTs-AuNPs-gelatin nanoparticles by ultrasonic assembly using gelatin, gold nanoparticles and single-walled carbon

nanotubes and successfully achieved in vitro fixation and highly sensitive electrochemical detection of human leukemia HL-60 cells.[36] Li and coworkers wrapped gelatin on the surface of Au nanoflowers (AuNFs) to produce AuNFs@gelatin and combined it with cationic conjugated materials to fix and image HeLa cells.[37]

Sensitive biosensors based on various electrochemical techniques have been widely used in the detection of cancer cells. In 2017, Feng *et al.* gave a detailed review of electrochemical detection of tumor cells, and summarized the analytical performance of biosensors used to detect cancer cells. In recent years it has been pointed out that the integration of electrochemical sensors into mobile sensitive detection devices is a widely explored direction in the future.[38] In addition, we still need to develop new electrochemical probes using new nanobiomaterials to improve the capture rate, develop new nanomaterial-modified electrodes to improve conductivity, and detect cancer cells with high sensitivity in a short time in a simple way.

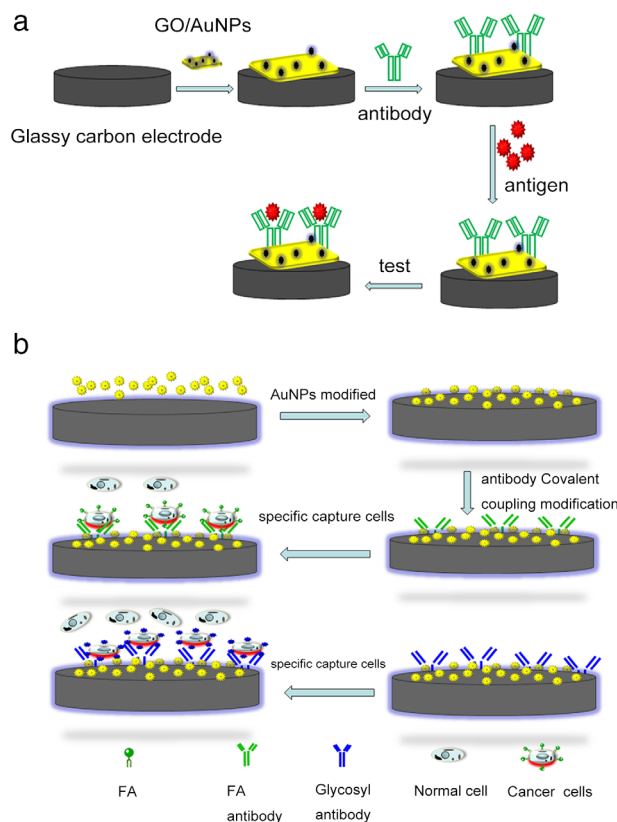## Electrochemical immunosensors in tumor cell detection

An electrochemical immunosensor is a product based on the combination of antigen-antibody specific reaction and electrochemical technology. The basic principle is that the antigen-antibody, as a molecular recognition element, is in direct contact with the electrochemical sensing element and converts the signal of a certain or a certain kind of

chemical concentration into the corresponding electrical signal through the sensing element (Fig 3a). Therefore, biosensors constructed on the basis of specific reactions between antigens and antibodies, specific recognition function of adapters and the cell impedance principle have successfully achieved high-sensitivity linear detection of various cancer cells, including human liver cancer cell line HepG2, human breast cancer cell line MCF-7, small cell lung cancer, lung adenocarcinoma, squamous cell cancer, skin cancer, prostate cancer and breast cancer.[39–41] In addition, glycosyls on the cell surface play an important role in cell recognition, adhesion, immune response, and the occurrence and migration of cancer cells.[42–44] Therefore, the cell electrochemical sensor based on surface-specific glycosyls of cancer cells has also successfully achieved the specific recognition and linear detection of HeLa cells, polysaccharide-rich K562 leukemia cells, and MDA-MB-231 cells.[14,45–48]

Folic acid (FA) receptor is a cell surface receptor that is excessively expressed on most human tumor cells and rarely expressed or not at all in normal organs. Therefore, FA is often used as a target for antitumor drugs.[49–52] Using

the high affinity of FA and FA receptors, researchers used hydrothermal synthesis of functionalized fiber nanosilica (KCC-1), which was then functionalized with FA molecules to produce KCC-1-NH2-FA nanoparticles. Based on the excellent bleaching stability and excellent surface area to volume ratio of KCC-1-NH2-FA nanoparticles, a more sensitive cell sensor was designed for the detection of cancer cells HT-29 with a detection range of 50 to 1.2×10^4 cells/mL and the lower limit of detection is 50 cells/mL (Fig 3b).[24]

As shown in Table 2, we briefly summarize the common and hazardous tumor markers, apart from the tumor markers mentioned in the article, the remaining tumor markers and tumor markers to be discovered provide broad prospects for the specific detection of tumor cells by electrochemical immunosensors. However, in the preparation of electrochemical immunosensors, the fixation of antigen and antibody is an important factor affecting the performance of the sensor, which directly affects the service life, reproducibility and detection limit of the sensor. Due to the specific reaction of antigens and antibodies, electrochemical immunosensors have higher specificity and selectivity than other biosensors, and have been widely used and applied.

