The role of tumor cell-derived extracellular vesicles in cancer diagnosis and immunotherapies

Xi Li

Abstract

Cancer is a genetic and metabolic disease caused by mutations of genes, dysregulated metabolism, or environmental factors. Since the cancer rate is among the highest in the 20th century and the incidence keeps elevating in the 21st century, it has been expected that 25% of the population will suffer from cancer during their lifetime. However, as the most promising strategy to treat cancer, only 15-20% of patients achieve durable results with immunotherapy. Thus, we still need to find a new therapeutic and detection strategy to make up for the shortcomings of immunotherapy. Tumor cell-derived extracellular vesicles (t-EVs) may be a great helper. This review summarizes the potentials of tumor cell-derived extracellular vesicles used as the cancer biomarkers in cancer early diagnosis and prognostic monitoring and introduces the combined therapy between t-EVs and existing cancer immunotherapies.

Keywords: Tumor cell-derived extracellular vesicles, Biomarker, Immunotherapy

Introduction:

Cancer, as the most critical public health problem, has been investigated. There were 1,958,310 new cases and 609,820 death cases in 2022 by the American Cancer Society, including 29 percent of men with prostate cancer and 31 percent of women with breast cancer1.

Immunotherapy of cancer is a milestone not only for cancer treatment but also for oncology. Immunotherapy aims to boost the internal immune response to eliminate tumor cells, including oncolytic virus therapy, cancer therapeutic vaccine, immune checkpoint blockade, adoptive cell transfer, and so on. Unfortunately, there are still some limitations to the clinical usage of novel immunotherapies, such as low response rates, low ability to predict clinical effects, and toxic side effects2including adoptive cell transfer (ACT. Therefore, it is essential to figure out the new therapeutic and detection strategies to eliminate these drawbacks of existing immunotherapies.

T-EVs are Nano-sized bilayer vesicles secreted by cancer cells, which can contain proteins, lipids, nucleic acids, and other bioactive molecules. T-EVs can be divided into exosomes (30-200nm) and micro-vesicles (100-1000nm) mainly by size and membrane molecules3. Novel methods to isolate EVs are as followsespecially small extracellular vesicles (sEVs (Figure 1).

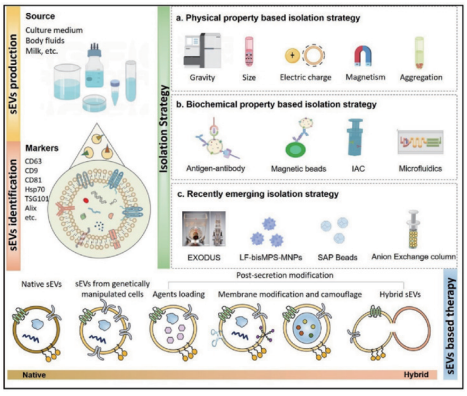

Figure 1. Novel methods of isolating EVs4especially small extracellular vesicles (sEVs

Different sizes depend on various processes of synthesis5. Exosomes originate from the endocytosis pathway (Figure 2a). After the materials are transported into the cell via endocytosis, they form late endosomes and become multi-vesicular bodies (MVB). During MVB formation, extra biomolecules are incorporated, including RNAs and proteins. The release of exosomes via the fusion between MVB and plasma membrane depends on the contact sites of the endoplasmic reticulum and late endosome membrane. On the other hand, micro-vesicles are derived from cell budding on the cell membrane directly (Figure 2b).

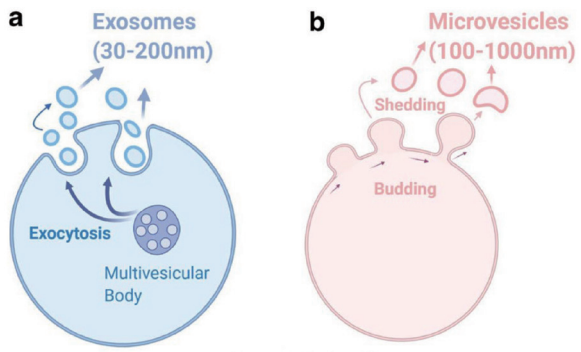

Figure 2. The synthesis of exosomes and micro-vesicles5

More and more evidence has proved that t-EVs play a significant role in immunosuppression and tumor microenvironment regulation. They can inhibit anti-tumor response because of the crosstalk between cancer cells and immune cells, what we call “changing hot tumor sites into cold ones”6. With accessibility, storage, passing through the blood-brain and blood tumor barriers, and loading various effector molecules, nowadays, t-EVs provide clinical researchers a newfangled way to overcome the safety deficiency and efficiency of traditional immunotherapies7.

