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Review

High-throughput analysis and functional interpretation of extracellular vesicle content in hematological malignancies

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ABSTRACT

Extracellular vesicles (EVs) are membrane-coated particles secreted by virtually all cell types in response to different stimuli, both in physiological and pathological conditions. Their content generally reflects their biological functions and includes a variety of molecules, such as nucleic acids, proteins and cellular components. The role of EVs as signaling vehicles has been widely demonstrated. In particular, they are actively involved in the pathogenesis of several hematological malignancies (HM), mainly interacting with a number of target cells and inducing functional and epigenetic changes. In this regard, by releasing their cargo, EVs play a pivotal role in the bilateral cross-talk between tumor microenvironment and cancer cells, thus facilitating mechanisms of immune escape and supporting tumor growth and progression. Recent advances in high-throughput technologies have allowed the deep characterization and functional interpretation of EV content. In this review, the current knowledge on the high-throughput technology-based characterization of EV cargo in HM is summarized.

Contents

1. Introduction

Extracellular vesicles (EVs) include a heterogeneous group of membrane-coated particles, with a size ranging from 15 nm to 10 μm, released by several types of cells in both normal and pathological conditions, including tumors [43]. According to their size, shape, and biogenesis, EVs are subclassified into exosomes (Exo, 20–150 nm), microvesicles (MVs, 50–1000 nm), and apoptotic bodies (50–5000 nm). The term "oncosomes" (up to 10 μm) has been used to describe small and large EVs released by cancer cells [50]. While exosomes are formed by inward budding of endoplasmic

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reticulum, microvesicles derive from the outward budding of plasma membrane [16,26].

Several factors can induce EVs release from normal or tumor cells, including microenvironmental signals, oxygen tension or intracellular Ca2+ concentrations [22]. EVs cargo generally reflects parental cells and includes proteins, lipids, and nucleic acids, but also metabolites and cellular organelles [20,60,72]. EVs act as cellular signaling vehicles and exert pleiotropic effects on target cells either through direct interaction with cell surface receptors or by releasing their cargo into the recipient cells. Once inside their target cells, EVs can induce functional and epigenetic changes, influencing different physiological and pathological processes and exerting immuno-regulatory effects by acting as both immune suppressors and stimulators [7,64,73,84,88].

In the context of hematological malignancies (HM) there is growing evidence of the capacity of tumor EVs to favor the cross-talk between tumor cells and bone marrow (BM) microenvironmental cells, thus enhancing tumor growth and proliferation; nevertheless, the underlying molecular mechanisms are still unclear [2,7,51]. EVs derived from malignant cells may suppress normal hematopoiesis, thus contributing to the formation of leukemia-modified niches. Furthermore, the immunomodulatory effect of EVs is involved in the mechanisms of immune escape adopted by neoplastic cells [6,63]. EVs exert their effects to target cells by delivering different bioactive molecules including growth factors, cytokines and chemokines, enzymes and other genetic materials [33]. Amongst them, microRNAs (miRs), which are non-coding single-stranded RNAs of approximately 19–24 nucleotides in length, are significantly represented in tumor-released EVs. In particular, aberrant levels of tumor-derived exosomal miRs have been reported in patients affected by HM, confirming their pathogenetic role [8]. Of note, because a single miR regulates multiple gene targets, the deregulation of miRs may lead to a wide range of transcript alterations and may modulate several molecular pathways [74–75].

Recent studies suggested that miRs are also involved in drug resistance, mainly by downregulating apoptotic genes or impairing cell differentiation [82].

In addition, long non-coding RNAs (lncRNAs) have emerged recently as essential gene regulators, with much evidence of their involvement in cancer development and progression. Unlike miRs, lncRNAs display high cell and tissue specificity, thus being suitable for diagnostic and prognostic purposes [12,52,86].

High-throughput technologies, including mass spectrometry-based approaches and next-generation sequencing, have permitted a deep characterization of EVs content by identifying a variety of miRs, lncRNAs and other molecules acting as potential disease biomarkers and putative therapeutic targets [69].

The aim of this review is to discuss the recent data regarding the functional role of EVs in HM, with a particular focus on multiple myeloma (MM), chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML).

2. High-throughput technologies, analysis and data interpretation of EV-associated molecules

EVs are considered a sub-repertoire of the cell of origin, because they can store various proteins, mRNAs, miRs, and lncRNAs, depending on the cell of origin; therefore, they can be exploited as rich reservoirs of disease biomarkers that can be released into body fluids. For this reason, the detection of tumor-derived exosomes (TEX) via blood tests are provided with clinical potential, offering a more comprehensive assessment of cancer diagnosis, prognosis, and progression [41,70].

The vast majority of the studies employed global high-throughput analyses to dissect EVs content even starting from a tiny material, thus identifying the genomic, transcriptomic, proteomic, lipidomic, and metabolomic profiles of EVs and leading to a massive generation of EV-related OMICS data that are now available in literature and in different free-to-use web databases. Among these, ExoCarta (http://www.exocarta.org), Vesiclepedia (http://www.microvesicles.org), and EVpedia (http://evpedia.info) include an integrated database of high-throughput datasets from both prokaryotic and eukaryotic vesicles. EVpedia also provides an array of tools for global analysis of EVs content, such as Gene Ontology analysis, network analysis of vesicular proteins and mRNAs, and a comparison of vesicular datasets by ortholog identification. These resources represent a fundamental repository to elucidate the novel functions of these complex extracellular organelles, underlying the molecular mechanisms of different disease conditions from which EVs are isolated.

Furthermore, as the purity of EVs pools and the consequent results strictly depend on the type of EVs isolation protocol, all these databases display the information regarding the isolation procedures employed. In addition, several free-to-use web-based and commercial software packages are available for the analysis of EVs datasets, in order to evaluate the biological functions of EVs components. Such tools provide biological annotations in the explored dataset, thus identifying the pathways and molecular processes that may be influenced by EVs; among these, DAVID is commonly used as Web-based enrichment analysis tool [31]. Cytoscape is an open-source tool for analysis and visualization of interaction networks among proteins [68]. IPA® and MetaCore™ are commercially available softwares providing multiple options for analyzing OMICS datasets. The peculiarity and reliability of both softwares rely on customized datasets integrated through the available scientific databases that can be updated with new data from the literature. This aspect represents the major strength of these software tools [25,53].

