



Review

Circulating extracellular vesicles are effective biomarkers for predicting response to cancer therapy

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ABSTRACT

Cancer remains one of the most challenging diseases, as many patients show limited therapeutic response to treatment. Liquid biopsy is a minimally invasive method that has the advantage of providing real-time disease information with the least damage to cancer patients. Extracellular vesicles (EVs) released by the parental cells and protected by lipid bilayer membrane structure represent an emerging liquid biopsy modality. Apart from promoting cell growth, proliferation, and migration, EVs and their cargos (mainly miRNAs and proteins) are also biomarkers for cancer diagnosis and prognosis. Furthermore, their alterations pre- and post-therapy can guide therapeutic strategy determinations for better-stratified therapy. In this review, we summarize the potential clinical significance of EVs and their cargos in therapeutic response monitoring and prediction in several cancers (mainly lung cancer, prostate cancer, breast cancer, melanoma, lymphoma, glioblastoma, and head and neck squamous cell carcinoma) and discuss the questions that require future investigation.

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1. Introduction

Cancer has been widely debated over the past few decades, and it continues to be a major cause of disease-related death worldwide [1].

Abbreviations: EVs, extracellular vesicles; ctDNA, circulating tumour DNA; CTCs, circulating tumour cells; miRNAs, microRNAs; MVs, microvesicles; MVBs, multivesicular bodies; MISEV2018, minimal information for studies of extracellular vesicles 2018; mRNA, messenger RNA; ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; AUC, area under the curve; ALK, anaplastic lymphoma kinase; TKI, tyrosine kinase inhibitor; lncRNAs, long noncoding RNAs; PD, progressive disease; PR, partial response; CIRT, carbon ion radiotherapy; AR-V7, androgen receptor splice variant 7; PFS, progression-free survival; OS, overall survival; PSA, prostate specific antigen; ADT, androgen deprivation therapy; PSMA, prostate-specific membrane antigen; 5T4, a oncofoetal glycoprotein; MDR, multi-drug resistance proteins; PABP4, poly(A)-binding protein4; MCSP, melanoma chondroitin sulphate proteoglycan; MCAM, melanoma cell adhesion molecule; LNGFR, low-affinity nerve growth factor receptor; ErbB3, receptor tyrosine-protein kinase; NAC, neoadjuvant chemotherapy; TRPC5, transient receptor potential channel 5; DLBCL, diffuse large B-cell lymphoma; GBM, glioblastoma; MGMT, O6-methylguanine DNA methyltransferase; APNG, alkylpurine-DNA-N-glycosylase; EGFR, epidermal growth factor receptor; EGFRvIII, a mutant subtype of epidermal growth factor receptor; HOTAIR, HOX transcript antisense RNA; sEVs, small extracellular vesicles; sEMPs, small-sized endothelial microparticles; CEA, carcinoma embryonic antigen; CA19-9, carbohydrate antigen 19-9; HNSCC, head and neck squamous cell carcinoma; CRT, chemoradiation therapy

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It is estimated that there will be 1806,590 new cancer cases and 606,520 cancer deaths in 2020 in the United States [1]. With the breakthrough of early screening and new therapeutic interventions, the overall mortality rate of cancer patients has been persistently declining since 1991, resulting in a decrease of 29% in mortality rate [1]. However, the presence of resistance to therapy, disease relapse, and individual differences continues to limit the survival of cancer patients and makes the disease impossible to cure [2,3]. It is suggested that therapeutic response assessment, especially treatment reaction prediction, is valuable to guide treatment strategy determinations and provide responsive therapy for better survival [2,3].

Liquid biopsy is a minimally invasive method that has the advantage of real-time monitoring with the least damage to cancer patients [2,3]. As a widely used technology in the clinic, liquid biopsy can not only help detect patients with a high risk of developing cancer but also help monitor disease progression or recurrence after treatment through representative molecules in the circulation [4], thus playing an early warning role. Circulating tumour DNA (ctDNA) and circulating tumour cells (CTCs) are two of the most researched molecules in this regard. In addition to functioning as potential therapeutic targets in cancer, cancer-related gene alterations in tumour tissues also achieve early screening and prevention [5]. Their recent discovery and applications in the circulation have conferred a new direction on therapy monitoring [5]. Apart from ctDNA, CTCs from the direct

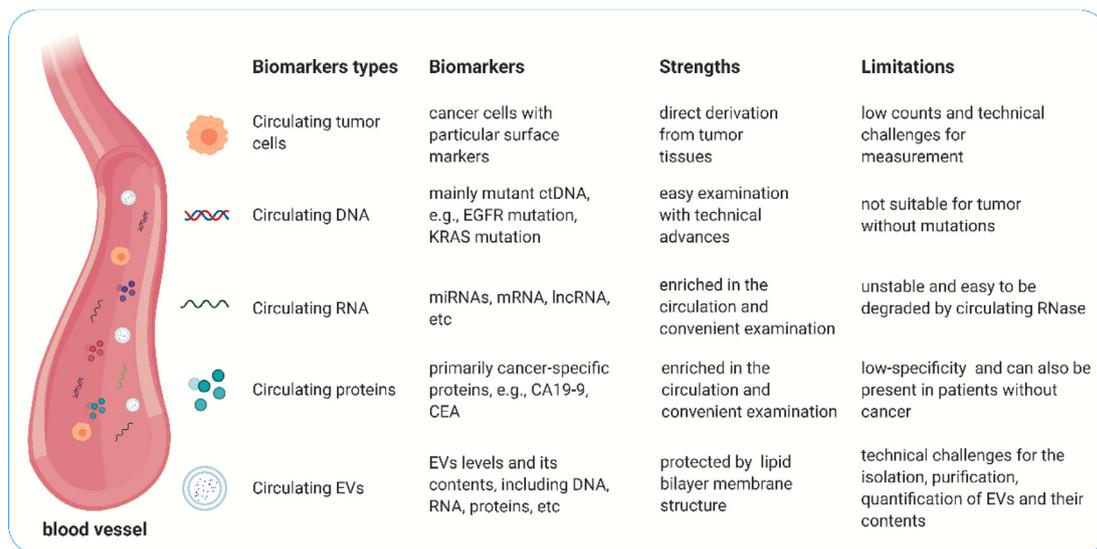


Fig. 1. The strengths and limitations of circulating biomarkers in cancer. Circulating tumour cells (CTCs), circulating DNA (mainly circulating tumour DNA, ctDNA), circulating RNA (miRNA, mRNA, etc.), circulating proteins (e.g., carcinoma embryonic antigen, CEA; carbohydrate antigen 19–9, CA19–9) and circulating extracellular vesicles (EVs) are important biomarkers for therapy response evaluation.

metastasis of cancer cells in primary tumours have also been detected as representative tools in this area and need to be optimized for enhanced detection efficiency and clinical applications [6]. Moreover, tumour-associated RNAs and proteins have also been researched in this field [3,7]. However, there are many challenges to be solved going forward, as summarized in Fig. 1.

Extracellular vesicles (EVs), as an emerging liquid biopsy form, have also been isolated from biological fluids. EVs are composed of a series of vesicles and nanoparticles with varying cellular origin, size, and content [8]. Studies suggest that EVs mediate multiple biological cancer processes, including cell growth, proliferation and migration, through their cargos transferred between different cells [9,10]. Based on their roles in carcinogenesis, attempts have been made to explore EVs in the clinic. With a favourable lipid bilayer structure, it has been identified that EVs are native carriers for delivering anti-tumour drugs with preferable biocompatibility in targeted therapy [11,12]. In addition, cancer-associated molecules found in EVs are also biomarkers for the diagnosis and prognosis of cancer patients [9,10]. Specifically, their alterations before and after therapy also show great potential for therapeutic response monitoring [13,14], promoting hierarchical management and individual therapy in cancer patients [13]. With the development of sequencing and omics technologies, microRNAs (miRNAs) and proteins in EVs are now widely studied.