Electrochemical nucleic acid biosensors in tumor cell detection

Electrochemical nucleic acid biosensors use nucleic acid molecules as molecular recognition elements, whose principle is to fix a single strand of oligonucleotides on the electrode and hybridize with the target DNA, and detection of target substances by detecting changes in electrochemical parameters before and after hybridization. The target substances can be DNA, miRNA, or other biological molecules (Fig 4a). Aptamer is a synthetic nucleic acid with high specificity and affinity, and ease of biological and chemical modification that has been screened by the screening technique SELEX in vitro (Systematic Evolution of Ligands by Exponential Enrichment). A nucleic acid adapter shows highly specific binding to tumor cell surface target molecules and is easy to use, which has been widely used in the construction of cell sensors in recent years,[62–65] greatly improving the target recognition ability of sensors and detection selectivity to tumor cells.[66–69] For example, Li *et al.* used MUC1 to bind an aptamer for detecting MUC1 proteins on the surface of tumor cells while identifying their CEA proteins with nanometer CdS-labeled carcinoembryonic antigen (CEA), which effectively reduced the occurrence of false positives in the detection of tumor cells.[58] Studies have shown that ITO electrodes with good light transmittance and electrical conductivity were first modified by the AS1141 aptamer, which can selectively bind to the overexpressed nucleolins on the
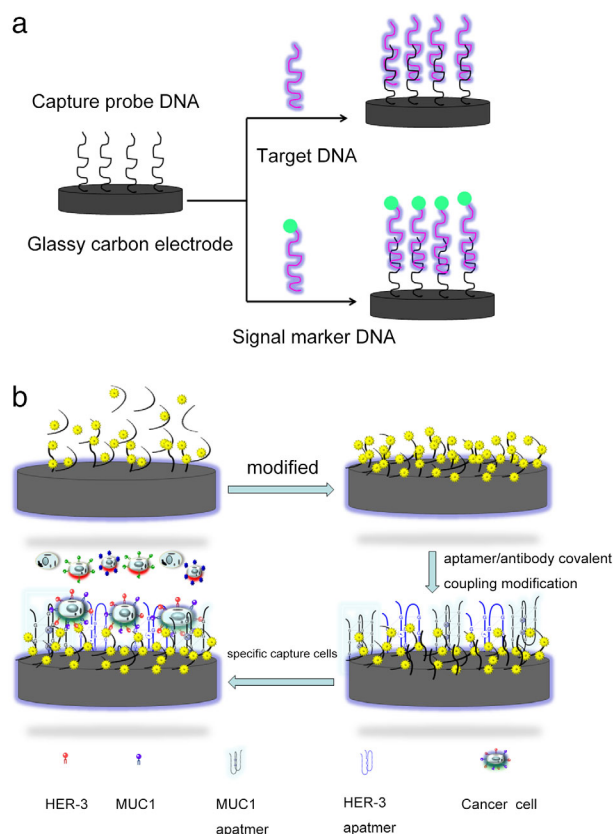

Figure 3 Schematic diagram: (**a**) Electrochemical immunosensor. (**b**) Specific antibody is covalently linked to a modified glassy carbon electrode for specific capture of cancer cells.

Table 2 Summary of the common and serous tumor markers

Tumor cells	Cell-surface/serum markers	Reference
Liver cancer stem cell	CD13	Sun et al.[53]
Hepatocellular carcinoma	Assessing serum α-fetoprotein (AFP)	Tsuchiya et al.[54]
	Des-γ-carboxyprothrombin (DCP)	
	AFP-L3	
	Glypican-3 (GPC3)	
	Golgi protein-73 (GP73)	
Lung cancer cell line	Carbonic anhydrase 9 (CA9)	Cohen et al.[55]
	G protein-coupled receptor 87 (GPR87)	
	LYPD3	
	SLC7A11	
	CXorf61	
Breast cancer cell line	Human epidermal growth factor receptor 3 (HER-3)	Lv et al.,[56] Chiu et al.[57]
Breast cancer cell line	Carbohydrate antigen125 (CA125)	Liu et al.,[58] Jafari et al.[59]
	Human epidermal growth factor receptor-2 (Her-2)	
	Cytokeratin5/6 (CK5/6)	
	E-cadherin (E-cad)	
	carcinoembryonic antigen (CEA)	
	MUC1	
Gastric cancer cell line	Folic acid (FA)	Liu et al.[60]
	GRP78	
	anti-CD146 MAb	
	BRCAA1 MAb	
Acute myeloid leukemia (AML)	CD123, CD45, CD34, CD38, MLL-AML, core binding factor, among others	Prada-Arismendy et al.[61]
HT-29	Folic acid (FA)	Soleymani et al.[24]

surface of breast cancer cells McF-7. Then, the mucin-1 antibody (anti-muc1) and DNA-AgNCs complexes with unique fluorescent and electrochemical properties template silver nanocluster (DNA-AgNCs) bind to the MUC1 on the surface of McF-7 cells to form the sandwich structure. The strong red fluorescence of DNA-AgNCs shows the presence of cancer cells, the strong conductivity of DNA-AgNCs can improve the sensitivity of quantitative analysis, making the detection limit up to 3 cells/mL.[15] In addition, the sensitivity and specificity of tumor cell detection can be improved by using the electrochemical sensor constructed by a dual-aptamer. For example, human epidermal growth factor receptor 3 (HER-3) binding aptamer and MUC1 aptamer were used to simultaneously detect MUC1 protein and HER-3 receptor on the surface of breast cancer cells, which not only kept the probe stable in the complex system, but also had good selectivity and sensitivity for the detection of MCF-7 cells. The linear calibration range of this electrochemical method was 1.0×10^2 to 1.0×10^6 cells/mL, and the detection limit 100 cells/mL (Fig 4b).[56] The research group of Qu *et al.* directly combined the aptamer TLS1c through the flexible linker and the aptamer TLS11a through the rigid linker to the surface of glassy carbon electrode (GCE) to capture MEAR cancer cells; the linear range of detection was 1–14 MEAR cells, and the detection limit was 1 MEAR cell in 10 whole blood cells.[70] In the study by Yazdanparast *et al.*

biocompatible nanocomposite consisting of multiwall carbon nanotubes (MWCNT) and poly(glutamic acid) was placed on a glassy carbon electrode (GCE), then a mucin 1 (MUC1)-binding aptamer was first immobilized on the surface of modified GCE. In order to enhance the selectivity, another aptamer (labeled with silver nanoparticles) was used for secondary MCF-7 cell recognition, which building a kind of electrochemical sensors with high selectivity, sensitivity, stability and reproducibility. Under optimal conditions, the detection range of the sensor was 1.0×10^2 to 1.0×10^7 cells/mL, and the detection limit was 25 cells/mL.[16]