3. Functional interpretation of EVs content in HM

3.1. Multiple myeloma

EVs from different cells of origin usually have a peculiar protein cargo. However, recent studies reported that EVs isolated from distinct cell lineages may share several proteins, irrespective of their parental cells [17,48].

By using shotgun proteomics, Harshman et al. characterized the protein composition of EVs derived from two different multiple myeloma cell lines (MM.1S and U266). They found a high reciprocal similarity in protein content, consistently with other proteomic studies [47,49]. Nevertheless, MM.1S and U266 differed for 32 (10%) and 13 (4%) proteins, respectively. Further application of label-free spectral count relative quantification allowed the evaluation of differences in protein abundance and showed that EVs had a different protein abundance compared to their cell of origin. These data suggest that EVs preserve a set of unique proteins depending on their cell of origin as well as their biological functions [24].

In order to define a specific set of MM-derived EV proteins, the same Authors further performed a global systematic proteomic analysis. This study aimed also at identifying circulating myeloma associated markers, showing that EVs isolated from patients' serum and MM cell lines had higher levels of Major Histocompatibility Complex Class I (MCHI) and its binding protein β2.

Microglobulin (β2-MG) as compared to healthy donors. Furthermore, EVs isolated from corticosteroid-resistant MM cell lines (MM.1R) and newly diagnosed MM patients showed higher expression of the single-chain transmembrane glycoprotein CD44 compared to corticosteroid-sensitive cell lines (MM.1S), thus

suggesting that serum CD44 could be a potential prognostic biomarker [23].

Several studies confirmed that the bone marrow microenvironment strongly supports tumor growth in the majority of HM. Growing evidence suggests that TEX are involved in the modulation of bone marrow microenvironment and can induce malignant transformation by transferring proteins and nucleic acids (miRs, DNA and non-coding RNA) to target cells, thus affecting their phenotype and function [11,27,57,90]. Roccaro et al. highlighted the contribution of MM bone marrow mesenchymal stromal cells (MM BM-MSCs)-derived exosomes in tumor growth and disease progression. They found that normal and MM BM-MSCs-derived exosomes differed in the miR profile, observing a lower miR-15a expression in MM versus normal BM-MSCs-derived exosomes. Lower levels of miR-15a were also detected in MM cells, suggesting its role as a possible tumor suppressor. Moreover, protein array analysis was used to characterize the protein content of MM BM-MSCs-derived EVs, thus showing an enrichment of oncogenic proteins and regulators of adhesion and migration. Although this study focused only on BM-MSCs-derived EVs and analyzed a limited spectrum of proteins, these results supported the hypothesis of an active participation of MM BM-MSCs-derived exosomes in MM growth and progression [65]. Subsequent studies have tried to elucidate the deregulation of miR expression in MM. Zhang et al. confirmed the participation of exosome-derived miRs in the in vivo intercellular cross-talk in MM patients. Using microarray profiling, this study highlighted the predictive value of serum exosome-associated miRs expression in drug resistance in a large cohort of MM patients. In particular, four exosomal miRs (miR-16, miR15a, miR-20a, and miR-17) were downregulated in bortezomib-resistant patients, suggesting their possible use as drug resistance biomarkers [90].

Further studies enhanced the prognostic significance of circulating exosomal miRs in MM (Table 1). A recent study assessed the relationship between miR levels and outcome in a cohort of 156 newly diagnosed patients, uniformly treated with bortezomib and dexamethasone as frontline regimen. Small RNA sequencing of serum circulating exosomes and subsequent quantitative reverse transcription-polymerase chain reaction (qRT-PCR) array allowed the identification of two circulating miRs, let-7b and miR-18a, both paired with dismal outcomes. In particular, the low expression of let-7b and miR-18a was significantly associated with decreased overall survival (OS) and progression free survival (PFS) [44]. Several studies have shown that miR-125b-5p directly regulates the expression of p53, thus supporting tumor cell proliferation (Fig. 1) [40,54,83].

Jiang et al. performed miR microarray analysis and found that 12 miRs were differentially expressed in MM patients (n = 6) and healthy controls (n = 6). Of note, high expression of miR-125b-5p was associated with extramedullary involvement and shorter event-free survival (EFS) in patients uniformly treated with Bortezomib -Thalidomide-Dexamethasone containing regimen [36].

Other miRs, such as miR-21, miR17-92, and miR-34, are altered in MM [13–14,37,42]. Considering their natural capability to transport miRs and anti-miRs [66], several preclinical and clinical trials have used exosomes to restore normal levels of tumor suppressor miRs (“miRs mimics”) or to inhibit overexpressed oncogenic miRs (“antagomiRs”) [1,21,32,80,89,91].

Further investigations are needed to assess the possible use of miRs as therapeutic targets in clinical practice.

3.2. Chronic lymphocytic leukemia (CLL)

Several studies explored the role of CLL-derived exosomes in the pathogenesis of this disease, strongly associated with both permissive microenvironment and disrupted immune response. As demonstrated for other HM, CLL-derived exosomes can modify the transcriptional profile of the recipient cells, thus enhancing

Table 1
EV-associated biomarkers in multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML).