On the one hand, cancer treatment alters the levels of circulating EVs and their compositions [15,16], and on the other hand, the dynamic examination of circulating EVs and their contents can provide real-time information on therapeutic responses in cancer patients, including a common challenge of therapy resistance [13,17]. Accordingly, combining the advantage of EVs and research demand on therapy efficacy follow-up, we summarize the potential predictive significance of circulating EVs and their cargos in cancer treatment evaluation and the challenges in this field that need to be solved in the future.

2. An overview of extracellular vesicles

Extracellular vesicles (EVs), also called membrane vesicles, have a subcellular structure with a lipid bilayer similar to a cell membrane [18] and are commonly classified as exosomes and microvesicles (MVs). Exosomes, 50–100 nm in diameter, are packaged in the late endosome and generated by the fusion of multivesicular bodies (MVBs) with the plasma membrane [18,19] (Fig. 2). MVs are usually

defined as vesicles of 100–1000 nm that are budding/blebbing from the plasma membrane [18,19]. In addition, ectosomes, membrane particles, exosome-like vesicles, and apoptotic vesicles are also components of EVs [18,19]. From a historical perspective, there is a lot of research on EVs, but an agreed definition has not been established [19–21]. According to the latest guide released by the International Society for Extracellular Vesicles in 2018 (also called MISEV2018), it is suggested that EV subpopulations should be classified according to their size, density, biochemical composition, and cell origin [20,21]. For example, small EVs are 100–200 nm, and medium/large EVs are >200 nm [21]; this classification may promote the standardization of EV-associated studies. For the purposes of this review, we regard all membrane-associated vesicles as EVs.

EVs are mainly isolated from cell culture supernatants and physiological fluids [21]. It is valuable to research EVs in the circulation, including plasma, serum, urine, milk, and bronchoalveolar lavage [21]. At present, differential centrifugation, size-exclusion chromatography, immunocapture, and commercialized reagent kits are common methods for the isolation and purification of circulating EVs [21–23], as summarized in

Table 1. However, the low purity of circulating EVs, usually accompanied by soluble secreted factors, remains a problem [22,24], and a widely agreed method is lacking. The purity of EVs is mainly assessed using size, morphology, concentration, membrane markers and contaminations [21,24], and high EV purity will promote accurate EV cargo analysis for functional research. Studies have illustrated that the high rotor speed and extended centrifugation time of differential centrifugations may enhance EV purity [25], and an optimized centrifugation protocol is needed. Recently, many emerging technologies have been explored. Microfluidics-based techniques have shown great potential for simultaneous EV isolation and detection through a single chip [26]. Chen et al. described an ultrafast-isolation system for EV detection, called EXODUS, which has the advantage of automated label-free purification with incomparable yield and purity [27]. The purity of EVs sorted using polychromatic flow cytometry combined with a lipophilic cationic dye is also over 90% [28]. Considering the contaminations in EVs, an analysis of EV characteristics before further functional research is necessary. Raman spectroscopy [29] and nanoflow cytometry [30] are two recommended methods.

As discussed above, EVs are important regulators for cell-cell communications, including a possible soma-to-germline delivery [31], and promote tumour progression, especially therapeutic failure

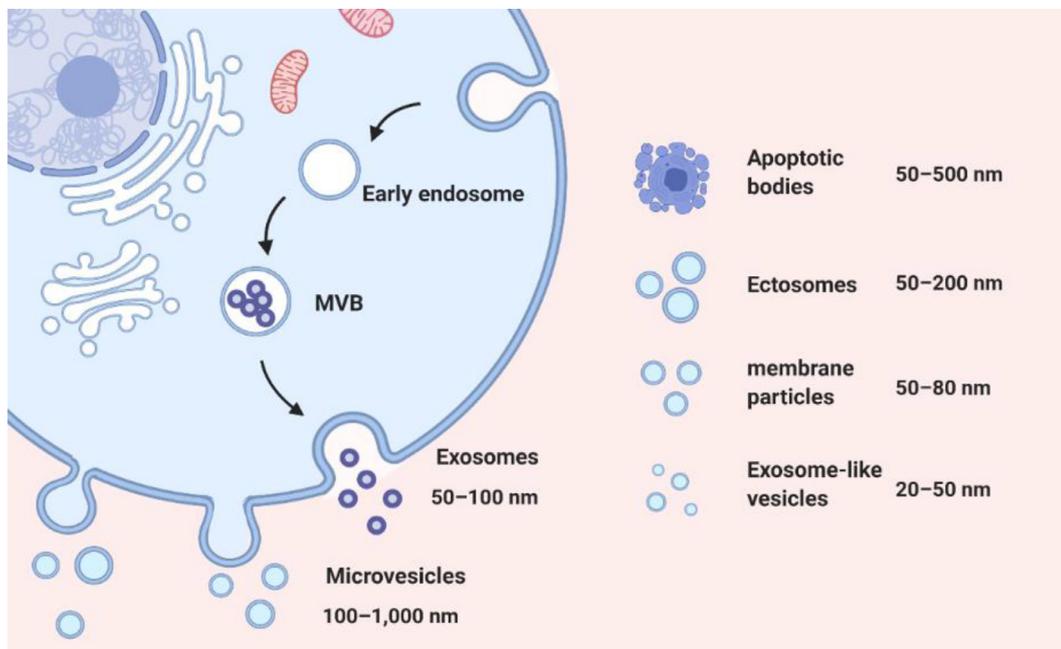


Fig. 2. Biogenesis of exosomes and microvesicles. Exosomes are packaged in the late endosome and generated by the fusion of multivesicular bodies (MVBs) with plasma membrane. Microvesicles (MVs) usually refer to vesicles budding/blebbing from the plasma membrane. In addition, ectosomes, membrane particles, exosome-like vesicles, and apoptotic vesicles are also components of extracellular vesicles (EVs) with different size.

Table 1
Representative isolation and purification methods of EV.

Methods	Principle	Category in MISEV2018	Advantages	Limitations	References
Successive differential centrifugation	Sedimentation velocity	Intermediate recovery, intermediate specificity	Widely application, high purity	Time consuming, low yield	[21,24,25]
Size-exclusion chromatography	Particle size	Intermediate recovery, intermediate specificity	Easy operation, relatively high yield	Contamination of proteins	[21,24]
Immunocapture	Specific EVs surface molecules	Low recovery, high specificity	Easy operation, high specificity, high purity	Low yield, only capture EV with specific molecules	[21,23]
Commercialized reagent kits	Precipitation	High recovery, low specificity	Easy operation, high yield	Expensive, low purity, contamination of reagent molecules	[21,30]
Microfluidic devices	Physical properties, specific EVs molecules	Not defined	Easy operation, high specificity, high purity	Low yield, only capture EVs with specific molecules and physical properties	[21,26]

[13,17]. It has been demonstrated that EVs are involved in resistance mechanisms [32] and may induce immune escape by releasing proapoptotic molecules [33]. Another study by Federici et al. has also illustrated that low pH-induced reduction of antitumor drug uptake and increased release of EVs with an antitumor drug are important mechanisms for the occurrence of drug resistance [34]. Therefore, EVs play an important role in pharmacological reactions. Amongst these cargos, miRNAs and proteins are commonly indicated. MiRNAs are the small noncoding RNA transcripts of gene expression, mainly participating in post-transcriptional modifications of messenger RNAs (mRNAs) [35]. Various studies have suggested that miRNAs in EVs released from cancer cells modulate the biological processes of tumour growth, invasion, metastasis, angiogenesis, and drug resistance and are potential therapeutic targets in cancer [35,36]. In addition, their involvement in drug resistance confers a predictive value in therapy response assessment (such as miR-34a and miR-21) [35], as summarized in Table 2. Low levels of miR-34a in EVs tend to be associated with poor survival in prostate cancer [37]. In contrast, higher hsa-miR-21-5p in serum EVs in high-risk prostate cancer patients was significantly associated with elevated radiation response [38]. In addition, various representative proteins in EVs also play a similar role (Table 3). CD31 is a marker for endothelial-derived EVs regulating T cell response [28]. It has been found that low levels of CD31+ endothelial-derived EVs in responders to immune

checkpoint inhibitor (ICI) correspond with the activation of an immune response, whereas high levels of these EVs might reflect an endothelial-induced tumour immune escape in non-responders with non-small cell lung cancer (NSCLC) [28]. Besides, similar to particular cancer markers in serum, such as prostate specific antigen (PSA) [39] and carcinoma embryonic antigen (CEA) [33], there is also a possibility of the existence in EVs with comparable roles. It has been found that the expression of PSA in EVs decreased after treatment in prostate cancer [39]. Moreover, an increased carbonic anhydrase IX expression and activity of EVs in an acidic microenvironment have also been reported [40]. However, the effects of EVs need to be investigated.