Quantum dots (QDs), also known as semiconductor nanocrystal, has been a hot new type of luminescent nanomaterials in recent years, with unique optical and electrical properties. In addition, QDs has active electrochemical properties, and its metal components can show very sharp redox peak signals after voltammetry analysis. Therefore, QDs can be used as an electroactive substance with signal amplification for the construction of a variety of biosensors. In recent years, for electrochemical cell sensors, QDs and aptamer are usually combined to capture cells, and electrochemical analysis of the metal components of QDs is used to achieve the purpose of quantitative detection of cells (Fig 5). For example, in 2011, Zhu *et al.* directly assembled complementary DNA (cDNA), aptamer and QDs onto the surface of the gold electrode to achieve

Figure 4 Schematic diagram: (**a**) Electrochemical nucleic acid biosensors. (**b**) Specific aptamers is covalently linked to the modified glassy carbon electrode to enhance the specificity of cancer cell capture.

Figure 5 Electrochemical probes based on quantum dot-aptamers were constructed to detect tumor cells. (**a**) Construction of electrochemical probe. (**b**) Working electrode was modified by nanomaterials. (c) The probe captures the tumor cells. Nitric acid digests probes that specifically bind to tumor cells for electrochemical detection.

highly sensitive detection of tumor cells with a detection limit of 100 cells/mL.[71] In recent years, in order to achieve high selectivity and sensitivity of cancer cells to capture and detect, glassy carbon electrodes were firstly modified by multiwall carbon nanotubes (MWCNT), gold nanoparticles (AuNPs), polydopamine (PDA), graphene oxide (GO), polyaniline (PANI) and concanavalin A (Con A) using a layer-by-layer technique. Subsequently, the aptamer-DNA concatamer-CdTe quantum dots (QDs) as the signal amplification probe was covalently connected to the surface of the modified electrode, making a sensor with high stability, selectivity and sensitivity, with a detection limit reaching 50–60 cells/mL.[20,72]

The detection technology of tumor cells based on an aptamer electrochemical probe has been widely used. In 2019, Chen *et al.* published a detailed review of the application of aptamer-based electrochemical cytosensors in tumor diagnosis.[73] However, in vitro screening technique SELEX is still needed to obtain more specific aptamers to improve the detection range and limit of electrochemical sensors.

Detection of circulating tumor cells by electrochemical biosensors

Circulating tumor cells (CTCs) were first discovered in 1869 by Ashworth who found similar tumor cells in peripheral blood during an autopsy of a patient who died of cancer. The main cause of death from malignant tumors is when tumor cells are released from a primary or metastatic lesion into the peripheral blood or lymphatic circulation, resulting in CTCs and metastasis in other parts. Previous studies have shown that CTCs can be a new diagnostic target for tumor staging and prognosis (fewer CTCs indicate longer survival) and can provide information for treatment evaluation.[74–77] Therefore, it is becoming more and more important to detect CTCs rapidly and accurately in peripheral blood for the clinical treatment of tumors and prognosis.

As a fast and efficient detection tool, electrochemical biosensors have been widely used in the detection of tumor cells. However, the average density of tumor cells in the blood is 200 cells/mL, which accounts for only 0.004% of the number of cells. Therefore, it is necessary to develop highly specific and sensitive tools to capture CTCs at low concentrations in the blood. In 2014, Costa *et al.* reviewed isolation and detection with high sensitivity to circulating tumor cells (CTCs) through various biosensors, and pointed out that compared with other biosensors, an electrochemical biosensor has higher sensitivity, simplicity and low cost. Different new nanomaterials are being used to modify electrodes to amplify biometric event signals, narrow detection range and improve detection sensitivity; at

the same time, more specific electrochemical probes need to be constructed to improve the ability to capture circulating tumor cells (CTCs); both are still challenges for researchers.[78] For example, in the report by Shen *et al.* a label-free electrochemical impedance biosensor based on the specific recognition between specific epithelial cell adhesion molecules (EpCAM) overexpressed on the cell membrane and EpCAM aptamer was constructed to detect CTCs. First, 6-mercapto 1-hexanol (MCH) was fixed on the gold electrode; second, the capture probe was directionally inserted in MCH interspaces; the detection range of the sensor was 30 to 10^6 cells/mL, and the detection limit was 10 cells/mL.[23] Tang *et al.* designed a novel ultrasensitive immunoassay protocol by using Pt@Ag nanoflowers (pt@AgNFs) and AuNPs/Acetylene black (AuNPs/AB) nanomaterial to detect CTCs.[17] Pt@AgNFs had high specific surface area and good biocompatibility, and were not only used as the carriers of signal antibodies (Ab_2) but also catalyzed the reduction of H_2O_2 to amplify the current signal. AuNPs/AB nanomaterial was used as a substrate material to increase the specific surface area and conductivity of the gold electrode. The detection range of the sensor was 20 to 1.0×10^6 cells/mL, and the detection limit was 3 cells/mL.[17] In addition, researchers have cleverly combined photoexcitation and electrochemical detection processes to construct photoelectrochemical (PEC) biosensors with higher detection sensitivity for the detection of circulating tumor cells. In this study, a PEC biosensor was proposed based on hexagonal carbon nitride tubes (HCNT) as photosensitive material, and magnetic Fe3O4 nanospheres were used for efficient magnetic capture of CTCs, and Cu2O nanoparticles were used for signal amplification, making the detection range of this sensor range from 3 to 3000 cells/mL, with a detection limit down to 1 cell/mL.[18]

