

Research progress of exosome biosensors

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Abstract: At present, cancer is still an important cause of high morbidity and mortality worldwide. Early diagnosis has become the key to improving the cure rate and life quality of patients. Exosomes are small extracellular vesicles that play important roles in intercellular communication. Exosomes contain a variety of biomarkers, such as DNA, RNA, proteins, lipids, and metabolites, which reflect the state of the parent cell. Since exosomes affect tumorigenesis and metastasis in cancer patients, they are excellent non-invasive potential indicators for early cancer detection. In addition, the growing understanding of the structure and function of cancer marker exosomes provides new solutions for early cancer detection. Nowadays, the main cancer screening methods (ultrasound, nuclear magnetic resonance, tissue biopsy, etc.) are expensive, time-consuming, and require professionals to operate imaging, etc. Exosome biosensors have become effective tools for early cancer diagnosis due to their advantages of simple operation and cost-effectiveness. Therefore, based on the structure and function of exosomes, this study summarizes the research progress of different types of biosensor detection, discusses the characteristics of current biosensors, and looks forward to future research trends.

Key words: Exosome, Biosensor, Detection.

1. Introduction

Cancer is a serious threat to human health and safety due to the deteriorating living environment [1]. Therefore, it is crucial to diagnose cancer early to prevent the disease and improve patients' prognosis. Access to cost-effective and accurate diagnostic cancer markers is essential to provide patients with appropriate information about their disease and optimize treatment strategies [2]. Exosomes released by cancer cells play a significant role in tumorigenesis, including immunosuppression, angiogenesis, and cell migration. Furthermore, exosomes carry information about the tumor microenvironment, making them an emerging cancer marker for non-invasive early detection and evaluation of therapeutic effects [3]. This paper summarizes the detection methods for cancer marker exosomes, compares their advantages and disadvantages, and discusses their future development trends.

2. Exosome

Exosomes, approximately 40-100 nm in diameter, are cell-secreted bionanoscale spherical lipid bilayer vesicles floating in sucrose density gradient solutions at a density of 1.13-1.19 g·mL⁻¹. In 1981, Trams [4] collectively referred to vesicles derived from the plasma membrane as exosomes and proposed the concept of "exosomes" for the first time, believing that exosomes were membrane vesicles derived from exudates from cultures of various cell lines and had 5'-nucleotidase activity with certain physiological functions. Exosomes (40-100 nm) were first discovered in sheep reticulocytes in 1983. Johnstone [5] tracked transferrin receptors during reticulocyte maturation and found that the deletion process of transferrin receptors in mature red blood cells would lead to the formation of exosomes. To distinguish them from other types of extracellular vesicles (EVs), they are named exosomes. However, it is worth noting that due to difficulties in isolation methods, even though the term "exosome" is widely used in the International Extracellular Vesicle Research guidelines [6], it is now recommended to replace it with the term "small extracellular vesicle (sEVs)". Studies have found that exosomes contain nucleic acids, proteins, lipids, cytokines, transcription factor receptors and other bioactive substances [7, 8]. Among them, exosomal protein components

are mainly divided into two categories, one is universal and participates in the process of vesicle formation and secretion. Including membrane transport and fusion related proteins (such as Rab, GTPases), heat shock proteins (such as HSP70, HSP90), four transmembrane protein superfamily (such as CD63, CD81), ESCRT complex related proteins (such as TSG101, Alix), integrins, etc. The other is specific components closely related to progenitor cells, such as CD45 and MHC-II derived from antigen-presenting cells. With the deepening of exosome research, its application is becoming more and more extensive. Exosomes can play a role in physiological and pathological processes as mediators of intercellular communication and material exchange. In addition, exosomes can deliver a variety of bioactive substances and components that are easily inactivated or easily degraded through a variety of pathways and sites, and safely transfer to target cells to participate in regulation, such as tissue repair, tumor diagnosis, therapy, and immune regulation.