3. Circulating EVs in lung cancer

Lung cancer remains one of the most common cancers in both men and women worldwide [1]. As illustrated above, EVs are important mediators of intercellular communications [32,34]. In lung cancer, circulating EV-miRNAs are the most commonly researched.

3.1. Circulating EV-miRNAs in lung cancer

Shukuya et al. found that there were various differentially expressed plasma miRNAs in responders and non-responders to anti-

Table 2
Circulating EV-miRNAs and mRNAs in cancer therapeutic response.

Cancer Types	Biological Fluids	Therapy Involved	Biomarkers in EVs	Therapeutic Changes	Potential application in cancer patients	Reference
Prostate cancer	Urine	Chemotherapy	miR-34a	Decreased	Indicating therapy failure	[37]
Prostate cancer	Serum	Radiotherapy	hsa-let-7a-5p hsa-miR-21-5p	Increased	Elevated radiation response	[38]
NSCLC	Plasma	Immunotherapy	hsa-miR-320d hsa-miR-320c hsa-miR-320b hsa-miR-125b-5p	Increased	Correlating with unfavourable response	[41]
NSCLC	Serum	Chemotherapy	miR-146a-5p	Decreased	Predicting recurrence	[46]
NSCLC	Serum	Chemotherapy	miR-425-3p	Increased	Predicting a low responsiveness	[47]
Prostate cancer	Serum	Radiotherapy	11 miRNAs	Increased	Predicting a better therapy efficacy	[49]
Prostate cancer	Plasma	Hormonal therapy	AR-V7 mRNA	NA	Predicting therapy resistance	[50]
Breast cancer	plasma	Neoadjuvant therapy	miR-155 miR-301	Decreased	Predicting improved pathological complete response	[57]
Breast cancer	Serum	Chemotherapy	8 miRNAs	NA	Predicting a poor response	[60]
Melanoma	Plasma	Targeted therapy	let-7g-5p miR-497-5p	Increased	Related with a better disease control	[63]
Melanoma	Plasma	Immunotherapy	variable transcripts	NA	Predicting treatment responses and monitoring tumour progression	[64]
Melanoma NSCLC	Plasma	Immunotherapy	PD-L1 mRNA	Decreased	Correlated with responsive response	[65]
DLBCL	Plasma	Chemotherapy	miR-155	Decreased	Predicting a good response	[71]
DLBCL	Serum	Immunotherapy chemotherapy	miR-451a	Increased	Evaluating therapy efficacy	[73]
DLBCL	Serum	Chemotherapy	miR-99a-5p miR-125b-5p	Increased	Predicting chemotherapeutic resistance	[76]
Hodgkin lymphoma	Plasma	Chemotherapy	5 miRNAs	Decreased	Monitoring treatment responses and predicting relapse	[79]
GBM	Serum	Chemotherapy	Δ MGMT mRNA Δ APNG mRNA	Increased	Predicting therapy resistance	[80]

Table 3
Circulating EV-proteins in cancer therapeutic response.

Cancer Types	Biological Fluids	Therapy Involved	Biomarkers in EVs	Therapeutic Changes	Potential application in cancer patients	Reference
NSCLC	Blood	Immunotherapy	10 proteins	Decreased	Activation of immune response	[28]
Prostate cancer	Urine	Hormonal therapy	PSA PSMA 5T4	Decreased	Monitoring treatment-related responses	[39]
Prostate cancer	Serum	Chemotherapy	MDR-1 MDR-3 Endophilin-A2 PABP4	NA	Predicting therapeutic resistance	[53]
Prostate cancer	Serum	Chemotherapy	P-glycoprotein	Increased	Predicting therapy resistance	[54]
Breast cancer	plasma	Chemotherapy	TRPC5	Increased	Predicting chemoresistance	[62]
Melanoma	Plasma	Immunotherapy	PD-L1	Increased	Predicting beneficial treatment responses	[67]
Melanoma	Plasma	Immunotherapy	Δ ExoPD-L1	Decreased	Predicting responsive therapy	[68]
Melanoma	Serum	Immunotherapy	PD-1 CD28	Increased	Predicting beneficial treatment responses	[69]
Melanoma	Serum	Targeted therapy	MCSF MCAM ErbB3 LNGFR	NA	Monitoring treatment responses	[70]
GBM	Plasma	Chemotherapy	CD63 EGFR EGFRvIII	Decreased	Monitoring and predicting therapy effectiveness	[83]
GBM	Plasma	Surgical resection	11 proteins	Increased	Monitoring treatment responses and predicting relapse	[84]
HNSCC	Plasma	Chemoradiation therapy	119 proteins in non-responders; 39 proteins in complete responders	NA	Predicting and assessing treatment responses	[86]

PD-1 or PD-L1 therapy in NSCLC [14]. It was indicated that miR-200c-3p, miR-21-5p, and miR-28-5p, which were observed to be higher in non-responders than in healthy controls, were all decreased in responders before therapy [14], implying an obvious association of decreased miRNAs with better immunotherapy responses. Furthermore, the combination of miR-199a-3p, miR-21-5p, and miR-28-5p achieved an area under the curve (AUC) of 0.925, while the AUC of PD-L1 expression by tissue biopsy was only 0.575 [14]. However, the differentially expressed miRNAs in plasma EVs have not been validated for limited studies [14]. Therefore, more studies need to be

conducted on the association of these EV-miRNAs with lung cancer and immunotherapy. Another similar study by Peng et al. indicated that the levels of hsa-miR-320d, hsa-miR-320c, and hsa-miR-320b in plasma EVs at baseline were significantly upregulated in the progressive disease (PD) group than in the partial response (PR) group, suggesting an unfavourable response of the above miRNAs to immunotherapy [41], evident in the negative prognosis of these miRNAs [42]. However, the roles of the miRNAs mentioned above have not been validated due to lack of plasma samples [41]. In addition, it was identified that hsa-miR-125b-5p, as a T-cell suppressor, had an

increasing trend in the PD group at baseline and was significantly decreased in plasma EVs of PR patients after treatments [41]. It is thought that down-regulation of hsa-miR-125b-5p in EVs during treatment may imply an increased T-cell function of patients and a favourable response to immunotherapy [41].

In addition, EVs, as carriers of various molecules associated with drug resistance, are also biomarkers for therapeutic failure. Kwok et al. found that EVs released from an anaplastic lymphoma kinase (ALK)-tyrosine kinase inhibitor (TKI)-resistant subclone of ALK-translocated lung adenocarcinoma cell lines can induce drug resistance in the originally sensitive one [43]. Furthermore, the differential expression of miR-21-5p, miR-486-3p, and long noncoding RNAs (lncRNAs), MEG3 and XIST, in EVs may take action [43], corresponding to the role of miR-21 in drug resistance through the targeting of apoptotic protease activating factor-1 [44]. In addition, the lncRNAs, XIST and MEG3, may take effect through the sponging of miR-21-5p [43]. Although a study has reported that miR-486-5p functioned as an oncomiR by promoting cancer aggressiveness [45], the effects of miR-486 on drug resistance need to be validated. Another study by Yuwen et al. found that the levels of miR-146a-5p in A549 cell-derived EVs gradually decreased during the process of cisplatin-induced resistance, and a lower level of miR-146a-5p in serum EVs indicated a higher recurrence rate of NSCLC patients after chemotherapy, which may be achieved by decreased autophagy inhibition via the targeting of autophagy-related gene12 [46]. The same team also found that higher expression of miR-425-3p in serum EVs was correlated with a low response to chemotherapy with increased autophagic levels [47]. This result may be attributed to the association of high basal autophagy levels with low responsiveness, and miR-425-3p upregulated the autophagic levels by targeting protein kinase B/AKT1 [47], thus resulting in a decreased therapeutic response.