In this review, we summarize the potentials of t-EVs used as the cancer bio-markers in cancer early diagnosis and prognostic monitoring according to their contents and the existing t-EVs based immunotherapies. Moreover, we also analyze the main limitations of t-EVs’ clinical extension and provide some solutions.

2. T-EVs used as biomarkers:

T-EVs carrying proteins, lipids, and nucleic acids can be obtained from the cancer patient's blood, urine, and saliva stably by ultracentrifugation, western blotting, and flow cytometry7. With these characteristics, T-EVs have become a promising cancer diagnosis and monitoring strategy.

2.1 Proteins from t-EVs:

Based on needless purification of membrane proteins on t-EVs, researchers have tried to detect the membrane proteins of exosomes and micro-vesicles. The surface of exosomes is enriched with CD63, CD9, and CD81. Accordingly, the surface of micro-vesicles is enriched with CD9 and CD818. These results have been viewed as a standard of EVs’ classification. However, some innovative ideas are also about characterizing the protein phenotype to establish a cancer indicators list.

Lapitz A et al. raised that CRP/FIBRINOGEN/FRIL-carbohydrate antigen 19-9, CRP/PIGR/VWF, and CRP/FRIL from patients' serum extracellular vesicles could be used to diagnose primary sclerosing cholangitis cholangiocarcinoma (PSC-CCA), non-PSC CCAs and CCAs regardless of etiology (Pan-CCA) respectively 9 (Figure 3). These proteins of t-EVs may help work out the unclear prognosis and poor accuracy of CCA.

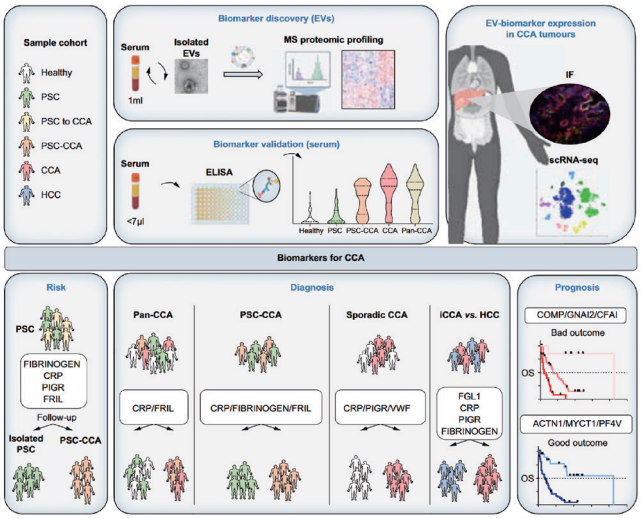

Figure 3. Liquid biopsy-based protein biomarkers of CCA9

2.2 Lipids from t-EVs:

The lipids in t-EV with a lipid bilayer structure have been ignored as biomarkers of cancer for the long term. However, there was increasing evidence indicating that lipid metabolism could also affect the biosynthesis of EVs and their interactions with recipients.

Yang P et al. have found that the EV contains long-chain fatty acids (LCFAs) mediated by CD36 can lead to crosstalk between tumor cells and tumor-associated macrophages (TAMs), which may decrease the anti-tumor effects of CD8+ T cell and help tumor proliferation in liver-metastasis C56/BL6 mouse model. The LCFAs-enriched t-EVs are isolated by differential centrifugation. Moreover, they also observed that in patients bearing liver metastases, high expression of CD36 correlates with M2-type MAMs infiltration, creating a highly immunosuppressive TME10. This result also means that the LCFAs t-EV may present in human liver metastasis patients. Reasonably, Lipids with t-EV have a promising future in diagnosing human beings' cancer metastasis.