3. Exosome classification

Exosomes contain high levels of proteins, including transmembrane proteins (CD9, CD63, and CD81), heat shock proteins (Hsp60, Hsp70, and Hsp90), transporters (TSG-101 and Alix), and cancer-related specific markers (GPC1, EpCAM, PD-L1, and EGFR). Exosomal proteins play a crucial role in pre-metastatic niche development and intercellular communication.

3.1. Tetraspanins

Tetraspanins are transmembrane proteins with short cytoplasmic n-terminus and C-terminus, one in each extracellular domain (EC1 and EC2) size, with a unique cysteine motif in the EC2 domain. Tetraspanins are mainly expressed on the cell surface or intracellular vesicles and are involved in cell motility, adhesion, morphological change, and cell and vesicle membrane fusion [9]. In addition, Tetraspanin forms networks with exosomes and other proteins that regulate signal transduction pathways and the formation of pre-metastatic sites in the cancer microenvironment. Tetraspanin plays a crucial role in target cell selection for exosome uptake, which may lead to reprogramming of target cells. The reprogrammed target cells contribute to the formation of the metastatic intracellular environment. The four major transmembrane proteins that are associated with exosomes and promote cancer cell movement, invasion, metastasis, tumorigenesis, progression and angiogenesis include CD9, CD8, CD82 and CD151[10]. Tetraspanins are also of great therapeutic value due to their functional roles in biogenesis, protein and RNA sorting, targeting, and uptake.

The CD63 gene is located on human chromosome 12q13 and encodes 60 amino acid residues with a molecular weight of 237-30 kDa [11]. CD63, as the first tetraspanin to be characterized [12], is structurally similar to other tetraspanin, consisting of four hydrophobic transmembrane domains, short cytoplasmic domains N-terminal and C-terminal, intracellular cross-links between transmembrane domains 2 and 3, and two extracellular domains/loops [13]. CD63 is expressed on all cell and tissue types, not only on the plasma membrane of cells, but also in late endosomes, lysosomes and polyvesicular bodies (MVB) [14]. At the same time, CD63 is also present in MVBs of platelet granules, melanosomes of melanocytes, cytotoxic granules of T cells, Weibel-Palade bodies of endothelial cells, and major histocompatibility Complex II (MHC-II) compartments of dendritic cells [15-19]. Stimulation of these cells causes the polyvesicles to fuse with the cell surface, releasing microvesicles called exosomes in the extracellular microenvironment. Therefore, CD63 is rich in exosomes from different cell types [20].

The main characteristic of Tetraspanins is its ability to interact with itself and with various cell surface proteins to form supramolecular complex networks [11]. When Tetraspanins bind to other proteins that form supramolecular complexes on the cell surface, its primary role is to trigger signal transduction pathways [21]. These supramolecular complexes mainly affect cell behavior, such as basic cellular processes such as cell proliferation, migration, adhesion, differentiation, and motility [21, 22]. Therefore, as a quadriprotein, CD63 can also directly or indirectly interact with a variety of proteins, such as integrins (mainly $\beta 1$ -integrin), other quadriproteins, cell surface receptors, adaptor

proteins, intracellular signaling molecules, and lysosomal proteins [23-27]. It has been found that CD63 is an important cofactor in the transport of p-selectin from Weibel-Palade bodies (WPB) of endothelial cells to the plasma membrane, because CD63-deficient mice have no P-selectin on the surface of endothelial cells. This suggests that CD63 plays a key but indirect role in the recruitment, rolling and exosmosis of white blood cells during inflammation [28]. In addition, it is also related to β 1-integrin and VEGFR2 in the plasma membrane of human umbilical vein endothelial cells (HUVECs), and loss of CD63 expression leads to reduced phosphorylation of FAK, thereby regulating β 1-integrin-induced intracellular signaling [29]. After stimulation, CD63 will transfer to the plasma membrane of T lymphocytes, and co-stimulate with T cell receptor (TCR) to induce the activation and proliferation of T cells and the production of IL-2 [30].