3.2. Circulating EV-proteins in lung cancer

CD31 is a transmembrane protein marker for endothelial-derived EVs regulating T cell response [28]. It has been found that low levels of CD31+ endothelial-derived EVs in responders to immune checkpoint inhibitors (ICIs) correspond with the activation of an immune response, whereas high levels of these EVs might reflect an endothelial-induced tumour immune escape in non-responders [28]. In addition, there are various EV-proteins differentially expressed between responders and non-responders to immunotherapy [28]. A functional enrichment analysis found that ten of them are associated with “neutrophil degranulation”, “defence response”, “immune response”, including the inhibition of immune system regulator interleukin-6, immune function regulator PR domain zinc finger protein 1, immune escape mediators oncostatin M, and EV release modulator Rho-associated protein kinase-2 [28]. However, CD31 was not detected due to its low abundance in EVs [28]. Moreover, it is implied that Annexin A2 and S100A9 are differentially regulated during immunotherapy of the two groups and may represent candidate EV-related biomarkers [28]. ALK has been found in EVs released from cancer cells [48]. Wu et al. found that after irradiation treatment, phosphorylated ALK expression was increased in EVs, and it activated the AKT, STAT3, and ERK pathways in recipient cells [48]. In addition, these irradiated EVs present a compromised therapeutic efficacy to ALK-specific inhibitor treatment *in vivo* and *in vitro* [48]. Thus, ALK is an important candidate in pharmacological assessment.

4. Circulating EVs in prostate cancer

Prostate cancer is one of the most common cancers in men, and there will be more than 20% new diagnoses by 2020 [1]. In addition to promoting tumour progression, EVs are also indicators of response to therapy [13,14]. In prostate cancer, circulating EV-RNAs and proteins are taken into wide consideration.

4.1. Circulating EV-RNAs in prostate cancer

As illustrated above, low levels of miR-34a in EVs tend to be associated with poor survival in prostate cancer patients [37], which may be associated with the regulation of EV-miR-34a in response to docetaxel through the targeting of B-cell lymphoma 2 [37]. However, most EV-miRNAs have found a protective role in prostate cancer. Malla et al. found that in addition to higher EV levels in patients after radiotherapy, higher hsa-let-7a-5p and hsa-miR-21-5p were also present in serum EVs of the high-risk group and were significantly associated with elevated radiation response, consistent with their anti-tumour roles in cancer [38]. Another similar study by Yu et al. also found that higher levels of several specific miRNAs (miR-493-5p, miR-323a-3p, miR-411-5p, miR-494-3p, miR-379-5p, miR-654-3p, miR-409-3p, miR-543, and miR-200c-3p) were delivered in serum EVs with a good response to carbon ion radiotherapy (CIRT), especially miR-654-3p and miR-379-5p [49]. It is demonstrated that their higher expression in EVs predicted a better therapy efficacy in patients after CIRT [49]. In addition, these miRNAs may work through the regulation of CIRT-related signalling pathways, such as MAPK, PI3k-AKT, and mTOR [49].

Androgen receptor splice variant 7 (AR-V7) is a variant of the androgen receptor [50]. Recently, Del Re et al. discovered that AR-V7 mRNA in plasma EVs are associated with shorter progression-free survival (PFS) and overall survival (OS) in metastatic castration resistance prostate cancer and may predict resistance to hormonal therapy [50].

4.2. Circulating EV-proteins in prostate cancer

Currently, the PSA test is the gold standard for early detection and functions as a tool for therapy response prediction in prostate cancer [7]. Logozzi et al. found that apart from increased EV secretion by cancer cells, the acidic tumour microenvironment also induced PSA expression in EVs [51], suggesting that the role of EV-PSA may be similar to that of serum PSA. A previous study has discovered that PSA in plasma EVs is a potential biomarker for prostate cancer diagnosis [52]. Recently, the clinical value of EV-PSA in therapeutic monitoring has also been researched. The results indicated that there was an approximately 2-fold reduction in urinary EVs levels after neoadjuvant androgen deprivation therapy (ADT), similar to the decreased serum PSA levels [39]. Moreover, the authors found that prostate cancer markers, PSA, PSMA (prostate-specific membrane antigen), and 5T4 (oncofoetal glycoprotein), were differentially positive in urinary EVs from cancer patients and negative in all healthy volunteers [39]. In addition, it was discovered that these markers were decreased after therapy [39]. Therefore, it is inferred that prostate cancer markers in EVs are promising biomarkers for follow-up in patients after therapy [39]. However, their effects in urinary EVs need to be validated in a larger cohort study.

In addition, various proteins associated with therapy resistance were demonstrated to be present in circulating EVs as well. Khara-ziha et al. found that EVs from therapy-resistant prostate cancer cells induced resistance to therapy through the transfer of multi-drug resistance (MDR) proteins, such as MDR-1/ p-glycoprotein, MDR-3, endophilin-A2, and poly(A)-binding protein-4 (PBP4) in EVs; this was further validated in serum EVs of prostate cancer patients [53]. These proteins mainly take action by mediating drug export [53]. Amongst these proteins, p-glycoprotein promotes chemoresistance through an ATP-dependant drug efflux pump [53]. Kato et al. also found that higher p-glycoprotein levels were in plasma EVs from docetaxel-resistant prostate cancer patients [54], functioning as an important biomarker for drug resistance.

5. Circulating EVs in breast cancer

Breast cancer is currently one of the major causes of cancer-related death in women [1]. Compared to other drug resistance

mechanisms, EVs are the emerging mechanism with the advantage of protection by the lipid bilayer membrane structure [32], mediating treatment response. In breast cancer, EV-miRNAs and proteins have been hotly debated around the world.

5.1. Circulating EV-miRNAs in breast cancer

Rodríguez-Martínez et al. found various miRNAs differentially expressed between breast cancer patients and healthy donors and eventually selected five of them [55]. It was implied that higher levels of miR-21, miR-222, and miR-155 in serum EVs were significantly related to the presence of CTCs [55]. In addition, an analysis of the association of miRNAs and clinical response after 3 months of treatment showed a lower expression of miR-221 and miR-21 in EVs, with the lower expression of miR-221 indicating a good prognosis [55]. However, there were no significant differences in EV-miRNA-21 amongst different groups in response to treatment [55], distinguished from its low expression in drug resistance control [56]. Thus, a large cohort is needed for validation. Moreover, another similar study by Stevic et al. found that decreased miR-155 and miR-301 in plasma EVs may predict pathological complete response to neoadjuvant therapy through uni- and multivariate models [57]. Prior works have studied the functional roles of these two miRNAs in facilitating tumour progression [58, 59]. However, further prospective studies are necessary for their predictive significance in therapeutic follow-up. For a comprehensive assessment, Salvador-Coloma et al. made a miRNA profile of EVs from non-responders and complete responders to neoadjuvant chemotherapy (NAC) in breast cancer [60]. It was revealed that lower expression of miR-185, miR-4283, miR-5008, and miR-3613 and higher levels of miR-1302, miR-4715, and miR-3144 were present in the EVs of non-responsive patients [60]. Speculative targeting of the genes of these miRNAs revealed that the enriched pathways were closely related to immune activation, immune suppression, and immune response [60], suggesting a possible immune inhibition in NAC response. Nevertheless, the mechanisms of these miRNAs in action need to be explored.

5.2. Circulating EV-proteins in breast cancer

Apart from EV-miRNAs, there are also studies on EV-proteins in breast cancer. The intercellular transfer of transient receptor potential channel 5 (TRPC5) in EVs stimulates multi-drug efflux transporter p-glycoprotein production, thus conferring chemoresistance on non-resistant cells [61]. Wang et al. found that increased expression of TRPC5 in plasma EVs can predict a low response to chemotherapy prior to tumour progression, determined by imaging examination [62]. Recently, a comparative proteomic analysis of plasma EVs was performed in healthy controls and breast cancer patients undergoing chemotherapy, radiotherapy, or after surgery [17]. The results suggested that various proteins were differentially expressed amongst groups [17], including focal adhesion kinase, mitogen-activated protein kinase kinase1, and fibronectin, for diagnosis, and heat shock protein 70 for recurrence risk prediction [17]. In addition, alterations in these proteins after treatments can provide information for drug efficacy evaluation [17].