2.3 Nucleic acids from tumor cell-derived EV:

The DNA of t-EVs, with nearly the same sequence as the tumor cell, could reflect the genetic status of the tumor, for example, a significant sequence amplification of the oncogenes. Therefore, the t-EV DNA in the circulation detected in patients’ blood, cerebrospinal fluid, or urine could be used as the biomarker among cancer patients. However, DNA packaging in EV may

be cell-type dependent, and the abundance of DNA in EV could be very low to detect11. According to Maire CL et al., genome-wide DNA methylation and copy number variations (CNV) were detected accurately in glioblastoma cell derived-EV by EV isolation techniques, such as transmission electron microscopy and super-resolution imaging12. It may lead to a better way to non-invasive tumor subtype classification, patients' fractionation, and monitor therapy among patients with glioblastoma.

RNA in T-EV that can regulate tumor microenvironment by reprogramming M2 TAMs mostly are micro-RNAs (miRNA) and long non-coding RNAs (Inc RNAs). These two kinds of non-coding RNA may lead to the material and information communication between tumor cells and immune cells, which can cause tumor proliferation, invasion, and metastasis13. Hu, Zongqiang, et al. have found that hepatocellular carcinoma (HCC) cell-derived circCCAR1, as a sponge of miR-127-5p, stimulates a feedback loop comprising circCCAR1/miR-127-5p/WTAP axis14. Increased circCCAR1 can lead to CD8+T cell dysfunction and anti-PD1 immunotherapy resistance in a mouse model combined with human immune system components (huNSG mice). Therefore, circCCAR1 may be used as a new biomarker to monitor the progression of HCC.

3.Engineered tumor cell-derived EV based immunotherapies:

With the ability to support cell-free immunotherapy, t-EVs based immunotherapies have got more attention to enhance anti-tumor immunity in patients bearing cancer, which could be divided into three main sections, containing t-EVs based anti-tumor vaccine, t-EVs based immune checkpoint strategy, and t-EVs immune reprogramming strategy15.

3.1T-EV based anti-tumor vaccines:

The main idea of the anti-tumor vaccine is stimulating the T-cell mediated anti-tumor vaccine in cancer patients. When the anti-tumor vaccines are injected into patients' bodies, they can filter into lymph nodes and then be absorbed and recognized by antigen-presenting cells (APCs). However, with the low antigen encapsulation efficiency, poor lymph node targeting, and weak lysosomal escape ability, the clinical application of anti-tumor vaccine is still limited16. Since t-EV contains more tumor-associated antigens and has higher tissue targeting ability than traditional anti-tumor vaccines. The possibility of engineering t-EVs in clinical treatment is considered.

To intervene in the process of DC endocytosis and enzymolysis of t-EVs, Morishita, Masaki, et al. combined melanoma cell derived-exosome with pH-sensitive fusogenic GALA peptide, called GALA-exo to achieve a cytosolic delivery of tumor antigens efficiently in mouse model. As a result, after injecting GALA-exo, DCs showed an increasing antigen presentation ability17(Figure 4).

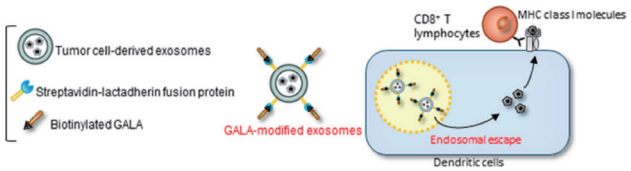

Figure 4. The process of GALA-exo synthesis17

3.2 T-EV based immune checkpoint blockade strategy:

Immune checkpoint blockade uses antibodies to inhibit immunosuppressive molecules, including CTLA-4 and PD-L1, to prevent termination of the body's immune response in the tumor environment and bring back CTLs from the depleted state. Although it has achieved high clinical satisfaction, the low response and disaster side effects still cannot be ignored. Using t-EV to increase tumor tissue infiltration and enrichment can solve these problems easily. Tian, Tian, et al. tried to use an engineered exosome with a brain-tumor-targeting cyclic RGDyK peptide (RGD-EV) and small interfering RNA (siRNA) against programmed cell death ligand-1 (PD-L1) to Break through the blood-brain barrier is difficult for traditional immune checkpoint suppression therapies in glioblastoma murine model18 (Figure 5).