MM	Methods	Source of EVs	Biomarker	Effect	Reference
	miRNA microarray analysis	Normal and MM BM MSCs	miR-15a	Tumor suppressor	[65]
	Proteomic analysis	MM cell lines, PB and BM from MM patients	CD44	Drug resistance	[23]
	miRNA microarray analysis	MM cell lines, serum from MM patients	miR-16-5p, miR-15a-5p, miR-20a-50, miR-17-5p	Drug resistance	[90]
	RNA-sequencing	Serum from MM patients and healthy donors	let7-b, miR-18a	Decreased OS and PFS	[44]
	miRNA microarray analysis	Plasma from MM patients and healthy donors	miR-125b-5p	Increased risk of extramedullary involvement, decreased EFS	[36]
CLL	RNA-sequencing	Primary CLL cells and CLL cell lines	miR-21, miR-146a	Enhanced MSC proliferation, EC angiogenic activity, CLL cell survival and proliferation	[55]
	LNA miRNA microarray analysis	Primary CLL cells and cell lines	miR-202-3p	Influence on clinical outcome	[15]
	nCounter miRNA expression assay	Plasma from CLL patients and healthy donors	miR-150, miR-155	Drug resistance	[87]
	Mass spectrometry	Plasma from CLL patients	S100-A9	Tumor growth	[59]
AML	miRNA microarray analysis	AML cell lines and AML-conditioned stroma, serum and plasma from AML patients and healthy donors	miR-150, miR-155, miR-1246	Correlation with disease status and minimal residual disease (MRD) persistence	[29]
	Bioanalyser electropherogram	BM MSCs and cells from AML patients and healthy donors	miR-155, miR-375	Drug resistance and increased risk of relapse	[79]
	miRNA microarray analysis	AML cell lines (HL60 and HL60/AR)	miR-19b, miR-20a	Drug resistance and tumor growth	[5]
	Next-generation sequencing	BM MSCs from AML patients and healthy donors	miR-26a-5p, miR-101-3p	Leukemogenesis	[3]

LEGEND: MM, multiple myeloma; **PB,** peripheral blood; **BM,** bone marrow; **MSCs,** mesenchymal stromal cells; **RNA-seq,** RNA sequencing; **OS,** overall survival; **PFS,** progression free survival; **EFS,** event free survival; **CLL,** chronic lymphocytic leukemia; **ECs,** endothelial cells; **AML,** acute myeloid leukemia; **MRD,** minimal residual disease.

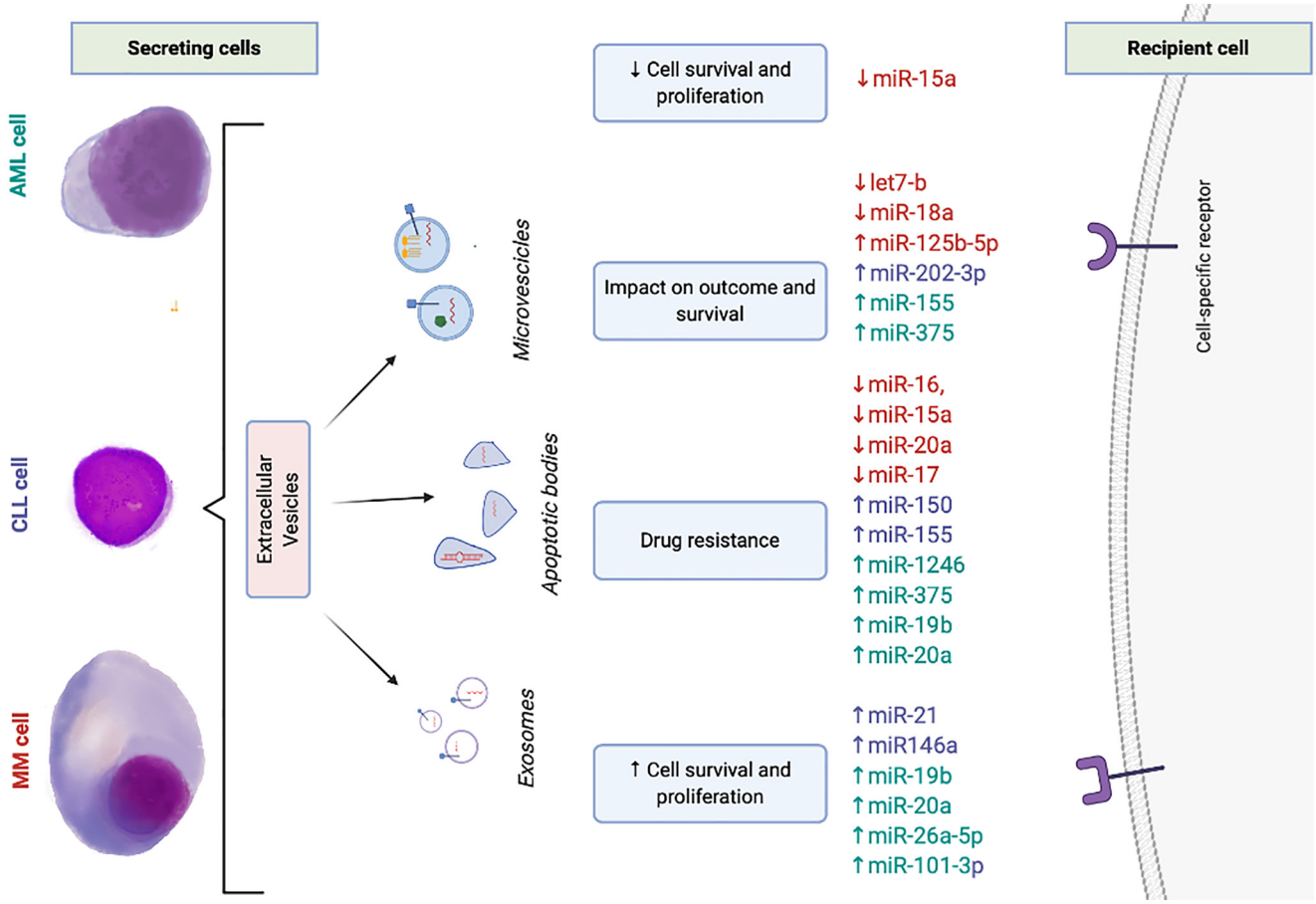

Fig. 1. Bioactive miRs released by EVs. Through the secretion of EVs, tumor cells may modulate several processes in recipient cells, including cell survival and proliferation. They are also able to influence drug sensitivity thus affecting patients' outcome and survival. miRs isolated from MM-derived EVs are highlighted in red, miRs from CLL and AML-derived EVs are in violet and green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tumor survival and favoring progression [18]. CLL-derived exosomes often show a peculiar miR profile by which they play a crucial role in the bidirectional cross-talk between CLL cells and their microenvironment, as previously shown for other HM.

In 2012, Willimott and Wagner performed a microarray analysis to compare miR expression profile of circulating CLL cells with that of cultured stromal cells, demonstrating that stromal cells induced the expression of 20 miRs that were undetectable in peripheral blood cells [81].