3.2. Heat shock proteins (Hsps)

The expression of Hsps is to participate in specific stress responses, and the accumulation of denator-protein is the proximal signal induced by it [31]. Hsps are overexpressed in a wide range of human cancers and are associated with tumor cell proliferation, differentiation, invasion, metastasis, death, and immune system recognition. It is not clear how Hsps are overexpressed in cancer, but one hypothesis is that the physiological and pathological features of the tumor microenvironment (low glucose, pH, and oxygen) stimulate Hsp expression [32, 33]. Although the level of Hsp is not of reference value in medical diagnosis, due to its overexpression in malignant cells and tissues, Hsps can be used as an effective biomarker for carcinogenesis in tissues, and its value also indicates the degree of differentiation and aggressiveness of certain types of cancer [34]. In addition, the levels of Hsps and their antibodies circulating in cancer patients may help in tumor diagnosis.

Several Hsps are associated with the prognosis of specific cancers, the most significant being Hsp27, whose expression is associated with poor prognosis of gastric cancer, liver cancer, prostate cancer and osteosarcoma, while Hsp70 is associated with poor prognosis of breast cancer, endometrial cancer, cervical cancer and bladder cancer [35]. Increased Hsp expression may also predict response to certain anti-cancer treatments. Although Hsp27 is associated with poor chemotherapy response in leukemia patients, Hsp70 expression in osteosarcoma predicts a better chemotherapy response [34]. In addition, due to its role as an immune adjuvant, Hsp can be used as an anti-cancer vaccine for targeted therapy in tumor progression and therapeutic response [36].

3.3. TSG101

The human TSG101 protein is 390 amino acid residues and contains four known structural moieties: the N-terminal UEV domain, the proline rich region (PRR), the spiral coil (CC) region, and the C-terminal alpha-helix/stable box (SB) domain. Each part of these mods has a unique structure and function, giving TSG101 multiple functions in cell function.

At the subcellular level, most TSG101 exists in the cytoplasm [37]. Some proteins can be detected in the nucleus in the late interdivision phase. During mitotic cell division, TSG101 colocalizes with the spindle [37-39]. The temporal distribution of TSG101 across multiple cellular compartments suggests that this multi-domain protein promotes a variety of biological processes, including transcriptional regulation [40, 41], cell proliferation and division [37, 42, 43], as well as ubiquitination and intracellular motility of other proteins [44].

TSG101 is overexpressed rather than lost in important subgroups of malignancies, including breast cancer [45], lung cancer [46], thyroid cancer [47], ovarian cancer [48, 49], and colon cancer [50]. Based on publicly available information in the Human Protein Atlas, most human cancers exhibit moderate cytoplasmic and membrane immunoreactivity to TSG101, and its staining strength may serve as a prognostic marker for certain cancer types, such as hepatocellular and renal tumors. TSG101 has been reported to be upregulated in human ovarian epithelial cells expressing carcinogenic HRAS or KRAS and in 70% of human ovarian cancers analyzed with tissue arrays [48, 49, 51]. Although TSG101 was not significantly expressed in normal human ovarian epithelium, elevated levels of TSG101 were found in both low-grade and high-grade tumors. What's more, when

cancer tissue exhibited higher levels of TSG101 compared to patients with low expression of this protein (53 percent), patients' 5-year survival was significantly reduced (33 percent). These findings suggest that TSG101 may be a prognostic marker for poor overall survival of ovarian cancer [49].

