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# The potential role of exosomal microRNAs as biomarkers source for solid and hematological cancers: a narrative review

**Anna Maria Zimbo[1,†], Nancy Nisticò[1,†], Domenico Maisano[2,*], Rossella Russo[3], Massimo Gentile[4], Enrica Antonia Martino[4], Ernesto Vigna[4], Antonella Bruzzese[4], Annamaria Aloisio[1], Giuseppe Fiume[1], Ileana Quinto[1], Selena Mimmi[1,*,‡] and Enrico Iaccino[1,‡]**

[1] Department of Experimental and Clinical Medicine, Magna Graecia University of Catanzaro, 88100 Catanzaro, Italy
[2] Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02215, USA
[3] Department of Pharmacy, Nutritional and Health Sciences, University of Calabria, Arcavacata di Rende, 87100 Cosenza, Italy
[4] Hematology Unit, Department of Onco-Hematology, A.O of Cosenza, 87100 Cosenza, Italy
† These authors share the first position.
‡ These authors share the senior position.

* Correspondence authors; E-mails: domenico_maisano@dfci.harvard.edu (D.M.); mimmi@unicz.it (S.M.).

**Abstract**: Background and Objective: Exosomes are extracellular vesicles that regulate communication between cells and participate in various physiological and pathological processes. These nanovesicles transport proteins, lipids, and nucleic acids, including microRNAs (miRNAs). miRNAs are small non-coding RNA molecules that regulate gene expression. Exosomal miRNAs (exo-miRNAs) modulate many pathways involved in tumor progression, growth, metastasis, and angiogenesis. Therefore, in this review we investigated the role of exosomal miRNAs in both solid and hematological tumors, with the purpose of highlighting their potential role as disease biomarkers. Methods: Published peer-reviewed papers used as references for this narrative review were selected from the PubMed database using “exosomes”, “microRNA” and “cancers” as keywords. The time frame adopted in the bibliographic research covers an 11-year period, from January 2012 to January 2023. Key Content and Findings: This review explores the role of exosomal miRNAs as disease biomarkers. An excursus on exosomes and miRNAs is initially made to then illustrate the mechanisms of miRNAs sorting in exosomes. Exo-miRNAs can communicate with cells and can modulate their actions. Their half-life is extended thanks to the protection provided by

exosomes. The role of exosomal miRNAs in different types of solid and hematological tumors is summarized highlighting the potential of disease staging and progression. Conclusions: All manuscripts studied suggest that exosomal miRNAs can be used as diagnostic and prognostic biomarkers iwn cancer. The lack of appropriate and usable biomarkers for cancer diagnosis and prognosis makes exosomal miRNAs good candidates to provide non-invasive information to guide disease management in different types of cancer.

**Keywords**: exosomes; microRNAs; exosomal miRNAs; cancer; biomarkers

## 1. Introduction

All cells can communicate with each other through the release of Extracellular Vesicles (EVs), a high-throughput transport system of which exosomes represent the smallest and most sophisticated form [1]. Exosomes are vesicles small enough to move freely in the body fluids as bottled messages, reaching and penetrating the target cells to deliver their content. Being exosomes nanospheres with a diameter of less than 150 nm surrounded by a lipid membrane protecting a cargo composed of nucleic acids (mRNA, DNA, miRNA), proteins, and lipids, the enormous potential of these particles has emerged only recently as they can influence the behavior of targeted cells. In particular, exosomal miRNAs can modulate gene expression in all targeted cells, both in normal and pathological contests such as cancer, modulating processes that interfere with cancer immunity and microenvironment and are significantly involved in tumor growth, invasion, metastasis, angiogenesis and drug resistance [2,3]. This peculiar ability opens up to relevant research scenarios from a diagnostic and therapeutic point of view [4] that make the field of extracellular vesicles – included in the liquid biopsy approach in oncology – deserving more effort and attention.

Here, we highlighted recently published advances in cancer-derived exosomes summarizing their role of miRNA carriers in order to better understand their potential as diagnostic and prognostic molecular markers. In particular, the present review discusses the expression and critical role of exo-miRNAs originating from solid tumors (breast, ovarian, colorectal, and lung) and hematologic cancers (multiple myeloma, malignant tumors lymphoma, and chronic lymphoblastic leukemia), chosen based on the projects we are following as a research team. We present this article in accordance with the Narrative Review reporting checklist.

## 2. Methods

Literature research was conducted through the PubMed database (https://pubmed.ncbi.nlm.nih.gov), selecting papers published in English from January 2012 to January 2023 in peer-reviewed journals. Search terms used were exosomes, miRNAs, exosomal miRNAs, liquid biopsy, breast cancer, lung cancer, colorectal cancer, ovarian cancer, and hematological malignancies. Table 1 summarizes the strategy adopted in the bibliographic research.

**Table 1.** The search strategy summary.

| Items | Specification |
|---|---|
| Date of Search | January 2023 |
| Databases and other sources searched | PubMed (https://pubmed.ncbi.nlm.nih.gov) |
| Search terms used | Exosomes, microRNAs, exosomal miRNAs, liquid biopsy, breast cancer, lung cancer, colorectal cancer, ovarian cancer, hematological malignancies |
| Timeframe | Manuscripts published from January 2012 to January 2023 |
| Inclusion and exclusion criteria | Only English original articles and reviews published between Jan 2012 and Jan 2023 were chosen focused on exosomal miRNAs involved in cancer pathology. Literature in a language other than English and dated before January 2012 was excluded |
| Selection process | The selection process was carried out by the author in order to meet the criteria of novelty and contribution to the field |

## 3. Results

### *3.1. Exosomes as the small extracellular vesicles*

EVs are a heterogeneous group of lipid bi-layered particles that are actively released into the extracellular space by all cell types. They are involved in multiple physiological and pathological processes and are now considered to outline an additional mechanism for intercellular communication, allowing cells to exchange proteins, lipids, and genetic material with each other [5,6]. EVs are classified according to their cellular origin and/or biological function, biogenesis, and size.

The three main classes of extracellular vesicles are microvesicles, apoptotic bodies, and exosomes [7]. Exosomes originate from the membranes of multivesicular bodies (MVB), cup-shaped, with diameters typically ranging from 30 to 150 nm. They are secreted by all cell types being widely present in all body fluids and tissues, including plasma, urine, semen, saliva, bronchial fluid, cerebrospinal fluid (CSF), breast milk, serum, amniotic fluid, synovial fluid, tears, lymph, bile and gastric acid [8,9]. The first discovery of exosomes dates back to 1980, when they were initially classified as non-functional "garbage bags" containing unwanted cellular constituents [10]. In the mid-1990s exosomes were gradually shown to play an important role in cell-cell communication both in normal physiological processes and disease pathogenesis, including cancer, cell proliferation and senescence, angiogenesis, extracellular matrix support and reorganization, and immunomodulation [11,12]. Through exosomes, donor cells can transfer exogenous substances, such as proteins, nucleic acids, and lipids, to recipient cells [13]. Moreover, they can interact with recipient cells via exosomes surface receptors and can be transferred from host to recipient cells, leading to the exchange of genetic information and reprogramming of cellular functions [14].

### *3.2. Biogenesis of exosomes*

Exosomes are constitutively generated by the Endosomal Sorting Complex Required function for Transport (ESCRT) starting from late endosomes. The cell membrane goes through a double invagination process that results in the formation of the intracellular multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) [15,16]. Table 2 shows the constitutive proteins of ESCRT that work cooperatively to facilitate MVB formation, vesicle formation, and protein cargo sorting [17].

**Table 2.** Constitutive proteins of ESCRT complex.

| Type | Function |
|---|---|
| ESCRT 0 | Plays a vital role in the generation of multivesicular bodies by recognition and sequestration of ubiquitin proteins and recruits ESCRT-I to the endosomal membrane |
| ESCRT I<br>ESCRT II | Interact together to form a complex, which promotes deformation of the endosomal membrane and forms initial buds containing sorted cargoes |
| ESCRT III | It is involved in the promotion of budding processes, resulting in the formation of ILVs in the MVB lumen. Finally, the ESCRT-III complex separates from the MVB membrane with energy provided by the sorting protein Vps4 |

ILVs are ultimately secreted as exosomes into the extracellular space after the fusion of the MVB with the plasma membrane [18]. Ras GTPase-related protein Rab, Sytenin-1, TSG101 (tumor susceptibility gene 101), ALIX (apoptosis-linked gene 2 interacting protein X), syndecan-1, heat shock proteins (HSP60, HSP70), proteins ESCRT, phospholipids, tetraspanins (CD9, CD63, and CD81), ceramides, sphingomyelinases, and SNARE complex proteins [soluble factor N attachment protein ethylmaleimide (NSF) receptor] are involved in the origin and biogenesis process of exosomes [16]. There is also an ESCRT-independent mechanism for ILV biogenesis that requires ceramide and sphingolipids, which allow for spontaneous membrane curvature [19].