Paggetti et al. demonstrated that CLL-derived exosomes could induce phenotypical changes in stromal cells, both in vivo and in vitro. After active internalization by BM-MSCs and endothelial cells (ECs), circulating exosomes deliver their cargo, including functional miRs and proteins, thus activating a variety of signaling pathways involved in leukemic cells survival. Moreover, stromal cells exposed to CLL-derived exosomes showed an inflammatory phenotype similar to cancer-associated fibroblasts and had higher proliferative properties. Through a small RNA sequencing, this study compared the miRs profile of CLL exosomes with that of the CLL cells of origin, showing that exosomes enriched in miR-21 and miR-146a were capable of inducing MSC proliferation and EC angiogenic activity, consequently promoting cell survival and proliferation. Furthermore, a proteomic characterization of exosomes through mass spectrometry analysis confirmed that exosomes are endowed in proteins implicated in several cellular processes, such as migration and RNA synthesis, and participate to the phenotypical modification of tumor microenvironment (Table 1) [55].

Farahani et al. performed a locked nucleic acids (LNA) array to compare exosomal miRs cargo to CLL intracellular miRs. CLL-derived exosomes exhibited similar miR profiles than parental CLL cells, but they were specifically enriched in miR-202-3p, miR-628-3p, and miR-1290. Moreover, this study showed that internalization of CLL exosomes by stromal cells promoted cell proliferation. miR-202-3p is associated with cell differentiation, by downregulating Sonic Hedgehog Signalling pathway (Hh) and increasing its target Sufu (Suppressor of Fused), as well as to poor prognosis in CLL. These findings suggest that the secretion of miR-202-3p and consequent uptake from recipient stromal cells may influence the disease aggressiveness by regulating Sufu levels in CLL cells [15].

Recently, Reiners et al. applied next-generation sequencing to characterize and compare the miR content in CLL cells and B cells from healthy donors. This study confirmed that CLL-derived EVs displayed a disease-related signature and were enriched in miRs encoding for genes frequently mutated in CLL, such as B-cell receptor (BCR) kinases, apoptosis-related genes, and splicing factors [62].

Interestingly, the secretion of CLL-derived exosomes seems to be influenced by the activation of the BCR signaling and is therefore sensitive to the therapeutic effect of Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor. Yeh et al. showed after 28 days of Ibrutinib therapy that CLL patients had significantly lower exosome concentration in plasma. Moreover, CLL patients displayed higher levels of exosomes and a unique microRNA signature compared to healthy donors. In particular, the nCounter microRNA

allowed the identification of two disease-associated miRs: miR-150 and miR-155, whose levels were significantly increased in CLL as compared to normal B cells [87].

All these studies have evaluated the content of CLL-derived exosomes irrespective of the clinical stage of the disease. To understand whether CLL-derived exosomes derive from a cargo modification according to the evolution of the disease, Prieto et al. performed a comprehensive proteomic analysis through liquid chromatography-tandem mass spectrometry of plasma-derived exosomes isolated from patients with both indolent and progressive disease. Intriguingly, exosomes isolated from progressive CLL exhibited a protein cargo associated with inflammation and tumor progression and had higher expression levels of S100-A9 as compared to exosomes from indolent disease. Furthermore, this study showed that increased expression of S100-A9 in CLL patients induced the activation of the canonical NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway, thus promoting tumor survival and proliferation [59].

Altogether, these data suggest that EVs are markedly implicated in the cross-talk between CLL cells and their microenvironment; in particular, EV protein and miR content seems to play an essential role in promoting tumor survival, proliferation, and eventually progression of CLL (Fig. 1).

3.3. Acute myeloid leukemia (AML)

Acute leukemia-derived EVs contain a variety of non-coding RNAs supporting leukemogenesis and influencing the outcome and response to therapy through the regulation of several genes involved in the pathogenesis of this disease [56]. In addition, several studies have highlighted the promising role of circulating miRs as biomarkers in acute myeloid leukemia (AML) (Table 1) [4,34,74,82,85]. For instance, Marcucci and colleagues, by using nCounter assay, demonstrated that miR-155 overexpression was independently associated to poor prognosis in a large cohort of adult AML patients with normal karyotype [45].

Leukemia cells can suppress normal hematopoiesis and transform the BM niche into a permissive niche through exosomes secretion [39]. The formation of a protective niche probably represents the underlying mechanism of late relapse, occurring months or years after first-line treatment in a significant proportion of AML patients. To identify the miRs involved in leukemic progression, Barrera-Ramirez and colleagues profiled miRs of MSCs from AML patients and healthy controls using next-generation sequencing. Five miRs were found to be differentially expressed, two of them being significantly overexpressed in AML-MSC-derived exosomes, i.e. miR-26a-5p and miR-101-3p. The quantification of their target genes expression levels allowed the recognition of three molecules, namely KRBA2, RRBP1, and HIST2H 2BE, which have not been previously associated with leukemogenesis. Consequently, miR profiling of AML-MSCs- derived exosomes allows the identification of new molecular pathways involved in the leukemic process [3].

Exosomes are released by both AML cells and components of the BM microenvironment. Hornick et al. performed a comparative microarray analysis of AML cells and stroma-derived exosomes and proposed a set of miRs related to disease status, providing preclinical evidence that serum exosomal miRs might represent a clinical tool for the detection of occult disease [29].

Numerous studies have established that miRs may be responsible for chemoresistance. Chen and colleagues, carried out microarray analysis of OCI-AML3 cells demonstrating that overexpression of CXCR4, whose expression has been associated to a higher risk of relapse and decreased survival in AML patients [10,38,71], was associated to let-7a downregulation and, consequently, to overexpression of antiapoptotic BCL-XL protein in AML cells [9]. Some studies showed that higher expression of anti-apoptotic proteins, i.e., BCL2, BCL-XL. MCL-1 and BAX, was associated with reduced disease-free survival (DFS) in AML patients [77–78].

Wojtuszkiewicz and colleagues compared through label-free comparative proteomics the secretome protein profile of AML cells resulting either apoptosis-resistant or sensitive, thus unraveling novel proteins with regulatory properties involved in the apoptotic process. Interestingly, this study showed that the secretomes of apoptosis-resistant AML cells were enriched in apoptosis-related proteins involved in global gene regulations. In particular, the most represented protein cluster was associated with miR splicing process, which is known to regulate apoptosis-related proteins. The second top cluster was represented by proteins mainly involved in RNA processing, including NPM1 (Nucleophosmin-1), whose overexpression leads to apoptosis resistance. These findings were subsequently endorsed by EVs proteomic analysis and suggested that vesicle-mediated transfer of apoptosis-regulatory proteins may represent a novel mechanism of apoptosis-resistance gain [82].