With the development of science and technology, the basic operating units such as sample preparation, reaction, separation and detection of biological, chemical, and medical analysis processes are integrated into microscale chips, which automatically completes the entire analysis process and combines with electrochemical sensing for the detection of CTCs. In 2007, Nagrath and coworkers used a microfluidic chip modified with an epithelial-specific adhesion molecule (EpCAM) antibody to successfully separate untreated peripheral blood CTCs for the first time.[79] At present, the microfluidic chip based on an antibody as the trapping probe has successfully detected CTCs.[80–83] At the same time, as an artificial small molecule that is easier to preserve and modify than antibodies, the aptamer is more suitable for functional modification of microfluidic chips. For example, the microfluidic chip modified by nucleic acid aptamer sgc8 has successfully achieved the separation and capture of target cells from many samples with a

capture efficiency of 80% and a specificity greater than 97%. In addition, different aptamers can be used to separate and capture different target cells.[84,85] On the basis of earlier studies, Soper *et al.* and Tsing *et al.* used aptamers that identified PSMA and A549 cells to perform functional modifications on microfluidic chips, and constructed microfluidic devices to capture tumor cells, and they were able to detect CTCs in the blood of cancer patients.[86,87] However, in these studies, the length of the aptamers modified on the surface of the microfluidic chip exposed to the solution was only a few nanometers, which makes microfluidic chip detection inefficient and difficult to capture cells in high-speed flowing liquid. To overcome this shortcoming, researchers continue to explore the use of cyclic DNA templates and connected primers at the end of the template, and then in the presence of polymerase and dNTP, each primer extends along the cyclic DNA template to finally generate a single-stranded DNA consisting of a plurality of aptamers in series as a capture probe, which can effectively enhance its ability to capture the CTCs.[21]

With the rapid development of micro/nano manufacturing technology, the analysis method based on three-dimensional (3D) bionic interface has become a hot research topic in nanotechnology and life sciences. Micro/nanostructure-based devices have been identified as the simplest and most effective technologies for capturing CTCs. Chen and coworkers showed a nickel (Ni) microcolumn cell sensor deposited by electro-textile nanofibers. First, ultralong poly (lactic-co-glycolic acid) (PLGA) nanofibers were laterally stacked on the surface of nickel micropillars by electrospinning to construct a 3D biomimetic interface for capturing CTCs, which would be connected with quantum dot (QD). The functionalized anti-EpCAM antibody (QD-EpCAM) was modified at the 3D biomimetic interface to successfully achieve the highly specific detection of MCF7 breast cancer cells as a CTC model. The detection range was 10^1 to 10^5 cells/mL, and the detection limit was 8 cells/mL.[19] In summary, the combination of electrochemical sensing technology and microfluidic chip technology can provide a powerful, rapid and easy-to-use tool for the clinical detection of CTCs.

Conclusions and perspectives

Compared with normal cells, tumor cells, especially malignant tumors, exhibit abnormal movement and migration capabilities, rapid cell division, and cytoskeletal abnormalities.[88–90] At the same time, existing studies have shown that persistent inflammation can also trigger and exacerbate malignant tumors.[91] These characteristics not only give researchers new ideas on how to treat cancer; for example, by inhibiting the activity of microtubule motor proteins, blocking mitosis or development of inflammatory

factor-related inhibitors (such as histone deacetylase 6 [HDAC] inhibitors), achieving the purpose of anticancer agents, they also provide valuable reference to assist with the early diagnosis of tumors.[63,92,93] Electrochemical biosensor technology, as a new type technology of tumor detection, has achieved breakthrough results after decades of development. In particular, the emergence of cell electrochemical sensors provides convenient tools for cell counting, cell classification, and the detection of tumor cells.[94,95] Among the many breakthrough results, the detection of tumor cells has not only achieved high sensitivity (limit of detection of 10 tumor cells in 250 µL samples[96]) and high specificity, but has also made it possible to detect double antigen on the tumor cell surface, successfully avoiding false positive results. However, in the process of sensor configuration and application research, some deficiencies and improvements have also been found.

First, with the advent of nucleic acid adapters and microfluidic chips, the problem of efficient and specific trapping of tumor cells has been solved. However, during the application of the electrochemical sensors, an irreversible chemical reaction occurs between recognition elements and target on the surface of tumor cells, or the recognition element is contaminated with blood impurities which greatly reduces the reuse rate of the sensors and recognition ability of the identification elements. Therefore, the recognition elements of the sensors are chemically treated with different regenerative solvents in different situations to restore the recognition function in time for the purpose of reuse.

Second, in the preparation of nanomaterials and the preparation of functionalized nanocomposites, nanomaterials with high catalytic properties should be combined with carbon nanotubes and peptide carbon nanotubes to improve sensitivity, selectivity and stability of nanocomposites. In terms of biocatalytic induction of nanomaterials, a new biosensor interface should be explored further for the assembly of biomolecules and nanometer microarray so as to achieve high specificity and high sensitivity detection of CTCs in complex blood samples. In addition, the manufacturing process of future sensors could be more delicate, which will not only improve the performance of future sensors but will also promote the miniaturization of sensors to meet the needs of specific situations.