Studies are in progress to understand the main interactors involved in this process, and recently few proteins, such as Rab11, Rab35, and Rab27 were identified as key players [20].

### *3.3. Exosomes isolation methods*

Since they participated in several processes involved in cancer progression, such as tumor metastasis, drug resistance, and immune response [14], exosomes have become a fruitful field of investigation to develop non-invasive approaches for disease staging and progression. The need to identify new exosome isolation techniques has now become one of the main aims of research in this field. Up to now, six classes of exosome separation strategies have been described: ultracentrifugation (UC), ultrafiltration, immunoaffinity capture, charge neutralization-based polymer precipitation, size exclusion chromatography, and microfluidic techniques [21].

The high levels of protein aggregate and lipoprotein contamination in exosomes samples obtained using UC impair their quantification and functional analysis; therefore other

methods have been developed to address the challenges associated with ultracentrifugation [21]. Even if ultracentrifugation remains the "gold standard" method for separating exosomes from biological fluids [22], the ubiquitarian expression of tetraspanins (CD9, CD63, and CD81), heat shock proteins (HSP60, HSP70) and ESCRT-associated components (Alix and TSG101) has been exploited in setting immunoaffinity-based protocols for exosomes identification and detection [23]. Several commercial kits are also used to isolate and study exosomes [24,25], taking into consideration the size and the morphology with Nanoparticle Tracking Analysis (NTA) and transmission electron microscopy (TEM). NTA, adopting z potential measures, can be also used to assess the purification's yield, whether, to assess protein expression, electron microscopy imaging, and western blot analysis are the gold standard. Nucleic acid content is evaluated by bioanalyzers and PCR techniques such as RT-qPCR or ddPCR [26]. However, because these technologies cannot isolate exclusively exosomes and produce complex mixtures of EVs with other extracellular components, the challenging goal for the effective adoption of exosomes in clinical practice remains the development of techniques that fulfill the need for rigor and standardization to shed light on exosome diversity.

### *3.4. miRNAs and exo-miRNAs*

miRNAs are small, non-coding, endogenous single-strand RNAs that play a key regulatory role in suppressing gene expression by inhibiting protein translation and promoting mRNA cleavage [27,28]. They are approximately between 21 and 25 nucleotides in length and are mainly transcribed by RNA polymerase II (Pol II) [29,30]. Since their discovery, several miRNAs have been identified, and the research has made extraordinary progress in studying their function and their translation from the bench to the bedside [29].

First, RNA polymerase II starts the miRNAs transcription in the nucleus resulting in the production of the primary miRNA (pri-miRNA) characterized by the presence of the polyA chain and showing one or more hairpin structures [28]. Then, the pri-miRNA is processed and cleaved into precursor miRNA (pre-miRNA) by the RNAse Drosha and its cofactor DGCR8. Hairpin pre-miRNAs are then transported into the cytoplasm by Exportin-5 through the nuclear pores, where the hairpin is cleaved by Dicer, another RNAse, which cuts the pre-miRNA producing a double-stranded mature miRNA. One of these strands is loaded into the RISC complex containing an Argonaute protein (Ago-2). Now the RISC complex can reach the 3′UTR of the target mRNA, resulting in mRNA cleavage (if the homology with the target mRNA is high) or translation inhibition (Figure 1) [28,31].

At the biological level, a single miRNA can target hundreds of target RNAs, influencing several downstream effects of many genes often involved in cancer formation and development. Precisely, they can, for example, mediate post-transcriptional gene silencing by binding to the 3′-untranslated region (UTR) or open reading frames (ORFs) of target mRNA [29,30] in various physiological and pathological processes [32]. It is now a fact that the presence of circulating miRNAs can provide prognostic significance in the broader fields of liquid biopsy.

Indeed, circulating miRNAs can be detected in various body fluids, such as blood, saliva, and urine, by using real-time polymerase chain reaction (RT-PCR) or microarrays in order to offer opportunities to use these molecules as biomarkers of diseases [33]. Circulating miRNAs can be dispersed in biological fluids, for example in the bloodstream, or enclosed in vesicles. Recent studies have identified miRNAs in exosomes (exo-miRNAs), which can be uptaken by nearby or distant cells, subsequently modulating the behavior of recipient cells [29], suggesting them as potential biomarkers for liquid biopsy and proposing their use in future clinical trials [34]. Moreover, exo-miRNAs are protected from RNase digestion thanks to the exosome's lipid bilayer. This characteristic improves exo-miRNAs half-life which is longer than the normal circulating miRNAs [35].

Mature miRNAs are integrated into exosomes via the following five potential pathways:

(1) *Neutral sphingomyelinase 2 (nSMase2)-dependent pathway:* nSMase2 is the first identified protein involved in the secretion of miRNAs into exosomes. It has been found that the overexpression of nSMase2 increases the levels of exo-miRNAs, while its downregulation/inhibition reduces the number of exo-miRNAs [36,37].

(2) *3′ miRNA sequence-dependent pathway*: 3' Uridine-rich miRNAs are mostly found in B cells-derived exosomes and those purified from urine whether 3' polyA-rich miRNAs are mainly present in B cells-derived exosomes.

(3) *miRNA motif and sumoylated hnRNPs-dependent pathway:*it has been studied that sumoylated HNRNPA2B1 can recognize the GGAG motif in the 3′ portion of miRNA sequences, causing the packaging of specific miRNAs into exosomes. Also, HNRNPA1 and hnRNPC can bind the exo-miRNAs [38].

(4) *miRNA-induced silencing complex (miRISC)-related pathway*: mature miRNAs can form the miRISC complex by interacting with assembly proteins. This complex mainly contains miRNAs, miRNA-repressible mRNAs, GW182, and AGO2. The binding of mRNAs by these miRNAs occurs mainly within their 3′ untranslated regions (3′UTRs). The mRNA/miRNA complex next suppresses translation by preventing initiation or increasing mRNA degradation. Recently published findings have demonstrated a potential correlation between AGO2 and exo-miRNAs sorting. Guduric-Fuchs *et al.* discovered that knockout of AGO2 could suppress miRNAs such as miRNA-150, miRNA-142-3p and miRNA-451 in HEK293T-derived exosomes [39].

(5) *Ceramide-related pathway*: it has been studied that treatment with ceramide C6 (C6-cer) increased the levels of some tumor-suppressive miRNAs in exosomes, suggesting that the ceramide pathway plays an essential role in miRNA incorporation into exosomes [40].

As well, some enzymes may control the sorting of exo-miRNAs, regardless of miRNA sequences.

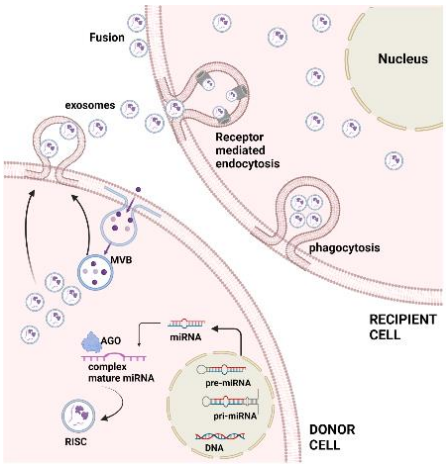


**Figure 1.** Schematic illustration of exosomal miRNA biogenesis and secretion with recipient cells. MiRNA genes in the nucleus are transcribed to generate pri-miRNAs, subsequently transmitted into pre-miRNAs that reach the cytoplasm where they are transformed into duplex miRNAs and subsequently converted into single-stranded miRNAs. These miRNAs bind to the AGO protein to be assembled in the RISC complex together with DICER. This complex can be loaded in the forming vesicles which, at this point, exosomes are released into the extracellular environment, and ready to be uptaken by the recipient cells through direct membrane fusion, receptor-mediated endocytosis, or phagocytosis. The exosomal miRNAs are then released in the recipient cells, thus playing its role in various biological and pathological processes.

### *3.5. Exo-miRNAs detection*

A wide variety of methods for the detection of exo-miRNAs have been reported. Among them, quantitative real-time PCR (qRT-PCR) has been defined as the gold standard for miRNA analysis due to its excellent sensitivity and flexibility [41]. Despite this, qRT-PCR presents several problems: the non-absolute quantification, the possibility of giving false positive results, the dependence on expensive equipment, and the need for numerous biological samples, which limit its application in routine clinical practice. The other standard methods used to quantify and profile miRNAs are digital PCR and next-generation sequencing (NGS) [42]. In addition, many biosensors have been designed to explore disease-associated markers. However, despite the superior performance of these methods, they have shortcomings compared to liquid biopsy. Indeed, attention has focused on the use of liquid biopsy for the detection of circulating miRNAs, which are readily available in a variety of body fluids, where they are released in the form of complexes with extracellular vesicles [43]. The abnormal expression of circulating miRNAs may contribute to differentiate healthy from diseased subjects, with a strong potential in the identification of the tumor's origin and subtypes [44,45]. These premises allow us to affirm the need to expand the knowledge in liquid biopsy, to increase the healthcare system's performance in early detection and prognosis profiling.