3.4. Cancer-related specific markers

3.4.1 PD-L1

Programmed death ligand 1 (PD-L1)/Programmed death 1 (PD-1) signaling pathway is a classical co-inhibitory signaling pathway that plays an important role in tumor cell immune escape and thymus dependent lymphocyte (T cell) failure. The overexpression of PD-L1 protein on the cell membrane of tumor cells can specifically recognize PD-1 from the cell membrane of T cells, and this interaction inhibits the activity of T cells, realizes the immune escape of tumor cells, and promotes the further growth of tumor cells [52-55]. The specific binding of PD-1 to PD-L1 induces the conformational change of the intracellular domain of PD-1, and the conformational change of PD-L1 leads to phosphorylation of ITIM and ITSM [56]. The Src homology 2 domain containing phosphatase 2 (SHP-2) is recruited to the ITSM domain, leading to dephosphorylation of signaling kinases such as ZAP70, resulting in an overall inhibitory effect on T cell expansion [57] due to the inactivation of the PI3K/Akt and Ras/MEK/ERK cascades [58]. This mechanism ultimately inhibits the transcription and translation of genes and cytokines required for T cell activation, thereby negatively regulating their activity, leading to T cell failure and immune escape of tumor cells. Therefore, new immunotherapies based on the PD-L1/PD-1 signaling pathway can fully utilize the patient's own immune system to fight tumor cells and thus treat cancer, which has attracted worldwide attention. The FDA has approved several PD-L1 inhibitors for malignancies such as non-small cell lung cancer NSCLC, Merkel cell carcinoma, and melanoma, such as atezolizumab, durvalumab, and avelumab[59].

3.4.2 EpCAM

EpCAM is a type I membrane protein containing 314 amino acids, consisting of an extracellular domain (EpEX), a transmembrane domain, and a short 26-amino acid intracellular domain (EpICD) [60]. Extracellular domains include an EGF-like domain, a thyroglobulin repeat domain, and a cysteine-deficient domain. The epidermal growth factor-like domain and thyroglobulin domain form a globular structure that is necessary for the homophil cell-cell adhesion of EpCAM [61].

The biological function of EpCAM is to eliminate e-cadherin-mediated cell-cell adhesion by disrupting the connection between A-catenin and F-actin [62]. In addition, the binding of EpCAM to clclin-7 interferes with EPCAM-mediated homotypic cell-cell adhesion and promotes cell motility, proliferation, survival, carcinogenesis and metastasis [63]. Studies have shown that the intracellular domain, as part of the transcription complex, induces the expression of c-myc and cyclin A and E during intramembrane proteolysis by EpCAM [64]. These findings are strong evidence in support of EpCAM as an oncogene. In contrast to its role in promoting tumor formation, EpCAM has also been described as a tumor suppressor protein. EpCAM was originally proposed as a cell adhesion molecule because EpCAM can mediate homophile adhesion interactions [65], thereby preventing cell scattering. Due to these adhesion properties, EpCAM may play a role in inhibiting invasion [65, 66].

3.5. RNA (microRNA, miRNAs)

miRNAs are endogenous non-coding RNAs involved in post-transcriptional regulation. Circulating miRNAs are highly stable and abundant, and are expected to become novel biomarkers in tumor screening. Huang [67] found that six miRNAs (miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p, miR296-5p) were overexpressed in the serum of patients with gastric cancer. The AUC values of the training stage set and the verification stage set were 0.764 and 0.702, respectively. In addition, Yuwen [68] isolated and used circulating exosome miRNAs for HiSeq deep sequencing analysis of serum samples from platinum-resistant or platinum-sensitive patients, and further verified the predictive efficacy of six kinds of exosome miRNAs in serum samples from 170

patients with advanced NSCLC by qRT-PCR. The regulatory role of clinically relevant miRNAs in reactivity was elucidated through acquisition and loss of function tests, and immunohistochemical analysis was performed on 203 NSCLC patients receiving platinum-based chemotherapy to evaluate the association between basal autophagy and reactivity in lung cancer tissues. The results showed that, compared with platinum-based NSCLC patients, The expression of miR-425-3p and Mir-4475-5p was higher in platinum-resistant patients, but the expression of miR-146a-5p was lower, confirming that the circulating exosome miR-425-3p is a potential biomarker for predicting clinical response to platinum-based chemotherapy in NSCLC patients. Han [69] used a ligation-activated loop-mediated isothermal amplification (LAMP) strategy. When the mutant target (miR-196a2T) was present, two substrates (HP1 and HP2) were specifically linked to form a dumbbell probe (DSP) to initiate subsequent LAMP. After DSP formation, preprimers and postprimers trigger automatic, continuously repeated polymeric extension and strand replacement DNA synthesis, initiating isothermal exponential amplification reactions to produce large amounts of double-stranded DNA (dsDNA). Using SYBR Green I as a fluorescent indicator, the generated dsDNA can be simply monitored without labels, and the real-time detection of miR-SNPs with zero background and no labels can be realized.