Another interesting study pointed out the ability of apoptosis-resistant leukemia cells to confer their chemoresistance to sensitive cells via EVs. Bouvy and colleagues performed a microRNA array analysis to compare the miR cargo of EVs derived from HL60 (chemo-sensitive) and HL60/AR (chemo-resistant) AML cell lines, respectively. Although both cell lines were capable of releasing EVs, there was a difference in the miR cargo of EVs released by either sensitive or chemo-resistant cells. In particular, among 29 microRNAs that were differentially expressed, miR-19b and miR-20a were more expressed in EVs from resistant cells. These two miRs, belonging to the miR-17–92 cluster, are overexpressed in solid cancers and seem to act as oncomiR by targeting the TGFβ signaling pathway and enhancing cell proliferation [58]. Furthermore, they may contribute to the constitutive activation of PI3 kinase/Akt signaling, frequently described in AML and associated with poor outcome, by targeting PTEN [5,46,76].

Viola and colleagues used a Bioanalyser electropherogram and evaluated the content of AML-MSC-derived exosomes, showing a statistically significant enrichment in miR-155 and miR-375 compared to parental cells and suggesting that exosomes released from AML-MSCs are endowed with prognostically significant miRs. Both miR-155 and miR-375 have been associated to the increased risk of relapse in AML patients [45,61]. The same study evaluated the exosomal cytokine concentration and showed that AML-MSCs-derived exosomes had a higher concentration of TGFβ1 as compared to normal BM MSCs. Furthermore, after exposure of FLT3-ITD + AML cells to exosomes from AML-MSCs and normal BM MSCs or control media, only AML-MSCs-derived exosomes provided protective effect from a tyrosine kinase inhibitor (AC220), confirming the hypothetic mechanism of extrinsic chemoresistance provided by exosomes trafficking [79].

Finally, chemo-resistance in AML cells may also derive from exosome-induced immune dysregulation, through the release of immunosuppressive proteins or inhibitory ligands [28]. Thanks to novel immunotherapeutic agents, these features are particularly interesting and might provide new insights into immunotherapy resistance.

3.4. Other HM

Jiang and colleagues recently explored the miRs expression profile in pediatric patients affected by acute lymphoblastic leukemia (ALL) using qRT-PCR-based TaqMan low-density microRNA arrays. Interestingly, newly diagnosed and relapsed patients had lower levels of circulating miR-652-3p than healthy controls, while its level was restored in patients achieving complete remission (CR). These results were confirmed in ALL cell lines, where overexpression of miR-652-3p significantly increased the sensitivity to

vincristine and cytarabine, indicating that this miR might enhance chemosensitivity and promote apoptosis in ALL cells [35].

Likewise, Giudice et al. screened a large number of circulating exosomal miRs through miRNA PCR array, in plasma samples from patients with aplastic anemia and myelodysplastic syndromes; miRs were differentially expressed, one of them (miR-126-5p) being negatively associated with therapy response in aplastic anemia [19].

Another recent study performed RNA-seq to identify five circulating miRs (miR-423-5p, miR-126-3p, miR-151a-3p, miR-125a-5p, and miR-199a-3p), whose expression had a predictive value in terms of response to hypomethylating agents [30].

The role of EVs in the pathogenesis of diffuse large B-cell lymphomas (DLBCLs) is mostly unknown. A recent study characterized the content of EVs secreted by five different DLBCL cell lines by using RNA sequencing, showing that EVs cargo contained a variety of coding and non-coding RNAs involved in B-cell development. Moreover, exome sequencing of DLBCL cell lines and DLBCL-derived EVs demonstrated that secreted EVs harbor the same mutational profile than their cell of origin, thus suggesting new strategies for disease monitoring [67].

4. Summary and outlook

In this review, we gave a quick overview on the current status of functional characterization of the EVs content by using high-throughput analysis. Altogether, these studies highlight the potential of EVs as promising biomarkers in HM, both as prognostic indicators and predictors of chemosensitivity. Table 1 A challenging issue is still the discrimination of tumor-derived EVs from their nonmalignant counterpart, while EVs reliability as biomarkers is still partial due to the lack of standardized protocols for collection and processing. Nevertheless, EVs present a number of peculiarities, due to their structural stability and long-lasting action, that may be exploited to overcome drug resistance and increase survival rates in hematological patients by delivering drugs directly to target cancer cells.

In conclusion, further validation is required to use of EVs as diagnostic and prognostic biological markers as well as novel targeted therapy; to this aim, high-throughput analysis may be employed for accurate functional characterization of EVs content in HM, thus providing new insights for future applications.

CRediT authorship contribution statement

Ilaria Tanasi: Conceptualization, Writing - original draft, Writing - review & editing. **Annalisa Adamo:** Conceptualization, Writing - original draft, Writing - review & editing. **Paul Takam Kamga:** Writing - review & editing. **Riccardo Bazzoni:** Writing - review & editing. **Mauro Krampera:** Conceptualization, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Ahmad N, Haider S, Jagannathan S, Anaissie E, Driscoll JJ. MicroRNA theragnostics for the clinical management of multiple myeloma. Leukemia 2014;28:732–8. https://doi.org/10.1038/leu.2013.262.

[2] Arendt BK, Walters DK, Wu X, Tschumper RC, Jelinek DF. Multiple myeloma dell-derived microvesicles are enriched in CD147 expression and enhance tumor cell proliferation. Oncotarget 2014;5:5686–99. https://doi.org/10.18632/oncotarget.2159.

[3] Barrera-Ramirez J, Lavoie JR, Maganti HB, Stanford WL, Ito C, Sabloff M, Brand M, Rosu-Myles M, Le Y, Allan DS. Micro-RNA profiling of exosomes from marrow-derived mesenchymal stromal cells in patients with acute myeloid leukemia: implications in leukemogenesis. Stem Cell Rev Rep 2017;13:817–25. https://doi.org/10.1007/s12015-017-9762-0.