### *3.6. Exo-miRNAs as biomarkers in different types of cancer*

The set of circulating components responsible for cell-cell communication is called the "circuloma" [46]. Under pathological conditions, circulatory components, as well as exo-miRNAs, can reflect the course of the disease. In order to be able to analyze the components of the circuloma, the liquid biopsy is used. Thanks to this non-invasive technique, it is possible to analyze these components from different fluids, such as peripheral blood, urine, liquor, and other body fluids [23,47]. Furthermore, liquid biopsy allows repeated testing over time, providing longitudinal monitoring of the tumor as well as its adaptation to anticancer treatment [48].

Recent studies have demonstrated that tumor-derived exosomes (TEX), present in all bodily fluids of cancer patients, are ideal biomarkers for early cancer diagnosis and therapeutic monitoring [49]. Exo-miRNAs, in addition to participating in various processes involved in tumorigenesis and tumor development, can influence the environment surrounding the tumor, influencing the extracellular matrix (ECM) as well as the activation and recruitment of the immune system [50]. Specifically, they mediate paracrine and endocrine pathways between different tissues in the tumor microenvironment to regulate gene expression and function of distal cells.

For these reasons, in this review, we discuss the expression and the key role of exo-miRNAs originating from a diverse type of cancer cells, such as solid tumors (breast, ovarian, colorectal, and lung) and hematologic tumors (multiple myeloma, malignant lymphoma, and chronic lymphoblastic leukemia) [51,52].

### *3.7. Exo-miRNAs in breast cancer*

Breast cancer (BC) accounting for 12.5% of all annual new cancer cases worldwide represents the second leading cause of cancer deaths among women [53]. Exo-miRNAs have emerged as a significant biomarker in BC diagnosis and clinical management. Eichelser and co-workers compared serum levels of circulating free and exosomal miRNAs and their involvement in the molecular subtypes of BC patients. Their data showed associations between increased serum exosomal miR-373 levels with receptor-negative tumors, thereby highlighting the potential role of this exo-miRNA as a blood biomarker for aggressive tumors such as triple-negative BC [54]. Exo-miR-122 has been extensively found in BC cells and has been associated with metastasis in BC patients as it inhibits glucose uptake by stromal cells through the downregulation of glycolytic enzymes, allowing tumor cells to absorb glucose preferentially [55]. miR-1246 and miR-21 were significantly enriched in the tumor-derived exosomes of sixteen BC patients compared with the plasma exosomes of healthy controls [56]. miR-21, miR-155, miR-182 and miR-373 were isolated from exosomes present in the serum of BC patients and were found to be up-regulated compared to healthy controls [57]. Specifically, exo-miR-155 can induce chemoresistance to doxorubicin and paclitaxel, two chemotherapeutic agents, and simultaneously act as an oncogenic signal that reprograms cancer metabolism [58]. Furthermore, the elevated levels of exo-miR-155 and exo-miR-1246 can be used to distinguish trastuzumab-resistant from sensitive patients [59]. The regulation

of miR-21 and its role in carcinogenesis has been extensively studied, and its involvement in almost all stages of BC development is now clear [60]. Specifically, the up-regulation of exo-miR-21 inhibits the expression of the phosphatase and tensin homolog deleted gene on chromosome ten (PTEN), which acts as a tumor suppressor gene of the phosphatidylinositol 3-kinase (PI3K) pathway, thereby suppressing the expression of the tumor suppressor gene Drg-1 (differentiation-related gene 1) [61]. As regards the regulatory mechanisms, it has been suggested that exo-miR-21 may directly inhibit the expression of some proteins, such as mammary serine protease inhibitor (maspin), the programmed cell death protein 4 (PDCD4) and the tumor suppressor gene tropomyosin 1 (TPM1) [62]. Upregulation of exo-miR-21 has been reinvestigated in several cancers [57], suggesting that some miRNAs are not specific for one type of cancer, but could be considered biomarkers for the presence or absence of cancer in general [50]. Recently, Yuan *et al.* confirmed the role of exo-miR-21 in osteoclastogenesis by regulating PDCD4 protein levels. Furthermore, they showed the highest expression levels of exo-miR-21 in serum exosomes of BC patients with bone metastases compared with those of other subpopulations [63]. Finally, Li et al in 2020, found that serum exo-miR-148a levels are significantly reduced in BC patients [64].

### *3.8. Exo-miRNA in ovarian cancer*

Ovarian cancer (OC) is among the most common gynecological cancers, after cervical and uterine cancer, but has the worst prognosis and the highest mortality rate [65]. Exo-miRNAs show diagnostic potential and can be used as an auxiliary tool also in the diagnosis of OC [66]. Taylor and Gercel-Taylor were the first to conduct a study in this field. They used sera derived from women diagnosed with serous OC, women with benign disease, and healthy women as control. By exo-miRNA analysis, a total of eight miRNAs (miRNA-21, miRNA-141, miRNA-200a, miRNA-200b, miRNA-200c, miRNA-203, miRNA-205 and miRNA-214) were found overexpressed in tumor samples and absent in healthy controls and patients with benign disease. These results indicated a possible clinical value of these exo-miRNAs in the early diagnosis of ovarian cancer [67]. Furthermore, some years later, Meng X *et al.* found higher levels of exosomal miR-200b and miR-200c in patients with OC at FIGO stages III-IV compared to patients with FIGO stages I-II. Increased miRNA-200b and miRNA-200c levels significantly correlated with carbohydrate antigen (CA-125) levels and shorter survival [68]. Exo-miR-1307 and exo-miR-375 were significantly up-regulated in serum exosomes of OC compared with patients with benign adenoma and healthy groups. Furthermore, these two miRNAs are associated with lymph node metastasis and tumor staging in OC, respectively [69]. Ying and colleagues reported up-regulated levels of exo-miR-222-3p in serum samples from patients with epithelial OC compared with healthy controls [70].

### *3.9. Exo-miRNAs in colorectal cancer*

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths worldwide [71,72]. In 2014 Ogata-Katawa *et al.* were the first to find that serum exosomal levels of seven miRNAs were significantly up-regulated

in CRC patients, even those newly diagnosed with the disease, compared to healthy controls. They selected and validated let-7a, miR-1229, miR-1246, miR-150, miR-21, miR-223 and miR-23a. They also found that their levels were downregulated after surgical resection of the tumors [73]. In the same year, Matsumura *et al.* analyzed the miRNA expression profile of two sets of serum samples by RT-qPCR. The reported results demonstrate that the expression level of exo-miR-19a was significantly increased in CRC patients compared with healthy individuals with gene amplification. CRC patients with high exosomal miR-19a expression showed poorer survival than the low expression group, thus concluding that abundant serum exo-miR-19a expression may be a potential prognostic marker and therapeutic target in CRC [74]. In 2020 Liu *et al.* found that the expression level of exo-miR-139-3p in the plasma of CRC patients was significantly downregulated compared to healthy controls. This result suggests the potential for exo-miR-139-3p to act as a biomarker for early diagnosis and monitoring of metastases in CRC patients [75]. In 2020 Liu *et al.* identified miR-106b-3p as a promising prognostic biomarker and therapeutic target, as it was shown that its serum exosomal expression level was significantly higher in CRC patients with metastases than in those without metastases [43]. In the same year, Zhang and colleagues also detected the serum expression of exo-miR-874 in CRC patients. They demonstrated that serum exo-miR-874 was markedly downregulated in CRC patients compared with healthy controls, concluding that serum exo-miR-874 expression could serve as a reliable marker for CRC diagnosis and prognosis prediction [76]. Exo-miR-122, according to Sun *et al.*, can be considered as an independent predictive biomarker of CRC, as its expression was significantly up-regulated in patients with CRC, especially in those with liver metastases (SCI) [77]. Shi *et al.* in 2021 found that four exo-miRNAs (miR-126, miR-1290, miR-23a, miR-940) showed a high ability to differentiate TNM stage I CRC patients from healthy controls. These data suggested that exo-miRNAs are new potential biomarkers for early diagnosis of CRC [72].

### *3.10. Exo-miRNAs in lung cancer*

Lung cancer (LC) is one of the most frequently diagnosed cancers and the leading cause of cancer-related deaths worldwide, with an estimated 2 million new cases and 1.76 million deaths annually [78]. The early stages of lung cancer are often asymptomatic, then it is diagnosed at a late stage, with poor survival outcomes. It would be very beneficial if LC could be diagnosed early using non-invasive molecular markers [79].