4. Biosensor detection of exosomes

There are many types of cancer markers and different detection methods. Currently, there are seven main detection methods: radioimmunoassay, chemiluminescent immunoassay, enzyme-linked immunosorbent assay, immunosensor, proteomics, molecular biological methods and liquid biopsy. As a new cancer detection method, biosensor detection has the advantages of simple operation, fast analysis speed and high detection throughput, and has become the most promising analysis technology in the field of life analysis.

Biosensor is an advanced analysis and detection device composed of immobilized bioactive substances (enzymes, proteins, microorganisms, DNA and biofilms, etc.) as sensitive elements organically combined with appropriate physical or chemical transducers. The working principle is: the substance to be tested enters the molecular recognition element (bioactive material) through the diffusion effect, the molecular recognition effect is specifically combined with the molecular recognition element, biochemical reaction occurs, and the biological information generated is converted into light signal or electrical signal that can be quantitatively processed through the corresponding signal conversion element, and then amplified, processed and output by the electronic measuring instrument. To achieve the purpose of analysis and detection [70-72]. Depending on the different types of sensors used, biosensors can be classified into electrochemical biosensors, piezoelectric biosensors, calorimetric biosensors, and optical biosensors (fluorescence, surface plasmon resonance, and surface-enhanced Raman scattering) [73]. The summary and comparison of different exosome detection biosensors are shown in Table 1.

4.1. Optical biosensors

4.1.1 Fluorescent biosensor

In recent years, fluorescence based sensing technology has been widely concerned because of its advantages such as high sensitivity, short response time, low cost and convenient detection. Researchers have designed and developed a variety of fluorescence sensing materials, of which common fluorescence elements include organic fluorescent dyes, semiconductor quantum dots, and rare earth fluorescent materials, as well as other fluorescent materials such as fluorescent proteins, organic polymer dots, and AIE nanodots. Fluorescent biosensors can be used to detect proteins [74] and nucleic acids [75, 76]. Li [77] designed a sandwich-based evanescent wave fluorescent biosensor (S-EWFB) in 2022. First, the fluorescent probe was incubated with exosomes. The Cy5.5 fluorescent probe of end-modified cholesterol was inserted into the phospholipid bilayer membrane of exosomes through hydrophobic interaction to achieve one-to-many connection between exosomes and signaling

molecules, thus amplifying the signal. Then, the fiber surface modified CD63 nucleic acid aptamers specifically capture exosomes carrying signaling molecules, in which when the fluorescence molecules carried by the exosomes are near the fiber surface, they are excited by evanescent waves to produce fluorescence, achieving online monitoring. In addition, the fluorescence signal peak was positively correlated with exosome concentration. Using S-EWFB can realize simple, specific, sensitive and reproducible rapid real-time exosome detection, which has good clinical application value.

4.1.2 Surface plasmon resonance

Biosensors based on surface plasmon resonance (SPR) rely on excitation of a cloud of electrons, called a plasma, which typically occurs at the interface of two materials (such as metal/air or metal/water) with different dielectric constant values. In SPR spectroscopy, gold layers are usually used. When the near-infrared incident light projected by the laser strikes the metal surface, the components of the incident light parallel to the surface are perfectly aligned with the surface plasma wave, and the oscillation amplification of the surface plasma produces electron resonance. When the metal surface size or dielectric constant changes, the resonance frequency will also change, resulting in different surface plasmon resonance effects [78]. Therefore, we can track protein accumulation in real time by rapidly changing the incidence Angle of projected light and monitoring the change in SPR Angle [79].