6. Circulating EVs in melanoma

Melanoma is the most prevalent cancer of the skin, with a relative survival rate of 92% [1]. Regarding melanoma, immunotherapy and targeted therapy are both recommended, and EVs have great potential for therapeutic monitoring [63, 64]. In melanoma, EV-RNAs and proteins have received global attention, and PD-L1 is the most researched.

6.1. Circulating EV-RNAs in melanoma

Let-7 and miR-497 are both regarded as tumour suppressors, and their up-regulation is associated with better chemo- or radiotherapy outcomes [63]. Svedman et al. indicated that the levels of let-7g-5p and miR-497-5p were elevated in plasma EVs after targeted therapy and correlated with better disease control in melanoma patients [63], consistent with their roles in inhibiting tumour progression.

Similar to the immune suppression role of PD-L1 in cancer, Del Re et al. discovered that after 2 months of treatment, decreased levels of PD-L1 mRNA in plasma EVs were found in the responsive melanoma group and NSCLC patients, while higher expression was found in evolutionary individuals, consistent with the poor prognosis associated with high PD-L1 expression [65].

For a comprehensive assessment of EV-RNAs, Shi et al. performed a transcriptome analysis of plasma EVs in melanoma patients after ICIs [64]. It was demonstrated that the enriched genes/pathways of these differentially expressed transcripts in EVs were correlated with ICI resistance, melanoma progression, and response to ICI therapy [64], providing reliable evidence. However, more studies are needed for validation.

6.2. Circulating EV-proteins in melanoma

Apart from RNAs in circulating EVs, EV-proteins, mainly PD-L1, are alternative biomarkers for treatment monitoring. PD-L1, as one of the immune checkpoints, is present in cancer cells and regarded as a potential therapeutic target and predictive biomarker in tumours [66]. A study by Chen et al. found that high levels of EV-PD-L1 may suggest a T cell “exhaustion” state before treatment, whereas, following the recovery of T cell functions, increased PD-L1 in EVs predicted a better anti-tumour immunity induced by immunotherapy in responders [67]. Furthermore, this change was not found in the non-responder group of melanoma patients [67]. In addition, another study by Cordonnier et al. found that although EV-PD-L1 expression levels at baseline were not related to clinical pathological characteristics, their alterations after treatment (Δ ExoPD-L1) correlated with therapeutic response [68]. With the Δ ExoPD-L1 cut-off set at > 100 , there was 83% sensitivity, 70% specificity, 91% positive predictive value, and 54% negative predictive value for disease progression [68], supporting the predictive significance of PD-L1 in EVs in therapeutic monitoring. Moreover, Tucci et al. found that higher expression of PD-1 and CD28 existed in immune cell-secreted EVs and may predict a good response to immunotherapy in metastatic melanoma [69], providing a new source of EVs. Wang et al. studied the role of several specific proteins associated with melanoma therapy and progression in EVs, including melanoma chondroitin sulphate proteoglycan (MCSP), melanoma cell adhesion molecule (MCAM), low-affinity nerve growth factor receptor (LNGFR), and receptor tyrosine-protein kinase (ErbB3) [70]. The results indicated that these particular EVs were changing in different melanoma patients during and after targeted therapy [70]. However, more studies are needed to evaluate their functions in patients receiving treatments.

7. Circulating EV-miRNAs in lymphomas

Lymphomas are a heterogeneous group of non-solid tumours, including Hodgkin's lymphoma and non-Hodgkin's lymphoma [1]; diffuse large B-cell lymphoma (DLBCL) is the most frequent subtype of non-Hodgkin's lymphoma [71]. In lymphoma/Hodgkin's lymphoma, miRNAs in EVs are mainly researched, and most of them have been demonstrated in previous studies.

As discussed above, miR-155 is a miRNA that promotes tumour progression [58]. Zare et al. found that compared to DLBCL patients responsive to R-CHOP therapy, higher EV levels and EV-associated miR-155 were present in the plasma of refractory/relapsed patients

[71]. It has been suggested that a relatively low expression of miR-155 in EVs may predict a good response to R-CHOP treatment [71], consistent with a higher complete remission rate, higher overall response rate, longer PFS time of low miR-155 expression in DLBCL patients [72]. Similarly, Xiao et al. found that the levels of miR-451a in serum EVs were increased in DLBCL patients treated with immunotherapy and chemotherapy and can be used for therapeutic efficacy evaluation with an AUC of 0.8038 [73], suggesting an anti-tumour role of miR-451a in cancer. Previous studies have demonstrated the dual role of miR-451a in cancer [74, 75], and further studies are needed for precise mechanisms of this miRNA in lymphoma. In contrast, Feng et al. found that elevated expression of miR-99a-5p and miR-125b-5p in serum EVs were associated with shorter PFS and predicted resistance to chemotherapy in DLBCL patients [76], consistent with their roles in chemotherapy sensitivity regulation [77, 78].

In Hodgkin's lymphoma patients, a series of similar miRNAs in EVs have also been observed. It has been found that decreased levels of miR-21-5p, miR-127-3p, let-7a-5p, miR-155-5p, and miR-24-3p in plasma EVs statistically matched changes in metabolic response after treatment, and these miRNAs were upregulated again when the disease relapsed [79], suggesting that these miRNAs are associated with poor prognosis.

8. Circulating EVs in glioblastoma (GBM)

Gliomas are thought to be the most common primary brain cancer in adults, and GBM is the most lethal subtype [80]. Studies have illustrated that EVs are important for follow-up [13, 14]. In GBM, EV-mRNAs and proteins are of focus.

Resistance to therapy has been a problem worldwide. It was found that O6-methylguanine DNA methyltransferase (MGMT) and alkyl-purine-DNA-N-glycosylase (APNG) were both enzymes associated with DNA damage repair, and their elevated levels in tumour tissues predicted poor therapeutic efficacy in GBM patients [81, 82]. Shao et al. also found that MGMT mRNA and APNG mRNA were present in serum EVs and their levels were differentially changed during treatment [80]. In addition, it has been demonstrated that there is no evident association of MGMT mRNA and APNG mRNA expression with clinical outcomes [80]. Further analysis concluded that the increased increment from serial measurements of MGMT mRNA and APNG mRNA in EVs could predict resistance to therapy in GBM patients [80].

In addition, Shao et al. found that after temozolomide therapy, the expression of CD63, epidermal growth factor receptor (EGFR), and EGFRVIII (a mutant subtype of EGFR) in EVs decreased in a dose-dependant manner [83]. Furthermore, GBM animal models also suggested a rapid increase in efficacy index after treatment, which was defined by the number and biomarker expression levels of EVs [83]. With the enhancement of proteomic profile, Osti et al. also found that in addition to alterations in EV levels during therapy (higher levels of circulating EV were present in GBM patients, whereas they decreased to a normal level after surgery and rose again when the disease recurred), 102 proteins in circulating EVs were also differentially expressed pre- and post-surgery [84]. Amongst these proteins, 11 common proteins (vWF, APCS, C4B, AMBP, APOD, AZGP1, C4BPPB, Serpin3, FTL, C3, and APOE) associated with coagulation cascade and iron metabolism are "the GBM EV protein signature", as named in the research, and the protein signature vanished after surgical resection [84]. However, more studies are needed for precise elucidation of the mechanisms of these proteins in therapeutic response.

9. Circulating EVs in head and neck squamous cell carcinoma (HNSCC)

HNSCC is the most common epithelial malignancy of head and neck cancers [85]. Multiple research has found that EVs are important

regulators of biological processes, including response to therapy [85], thus functioning as candidates for therapeutic monitoring. In HNSCC, EV-proteins are widely studied.