Several studies aimed to identify exo-miRNAs up-regulated in the exosomes of LC patients compared to healthy individuals. Cavoli *et al.* demonstrated that four exo-miRNAs (miR-378a, miR-379, miR-139-5p, and miR-200b-5p) can be used to divide the population into healthy controls and diseased patients with lung adenocarcinomas. Subsequently, six exo-miRNAs (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p) were selected for a second assay, to discriminate between lung adenocarcinoma and granuloma [80]. Regarding the functional role of exo-miRNAs in lung carcinogenesis, recent evidence demonstrated that miR-23a was significantly up-regulated in LC exosomes under hypoxic conditions, capable of enhancing angiogenesis and vascular permeability through its

targets prolyl hydroxylase (PHD) and tight junction protein -1 (also known as zonula occludens-1, ZO-1), revealing the clinical importance and prognostic value of this exo-miRNA in patients with LC [81]. In 2017, a noteworthy study aimed to identify exo-miRNAs as biomarkers capable of discriminating between adenocarcinoma and squamous cell carcinoma (SCC), as a non-invasive method in the early diagnosis of non-small cell lung cancer (NSCLC). They found that miR-181-5p, miR-30a-3p, miR-30e-3p, and miR-361-5p were specific for adenocarcinoma and miR-10b-5p, miR-15b-5p and miR-320b were Specific to SCC [82]. Serum exo-miR-378 was highly overexpressed in NSCLC patients compared with control subjects, and correlated with positive lymph node metastases and advanced TNM stage, showing the potential of exo-miR-378 as a prognostic biomarker [83]. In 2020 Zhang *et al.* screened 22 exo-miRNAs, to identify specific exo-miRNAs with diagnostic and prognostic potential in NSCLC patients, demonstrating that exo-miR-5684 and exo-miR-125b-5p were significantly downregulated in NSCLC patients compared with healthy donors. Furthermore, exo-miR-125b-5p was related to metastasis, chemotherapy impact, and survival. Thus, these two exo-miRNAs may serve as promising diagnostic and prognostic biomarkers for NSCLC [84]. In the same year, Huang et al investigated the correlations between serum exo-miR-1246 level and NSCLC prognosis in 105 patients compared with healthy controls. The results demonstrated that the expression of serum exosomal miR-1246 was markedly higher in NSCLC patients than in other subjects. Furthermore, the amount of serum exo-miR-1246 was substantially linked to the TNM stage [85]. Finally, Zheng et al 2021, selected six plasma exo-miRNAs (miR-21, miR-214, miR-1246, miR-210, miR-96, and let-7 g) to validate their diagnostic ability. Exo-miR-96 and exo-miR-1246 were shown to be independent NSCLC diagnostic biomarkers in 52 NSCLC patients compared with 45 healthy volunteers. In particular, miR-96 was found to be a promising diagnostic and prognostic biomarker for radioresistant NSCLC [86].

### *3.11. Exo-miRNAs in multiple myeloma*

Multiple myeloma (MM) is a hematologic malignancy, accounting for more than 10% of hematologic cancers, characterized by the expansion and clonal proliferation of plasma cells in the bone marrow microenvironment. MM is largely incurable due to its aggressiveness and drug-resistance characteristics [11]. Specifically, several studies reported that some MM patients showed an innate resistance to bortezomib, the anticancer drug commonly used to treat newly diagnosed and relapsed/refractory multiple myeloma, either as a single agent or in combination with other therapies. This resistance to drugs leads a large number of researchers to be interested in the early prediction of drug resistance in patients with MM to improve the therapy results of this pathology. Several studies correlated exo-miRNA expression with drug resistance in MM. In a predictive study, ten miRNAs with the greatest changes were superimposed on miRNAs based on the literature. These ten miRNAs were thought to have the potential to be used as predictive panels for drug resistance of MM patients [87]. Subsequently, this panel showed that downregulation of exo-miR-16-5p, miR15a-5p, miR-20a-5p, and miR-17-5p was correlated with bortezomib resistance in

patients with MM [88]. Manier and colleagues in 2017 characterized circulating exo-miRNAs in MM and determined their impact on patient outcomes. In particular, they focused on progression-free survival (PFS) and overall survival (OS), in order to determine a new biomarker to improve the prediction of survival rates in MM patients. This analysis showed that two exo-miRNAs, let-7b and miR-18a, are closely correlated with PFS and OS in newly diagnosed MM patients independently [89]. In 2018 Zang *et al.* explored the differential expression of serum exo-miRNAs between MM patients and healthy controls (HCs). They also investigated the relevance of exo-miRNAs to clinical symptoms. The results of exo-miRNA profiling showed that the expression levels of let-7d-5p, miR-140-3p and miR-425-5p were significantly lower in MM patients than inHC. The let-7 family may play a role in myeloma pathogenesis, while a low miR-140-3p level is associated with osteoporosis [90].

### *3.12. Exo-miRNAs in lymphomas*

Non-Hodgkin's lymphoma (NHL) is the most common hematologic malignancy worldwide. There are over 40 major subtypes of NHL. The most common subtype is diffuse large B-cell lymphoma (DLBCL), which accounts for 30-40% of all NHL globally [91,92]. The implication of exo-miRNA profiling in the diagnosis of DLBCL has been investigated by several studies. Feng et colleagues, in 2019, described exo-miR-99a-5p and exo-miR-125b-p as potential prognostic predictors of chemoresistance in the serum of DLBCL patients. The results showed that exo-miR-99a-5p and exo-miR-125b-5p were increased in the serum of DLBCL patients and were associated with shorter and lower PFS. Therefore, they can be used as good predictors of prognosis and chemotherapy efficacy [93]. Exo-mir-451a was found in more than one of these studies. Xiao *et al.* analyzed the expression level of exo-miR-451a in DLBCL patients treated with rituximab in combination with chemotherapy compared with healthy controls. The Statistical analysis results established that the expression level of miR-451a in the DLBCL group was significantly lower than that of the control group [94]. Cao and colleagues enrolled 256 individuals, including 133 DLBCL patients and 94 healthy controls, divided into three phases: screening, training, and testing. At the end of the steps, using microarray and qRT-PCR analysis, they identified five exo-miRNAs (miR-379-5p, miR-135a-3p, miR-4476, miR-483-3p and miR-451a) that can be used as noninvasive biomarkers for the diagnosis of DLBCL. Among them, exo-miR-451a is the only one that has prognostic value in patients with DLBCL [92]. Based on the concordance result of these studies, the downregulation of exo-miR-451a may be a useful biomarker. Liu and colleagues, in their study, provided evidence of the diagnostic value of three exo-miRNAs (miR-107, miR-375-3p, and miR-485-3p) in patients with DLBCL. Specifically, they identified that exo-miR-107 and exo-miR-375-3p were downregulated, and miR-485-3p was up-regulated in the plasma of DLBCL patients. They also identified interactions between miR-107 and 14-3-3η and demonstrated that in DLBCL, up-regulated 14-3-3η plays a stimulatory role in DLBCL progression. Still, they suggest that exo-miR-107 could abrogate DLBCL progression through the downregulation of 14-3-3η [95]. Finally,

Rinaldi and colleagues validated serum miR-22, an evolutionarily conserved miRNA, as a predictor of therapeutic outcome and response in patients with DLBCL [96].

### *3.13. Exo-miRNAs in chronic lymphocytic leukemia*

Chronic lymphocytic leukemia (CLL) represents a very heterogeneous type of adult leukemia, with a variable course of the disease. miRNAs were first identified as plasma-circulating molecules influencing the pathogenesis and survival of leukemia cells [97]. Such a contribution may involve specific communication in the tumor microenvironment, promoting proliferation and survival [98]. The spindle homolog suppressor (SUFU) is the target of miR-202-3p and is a gene encoding a protein that serves as a negative regulator of the hedgehog signaling pathway. SUFU is impaired in 0.87% of all tumors (The AACR Project GENIE Consortium) and has been implicated in pattern formation and cell proliferation during development. miR-202-3p and the "Sufu" gene are inversely proportional, so when the levels of the first decrease, there is an increase in expression of the second, with a consequent increase in survival and progression of tumor cells [99]. Expression levels of miRNAs (intracellular or serum levels) are often associated with the common phenotypic features of aggressive CLL [100]. A research study detected a variety of miRNAs in CLL exosomes, and the five most abundant miRNAs (miR-21, miR-155, miR-146a, miR-148a, and let-7g) accounted for 65% of all miRNA reads [101]. miR-155 is highly expressed in B cells and plays a critical role in various physiological and pathological processes and in cancer [102]. miR-155 is abundant in CLL-derived exosomes, and in this context has the function of inducing and retaining myeloid-derived suppressor cells (MDSCs) [103]. Overexpression of miR-21 has been associated with oncogenesis in many cancers [104]. Two other CLL-derived exosomal miRNAs are miR-146a and miR-451, which induce the transition of stromal cells to cancer-associated fibroblasts (CAF) [101], resulting in the secretion of inflammatory molecules that promote tumor progression [105]. Another exo-miRNA that considers the molecular characteristics of CLL is miR-150. This miRNA is associated with ZAP-70 gene expression or unmutated CLL phenotype [106]. CLL-derived exosomes support the pro-tumorigenic action of miR-150 [107], while miR-29 appears to play a key role in hematological malignancies through the regulation of TCL1, MCL1 and DNA-methyltransferase [108]. CLL-derived exosomes show elevated levels of miR-29 compared to normal exosomes [109]. Finally, exo-miR-223 is downregulated in aggressive stages of CLL and is associated with poor prognostic factors [110].