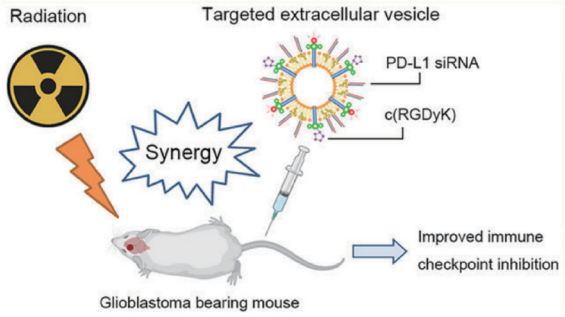

Figure 5. Immune checkpoint Inhibition Primed with Radiation by EVs18

3.3 T-EV based immune reprogramming strategy

The immune reprogramming strategy is an immunotherapy that regulates the pathway to reshape the tumor immune microenvironment. Because of the inheritance of t-EV from the tumor tissue, it contains a lot of bioactive substances to mediate the establishment of the tumor

microenvironment.

B16F10 murine melanoma-derived exosome (C-PMet), which is combined with CD39 antagonist POM1 and AMP-activated protein kinase (AMPK), has been synthesized by Wu, Long et al. (Figure 6). This engineered exosome can increase the level of extracellular ATP prevent the accumulation of immunosuppressive adenosine, and alleviate hypoxia. The former can trigger the activation of P2X7-NLRP3-inflammasome to drive macrophage pyro-ptosis, potentiate the maturation and antigen capacity of dendritic cells (DCs) to enhance the cytotoxic function of T cells and natural killer (NK) cells. Eventually, it can suppress tumor progress metastases and prevent anti-PD-1 resistance19.

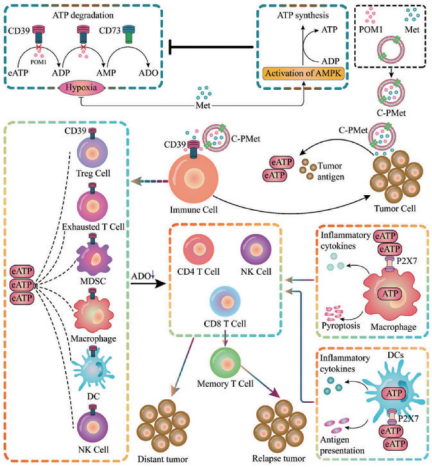

Figure 6. Anti-tumor immune responses induced by C-PMet-based immunometabolic therapy

4.Conclusion:

T-EVs play an essential role in tumor cell-cell communication and are involved in a variety of pathological processes. As an emerging cancer biomarker, t-EVs have a broad potential could be used in cancer targeting therapy. Simultaneously, the strategy of engineered EVs offers an opportunity to make up for the shortcomings of traditional tumor immunotherapy. In this paper, the research progress of t-EVs biomarkers is reviewed. Representative cases analyze the train of thought about engineered t-EVs in cancer immunotherapies. However, the research of t-EVs remains to be explored, and some limitations must be addressed.

Firstly, the biochemistry of EVs still needs to be entirely determined due to their complexity, like their subtypes, and their interaction with target cells16. For instance, many studies have found the mediator role of t-EVs. However, the specific mechanism of synthesis and release is still thoroughly unknown, which could help to develop new ideas of therapeutic strategies to treat cancer.

Secondly, the heterogeneity of t-EVs. Since we need to use t-EVs as clinical products, the standardization methods of EVs and analysis techniques are required. The isolation and modification of t-EVs with the characteristic of heterogeneity is still challenging. Both of them are the cornerstones of clinical application and industrial production. Some researchers have found strategies that obtain EVs by cell engineering. Moreover, the EVs can also be isolated by negative immune-magnetic separation20. The development of these new technologies will overcome the limitations of natural vesicles and generate more possibilities.

Moreover, the needs of different cancer patients. Since both different environments and natural traps will affect the efficacy and the components of EVs. With further studies of t-EVs' function and mechanism, a considerable EVs database could be established16. Artificial intelligence could also help clinical doctors recognize the best treatment and strategy for each patient from many basic studies and predict biological effects.

To sum up, the emergence of t-EVs in cancer detection and immunotherapies will appeal to more researchers to devote themselves before they can be used as a stable clinical standard and treatments.

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