Rodrigues-Junior et al. performed a plasma EVs proteomic comparison of responders and non-responders to chemoradiation therapy (CRT) in HNSCC patients [86]. The results indicated that there were 119 proteins specific to non-responders and 38 proteins specific to responders, some of which were associated with key pathways caused by CRT [86]. A further protein enrichment pathways analysis suggested that factor-associated suicide, p53, apoptosis, and cadherin signalling pathways were the main primary cancer networks in responsive patients [86]. In contrast, tumorigenesis-signalling pathways, including MAPK, protein kinase B, interleukin vascular endothelial growth factor, endothelial growth factor, and angiogenesis, were the main pathways in non-responders after CRT [86], providing a comprehensive study for further mechanisms exploration.

In addition, another study by Theodoraki et al. investigated the dynamic changes in tumour-derived EVs and T cell-derived EVs during therapy in HNSCC patients [87]. It was revealed that elevated levels of total EV proteins, tumour-derived EVs/total EV ratios, total CD3+, CD3(-) PD-L1+, and CD3+ CD15s+ (regulatory T-derived) EVs were present in HNSCC patients with recurrence [87]. Furthermore, total EV proteins and tumour-derived EV levels declined, CD3+ and CD3+ CD15s+ EVs stabilized, and CD3+ CTLA4+ EVs decreased after therapy in patients without disease recurrence [87], implying a potential value of EV subtypes in this field.

10. Outstanding questions

As discussed above, EVs released from cancer cells not only promote cancer progression through the delivery of cancer-associated molecules but also reflect alterations in the state of diseases during therapy and are promising biomarkers for therapeutic response evaluation, especially resistance to therapy [10, 32]. According to the literature in this field, miRNAs and proteins in EVs have received attention because of the privilege of enhanced technical developments in sequencing and proteomics. Although multiple clinical studies on EVs have conducted [41, 64], the underlying mechanisms of these miRNAs and proteins in cancer need to be further validated in the future.

In addition, there are limited studies on other EV-associated molecules in this field, such as DNA and lncRNA. It has been found that an increase in KRAS mutations in plasma-derived EVs predicted shorter PFS and OS in pancreatic cancer patients after neoadjuvant therapy, consistent with the pro-tumorigenesis role of KRAS mutations [88]. EGFR T790M associated with a good response to third-generation TKIs was also found in EVs of NSCLC patients [89]. lncRNAs are a class of noncoding RNAs that cannot be translated into proteins and play an important role in epigenetic regulation [90]. Tang et al. also found that the lncRNA, HOX (a gene family) transcript antisense RNA (HOTAIR), was significantly decreased in serum EVs after surgery, and its increase predicted poor therapeutic response to NAC and hormone therapy in breast cancer patients [91], making it an emerging biomarker in this field.

Moreover, there are also researches on the similar role of EV levels in therapeutic response assessment. Small-sized endothelial micro-particles (sEMPs) in plasma decreased after chemotherapy and were associated with better prognoses of breast cancer patients [92]. In contrast, the levels of small extracellular vesicles (sEVs) in acute myeloid leukaemia remained high after chemotherapy, predicting possible therapy resistance [93]. Furthermore, the dynamic concentration alterations in EVs during therapy also suggest disease progression [13, 84] and lower EV concentrations after NAC suggest the possible presence of minimal residual disease [13]. However, the possibility of using the number of EVs for a clinical follow-up of tumour patients has not been unified [94].

Furthermore, with the development of EV detection technologies, it is possible to use known biomarker-positive EVs, such as CD31+ endothelial-derived EVs [28], for both screening and clinical follow-up as mentioned above [95]. However, their use in almost all cancers has been restricted due to limited technology for isolation and detection. Currently, the precaution for the clinical use of circulating EVs as biomarkers is the purity of EVs [21, 24]. Although many methods have been explored, a standardized method for EV isolation and purification is necessary. In addition, a consensus is needed on the collection and analysis of EVs from biological fluid samples [96].

10.1. Search strategy and selection criteria

Data for this Review were identified by searches in MEDLINE, Current Contents, PubMed, and references from relevant articles using the terms “extracellular vesicles”, “extracellular particle”, “exosomes”, “liquid biopsy”, “therapeutic response”, “cancer” and “prediction”. Only articles published in English between 2005 and 2021 were included.

Contributors

ZE, LYM and JY participated in the design the manuscript. ZE and LYM wrote the manuscript, contributed equally to this work. WF and GMF contributed to making figures. XJ and WSF made the tables. TQ, MP and SSW contributed in revision. All authors reviewed and agree with the content of the manuscript. All authors have read and approved the final version of the manuscript. Figures were created with BioRender.Com.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

- [1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70(1):7–30.
- [2] Pantel K, Alix-Panabieres C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. *Nat Rev Clin Oncol* 2019;16(7):409–24.
- [3] Kilgour E, Rothwell DG, Brady G, Dive C. Liquid biopsy-based biomarkers of treatment response and resistance. *Cancer Cell* 2020;37(4):485–95.
- [4] Serrano MJ, Garrido-Navas MC, Diaz Mochon JJ, et al. Precision prevention and cancer interception: the new challenges of liquid biopsy. *Cancer Discov* 2020;10(11):1635–44.
- [5] Rolfo C, Cardona AF, Cristofanilli M, et al. Challenges and opportunities of cfDNA analysis implementation in clinical practice: perspective of the International Society of Liquid Biopsy (ISLB). *Crit Rev Oncol Hematol* 2020;151:102978.
- [6] Lin E, Cao T, Nagrath S, King MR. Circulating tumor cells: diagnostic and therapeutic applications. *Annu Rev Biomed Eng* 2018;20:329–52.
- [7] Gonzales JC, Fink LM, Goodman Jr. OB, Symanowski JT, Vogelzang NJ, Ward DC. Comparison of circulating MicroRNA 141 to circulating tumor cells, lactate dehydrogenase, and prostate-specific antigen for determining treatment response in patients with metastatic prostate cancer. *Clin Genitourin Cancer* 2011;9(1):39–45.
- [8] Choi D, Montermini L, Jeong H, Sharma S, Meehan B, Rak J. Mapping subpopulations of cancer cell-derived extracellular vesicles and particles by nano-flow cytometry. *ACS Nano* 2019;13(9):10499–511.
- [9] An T, Qin S, Xu Y, et al. Exosomes serve as tumour markers for personalized diagnostics owing to their important role in cancer metastasis. *J Extracell Vesicles* 2015;4:27522.
- [10] Lane RE, Korbie D, Hill MM, Trau M. Extracellular vesicles as circulating cancer biomarkers: opportunities and challenges. *Clin Transl Med* 2018;7(1):14.