## 4. Conclusion

Nowadays, the lack of appropriate biomarkers for early cancer diagnosis and prognosis limits the reduction of cancer mortality rate [43]. Modern medicine aims to find new options for more precise diagnosis and therapy of cancer [50]. Liquid biopsy is an innovative approach in clinical oncology that offers the opportunity of detecting, analyzing, and monitoring cancer in various body fluids. This non-invasive group of techniques allows for analyzing the tumor heterogeneity and it has the advantages of minimal invasiveness and easy sample acquisition.

A large variety of studies have been performed regarding the use of exosomes as promising disease biomarkers and therapeutic tools for treatment of cancers and other pathologies. In addition, the potential of exo-miRNAs as liquid biopsy biomarkers and valuable therapeutic agents was explored. Exosomes function as messengers transporting miRNAs between cells. This cell-to-cell communication plays an important role in regulating physiological as well as pathological processes, including the reprogramming of target cells, in terms of tumor invasion and tumor growth and angiogenesis [66]. Furthermore, exosomes prevent the miRNAs damage because of their double-layered membrane [45]. As described in our review, exo-miRNAs have been shown to have diagnostic and prognostic value in various cancer types, including lung, colorectal, ovarian, breast and hematological cancers. Exo-miRNAs can be used as ideal tools for non-invasive screening and targeting of cancer cells in the clinic, classifying cancers, and forecast responses to specific drug treatments. However, although many studies have shown these significant roles of exo-miRNAs, more research and further investigations are required, to obtain more validating data on the utility and effectiveness of utilizing exo-miRNAs as a diagnostic, prognostic, and monitoring tool for cancers. Indeed, one of the limitations of exo-miRNAs tumor screening is due to the correlation of tumor-related exo-miRNAs with changes related to tumor progression in terms of quality and quantity. Exo-miRNAs should not be considered specific tumor markers since each patient can have several parallel tumors, producing a series of exo-miRNAs. For example, obesity may affect serum exo-miRNA measurements [111–114]. In addition to obesity, chronic inflammation, and metabolic diseases can interfere with exo-miRNA signals [115–119]. The diet also induces circulating exosomal miRNAs, especially the consumption of pasteurized cow's milk which transmits milk exosomes and miRNAs into the circulation and peripheral tissues [120–124]. Cow milk consumption, for example, increases serum levels of oncogenic microRNA-21 [124]. In addition to this, there is recent concern that bovine milk-derived exo-miRNAs from pasteurized cow's milk promote cancerogenesis and lymphomagenesis [125–129].

Moreover, another challenge is the identification of easier and cheaper, robust, and specific technologies for exosomes isolation and exo-miRNAs detection. Based on these difficulties, new efforts in the settings of target-guided approaches are needed, selecting and characterizing exosomal subpopulations related to the expression of cell-specific proteins so that they can be used as baits for exo-miRNAs screening [130]. In conclusion, deeper knowledge of these exo-miRNAs as circulating biomarkers may lead not only to better comprehension of tumor diagnosis and progression but also to the newest therapeutic approaches in clinical oncology (Figure 2).

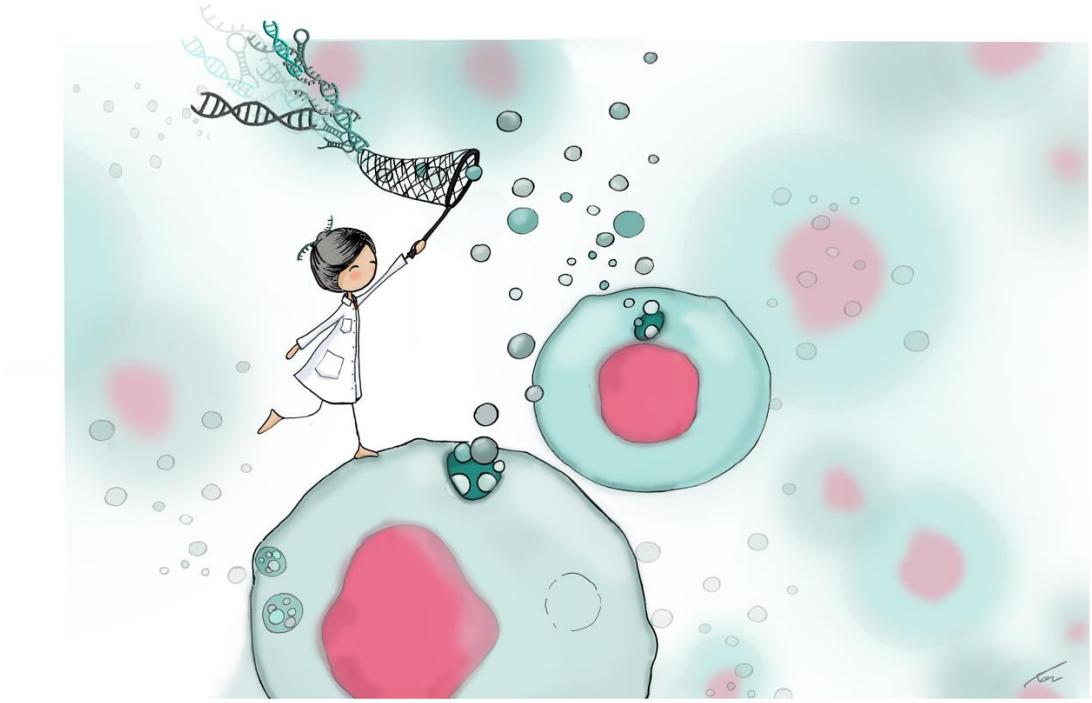

**Figure 2.** The exo-miRNAs hunter. In this captivating image, a skilled scientist gracefully captures tumor-derived exosomes, depicted as bubbles originating from cancer cells, using a fine net. These exosomes carry miRNAs (microRNAs), tiny molecular messengers with immense potential for understanding and combating cancer. The intricate process of netting these exosomes unveils a delicate dance of discovery, as researchers strive to unravel the secrets held within these tiny vesicles. Through their tireless efforts, scientists hope to harness the power of these miRNA-containing exosomes to unlock new insights, diagnostic tools, and therapeutic strategies in the ongoing fight against cancer.

## Conflicts of Interests

The authors declare no conflict of interest.

## Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## Authors' Contribution

Conceptualization: E.I., S.M., D.M, A.M.Z. and N.N.; Collection and assembly of data: A.M.Z., N.N., R.R., M.G., E.M., E.V., A.B., A.A. and G.F.;Data analysis and interpretation: E.I., S.M., D.M, A.M.Z., N.N., I.Q. and M.G.; Writing – Original Draft: A.M.Z., N.N., D.M., R.R., M.G., E.A.M., E.V., A.B., A.A., G.F., I.Q., S.M. and E.I.; Writing—review and editing: A.M.Z., N.N., D.M., R.R., M.G., E.A.M., E.V., A.B., A.A., G.F., I.Q., S.M. and E.I.; All authors have read and agreed to the published version of the manuscript.

## References


[1] Maia J, Caja S, Strano Moraes MC, Couto N, Costa-Silva B. Exosome-Based Cell-Cell Communication in the Tumor Microenvironment. *Front. Cell Dev. Biol.* 2018, 6:18.

[2] Hannafon BN, Ding WQ. Intercellular Communication by Exosome-Derived microRNAs in Cancer. *Int. J. Mol. Sci.* 2013, 14(7):14240–14269.

[3] Sun Z, Shi K, Yang S, Liu J, Zhou Q, *et al.* Effect of exosomal miRNA on cancer biology and clinical applications. *Mol. Cancer*. 2018, 17(1):147.

[4] Saleem T, Sumrin A, Bilal M, Bashir H, Khawar MB. Tumor-derived extracellular vesicles: Potential tool for cancer diagnosis, prognosis, and therapy. *Saudi. J. Biol. Sci.* 2022, 29(4):2063–2071.

[5] van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* 2018, 19(4):213–228.

[6] Subedi P, Schneider M, Philipp J, Azimzadeh O, Metzger F, *et al.* Comparison of methods to isolate proteins from extracellular vesicles for mass spectrometry-based proteomic analyses. *Anal. Biochem.* 2019, 584:113390.

[7] Veziroglu EM, Mias GI. Characterizing Extracellular Vesicles and Their Diverse RNA Contents. *Front. Genet.* 2020, 11:700.

[8] Doyle LM, Wang MZ. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells* 2019, 8(7):727.

[9] Li J, He X, Deng Y, Yang C. An Update on Isolation Methods for Proteomic Studies of Extracellular Vesicles in Biofluids. *Molecules* 2019, 24(19):3516.

[10] Pan BT, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes *in vitro*: Selective externalization of the receptor. *Cell* 1983, 33(3):967–978.

[11] Iaccino E, Mimmi S, Dattilo V, Marino F, Candeloro P, *et al.* Monitoring multiple myeloma by idiotype-specific peptide binders of tumor-derived exosomes. *Mol. Cancer* 2017, 16(1):159.