Acknowledgments

Disclosure

The authors have no conflicts of interest to declare.

References

1 Chen M, Xie S. Therapeutic targeting of cellular stress responses in cancer. *Thorac Cancer* 2018; **9** (12): 1575–82.

2 Sun S, Zhou J. Molecular mechanisms underlying stress response and adaptation. *Thorac Cancer* 2018; **9** (2): 218–27.

3 Du X, Zhang Z, Zheng X *et al.* An electrochemical biosensor for the detection of epithelial-mesenchymal transition. *Nat Commun* 2020; **11** (1): 192.

4 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68** (6): 394–424.

5 Fan Z, Sun L, Huang Y, Wang Y, Zhang M. Bioinspired fluorescent dipeptide nanoparticles for targeted cancer cell imaging and real-time monitoring of drug release. *Nat Nanotechnol* 2016; **11** (4): 388–94.

6 Kelley LC, Wang Z, Hagedorn EJ *et al.* Live-cell confocal microscopy and quantitative 4D image analysis of anchor-cell invasion through the basement membrane in *Caenorhabditis elegans*. *Nat Protoc* 2017; **12** (10): 2081–96.

7 Tao Z, Dang X, Huang X *et al.* Early tumor detection afforded by in vivo imaging of near-infrared II fluorescence. *Biomaterials* 2017; **134**: 202–15.

8 Sun D, Lu J, Luo Z, Zhang L, Liu P, Chen Z. Competitive electrochemical platform for ultrasensitive cytosensing of liver cancer cells by using nanotetrahedra structure with rolling circle amplification. *Biosens Bioelectron* 2018; **120**: 8–14.

9 Wan Y, Zhou YG, Poudineh M *et al.* Highly specific electrochemical analysis of cancer cells using multi-nanoparticle labeling. *Angewandte Chemie* 2014; **53** (48): 13145–9 (In German.).

10 Zhao J, Tang Y, Cao Y *et al.* Amplified electrochemical detection of surface biomarker in breast cancer stem cell using self-assembled supramolecular nanocomposites. *Electrochim Acta* 2018; **283**: 1072–8.

11 Wang SS, Zhao XP, Liu FF, Younis MR, Xia XH, Wang C. Direct Plasmon-enhanced electrochemistry for enabling ultrasensitive and label-free detection of circulating tumor cells in blood. *Anal Chem* 2019; **91** (7): 4413–20.

12 Liu M, Xu Y, Huang C *et al.* Hyaluronic acid-grafted three-dimensional MWCNT array as biosensing interface for chronocoulometric detection and fluorometric imaging of CD44-overexpressing cancer cells. *Mikrochim Acta* 2018; **185** (7): 338.

13 Xu Y, Wu H, Huang C *et al.* Sensitive detection of tumor cells by a new cytosensor with 3D-MWCNTs array based on vicinal-dithiol-containing proteins (VDPs). *Biosens Bioelectron* 2015; **66**: 321–6.

14 Ding C, Qian S, Wang Z, Qu B. Electrochemical cytosensor based on gold nanoparticles for the determination of carbohydrate on cell surface. *Anal Biochem* 2011; **414** (1): 84–7.

15 Cao Y, Dai Y, Chen H *et al*. Integration of fluorescence imaging and electrochemical biosensing for both qualitative location and quantitative detection of cancer cells. *Biosens Bioelectron* 2019; **130**: 132–8.

16 Yazdanparast S, Benvidi A, Banaei M, Nikukar H, Tezerjani MD, Azimzadeh M. Dual-aptamer based electrochemical sandwich biosensor for MCF-7 human breast cancer cells using silver nanoparticle labels and a poly(glutamic acid)/MWNT nanocomposite. *Mikrochim Acta* 2018; **185** (9): 405.

17 Tang S, Shen H, Hao Y *et al*. A novel cytosensor based on Pt@Ag nanoflowers and AuNPs/acetylene black for ultrasensitive and highly specific detection of circulating tumor cells. *Biosens Bioelectron* 2018; **104**: 72–8.

18 Luo J, Liang D, Zhao D, Yang M. Photoelectrochemical detection of circulating tumor cells based on aptamer conjugated Cu2O as signal probe. *Biosens Bioelectron* 2020; **151**: 111976.

19 Wu X, Xiao T, Luo Z *et al*. A micro−/nano-chip and quantum dots-based 3D cytosensor for quantitative analysis of circulating tumor cells. *J Nanobiotechnol* 2018; **16** (1): 65.

20 Zheng Y, Wang X, He S *et al*. Aptamer-DNA concatemer-quantum dots based electrochemical biosensing strategy for green and ultrasensitive detection of tumor cells via mercury-free anodic stripping voltammetry. *Biosens Bioelectron* 2019; **126**: 261–8.

21 Wang Y, Chang K, Yang C *et al*. Highly sensitive electrochemical biosensor for circulating tumor cells detection via dual-Aptamer capture and rolling circle amplification strategy. *J Biomed Nanotechnol* 2019; **15** (7): 1568–77.

22 Liu Y, Zhu F, Dan W, Fu Y, Liu S. Construction of carbon nanotube based nanoarchitectures for selective impedimetric detection of cancer cells in whole blood. *Analyst* 2014; **139** (20): 5086–92.

23 Shen H, Yang J, Chen Z *et al*. A novel label-free and reusable electrochemical cytosensor for highly sensitive detection and specific collection of CTCs. *Biosens Bioelectron* 2016; **81**: 495–502.