Compared to conventional SPR biosensors, nano-biosensors are limited by the fact that nanomaterials are expensive and difficult to obtain. Liu [80] developed a compact SPR biosensor (25 cm×10 cm×25 cm) with adjustable strength, which uses conventional SPR sensing mechanism and does not require nanostructure fabrication. With a sensitivity of $9.258 \times 10^3\%$ /RIU and a resolution of 8.311×10^{-6} RIU, exosome epidermal growth factor receptor (EGFR) and programmed death Helper 1 (PD-L1) can be used as biomarkers for in vitro diagnostic testing of cancer.

4.1.3 Surface-enhanced Raman spectroscopy

surface-enhanced Raman spectroscopy (SERS) is a powerful vibrational spectroscopy technique that can rapidly detect low-concentration analytes by amplifying the electromagnetic field generated by local surface plasma excitation. The detection accuracy of Raman spectroscopy mainly depends on the wavelength of the excitation light source used. If the sample under test contains objects smaller in size than the wavelength of the laser source used, the detection will be problematic. If the length of the excited light wave is at the wavelength of the visible band, various proteins and tiny particles can be identified. In addition, if the excitation wave used is larger than the wavelength of visible light, it is easier to detect. It has the characteristics of good selectivity, small sample size, high sensitivity, fast collection speed, non-destructive analysis and characteristic spectrum, and can be used to detect a variety of biological samples [81]. Examples include glucose [82], lipids [83], oligonucleotides [84], IgG [85], neurotransmitter dopamine [86], Shiga toxin [87], pyocyanin [88], and Escherichia coli O157: H7 [89]. Kim [90] used a portable Raman spectrometer with an identification algorithm based on multivariate statistics to detect or predict asymptomatic breast cancer from human tears. To develop a non-invasive real-time screening technique for detecting asymptomatic tumors and preventing tumor recurrence by fabricating polystyrene (Au/HCP-PS) nanospheres monolayer plasma SERS substrates packaged by hexagonal mucosa.

4.2. Electrochemical sensor

The electrochemical sensor combines biological or chemical materials with electronic detection technology, and uses the electrode as a conversion element and fixed carrier. The biological sensitive substances, such as antigens, antibodies, enzymes, hormones, etc., or the organism itself as sensitive elements are fixed on the electrode. Such as capacitance, current, potential and conductivity, etc., so as to achieve qualitative or quantitative detection of target analytes [91]. The detection targets of electrochemical sensors include cancer markers, exosomes and cells.

Electrochemical biosensors can simulate the electron transport and metabolism process in vivo, and the electrode acts as the electron donor and acceptor, so as to obtain the thermodynamic and kinetic parameters. The combination of the high specificity between biomolecules and the strong sensitivity of electrochemical analysis can provide strong support for trace detection of biological samples. Electrochemical sensors are usually composed of counter electrodes (CE) (such as graphite, platinum wire), reference electrodes (RE) (such as Ag/AgCL, calomel), and working electrodes (WE). Common working electrodes include carbon material electrodes (carbon fiber, graphene and glassy carbon, etc.), gold nanomaterials electrodes (such as gold nanoparticles, gold wires and gold films) and screen-printed electrodes. Liu [92] constructed a paper-based electrochemical biosensor for breast cancer exosome detection, which combined Metal-organic framework (MOF) functionalized paper and screen-printed electrodes. The recognition of Zr-MOF and aptamer exosomes is relied upon to initiate a hybrid chain reaction and form DNase for signal amplification. This method has a good linear range for the identification of exosomes, and the detection limit is as low as 5×10^3 particles /mL. In addition, the paper-based biosensor also has the advantages of low cost, simple operation, and high sensitivity, enabling convenient and reliable point-of-care diagnosis under resource-limited conditions.