[12] Ramasubramanian L, Kumar P, Wang A. Engineering Extracellular Vesicles as Nanotherapeutics for Regenerative Medicine. *Biomolecules* 2019, 10(1):48.

[13] Liang Y, Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. *Theranostics* 2021, 11(7):3183–3195.

[14] Zhu L, Sun HT, Wang S, Huang SL, Zheng Y, Wang CQ, *et al.* Isolation and characterization of exosomes for cancer research. *J. Hematol. Oncol.* 2020, 13(1):152.

[15] Henne WM, Buchkovich NJ, Emr SD. The ESCRT Pathway. *Dev. Cell* 2011, 21(1):77–91.

[16] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science* 2020, 367(6478):eaau6977.

[17] Lee C, Han J, Jung Y. Pathological Contribution of Extracellular Vesicles and Their MicroRNAs to Progression of Chronic Liver Disease. *Biology* 2022, 11(5):637.

[18] Zhang L, Yu D. Exosomes in cancer development, metastasis, and immunity. *Biochim. Biophys. Acta Rev. Cancer*. 2019, 1871(2):455–468.

[19] Xie S, Zhang Q, Jiang L. Current Knowledge on Exosome Biogenesis, Cargo-Sorting Mechanism and Therapeutic Implications. *Membranes* 2022, 12(5):498.

[20] Wei D, Zhan W, Gao Y, Huang L, Gong R, Wang W, *et al.* RAB31 marks and controls an ESCRT-independent exosome pathway. *Cell Res.* 2021, 31(2):157–177.

[21] Yang D, Zhang W, Zhang H, Zhang F, Chen L, Ma L, *et al.* Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. *Theranostics* 2020, 10(8):3684–3707.

[22] Jeppesen DK, Hvam ML, Primdahl-Bengtson B, Boysen AT, Whitehead B, *et al.* Comparative analysis of discrete exosome fractions obtained by differential centrifugation. *J. Extracell. Vesicles* 2014, 3(1):25011.

[23] Yu D, Li Y, Wang M, Gu J, Xu W, *et al.* Exosomes as a new frontier of cancer liquid biopsy. *Mol. Cancer* 2022, 21:56.
[24] Andreu Z, Rivas E, Sanguino-Pascual A, Lamana A, Marazuela M, *et al.* Comparative analysis of EV isolation procedures for miRNAs detection in serum samples. *J. Extracell. Vesicles* 2016, 5:31655.
[25] Crossland RE, Norden J, Bibby LA, Davis J, Dickinson AM. Evaluation of optimal extracellular vesicle small RNA isolation and qRT-PCR normalisation for serum and urine. *J. Immunol. Methods* 2016, 429:39–49.
[26] Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, *et al.* A Comparative Study of Serum Exosome Isolation Using Differential Ultracentrifugation and Three Commercial Reagents. *PLOS ONE* 2017, 12(1):e0170628.
[27] Ha M, Kim VN. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* 2014, 15(8):509–524.
[28] Ho PTB, Clark IM, Le LTT. MicroRNA-Based Diagnosis and Therapy. I*nt. J. Mol. Sci.* 2022, 23(13):7167.
[29] Du S, Ling H, Guo Z, Cao Q, Song C. Roles of exosomal miRNA in vascular aging. *Pharmacol. Res.* 2021, 165:105278.
[30] Manna I, De Benedittis S, Quattrone A, Maisano D, Iaccino E, Quattrone A. Exosomal miRNAs as Potential Diagnostic Biomarkers in Alzheimer's Disease. *Pharmaceuticals* 2020, 13(9):243.
[31] Yu X, Odenthal M, Fries JWU. Exosomes as miRNA Carriers: Formation–Function–Future. *Int. J. Mol. Sci.* 2016, 17(12):2028.
[32] Yang F, Ning Z, Ma L, Liu W, Shao C, Shu Y, *et al.* Exosomal miRNAs and miRNA dysregulation in cancer-associated fibroblasts. *Mol. Cancer* 2017, 16(1):148.
[33] Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, *et al.* MicroRNAs in body fluids—the mix of hormones and biomarkers. *Nat. Rev. Clin. Oncol.* 2011, 8(8):467–477.
[34] Wang X, Zhou Y, Gao Q, Ping D, Wang Y, *et al.* The Role of Exosomal microRNAs and Oxidative Stress in Neurodegenerative Diseases. *Oxid. Med. Cell Longev.* 2020, 2020:e3232869.
[35] Dilsiz N. Role of exosomes and exosomal microRNAs in cancer. *Future Sci. OA* 2020, 6(4):FSO465.
[36] Zhu J, Lu K, Zhang N, Zhao Y, Ma Q, *et al.* Myocardial reparative functions of exosomes from mesenchymal stem cells are enhanced by hypoxia treatment of the cells via transferring microRNA-210 in an nSMase2-dependent way. *Artif. Cells Nanomedicine Biotechnol.* 2018, 46(8):1659–1670.
[37] Kosaka N, Iguchi H, Hagiwara K, Yoshioka Y, Takeshita F, *et al.* Neutral Sphingomyelinase 2 (nSMase2)-dependent Exosomal Transfer of Angiogenic MicroRNAs Regulate Cancer Cell Metastasis*. *J. Biol. Chem.* 2013, 288(15):10849–10859.
[38] Mahmoudi A, Butler AE, Jamialahmadi T, Sahebkar A. The role of exosomal miRNA in nonalcoholic fatty liver disease. *J. Cell Physiol.* 2022, 237(4):2078–2094.
[39] Guduric-Fuchs J, O'Connor A, Camp B, O'Neill CL, Medina RJ, Simpson DA. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. *BMC Genomics* 2012, 13:357.
[40] Cheng Q, Li X, Wang Y, Dong M, Zhan F, Liu J. The ceramide pathway is involved in the survival, apoptosis and exosome functions of human multiple myeloma cells *in vitro*. *Acta Pharmacol. Sin.* 2018, 39(4):561–568.
[41] Rekker K, Saare M, Roost AM, Kubo AL, Zarovni N, *et al.* Comparison of serum exosome isolation methods for microRNA profiling. *Clin. Biochem.* 2014, 47(1):135–138.

[42] Coenen-Stass AML, Magen I, Brooks T, Ben-Dov IZ, Greensmith L, *et al.* Evaluation of methodologies for microRNA biomarker detection by next generation sequencing. *RNA Biol.* 2018, 15(8):1133–1145.
[43] Preethi KA, Selvakumar SC, Ross K, Jayaraman S, Tusubira D, *et al.* Liquid biopsy: Exosomal microRNAs as novel diagnostic and prognostic biomarkers in cancer. *Mol. Cancer* 2022, 21(1):54.s
[44] Lin B, Jiang J, Jia J, Zhou X. Recent Advances in Exosomal miRNA Biosensing for Liquid Biopsy. *Molecules* 2022, 27(21):7145.
[45] Li C, Zhou T, Chen J, Li R, Chen H, *et al.* The role of Exosomal miRNAs in cancer. *J. Transl. Med.* 2022, 20:6.
[46] Wu J, Hu S, Zhang L, Xin J, Sun C, *et al.* Tumor circulome in the liquid biopsies for cancer diagnosis and prognosis. *Theranostics* 2020, 10(10):4544–4556.
[47] Maisano D, Mimmi S, Russo R, Fioravanti A, Fiume G, *et al.* Uncovering the Exosomes Diversity: A Window of Opportunity for Tumor Progression Monitoring. *Pharmaceuticals* 2020, 13(8):180.
[48] Tay TKY, Tan PH. Liquid Biopsy in Breast Cancer: A Focused Review. *Arch. Pathol. Lab. Med.* 2020, 145(6):678–686.
[49] Whiteside TL, Diergaarde B, Hong CS. Tumor-Derived Exosomes (TEX) and Their Role in Immuno-Oncology. *Int. J. Mol. Sci.* 2021, 22(12):6234.
[50] Ingenito F, Roscigno G, Affinito A, Nuzzo S, Scognamiglio I, *et al.* The Role of Exo-miRNAs in Cancer: A Focus on Therapeutic and Diagnostic Applications. *Int. J. Mol. Sci.* 2019, 20(19):4687.
[51] Moloudizargari M, Hekmatirad S, Mofarahe ZS, Asghari MH. Exosomal microRNA panels as biomarkers for hematological malignancies. *Curr. Probl. Cancer* 2021, 45(5):100726.
[52]Rodriguez-Abreu D, Bordoni A, Zucca E. Epidemiology of hematological malignancies. *Ann. Oncol.* 2007, 18:i3–i8.
[53] Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, *et al.* Breast Cancer Statistics, 2022. *CA Cancer J. Clin.* 2022, 72(6):524–541.
[54] Eichelser C, Stückrath I, Müller V, Milde-Langosch K, Wikman H, *et al.* Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients. *Oncotarget* 2014, 5(20):9650–9663.
[55] Fong MY, Zhou W, Liu L, Alontaga AY, Chandra M, *et al.* Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. *Nat. Cell Biol.* 2015, 17(2):183–194.
[56] Hannafon BN, Trigoso YD, Calloway CL, Zhao YD, Lum DH, Welm AL, *et al.* Plasma exosome microRNAs are indicative of breast cancer. *Breast Cancer Res.* 2016, 18(1):90.
[57] Asgari R, Rezaie J. Differential Expression of Serum Exosomal miRNAs in Breast Cancer Patients and Healthy Controls. *Adv. Pharm. Bull.* 2022, 12(4):858–862.
[58] Santos JC, Lima N da S, Sarian LO, Matheu A, Ribeiro ML, Derchain SFM. Exosome-mediated breast cancer chemoresistance via miR-155 transfer. *Sci. Rep.* 2018, 8(1):829.
[59] Zhang Z, Zhang L, Yu G, Sun Z, Wang T, *et al.* Exosomal miR-1246 and miR-155 as predictive and prognostic biomarkers for trastuzumab-based therapy resistance in HER2-positive breast cancer. *Cancer Chemother. Pharmacol.* 2020, 86(6):761–772.
[60] Feng YH, Tsao CJ. Emerging role of microRNA-21 in cancer. *Biomed. Rep.* 2016, 5(4):395–402.
[61] Menezes SV, Sahni S, Kovacevic Z, Richardson DR. Interplay of the iron-regulated metastasis suppressor NDRG1 with epidermal growth factor receptor (EGFR) and oncogenic signaling. *J. Biol. Chem.* 2017, 292(31):12772.