- [11] Iessi E, Logozzi M, Lugini L, et al. Acridine Orange/exosomes increase the delivery and the effectiveness of Acridine Orange in human melanoma cells: a new prototype for theranostics of tumors. *J Enzyme Inhib Med Chem* 2017;32(1):648–57.
- [12] Lener T, Gimona M, Aigner L, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. *J Extracell Vesicles* 2015;4:30087.
- [13] Konig L, Kasimir-Bauer S, Bittner AK, et al. Elevated levels of extracellular vesicles are associated with therapy failure and disease progression in breast cancer patients undergoing neoadjuvant chemotherapy. *Oncoimmunology* 2017;7(1):e1376153.
- [14] Shukuya T, Ghai V, Amann JM, et al. Circulating micromas and extracellular vesicle-containing microRNAs as response biomarkers of anti-programmed cell death protein 1 or programmed death-ligand 1 therapy in NSCLC. *J Thorac Oncol* 2020;15(11):1773–81.
- [15] Aubertin K, Silva AK, Luciani N, et al. Massive release of extracellular vesicles from cancer cells after photodynamic treatment or chemotherapy. *Sci Rep* 2016;6:35376.
- [16] van Dommelen S, van der Meel R, van Solinge W, Coimbra M, Vader P, Schiffelers RJN. Cetuximab treatment alters the content of extracellular vesicles released from tumor cells. *Nanomedicine (Lond)* 2016;11(8):881–90.
- [17] Vinik Y, Ortega F, Mills G, et al. Proteomic analysis of circulating extracellular vesicles identifies potential markers of breast cancer progression, recurrence, and response. *Sci Adv* 2020;6(40).
- [18] Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol* 2009;9(8):581–93.
- [19] Colombo M, Raposo G, Thery C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 2014;30:255–89.
- [20] Witwer KW, Thery C. Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *J Extracell Vesicles* 2019;8(1):1648167.
- [21] Thery C, Witwer K, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018;7(1):1535750.
- [22] Hu T, Wolfram J, Srivastava S. Extracellular vesicles in cancer detection: hopes and hypes. *Trends Cancer* 2021;7(2):122–33.
- [23] Campos-Silva C, Caceres-Martell Y, Lopez-Cobo S, et al. An immunocapture-based assay for detecting multiple antigens in melanoma-derived extracellular vesicles. *Methods Mol Biol* 2021;2265:323–44.
- [24] Shu S, Yang Y, Allen CL, et al. Purity and yield of melanoma exosomes are dependent on isolation method. *J Extracell Vesicles* 2020;9(1):1692401.
- [25] Cvjetkovic A, Lötval J, Lässer C. The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. *J Extracell Vesicles* 2014;3(1).
- [26] Lin S, Yu Z, Chen D, et al. Progress in microfluidics-based exosome separation and detection technologies for diagnostic applications. *Small* 2020;16(9):e1903916.
- [27] Chen Y, Zhu Q, Cheng L, et al. Exosome detection via the ultrafast-isolation system: EXODUS. *Nat Methods* 2021;18(2):212–8.
- [28] Brocco D, Lanuti P, Pieragostino D, et al. Phenotypic and proteomic analysis identifies hallmarks of blood circulating extracellular vesicles in NSCLC responders to immune checkpoint inhibitors. *Cancers (Basel)* 2021;13(4).
- [29] Gualerzi A, Kooijmans SAA, Niada S, et al. Raman spectroscopy as a quick tool to assess purity of extracellular vesicle preparations and predict their functionality. *J Extracell Vesicles* 2019;8(1):1568780.
- [30] Tian Y, Gong M, Hu Y, et al. Quality and efficiency assessment of six extracellular vesicle isolation methods by nano-flow cytometry. *J Extracell Vesicles* 2020;9(1):1697028.
- [31] Cossetti C, Lugini L, Astrologo L, Saggio I, Fais S, Spadafora C. Soma-to-germline transmission of RNA in mice xenografted with human tumour cells: possible transport by exosomes. *PLoS ONE* 2014;9(7):e101629.
- [32] Dong X, Bai X, Ni J, et al. Exosomes and breast cancer drug resistance. *Cell Death Dis* 2020;11(11):987.
- [33] Huber V, Fais S, Iero M, et al. Human colorectal cancer cells induce T-cell death through release of proapoptotic microvesicles: role in immune escape. *Gastroenterology* 2005;128(7):1796–804.
- [34] Federici C, Petrucci F, Caimi S, et al. Exosome release and low pH belong to a framework of resistance of human melanoma cells to cisplatin. *PLoS ONE* 2014;9(2):e88193.
- [35] Sun Z, Shi K, Yang S, et al. Effect of exosomal miRNA on cancer biology and clinical applications. *Mol Cancer* 2018;17(1):147.
- [36] Thind A, Wilson C. Exosomal miRNAs as cancer biomarkers and therapeutic targets. *J Extracell Vesicles* 2016;5:31292.
- [37] Corcoran C, Rani S, O'Driscoll L. miR-34a is an intracellular and exosomal predictive biomarker for response to docetaxel with clinical relevance to prostate cancer progression. *Prostate* 2014;74(13):1320–34.
- [38] Malla B, Aebbersold DM, Dal Pra A. Protocol for serum exosomal miRNAs analysis in prostate cancer patients treated with radiotherapy. *J Transl Med* 2018;16(1):223.
- [39] Mitchell PJ, Welton J, Staffurth J, et al. Can urinary exosomes act as treatment response markers in prostate cancer? *J Transl Med* 2009;7:4.
- [40] Logozzi M, Mizzoni D, Capasso C, et al. Plasmatic exosomes from prostate cancer patients show increased carbonic anhydrase IX expression and activity and low pH. *J Enzyme Inhib Med Chem* 2020;35(1):280–8.
- [41] Peng XX, Yu R, Wu X, et al. Correlation of plasma exosomal microRNAs with the efficacy of immunotherapy in EGFR/ALK wild-type advanced non-small cell lung cancer. *J Immunother Cancer* 2020;8(1).