24 Soleymani J, Hasanzadeh M, Somi MH, Shadjou N, Jouyban A. Highly sensitive and specific cytosensing of HT 29 colorectal cancer cells using folic acid functionalized-KCC-1 nanoparticles. *Biosens Bioelectron* 2019; **132**: 122–31.

25 Eremeev SI, Krychenkov DA. Applicability of semi-conductor zinc oxide gas sensors to detection of low ozone concentrations in pressurized modules. *Aviakosm Ekolog Med* 2005; **39** (3): 53–5.

26 Cai C, Guo Z, Cao Y, Zhang W, Chen Y. A dual biomarker detection platform for quantitating circulating tumor DNA (ctDNA). *Nanotheranostics* 2018; **2** (1): 12–20.

27 Flampouri E, Imar S, OConnell K, Singh B. Spheroid-3D and monolayer-2D intestinal electrochemical biosensor for toxicity/viability testing: Applications in drug screening, food safety, and environmental pollutant analysis. *ACS Sens* 2019; **4** (3): 660–9.

28 Zhang Z, Zhou J, Du X. Electrochemical biosensors for detection of foodborne pathogens. *Micromachines* 2019; **10** (4): 1–9.

29 Du X, Zhou J. Application of biosensors to detection of epidemic diseases in animals. *Res Vet Sci* 2018; **118**: 444–8.

30 Zhang Z, Cong Y, Huang Y, Du X. Nanomaterials-based electrochemical Immunosensors. *Micromachines* 2019; **10** (6): 1–8.

31 Jing A, Zhang C, Liang G, Feng W, Tian Z, Jing C. Hyaluronate-functionalized graphene for label-free electrochemical cytosensing. *Micromachines* 2018; **9** (12): 1–11.

32 Xu J, Wang X, Yan C, Chen W. A Polyamidoamine dendrimer-based electrochemical Immunosensor for label-free determination of epithelial cell adhesion molecule-expressing cancer cells. *Sensors* 2019; **19** (8): 1879.

33 Liu JX, Bao N, Luo X, Ding SN. Nonenzymatic amperometric aptamer cytosensor for ultrasensitive detection of circulating tumor cells and dynamic evaluation of cell surface N-glycan expression. *ACS Omega* 2018; **3** (8): 8595–604.

34 Mulchandani A, Kaneva I, Chen W. Biosensor for direct determination of organophosphate nerve agents using recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. 2. Fiber-optic microbial biosensor. *Anal Chem* 1998; **70** (23): 5042–6.

35 Zheng J, Northrup SR, Hornsby PJ. Modification of materials formed from poly(L-lactic acid) to enable covalent binding of biopolymers: Application to high-density three-dimensional cell culture in foams with attached collagen. *In Vitro Cell Dev Biol Anim* 1998; **34** (9): 679–84.

36 Zhang JJ, Gu MM, Zheng TT, Zhu JJ. Synthesis of gelatin-stabilized gold nanoparticles and assembly of carboxylic single-walled carbon nanotubes/Au composites for cytosensing and drug uptake. *Anal Chem* 2009; **81** (16): 6641–8.

37 Cui Q, He F, Wang X, Xia B, Li L. Gold nanoflower@gelatin core-shell nanoparticles loaded with conjugated polymer applied for cellular imaging. *ACS Appl Mater Interfaces* 2013; **5** (1): 213–9.

38 Zhao F. Electrochemical techniques for the detection of cancer cells and cell-surface glycan expression: A mini review. *Int J Electrochem Sci* 2017; **12** (8): 7580–96.

39 Yang Y, Fu Y, Su H, Mao L, Chen M. Sensitive detection of MCF-7 human breast cancer cells by using a novel DNA-labeled sandwich electrochemical biosensor. *Biosens Bioelectron* 2018; **122**: 175–82.

40 Wang K, He MQ, Zhai FH, He RH, Yu YL. A novel electrochemical biosensor based on polyadenine modified aptamer for label-free and ultrasensitive detection of human breast cancer cells. *Talanta* 2017; **166**: 87–92.

41 Aydin EB, Sezginturk MK. A sensitive and disposable electrochemical immunosensor for detection of SOX2, a biomarker of cancer. *Talanta* 2017; **172**: 162–70.

42 Wasim L, Buhari FHM, Yoganathan M *et al*. N-linked glycosylation regulates CD22 organization and function. *Front Immunol* 2019; **10**: 699.

43 Swaminathan V, Mythreye K, O'Brien ET, Berchuck A, Blobe GC, Superfine R. Mechanical stiffness grades metastatic potential in patient tumor cells and in cancer cell lines. *Cancer Res* 2011; **71** (15): 5075–80.

44 Zhou SM, Cheng L, Guo SJ *et al.* Lectin RCA-I specifically binds to metastasis-associated cell surface glycans in triple-negative breast cancer. *Breast Cancer Res* 2015; **17**: 36.

45 Tang YH, Lin HC, Lai CL, Chen PY, Lai CH. Mannosyl electrochemical impedance cytosensor for label-free MDA-MB-231 cancer cell detection. *Biosens Bioelectron* 2018; **116**: 100–7.

46 Wang Y, Chen Z, Liu Y, Li J. A functional glycoprotein competitive recognition and signal amplification strategy for carbohydrate-protein interaction profiling and cell surface carbohydrate expression evaluation. *Nanoscale* 2013; **5** (16): 7349–55.

47 Zhang JJ, Cheng FF, Zheng TT, Zhu JJ. Design and implementation of electrochemical cytosensor for evaluation of cell surface carbohydrate and glycoprotein. *Anal Chem* 2010; **82** (9): 3547–55.