[62] Weidle UH, Dickopf S, Hintermair C, Kollmorgen G, Birzele F, *et al.* The Role of micro RNAs in Breast Cancer Metastasis: Preclinical Validation and Potential Therapeutic Targets. *Cancer Genomics Proteomics* 2018, 15(1):17–39.
[63] Yuan X, Qian N, Ling S, Li Y, Sun W, *et al.* Breast cancer exosomes contribute to pre-metastatic niche formation and promote bone metastasis of tumor cells. *Theranostics* 2021, 11(3):1429–1445.
[64] Li D, Wang J, Ma LJ, Yang HB, Jing JF, *et al.* Identification of serum exosomal miR-148a as a novel prognostic biomarker for breast cancer. *Eur. Rev. Med. Pharmacol. Sci.* 2020, 24(13):7303–7309.
[65] Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. *Int. J. Women's Health* 2019, 11:287–299.
[66] Padda J, Khalid K, Khedr A, Patel V, Al-Ewaidat OA, *et al.* Exosome-Derived microRNA: Efficacy in Cancer. *Cureus* 2021, 13(8):e17441.
[67] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. *Gynecol. Oncol.* 2008, 110(1):13–21.
[68] Meng X, Müller V, Milde-Langosch K, Trillsch F, Pantel K, *et al.* Diagnostic and prognostic relevance of circulating exosomal miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. *Oncotarget* 2016, 7(13):16923–16935.
[69] Su YY, Sun L, Guo ZR, Li JC, Bai TT, *et al.* Upregulated expression of serum exosomal miR-375 and miR-1307 enhance the diagnostic power of CA125 for ovarian cancer. *J. Ovarian Res.* 2019, 12:6.
[70] Ying X, Wu Q, Wu X, Zhu Q, Wang X, *et al.* Epithelial ovarian cancer-secreted exosomal miR-222-3p induces polarization of tumor-associated macrophages. *Oncotarget* 2016, 7(28):43076–43087.
[71] Keum N, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat. Rev. Gastroenterol. Hepatol.* 2019, 16(12):713–732.
[72] Shi Y, Zhuang Y, Zhang J, Chen M, Wu S. Four circulating exosomal miRNAs as novel potential biomarkers for the early diagnosis of human colorectal cancer. *Tissue Cell* 2021, 70:101499.
[73] Ogata-Kawata H, Izumiya M, Kurioka D, Honma Y, Yamada Y, *et al.* Circulating Exosomal microRNAs as Biomarkers of Colon Cancer. *PLOS ONE* 2014, 9(4):e92921.
[74] Matsumura T, Sugimachi K, Iinuma H, Takahashi Y, Kurashige J, *et al.* Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. *Br. J. Cancer* 2015, 113(2):275–281.
[75] Liu W, Yang D, Chen L, Liu Q, Wang W, *et al.* Plasma Exosomal miRNA-139-3p is a Novel Biomarker of Colorectal Cancer. *J. Cancer* 2020, 11(16):4899–4906.
[76] Zhang N, Zhang PP, Huang JJ, Wang ZY, Zhang ZH, *et al.* Reduced serum exosomal miR-874 expression predicts poor prognosis in colorectal cancer. *Eur. Rev. Med. Pharmacol. Sci.* 2020, 24(2):664–672.
[77] Sun L, Liu X, Pan B, Hu X, Zhu Y, *et al.* Serum exosomal miR-122 as a potential diagnostic and prognostic biomarker of colorectal cancer with liver metastasis. *J. Cancer* 2020, 11(3):630–637.
[78] Thai AA, Solomon BJ, Sequist LV, Gainor JF, Heist RS. Lung cancer. *The Lancet* 2021, 398(10299):535–554.
[79] Masaoutis C, Mihailidou C, Tsourouflis G, Theocharis S. Exosomes in lung cancer diagnosis and treatment. From the translating research into future clinical practice. *Biochimie* 2018, 151:27–36.
[80] Cazzoli R, Buttitta F, Nicola MD, Malatesta S, Marchetti A, *et al.* microRNAs Derived from Circulating Exosomes as Noninvasive Biomarkers for Screening and Diagnosing Lung Cancer. *J. Thorac. Oncol.* 2013, 8(9):1156–1162.

[81] Hsu YL, Hung JY, Chang WA, Lin YS, Pan YC, *et al.* Hypoxic lung cancer-secreted exosomal miR-23a increased angiogenesis and vascular permeability by targeting prolyl hydroxylase and tight junction protein ZO-1. *Oncogene* 2017, 36(34):4929–4942.
[82] Jin X, Chen Y, Chen H, Fei S, Chen D, *et al.* Evaluation of Tumor-Derived Exosomal miRNA as Potential Diagnostic Biomarkers for Early-Stage Non–Small Cell Lung Cancer Using Next-Generation Sequencing. *Clin. Cancer Res.* 2017, 23(17):5311–5319.
[83] Zhang Y, Xu H. Serum exosomal miR-378 upregulation is associated with poor prognosis in non-small-cell lung cancer patients. *J. Clin. Lab. Anal.* 2020, 34(6):e23237.
[84] Zhang Z, Tang Y, Song X, Xie L, Zhao S, *et al.* Tumor-Derived Exosomal miRNAs as Diagnostic Biomarkers in Non-Small Cell Lung Cancer. *Front. Oncol.* 2020, 10:560025
[85] Huang D, Qu D. Early diagnostic and prognostic value of serum exosomal miR-1246 in non-small cell lung cancer. *Int. J. Clin. Exp. Pathol.* 2020, 13(7):1601–1607.
[86] Zheng Q, Ding H, Wang L, Yan Y, Wan Y, *et al.* Circulating Exosomal miR-96 as a Novel Biomarker for Radioresistant Non-Small-Cell Lung Cancer. *J. Oncol.* 2021, 2021:e5893981.
[87] Zhang L, Pan L, Xiang B, Zhu H, Wu Y, *et al.* Potential role of exosome-associated microRNA panels and *in vivo* environment to predict drug resistance for patients with multiple myeloma. *Oncotarget* 2016, 7(21):30876–30891.
[88] Wang X, He L, Huang X, Zhang S, Cao W, *et al.* Recent Progress of Exosomes in Multiple Myeloma: Pathogenesis, Diagnosis, Prognosis and Therapeutic Strategies. *Cancers* 2021, 13(7):1635.
[89] Manier S, Liu CJ, Avet-Loiseau H, Park J, Shi J, *et al.* Prognostic role of circulating exosomal miRNAs in multiple myeloma. *Blood* 2017, 129(17):2429–2436.
[90] Zhang Z, Li Y, Geng C, Wang H, Chen W. Potential Relationship between Clinical Significance and Serum Exosomal miRNAs in Patients with Multiple Myeloma. *BioMed Res. Int.* 2019, 2019:1575468.
[91] Al-Naeeb AB, Ajithkumar T, Behan S, Hodson DJ. Non-Hodgkin lymphoma. *BMJ* 2018, 362:k3204.
[92] Cao D, Cao X, Jiang Y, Xu J, Zheng Y, *et al.* Circulating exosomal microRNAs as diagnostic and prognostic biomarkers in patients with diffuse large B-cell lymphoma. *Hematol. Oncol.* 2022, 40(2):172–180.
[93] Feng Y, Zhong M, Zeng S, Wang L, Liu P, *et al.* Exosome-derived miRNAs as predictive biomarkers for diffuse large B-cell lymphoma chemotherapy resistance. *Epigenomics* 2019, 11(1):35–51.
[94] Xiao XB, Gu Y, Sun DL, Ding LY, Yuan XG, *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):1620–1625.
[95] Liu J, Han Y, Hu S, Cai Y, Yang J, *et al.* Circulating Exosomal MiR-107 Restrains Tumorigenesis in Diffuse Large B-Cell Lymphoma by Targeting 14-3-3η. *Front. Cell Dev. Biol.* 2021, 9:667800.
[96] Rinaldi F, Marchesi F, Palombi F, Pelosi A, Di Pace AL, *et al.* MiR-22, a serum predictor of poor outcome and therapy response in diffuse large B-cell lymphoma patients. *Br. J. Haematol.* 2021, 195(3):399–404.
[97] Fathullahzadeh S, Mirzaei H, Honardoost MA, Sahebkar A, Salehi M. Circulating microRNA-192 as a diagnostic biomarker in human chronic lymphocytic leukemia. *Cancer Gene. Ther.* 2016, 23(10):327–332.
[98] Filip AA, Grenda A, Popek S, Koczkodaj D, Michalak-Wojnowska M, *et al.* Expression of circulating miRNAs associated with lymphocyte differentiation and activation in CLL—another piece in the puzzle. *Ann. Hematol.* 2017, 96(1):33–50.
[99] Hegde GV, Peterson KJ, Emanuel K, Mittal AK, Joshi AD, *et al.* Hedgehog-Induced Survival of B-Cell Chronic Lymphocytic Leukemia Cells in a Stromal Cell