[4] Benites BD, da Silva Santos Duarte A, Longhini ALF, Santos I, Alvarez MC, de Morais Ribeiro LN, de Paula E, Saad STO. Exosomes in the serum of Acute Myeloid Leukemia patients induce dendritic cell tolerance: Implications for immunotherapy. Vaccine 2019;37:1377–83. https://doi.org/10.1016/j.vaccine.2019.01.079.

[5] Bouvy C, Wannez A, Laloy J, Chatelain C, Dogné J-M. Transfer of multidrug resistance among acute myeloid leukemia cells via extracellular vesicles and their microRNA cargo. Leuk. Res. 2017;62:70–6. https://doi.org/10.1016/j.leukres.2017.09.014.

[6] Bruno S, Deregibus MC, Camussi G. The secretome of mesenchymal stromal cells: role of extracellular vesicles in immunomodulation. Immunol. Lett. 2015;168:154–8. https://doi.org/10.1016/j.imlet.2015.06.007.

[7] Caivano A, La Rocca F, Laurenzana I, Trino S, De Luca L, Lamorte D, Del Vecchio L, Musto P. Extracellular vesicles in hematological malignancies: from biology to therapy. Int. J. Mol. Sci. 2017;18. https://doi.org/10.3390/ijms18061183.

[8] Caivano A, Laurenzana I, De Luca L, La Rocca F, Simeon V, Trino S, D'Auria F, Traficante A, Maietti M, Izzo T, D'Arena G, Mansueto G, Pietrantuono G, Laurenti L, Musto P, Del Vecchio L. High serum levels of extracellular vesicles expressing malignancy-related markers are released in patients with various types of hematological neoplastic disorders. Tumour Biol. 2015;36:9739–52. https://doi.org/10.1007/s13277-015-3741-3.

[9] Chen Y, Jacamo R, Konopleva M, Garzon R, Croce C, Andreeff M. CXCR4 downregulation of let-7a drives chemoresistance in acute myeloid leukemia. J Clin Invest 2013;123:2395–407. https://doi.org/10.1172/JCI66553.

[10] Colmone A, Amorim M, Pontier AL, Wang S, Jablonski E, Sipkins DA. Leukemic cells create bone marrow niches that disrupt the behavior of normal hematopoietic progenitor cells. Science 2008;322:1861–5. https://doi.org/10.1126/science.1164390.

[11] Corcoran C, Rani S, O'Brien K, O'Neill A, Prencipe M, Sheikh R, Webb G, McDermott R, Watson W, Crown J, O'Driscoll L. Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer via exosomes. PLoS ONE 2012;7. https://doi.org/10.1371/journal.pone.0050999.

[12] Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JB, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Shiekhattar R, Gingeras TR, Hubbard TJ, Notredame C, Harrow J, Guigó R. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012;22:1775–89. https://doi.org/10.1101/gr.132159.111.

[13] Desantis V, Saltarella I, Lamanuzzi A, Melaccio A, Solimando AG, Mariggiò MA, Racanelli V, Paradiso A, Vacca A, Frassanito MA. MicroRNAs-based nano-strategies as new therapeutic approach in multiple myeloma to overcome disease progression and drug resistance. Int. J. Mol. Sci. 2020;21. https://doi.org/10.3390/ijms21093084.

[14] Di Martino MT, Campani V, Misso G, Gallo Cantafio ME, Gullà A, Foresta U, Guzzi PH, Castellano M, Grimaldi A, Gigantino V, Franco R, Lusa S, Cannataro M, Tagliaferri P, De Rosa G, Tassone P, Caraglia M. In vivo activity of miR-34a mimics delivered by stable nucleic acid lipid particles (SNALPs) against multiple myeloma. PLoS ONE 2014;9. https://doi.org/10.1371/journal.pone.0090005.

[15] Farahani M, Rubbi C, Liu L, Slupsky JR, Kalakonda N. CLL exosomes modulate the transcriptome and behaviour of recipient stromal cells and are selectively enriched in miR-202-3p. PLoS ONE 2015;10. https://doi.org/10.1371/journal.pone.0141429.

[16] Farooqi AA, Desai NN, Qureshi MZ, Librelotto DRN, Gasparri ML, Bishayee A, Nabavi SM, Curti V, Daglia M. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds. Biotechnol Adv 2018;36:328–34. https://doi.org/10.1016/j.biotechadv.2017.12.010.

[17] Février B, Raposo G. Exosomes: endosomal-derived vesicles shipping extracellular messages. Curr. Opin. Cell Biol. 2004;16:415–21. https://doi.org/10.1016/j.ceb.2004.06.003.

[18] Ghosh AK, Secreto CR, Knox TR, Ding W, Mukhopadhyay D, Kay NE. Circulating microvesicles in B-cell chronic lymphocytic leukemia can stimulate marrow stromal cells: implications for disease progression. Blood 2010;115:1755–64. https://doi.org/10.1182/blood-2009-09-242719.

[19] Giudice V, Banaszak LG, Gutierrez-Rodrigues F, Kajigaya S, Panjwani R, Ibanez MDPF, Rios O, Bleck CK, Stempinski ES, Raffo DQ, Townsley DM, Young NS. Circulating exosomal microRNAs in acquired aplastic anemia and myelodysplastic syndromes. Haematologica 2018;103:1150–9. https://doi.org/10.3324/haematol.2017.182824.

[20] Griessinger E, Moschoi R, Biondani G, Peyron J-F. Mitochondrial transfer in the leukemia microenvironment. Trends Cancer 2017;3:828–39. https://doi.org/10.1016/j.trecan.2017.10.003.

[21] Gullà A, Di Martino MT, Gallo Cantafio ME, Morelli E, Amodio N, Botta C, Pitari MR, Lio SG, Britti D, Stamato MA, Hideshima T, Munshi NC, Anderson KC, Tagliaferri P, Tassone P. A 13 mer LNA-i-miR-221 inhibitor restores drug sensitivity in melphalan-refractory multiple myeloma cells. Clin. Cancer Res. 2016;22:1222–33. https://doi.org/10.1158/1078-0432.CCR-15-0489.