48 Zhang X, Lu W, Shen J *et al.* Carbohydrate derivative-functionalized biosensing toward highly sensitive electrochemical detection of cell surface glycan expression as cancer biomarker. *Biosens Bioelectron* 2015; **74**: 291–8.

49 Lebret V, Raehm L, Durand JO *et al.* Folic acid-targeted mesoporous silica nanoparticles for two-photon fluorescence. *J Biomed Nanotechnol* 2010; **6** (2): 176–80.

50 Vlahov IR, Vite GD, Kleindl PJ *et al.* Regioselective synthesis of folate receptor-targeted agents derived from epothilone analogs and folic acid. *Bioorg Med Chem Lett* 2010; **20** (15): 4578–81.

51 Li N, Zhong D, Chen H *et al.* The utility of folate receptor-positive circulating tumor cell in cancer diagnosis in the elderly population. *Cancer Manag Res* 2019; **11**: 4097–107.

52 Zhang Z, Jia J, Lai Y, Ma Y, Weng J, Sun L. Conjugating folic acid to gold nanoparticles through glutathione for targeting and detecting cancer cells. *Bioorg Med Chem* 2010; **18** (15): 5528–34.

53 Sun JH, Luo Q, Liu LL, Song GB. Liver cancer stem cell markers: Progression and therapeutic implications. *World J Gastroenterol* 2016; **22** (13): 3547–57.

54 Tsuchiya N, Sawada Y, Endo I, Saito K, Uemura Y, Nakatsura T. Biomarkers for the early diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2015; **21** (37): 10573–83.

55 Cohen AS, Khalil FK, Welsh EA *et al.* Cell-surface marker discovery for lung cancer. *Oncotarget* 2017; **8** (69): 113373–402.

56 Lv S, Guan Y, Wang D, Du Y. Aptamer based strategy for cytosensing and evaluation of HER-3 on the surface of MCF-7 cells by using the signal amplification of nucleic acid-functionalized nanocrystals. *Anal Chim Acta* 2013; **772**: 26–32.

57 Chiu CG, Masoudi H, Leung S *et al.* HER-3 overexpression is prognostic of reduced breast cancer survival: A study of 4046 patients. *Ann Surg* 2010; **251** (6): 1107–16.

58 Li T, Fan Q, Liu T, Zhu X, Zhao J, Li G. Detection of breast cancer cells specially and accurately by an electrochemical method. *Biosens Bioelectron* 2010; **25** (12): 2686–9.

59 Jafari SH, Saadatpour Z, Salmaninejad A *et al.* Breast cancer diagnosis: Imaging techniques and biochemical markers. *J Cell Physiol* 2018; **233** (7): 5200–13.

60 Li R, Liu B, Gao J. The application of nanoparticles in diagnosis and theranostics of gastric cancer. *Cancer Lett* 2017; **386**: 123–30.

61 Prada-Arismendy J, Arroyave JC, Rothlisberger S. Molecular biomarkers in acute myeloid leukemia. *Blood Rev* 2017; **31** (1): 63–76.

62 Yang Y, Hao HJ, Wu XF *et al.* Mixed-lineage leukemia protein 2 suppresses ciliary assembly by the modulation of actin dynamics and vesicle transport. *Cell Discov* 2019; **5**: 33.

63 Liu M, Ran J, Zhou J. Non-canonical functions of the mitotic kinesin Eg5. *Thorac Cancer* 2018; **9** (8): 904–10.

64 Yu F, Guo S, Li T *et al.* Ciliary defects caused by dysregulation of O-GlcNAc modification are associated with diabetic complications. *Cell Res* 2019; **29** (2): 171–3.

65 Xie W, Yang Y, Gao S *et al.* The tumor suppressor CYLD controls epithelial morphogenesis and homeostasis by regulating mitotic spindle behavior and adherens junction assembly. *J Genet Genomics* 2017; **44** (7): 343–53.

66 Sun D, Lu J, Zhong Y *et al.* Sensitive electrochemical aptamer cytosensor for highly specific detection of cancer cells based on the hybrid nanoelectrocatalysts and enzyme for signal amplification. *Biosens Bioelectron* 2016; **75**: 301–7.

67 Liu J, Cui M, Niu L, Zhou H, Zhang S. Enhanced peroxidase-like properties of Graphene-Hemin-composite decorated with au Nanoflowers as electrochemical Aptamer biosensor for the detection of K562 Leukemia cancer cells. *Chemistry* 2016; **22** (50): 18001–8.

68 Yi Z, Li XY, Gao Q, Tang LJ, Chu X. Aptamer-aided target capturing with biocatalytic metal deposition: An electrochemical platform for sensitive detection of cancer cells. *Analyst* 2013; **138** (7): 2032–7.

69 Amouzadeh Tabrizi M, Shamsipur M, Saber R, Sarkar S. Isolation of HL-60 cancer cells from the human serum sample using MnO2-PEI/Ni/Au/aptamer as a novel nanomotor and electrochemical determination of thereof by aptamer/gold nanoparticles-poly(3,4-ethylene dioxythiophene) modified GC electrode. *Biosens Bioelectron* 2018; **110**: 141–6.

70 Qu L, Xu J, Tan X, Liu Z, Xu L, Peng R. Dual-aptamer modification generates a unique interface for highly sensitive and specific electrochemical detection of tumor cells. *ACS Appl Mater Interfaces* 2014; **6** (10): 7309–15.

71 Li J, Xu M, Huang H *et al.* Aptamer-quantum dots conjugates-based ultrasensitive competitive electrochemical cytosensor for the detection of tumor cell. *Talanta* 2011; **85** (4): 2113–20.