Microenvironment: A Potential New Therapeutic Target. *Mol. Cancer Res.* 2008, 6(12):1928–1936.
[100]Nisticò N, Maisano D, Iaccino E, Vecchio E, Fiume G, *et al.* Role of Chronic Lymphocytic Leukemia (CLL)-Derived Exosomes in Tumor Progression and Survival. *Pharmaceuticals* 2020, 13(9):244.
[101]Paggetti J, Haderk F, Seiffert M, Janji B, Distler U, *et al.* Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. *Blood* 2015, 126(9):1106–1117.
[102]Elton TS, Selemon H, Elton SM, Parinandi NL. Regulation of the MIR155 host gene in physiological and pathological processes. *Gene* 2013, 532(1):1–12.
[103]Bruns H, Büttcher M, Qorraj M, Fabri M, Jitschin S, *et al.* CLL-cell-mediated MDSC induction by exosomal miR-155 transfer is disrupted by vitamin D. *Leukemia* 2017, 31(4):985–988.
[104]Selcuklu SD, Donoghue MTA, Spillane C. miR-21 as a key regulator of oncogenic processes. *Biochem. Soc. Trans.* 2009, 37(4):918–925.
[105]Méndez-Ferrer S, Bonnet D, Steensma DP, Hasserjian RP, Ghobrial IM, *et al.* Bone marrow niches in haematological malignancies. *Nat. Rev. Cancer* 2020, 20(5):285–298.
[106]Mraz M, Chen L, Rassenti LZ, Ghia EM, Li H, *et al.* miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1. *Blood* 2014, 124(1):84–95.
[107]Stamatopoulos B, Van Damme M, Crompot E, Dessars B, Housni HE, *et al.* Opposite Prognostic Significance of Cellular and Serum Circulating MicroRNA-150 in Patients with Chronic Lymphocytic Leukemia. *Mol. Med.* 2015, 21(1):123–133.
[108]Kollinerova S, Vassanelli S, Modriansky M. The role of miR-29 family members in malignant hematopoiesis. *Biomed. Pap.* 2014, 158(4):489–501.
[109]Yeh YY, Ozer HG, Lehman AM, Maddocks K, Yu L, *et al.* Characterization of CLL exosomes reveals a distinct microRNA signature and enhanced secretion by activation of BCR signaling. *Blood* 2015, 125(21):3297–3305.
[110]Favero A, Segatto I, Perin T, Belletti B. The many facets of miR-223 in cancer: Oncosuppressor, oncogenic driver, therapeutic target, and biomarker of response. *Wiley Interdiscip. Rev. RNA* 2021, 12(6):e1659.
[111]Castaño C, Kalko S, Novials A, Párrizas M. Obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice. *Proc. Natl. Acad. Sci.* 2018, 115(48):12158–12163.
[112]Earle A, Bessonny M, Benito J, Huang K, Parker H, *et al.* Urinary Exosomal MicroRNAs as Biomarkers for Obesity-Associated Chronic Kidney Disease. *J. Clin. Med.* 2022, 11(18):5271.
[113]Ji C, Guo X. The clinical potential of circulating microRNAs in obesity. *Nat. Rev. Endocrinol.* 2019, 15(12):731–743.
[114]Ortiz-Dosal A, Rodil-Garcia P, Salazar-Olivo LA. Circulating microRNAs in human obesity: a systematic review. *Biomarkers* 2019, 24(6): 499–509.
[115]Povero D, Eguchi A, Li H, Johnson CD, Papouchado BG, *et al.* Circulating Extracellular Vesicles with Specific Proteome and Liver MicroRNAs Are Potential Biomarkers for Liver Injury in Experimental Fatty Liver Disease. *PLOS ONE* 2014, 9(12):e113651.
[116] Yao ZY, Chen WB, Shao SS, Ma SZ, Yang CB, *et al.* Role of exosome-associated microRNA in diagnostic and therapeutic applications to metabolic disorders. *J. Zhejiang Univ. Sci. B* 2018, 19(3):183–198.
[117]Szabo G. Exosomes and MicroRNA-223 at the Intersection of Inflammation and Fibrosis in NAFLD. *Hepatology* 2021, 74(1):5.

[118]Hou X, Yin S, Ren R, Liu S, Yong L, *et al.* Myeloid-Cell–Specific IL-6 Signaling Promotes MicroRNA-223-Enriched Exosome Production to Attenuate NAFLD-Associated Fibrosis. *Hepatology* 2021, 74(1):116.
[119]Devhare PB, Ray RB. Extracellular vesicles: Novel mediator for cell to cell communications in liver pathogenesis. *Mol. Aspects Med.* 2018, 60:115–122.
[120]Baier SR, Nguyen C, Xie F, Wood JR, Zempleni J. MicroRNAs Are Absorbed in Biologically Meaningful Amounts from Nutritionally Relevant Doses of Cow Milk and Affect Gene Expression in Peripheral Blood Mononuclear Cells, HEK-293 Kidney Cell Cultures, and Mouse Livers. *J. Nutr.* 2014, 144(10):1495–1500.
[121]Manca S, Upadhyaya B, Mutai E, Desaulniers AT, Cederberg RA, *et al.* Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. *Sci. Rep.* 2018, 8(1):11321.
[122]Melnik BC, Weiskirchen R, Schmitz G. Milk exosomal microRNAs: friend or foe?—a narrative review. *ExRNA*, 2022, 4: 22.
[123]Weil PP, Reincke S, Hirsch CA, Giachero F, Aydin M, *et al.* Uncovering the gastrointestinal passage, intestinal epithelial cellular uptake, and AGO2 loading of milk miRNAs in neonates using xenomiRs as tracers. *Am. J. Clin. Nutr.* 2023, 117(6):1195–1210.
[124]Mutai E, Ramer-Tait AE, Zempleni J. MicroRNAs in bovine milk exosomes are bioavailable in humans but do not elicit a robust pro-inflammatory cytokine response. *ExRNA* 2020, 2(1):2.
[125]Melnik BC, John SM, Carrera-Bastos P, Cordain L, Leitzmann C, *et al.* The Role of Cow's Milk Consumption in Breast Cancer Initiation and Progression. *Curr. Nutr. Rep.* 2023, 12(1):122–140.
[126]Melnik BC, John SM, Carrera-Bastos P, Schmitz G. MicroRNA-21-Enriched Exosomes as Epigenetic Regulators in Melanomagenesis and Melanoma Progression: The Impact of Western Lifestyle Factors. *Cancers* 2020, 12(8):2111.
[127]Melnik BC, John SM, Weiskirchen R, Schmitz G. The endocrine and epigenetic impact of persistent cow milk consumption on prostate carcinogenesis. *J. Transl. Genet. Genom.* 2022, 6: 1–45.
[128]Melnik BC. Dairy consumption and hepatocellular carcinoma risk. *Ann. Transl. Med.* 2021, 9(8):736.
[129]Melnik BC, Stadler R, Weiskirchen R, Leitzmann C, Schmitz G. Potential Pathogenic Impact of Cow's Milk Consumption and Bovine Milk-Derived Exosomal MicroRNAs in Diffuse Large B-Cell Lymphoma. *Int. J. Mol. Sci.* 2023, 24(7):6102.
[130]Mimmi S, Zimbo AM, Rotundo S, Cione E, Nistic ò N, *et al.* SARS CoV-2 spike protein-guided exosome isolation facilitates detection of potential miRNA biomarkers in COVID-19 infections. *Clin. Chem. Lab. Med.* 2023, 61(8): 1518–1524.