[22] Guo W, Gao Y, Li N, Shao F, Wang C, Wang P, Yang Z, Li R, He J. Exosomes: new players in cancer (Review). Oncol Rep 2017;38:665–75. https://doi.org/10.3892/or.2017.5714.

[23] Harshman SW, Canella A, Ciarlariello PD, Agarwal K, Branson OE, Rocci A, Cordero H, Phelps MA, Hade EM, Dubovsky JA, Palumbo A, Rosko A, Byrd JC, Hofmeister CC, Benson DM, Paulaitis ME, Freitas MA, Pichiorri F. Proteomic characterization of circulating extracellular vesicles identifies novel serum myeloma associated markers. J Proteomics 2016;136:89–98. https://doi.org/10.1016/j.jprot.2015.12.016.

[24] Harshman SW, Canella A, Ciarlariello PD, Rocci A, Agarwal K, Smith EM, Talabere T, Efebera YA, Hofmeister CC, Benson DM, Paulaitis ME, Freitas MA, Pichiorri F. Characterization of multiple myeloma vesicles by label-free relative quantitation. Proteomics 2013;13:3013–29. https://doi.org/10.1002/pmic.201300142.

[25] Henderson-Maclennan NK, Papp JC, Talbot CC, McCabe ERB, Presson AP. Pathway analysis software: annotation errors and solutions. Mol. Genet. Metab. 2010;101:134–40. https://doi.org/10.1016/j.ymgme.2010.06.005.

[26] Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell. Mol. Life Sci. 2018;75:193–208. https://doi.org/10.1007/s00018-017-2595-9.

[27] Hong CS, Muller L, Boyiadzis M, Whiteside TL. Isolation and characterization of CD34+ blast-derived exosomes in acute myeloid leukemia. PLoS ONE 2014;9. https://doi.org/10.1371/journal.pone.0103310.

[28] Hong C-S, Sharma P, Yerneni SS, Simms P, Jackson EK, Whiteside TL, Boyiadzis M. Circulating exosomes carrying an immunosuppressive cargo interfere with cellular immunotherapy in acute myeloid leukemia. Sci. Rep. 2017;7:14684. https://doi.org/10.1038/s41598-017-14661-w.

[29] Hornick NI, Huan J, Doron B, Goloviznina NA, Lapidus J, Chang BH, Kurre P. Serum exosome MicroRNA as a minimally-invasive early biomarker of AML. Sci Rep 2015;5:11295. https://doi.org/10.1038/srep11295.

[30] Hrustincova A, Krejcik Z, Kundrat D, Szikszai K, Belickova M, Pecherkova P, Klema J, Vesela J, Hruba M, Cermak J, Hrdinova T, Krijt M, Valka J, Jonasova A, Dostalova Merkerova M. Circulating small noncoding RNAs have specific expression patterns in plasma and extracellular vesicles in myelodysplastic syndromes and are predictive of patient outcome. Cells 2020;9. https://doi.org/10.3390/cells9040794.

[31] Uang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat. Protoc. 2009;4:44–57. https://doi.org/10.1038/nprot.2008.211.

[32] Jagannathan S, Vad N, Vallabhapurapu S, Vallabhapurapu S, Anderson KC, Driscoll JJ. MiR-29b replacement inhibits proteasomes and disrupts aggresome +autophagosome formation to enhance the antimyeloma benefit of bortezomib. Leukemia 2015;29:727–38. https://doi.org/10.1038/leu.2014.279.

[33] Javeed N, Mukhopadhyay D. Exosomes and their role in the micro-/macro-environment: a comprehensive review. J. Biomed. Res. 2017;31:386–94. https://doi.org/10.7555/JBR.30.20150162.

[34] Jiang L, Deng T, Wang D, Xiao Y. Elevated serum exosomal miR-125b level as a potential marker for poor prognosis in intermediate-risk acute myeloid leukemia. Acta Haematol. 2018;140:183–92. https://doi.org/10.1159/000491584.

[35] Jiang Q, Lu X, Huang P, Gao C, Zhao X, Xing T, Li G, Bao S, Zheng H. Expression of miR-652-3p and effect on apoptosis and drug sensitivity in pediatric acute lymphoblastic leukemia. Biomed. Res. Int. 2018;2018:5724686. https://doi.org/10.1155/2018/5724686.

[36] Jiang Y, Luan Y, Chang H, Chen G. The diagnostic and prognostic value of plasma microRNA-125b-5p in patients with multiple myeloma. Oncol. Lett. 2018;16:4001–7. https://doi.org/10.3892/ol.2018.9128.

[37] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, Moreaux J. Global miRNA expression analysis identifies novel key regulators of plasma cell differentiation and malignant plasma cell. Nucleic Acids Res. 2017;45:5639–52. https://doi.org/10.1093/nar/gkx327.

[38] Konopleva MY, Jordan CT. Leukemia stem cells and microenvironment: biology and therapeutic targeting. J. Clin. Oncol. 2011;29:591–9. https://doi.org/10.1200/JCO.2010.31.0904.

[39] Kumar B, Garcia M, Weng L, Jung X, Murakami JL, Hu X, McDonald T, Lin A, Kumar AR, DiGiusto DL, Stein AS, Pullarkat VA, Hui SK, Carlesso N, Kuo Y-H, Bhatia R, Marcucci G, Chen C-C. Acute myeloid leukemia transforms the bone marrow niche into a leukemia-permissive microenvironment through exosome secretion. Leukemia 2018;32:575–87. https://doi.org/10.1038/leu.2017.259.

[40] Le MTN, Teh C, Shyh-Chang N, Xie H, Zhou B, Korzh V, Lodish HF, Lim B. MicroRNA-125b is a novel negative regulator of p53. Genes Dev. 2009;23:862–76. https://doi.org/10.1101/gad.1767609.

[41] LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in cancer. Trends Cancer 2020. https://doi.org/10.1016/j.trecan.2020.03.007.

[42] Leone E, Morelli E, Di Martino MT, Amodio N, Foresta U, Gullà A, Rossi M, Neri A, Giordano A, Munshi NC, Anderson KC, Tagliaferri P, Tassone P. Targeting miR-21 inhibits in vitro and in vivo multiple myeloma cell growth. Clin. Cancer Res. 2013;19:2096–106. https://doi.org/10.1158/1078-0432.CCR-12-3325.