72 Liu H, Xu S, He Z, Deng A, Zhu JJ. Supersandwich cytosensor for selective and ultrasensitive detection of cancer cells using aptamer-DNA concatamer-quantum dots probes. *Anal Chem* 2013; **85** (6): 3385–92.

73 Sun D, Lu J, Zhang L, Chen Z. Aptamer-based electrochemical cytosensors for tumor cell detection in cancer diagnosis: A review. *Anal Chim Acta* 2019; **1082**: 1–17.

74 Seeberg LT, Waage A, Brunborg C *et al.* Circulating tumor cells in patients with colorectal liver metastasis predict impaired survival. *Ann Surg* 2015; **261** (1): 164–71.

75 Shao C, Liao CP, Hu P *et al.* Detection of live circulating tumor cells by a class of near-infrared heptamethine carbocyanine dyes in patients with localized and metastatic prostate cancer. *PLOS One* 2014; **9** (2): e88967.

76 Mikulova V, Cabinakova M, Janatkova I, Mestek O, Zima T, Tesarova P. Detection of circulating tumor cells during follow-up of patients with early breast cancer: Clinical utility for monitoring of therapy efficacy. *Scand J Clin Lab Invest* 2014; **74** (2): 132–42.

77 Muinelo-Romay L, Vieito M, Abalo A *et al.* Evaluation of circulating tumor cells and related events as prognostic factors and surrogate biomarkers in advanced NSCLC patients receiving first-line systemic treatment. *Cancers (Basel)* 2014; **6** (1): 153–65.

78 Costa C, Abal M, Lopez-Lopez R, Muinelo-Romay L. Biosensors for the detection of circulating tumour cells. *Sensors (Basel)* 2014; **14** (3): 4856–75.

79 Nagrath S, Sequist LV, Maheswaran S *et al.* Isolation of rare circulating tumour cells in cancer patients by microchip technology. *Nature* 2007; **450** (7173): 1235–9.

80 Yu CC, Ho BC, Juang RS *et al.* Poly (3,4-ethylenedioxythiophene)-based nanofiber mats as an organic bioelectronic platform for programming multiple capture/release cycles of circulating tumor cells. *ACS Appl Mater Interfaces* 2017; **9** (36): 30329–42.

81 Zhang L, Xu Z, Kang Y, Xue P. Three-dimensional microfluidic chip with twin-layer herringbone structure for high efficient tumor cell capture and release via antibody-conjugated magnetic microbeads. *Electrophoresis* 2018; **39** (12): 1452–9.

82 Qasaimeh MA, Wu YC, Bose S *et al.* Isolation of circulating plasma cells in multiple myeloma using CD138 antibody-based capture in a microfluidic device. *Sci Rep* 2017; **7**: 45681.

83 Huang W, Chang CL, Brault ND *et al.* Separation and dual detection of prostate cancer cells and protein biomarkers using a microchip device. *Lab Chip* 2017; **17** (3): 415–28.

84 Yeong Won J, Choi JW, Min J. Micro-fluidic chip platform for the characterization of breast cancer cells using aptamer-assisted immunohistochemistry. *Biosens Bioelectron* 2013; **40** (1): 161–6.

85 Ye X. Aptamer-based microfluidic device for enrichment, sorting, and detection of multiple cancer cells. *Anal Chem* 2009; **81**: 7436–7442

86 Dharmasiri U, Balamurugan S, Adams A *et al.* Highly efficient capture and enumeration of low abundance prostate cancer cells using prostate-specific membrane antigen aptamers immobilized to a polymeric microfluidic device. *Electrophoresis* 2009; **30** (18): 3289–300.

87 Shen Q, Xu L, Zhao L *et al.* Specific capture and release of circulating tumor cells using aptamer-modified nanosubstrates. *Adv Mater* 2013; **25** (16): 2368–73.

88 Shi X, Wang Y, Sun X *et al.* Centrosomal protein 70 is a mediator of paclitaxel sensitivity. *Int J Mol Sci* 2017; **18** (6): 1267.

89 Chen M, Li Y, Liu Z *et al.* Exopolysaccharides from a *Codonopsis pilosula* endophyte activate macrophages and inhibit cancer cell proliferation and migration. *Thorac Cancer* 2018; **9** (5): 630–9.

90 Yang Y, Mu T, Li T *et al.* Effects of FSTL1 on the proliferation and motility of breast cancer cells and vascular endothelial cells. *Thorac Cancer* 2017; **8** (6): 606–12.

91 Bishayee A. The role of inflammation and liver cancer. *Adv Exp Med Biol* 2014; **816**: 401–35.

92 He X, Liu Z, He Q *et al.* Identification of novel microtubule-binding proteins by taxol-mediated microtubule stabilization and mass spectrometry analysis. *Thorac Cancer* 2015; **6** (5): 649–54.

93 Ran J, Zhou J. Targeted inhibition of histone deacetylase 6 in inflammatory diseases. *Thorac Cancer* 2019; **10** (3): 405–12.

94 KumeriaT KMD, Diener KR, Parkinson L, Losic D. Label-free reflectometric interference microchip biosensor based on nanoporous alumina for detection of circulating tumour cells. *Biosens Bioelectron* 2012; **35** (1): 167–73.

95 Sepunaru L, Sokolov SV, Holter J, Young NP, Compton RG. Electrochemical red blood cell counting: One at a time. *Angew Chem Int Ed Engl* 2016; **55** (33): 9768–71.

96 Bamrungsap S, Chen T, Shukoor MI *et al.* Pattern recognition of cancer cells using aptamer-conjugated magnetic nanoparticles. *ACS Nano* 2012; **6** (5): 3974–81.