- [42] Wang W, Yang J, Xiang YY, Pi J, Bian J. Overexpression of Hsa-miR-320 is associated with invasion and metastasis of ovarian cancer. *J Cell Biochem* 2017;118(11):3654–61.
- [43] Kwok HH, Ning Z, Chong PW, et al. Transfer of extracellular vesicle-associated-RNAs induces drug resistance in ALK-translocated lung adenocarcinoma. *Cancers (Basel)* 2019;11(1).
- [44] Au Yeung CL, Co NN, Tsuruga T, et al. Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. *Nat Commun* 2016;7:11150.
- [45] Song L, Lin C, Gong H, et al. miR-486 sustains NF-kappaB activity by disrupting multiple NF-kappaB-negative feedback loops. *Cell Res* 2013;23(2):274–89.
- [46] Yuwen D, Sheng B, Liu J, Wenyu W, YJErM Shu. sciences p. MiR-146a-5p level in serum exosomes predicts therapeutic effect of cisplatin in non-small cell lung cancer. *Eur Rev Med Pharmacol Sci* 2017;21(11):2650–8.
- [47] Yuwen D, Ma Y, Wang D, et al. Prognostic role of circulating exosomal miR-425-3p for the response of NSCLC to platinum-based chemotherapy. *Cancer Epidemiol Biomarkers Prev* 2019;28(1):163–73.
- [48] Wu H, Zeng C, Ye Y, et al. Exosomes from irradiated nonsmall cell lung cancer cells reduced sensitivity of recipient cells to anaplastic lymphoma kinase inhibitors. *Mol. Pharm.* 2018;15(5):1892–900.
- [49] Yu Q, Li P, Weng M, et al. Nano-vesicles are a potential tool to monitor therapeutic efficacy of carbon ion radiotherapy in prostate cancer. *J Biomed Nanotechnol* 2018;14(1):168–78.
- [50] Del Re M, Biasco E, Crucitta S, et al. The detection of androgen receptor splice variant 7 in plasma-derived exosomal RNA strongly predicts resistance to hormonal therapy in metastatic prostate cancer patients. *Eur Urol* 2017;71(4):680–7.
- [51] Logozzi M, Angelini DF, Iessi E, et al. Increased PSA expression on prostate cancer exosomes in in vitro condition and in cancer patients. *Cancer Lett* 2017;403:318–29.
- [52] Logozzi M, Angelini DF, Giuliani A, et al. Increased plasmatic levels of PSA-expressing exosomes distinguish prostate cancer patients from benign prostatic hyperplasia: a prospective study. *Cancers (Basel)* 2019;11(10).
- [53] Kharaziha P, Chioureas D, Rutishauser D, et al. Molecular profiling of prostate cancer derived exosomes may reveal a predictive signature for response to docetaxel. *Oncotarget* 2015;6(25):21740–54.
- [54] Kato T, Mizutani K, Kameyama K, et al. Serum exosomal P-glycoprotein is a potential marker to diagnose docetaxel resistance and select a taxoid for patients with prostate cancer. *Urol Oncol* 2015;33(9):385. e15–20.
- [55] Rodriguez-Martinez A, de Miguel-Perez D, Ortega FG, et al. Exosomal miRNA profile as complementary tool in the diagnostic and prediction of treatment response in localized breast cancer under neoadjuvant chemotherapy. *Breast Cancer Res* 2019;21(1):21.
- [56] Gong C, Yao Y, Wang Y, et al. Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. *J Biol Chem* 2011;286(21):19127–37.
- [57] Stevic I, Muller V, Weber K, et al. Specific microRNA signatures in exosomes of triple-negative and HER2-positive breast cancer patients undergoing neoadjuvant therapy within the GeparSixto trial. *BMC Med* 2018;16(1):179.
- [58] He XH, Zhu W, Yuan P, et al. miR-155 downregulates ErbB2 and suppresses ErbB2-induced malignant transformation of breast epithelial cells. *Oncogene* 2016;35(46):6015–25.
- [59] Shi W, Gerster K, Alajez NM, et al. MicroRNA-301 mediates proliferation and invasion in human breast cancer. *Cancer Res* 2011;71(8):2926–37.
- [60] Salvador-Coloma C, Santaballa A, Sanmartin E, et al. Immunosuppressive profiles in liquid biopsy at diagnosis predict response to neoadjuvant chemotherapy in triple-negative breast cancer. *Eur J Cancer* 2020;139:119–34.
- [61] Ma X, Chen Z, Hua D, et al. Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance. *Proc Natl Acad Sci U S A* 2014;111(17):6389–94.
- [62] Wang T, Ning K, Lu TX, et al. Increasing circulating exosomes-carrying TRPC5 predicts chemoresistance in metastatic breast cancer patients. *Cancer Sci* 2017;108(3):448–54.
- [63] Svedman FC, Lohcharoenkal W, Bottai M, et al. Extracellular microvesicle microRNAs as predictive biomarkers for targeted therapy in metastatic cutaneous malignant melanoma. *PLoS ONE* 2018;13(11):e0206942.
- [64] Shi A, Kasumova G, Michaud W, et al. Plasma-derived extracellular vesicle analysis and deconvolution enable prediction and tracking of melanoma checkpoint blockade outcome. *Sci Adv* 2020;6(46).
- [65] Del Re M, Marconcini R, Pasquini G, et al. PD-L1 mRNA expression in plasma-derived exosomes is associated with response to anti-PD-1 antibodies in melanoma and NSCLC. *Br J Cancer* 2018;118(6):820–4.
- [66] Doroshow DB, Bhalla S, Beasley MB, et al. PD-L1 as a biomarker of response to immune-checkpoint inhibitors. *Nat Rev Clin Oncol* 2021.
- [67] Chen G, Huang AC, Zhang W, et al. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. *Nature* 2018;560(7718):382–6.
- [68] Cordonnier M, Nardin C, Chanteloup G, et al. Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *J Extracell Vesicles* 2020;9(1):1710899.
- [69] Tucci M, Passarelli A, Mannavola F, et al. Serum exosomes as predictors of clinical response to ipilimumab in metastatic melanoma. *Oncoimmunology* 2018;7(2):e1387706.
- [70] Wang J, Wuethrich A, Sina A, et al. Tracking extracellular vesicle phenotypic changes enables treatment monitoring in melanoma. *Sci Adv* 2020;6(9):eaax3223.
- [71] Zare N, Haghjooy Javanmard S, Mehrzad V, Eskandari N, Kefayat A. Evaluation of exosomal miR-155, let-7g and let-7i levels as a potential noninvasive biomarker among refractory/relapsed patients, responsive patients and patients receiving R-CHOP. *Leuk Lymphoma* 2019;60(8):1877–89.
- [72] Zhong H, Xu L, Zhong JH, et al. Clinical and prognostic significance of miR-155 and miR-146a expression levels in formalin-fixed/paraffin-embedded tissue of patients with diffuse large B-cell lymphoma. *Exp Ther Med* 2012;3(5):763–70.
- [73] Xiao X, Gu Y, Sun D, et al. Effect of rituximab combined with chemotherapy on the expression of serum exosome miR-451a in patients with diffuse large b-cell lymphoma. *Eur Rev Med Pharmacol Sci* 2019;23(4):1620–5.
- [74] Su Z, Ni L, Yu W, et al. MicroRNA-451a is associated with cell proliferation, migration and apoptosis in renal cell carcinoma. *Mol Med Rep* 2015;11(3):2248–54.
- [75] Wang R, Wang ZX, Yang JS, Pan X, De W, Chen LB. MicroRNA-451 functions as a tumor suppressor in human non-small cell lung cancer by targeting ras-related protein 14 (RAB14). *Oncogene* 2011;30(23):2644–58.
- [76] Feng Y, Zhong M, Zeng S, et al. Exosome-derived miRNAs as predictive biomarkers for diffuse large B-cell lymphoma chemotherapy resistance. *Epigenomics* 2019;11(1):35–51.
- [77] Zhang Y, Xu W, Ni P, Li A, Zhou J, Xu S. MiR-99a and MiR-491 regulate cisplatin resistance in human gastric cancer cells by targeting CAPSN1. *Int J Biol Sci* 2016;12(12):1437–47.
- [78] Iida K, Fukushi J, Matsumoto Y, et al. miR-125b develops chemoresistance in Ewing sarcoma/primitive neuroectodermal tumor. *Cancer Cell Int* 2013;13(1):21.
- [79] van Eijndhoven MA, Zijlstra JM, Groenewegen NJ, et al. Plasma vesicle miRNAs for therapy response monitoring in Hodgkin lymphoma patients. *JCI Insight* 2016;1(19):e89631.
- [80] Shao H, Chung J, Lee K, et al. Chip-based analysis of exosomal mRNA mediating drug resistance in glioblastoma. *Nat Commun* 2015;6:6999.
- [81] Hegi ME, Diserens AC, Gorlia T, et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005;352(10):997–1003.
- [82] Agnihotri S, Gajadhar AS, Ternamian C, et al. Alkylpurine-DNA-N-glycosylase confers resistance to temozolomide in xenograft models of glioblastoma multiforme and is associated with poor survival in patients. *J Clin Invest* 2012;122(1):253–66.
- [83] Shao H, Chung J, Balaj L, et al. Protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy. *Nat Med* 2012;18(12):1835–40.
- [84] Osti D, Del Bene M, Rappa G, et al. Clinical significance of extracellular vesicles in plasma from glioblastoma patients. *Clin Cancer Res* 2019;25(1):266–76.
- [86] Rodrigues-Junior DM, Tan SS, de Souza Viana L, et al. A preliminary investigation of circulating extracellular vesicles and biomarker discovery associated with treatment response in head and neck squamous cell carcinoma. *BMC Cancer* 2019;19(1):373.
- [87] Theodoraki MN, Yermeni S, Gooding WE, et al. Circulating exosomes measure responses to therapy in head and neck cancer patients treated with cetuximab, ipilimumab, and IMRT. *Oncoimmunology* 2019;8(7):1593805.
- [88] Bernard V, Kim DU, San Lucas FA, et al. Circulating Nucleic acids are associated with outcomes of patients with pancreatic cancer. *Gastroenterology* 2019;156(1):108–18 e4.
- [89] Castellanos-Rizaldos E, Grimm D, Tadigotla V, et al. EGFRExosome-based detection of T790M in plasma from non-small cell lung cancer patients. *Clin Cancer Res* 2018;24(12):2944–50.
- [90] Lee J. Epigenetic regulation by long noncoding RNAs. *Science* 2012;338(6113):1435–9.
- [91] Tang S, Zheng K, Tang Y, Li Z, Zou T, Liu D. Overexpression of serum exosomal HOTAIR is correlated with poor survival and poor response to chemotherapy in breast cancer patients. *J Biosci.* 2019;44(2).
- [92] Garcia Garre E, Luengo Gil G, Montoro Garcia S, et al. Circulating small-sized endothelial microparticles as predictors of clinical outcome after chemotherapy for breast cancer: an exploratory analysis. *Breast Cancer Res Treat* 2018;169(1):83–92.
- [93] Hong CS, Jeong E, Boyiadzis M, Whiteside TL. Increased small extracellular vesicle secretion after chemotherapy via upregulation of cholesterol metabolism in acute myeloid leukaemia. *J Extracell Vesicles* 2020;9(1):1800979.
- [94] Cappello F, Logozzi M, Campanella C, et al. Exosome levels in human body fluids: a tumor marker by themselves? *Eur J Pharm Sci* 2017;96:93–8.
- [95] Logozzi M, Mizzoni D, Di Raimo R, Fais S. Exosomes: a source for new and old biomarkers in cancer. *Cancers (Basel)* 2020;12(9).
- [96] Connors D, Allen J, Alvarez JD, et al. International liquid biopsy standardization alliance white paper. *Crit Rev Oncol Hematol* 2020;156:103112.