In addition to being simple and fast, electrochemical biosensors are also important for their ability to be reproducible. Liu [93] constructed a split-aptamer mediated regenerable temperature-sensitive electrochemical biosensor (split-aptamer mediated regenerable temperature-sensitive SMRT) for detecting tumor exosomes, and the principle is shown in Figure 1. In the presence of exosomes, split aptamers (split a and split b) consisting of two nucleic acid fragments move close to each other to capture targets and form a recognition configuration. When the REDOX part of methylene blue on split b is close to the electrode, the specific and sensitive detection of exosomes can be achieved, so as to generate REDOX signals and realize the quantitative detection of exosomes. Because split aptamers lose recognition at higher temperatures, the recognition configuration can be destroyed by flushing the electrode in PBS buffer at 37°C. Therefore, the electrode can be regenerated in as little as 30 seconds and used directly for subsequent testing. The limit of detection (LOD) of the method was 1.5×10^6 particles /mL, and the electrochemical biosensor maintained its original discrimination efficiency after five cycles with a relative standard deviation between 3.0% and 10.6%. Therefore, SMRT biosensor, as a simple, effective and reproducible biosensor for specific detection of tumor exosomes, can provide an innovative strategy for the design of cost-effective regenerable electrochemical biosensors.

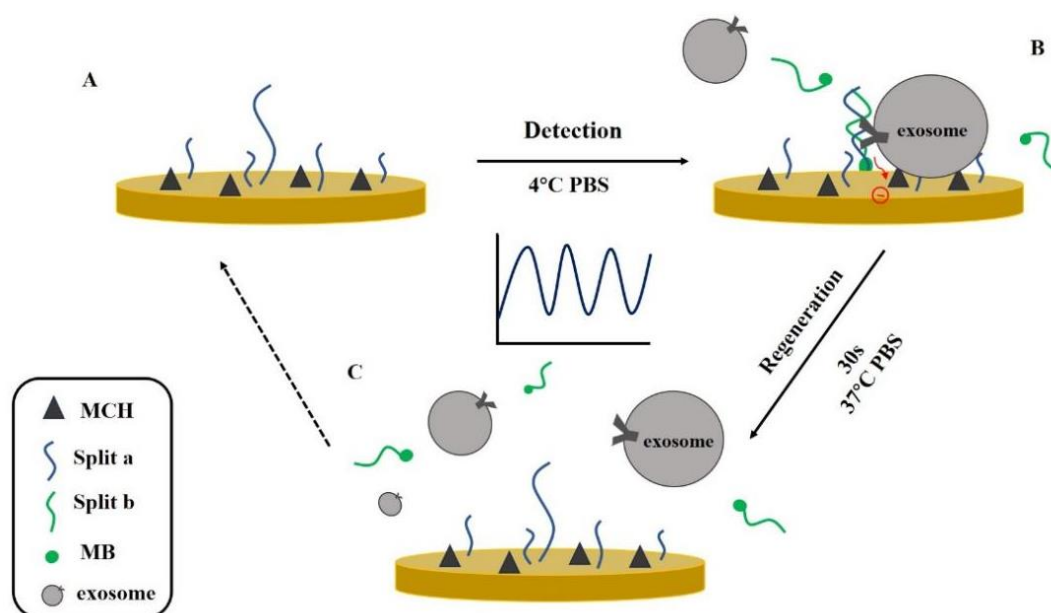


Fig. 1 Schematic illustration of split-aptamer mediated regenerable temperature-sensitive (SMRT) electrochemical biosensor for the detection of tumor exosomes

The rise of supramolecular materials also brings new inspiration for the detection of exosomes by electrochemical biosensors. Lin [94] synthesized a spherical covalent organic framework (COF) with regular shape and good dispersion, and used this material to prepare an electrochemical biosensor for exosome detection. For better biosensor performance, the COF was designed to load large quantities of a polymer named Histostar. Histostar can couple a variety of secondary antibodies and HRP on a single chain, and this structure can increase the availability of HRP at antigenic sites and signal amplification ability. In addition, due to the high porosity of COFs, COFs can load a large number of Histostar. At the same time, the exoskeleton of COFs can maintain the function of Histostar and further improve the sensitivity. This method has a wide linear range, low detection limit and superior performance. This opens up a new way for the application of COFs in biosensing, provides a simple and effective method for exosome detection, and promotes the development of biosensors based on metal-free porous crystal materials.

5. Summary

So far, cancer marker detection technology is almost the only way to detect asymptomatic microfocal tumors early. The development of non-invasive detection methods for cancer markers is also a research hotspot in the early diagnosis of cancer. At present, the main detection methods for cancer markers include radioimmunoassay, chemiluminescence immunoassay, enzyme-linked immunosorbent assay, immunosensor, proteomics, molecular biological methods and liquid biopsy technology, etc. All of these are non-invasive detection methods for cancer markers, with advantages of strong specificity, high sensitivity and rapid detection. However, it also has the disadvantages of expensive instrument, short luminescence process and high operation difficulty. In addition, we can use liquid biopsy technology to sample non-solid samples such as blood and obtain tumor cell information based on circulating novel biomarkers (including circulating tumor cells, DNA, RNA and exosomes) to achieve non-invasive detection and diagnosis of cancer. Compared with tissue biopsy, liquid biopsy can conduct early screening and detection of tumor markers, overcome the spatial and temporal heterogeneity of tumors, and has the advantages of non-invasive, easy to repeat sampling, simple operation and real-time monitoring, but it also has disadvantages such as expensive and inconsistent detection standards. The immunosensor that combines specific immune response with biosensing technology is proposed today. Its biometric recognition part comes from the specific recognition and combination of antigen and antibody, and the biological signal is converted into electrical signal for detection by physicochemical transducers and signal amplifiers. Nano materials with excellent properties are introduced to further improve the sensing performance through functional modification; At the same time, biological materials are also added to enhance its anti-interference ability, realize the detection of cancer markers with high selectivity, high sensitivity, convenient operation and low cost, and realize the real-time dynamic monitoring of cancer, which is of great significance for the prognostic treatment of patients.

Table 1. Summary and comparison of different exosome detection biosensors

| tool | Scheme | Target element | Recognition element | sample | Reference | Advantages | Disadvantages |
|-------------------------------------|--|---------------------------------|----------------------------|--------------------------|-----------|--|---|
| Fluorescent biosensors | Cadmium-Free Quantum Dots as Fluorescent Labels for Exosomes | CD63 | antibody | - | [95] | High sensitivity; High flexibility; With multiplexing capability | Expensive; Fluorescent labels may interfere with the recognition process |
| | A sandwich-based evanescent wave fluorescent biosensor for simple, real-time exosome detection | CD63 aptamer | aptamer | - | [77] | | |
| Optical biosensors | Sensitive detection of exosomal proteins via a compact surface plasmon resonance biosensor for cancer diagnosis | EGFR、PD-L1 | antibody | NSCLC | [80] | High sensitivity; Real-time detection; No label system | huge equipment; interference |
| | Label-free detection of exosomes using a surface plasmon resonance biosensor | HER2 | Anti-her2 antibody | Breast cancer | [96] | | |
| surface enhanced Raman spectroscopy | Innovative model of surface-enhanced raman spectroscopy for exosomes identification: An approach for the diagnosis of hepatocellular carcinoma | HCC exosome | antibody | Hepatocellular carcinoma | [97] | High sensitivity; Strong reuse ability; Suitable for complex biological samples | High cost, expensive equipment; The fabrication of SERS nanoprobe is difficult. Complex data analysis |
| | An ultrasensitive polydopamine bi-functionalized sers immunoassay for exosome-based diagnosis and classification of pancreatic cancer | CD9,CD63,MIF, GPC1, EGFR,E pCAM | antibody | Pancreatic cancer | [98] | | |
| Non-optical sensor | Metal-organic framework-functionalized paper-based electrochemical biosensor for ultrasensitive exosome assay | CD63 | aptamer | Breast cancer | [92] | High sensitivity; Quick response; Economical and efficient; Strong system multiplexing capability; Easy miniaturization of devices | Surface functionalization is challenging; Sample matrix effect; Difficulty of reproduction |
| | Split-aptamer mediated regenerable temperature-sensitive electrochemical biosensor for the detection of tumour exosomes | SMMC-7721 exosome | Split-aptamer | Liver cancer | [93] | | |
| | Histostar-functionalized covalent organic framework for electrochemical detection of exosomes | CD63 | Covalent organic framework | - | [94] | | |

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