[43] Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: unique intercellular delivery vehicles. Trends Cell Biol 2017;27:172–88. https://doi.org/10.1016/j.tcb.2016.11.003.

[44] Manier S, Liu C-J, Avet-Loiseau H, Park J, Shi J, Campigotto F, Salem KZ, Huynh D, Glavey SV, Rivotto B, Sacco A, Roccaro AM, Bouyssou J, Minvielle S, Moreau P, Facon T, Leleu X, Weller E, Trippa L, Ghobrial IM. Prognostic role of circulating exosomal miRNAs in multiple myeloma. Blood 2017;129:2429–36. https://doi.org/10.1182/blood-2016-09-742296.

[45] Marcucci G, Maharry KS, Metzeler KH, Volinia S, Wu Y-Z, Mrózek K, Nicolet D, Kohlschmidt J, Whitman SP, Mendler JH, Schwind S, Becker H, Eisfeld A-K, Carroll AJ, Powell BL, Kolitz JE, Garzon R, Caligiuri MA, Stone RM, Bloomfield CD. Clinical role of microRNAs in cytogenetically normal acute myeloid leukemia: miR-155 upregulation independently identifies high-risk patients. J. Clin. Oncol. 2013;31:2086–93. https://doi.org/10.1200/JCO.2012.45.6228.

[46] Martelli AM, Nyåkern M, Tabellini G, Bortul R, Tazzari PL, Evangelisti C, Cocco L. Phosphoinositide 3-kinase/Akt signaling pathway and its therapeutical implications for human acute myeloid leukemia. Leukemia 2006;20:911–28. https://doi.org/10.1038/sj.leu.2404245.

[47] Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res. 2012;40:D1241–1244. https://doi.org/10.1093/nar/gkr828.

[48] Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. J. Proteomics 2010;73:1907–20. https://doi.org/10.1016/j.jprot.2010.06.006.

[49] Mathivanan S, Simpson RJ. ExoCarta: a compendium of exosomal proteins and RNA. Proteomics 2009;9:4997–5000. https://doi.org/10.1002/pmic.200900351.

[50] Meehan B, Rak J, Vizio DD. Oncosomes – large and small: what are they, where they came from?. J. of Extracellular Vesicles 2016;5:33109. https://doi.org/10.3402/jev.v5.33109.

[51] Milani G, Lana T, Bresolin S, Aveic S, Pastò A, Frasson C, Te Kronnie G. Expression profiling of circulating microvesicles reveals intercellular transmission of oncogenic pathways. Mol. Cancer Res. 2017;15:683–95. https://doi.org/10.1158/1541-7786.MCR-16-0307.

[52] Morlando M, Ballarino M, Fatica A. Long non-coding RNAs: new players in hematopoiesis and leukemia. Front. Med. (Lausanne) 2015;2. https://doi.org/10.3389/fmed.2015.00023.

[53] Müller T, Schrötter A, Loosse C, Helling S, Stephan C, Ahrens M, Uszkoreit J, Eisenacher M, Meyer HE, Marcus K. Sense and nonsense of pathway analysis software in proteomics. J. Proteome Res. 2011;10:5398–408. https://doi.org/10.1021/pr200654k.

[54] Murray MY, Rushworth SA, Zaitseva L, Bowles KM, MacEwan DJ. Attenuation of dexamethasone-induced cell death in multiple myeloma is mediated by miR-125b expression. Cell Cycle 2013;12:2144–53. https://doi.org/10.4161/cc.25251.

[55] Paggetti J, Haderk F, Seiffert M, Janji B, Distler U, Ammerlaan W, Kim YJ, Adam J, Lichter P, Solary E, Berchem G, Moussay E. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. Blood 2015;126:1106–17. https://doi.org/10.1182/blood-2014-12-618025.

[56] Pando A, Reagan JL, Quesenberry P, Fast LD. Extracellular vesicles in leukemia. Leuk. Res. 2018;64:52–60. https://doi.org/10.1016/j.leukres.2017.11.011.

[57] Peinado H, Alečković M, Lavotshkin S, Matei I, Costa-Silva B, Moreno-Bueno G, Hergueta-Redondo M, Williams C, García-Santos G, Ghajar C, Nitadori-Hoshino A, Hoffman C, Badal K, Garcia BA, Callahan MK, Yuan J, Martins VR, Skog J, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. Nat. Med. 2012;18:883–91. https://doi.org/10.1038/nm.2753.

[58] Petrocca F, Vecchione A, Croce CM. Emerging role of miR-106b-25/miR-17-92 clusters in the control of transforming growth factor β signaling. Cancer Res. 2008;68:8191–4. https://doi.org/10.1158/0008-5472.CAN-08-1768.

[59] Prieto D, Sotelo N, Seija N, Sernbo S, Abreu C, Durán R, Gil M, Sicco E, Irigoin V, Oliver C, Landoni AI, Gabus R, Dighiero G, Oppezzo P. S100–A9 protein in exosomes from chronic lymphocytic leukemia cells promotes NF-κB activity during disease progression. Blood 2017;130:777–88. https://doi.org/10.1182/blood-2017-02-769851.

[60] Puhka M, Takatalo M, Nordberg M-E, Valkonen S, Nandania J, Aatonen M, Yliperttula M, Laitinen S, Velagapudi V, Mirtti T, Kallioniemi O, Rannikko A, Siljander PR-M, af Hällström TM. Metabolomic profiling of extracellular vesicles and alternative normalization methods reveal enriched metabolites and strategies to study prostate cancer-related changes. Theranostics 2017;7:3824–41. https://doi.org/10.7150/thno.19890.

[61] Ramamurthy R, Hughes M, Morris V, Bolouri H, Gerbing RB, Wang Y-C, Loken MR, Raimondi SC, Hirsch BA, Gamis AS, Oehler VG, Alonzo TA, Meshinchi S. miR-155 expression and correlation with clinical outcome in pediatric AML: a report from Children's Oncology Group. Pediatr. Blood Cancer 2016;63:2096–103. https://doi.org/10.1002/pbc.26157.

[62] Reiners KS, Shatnyeva O, Vasyutina E, Bösl T, Hansen HP, Hallek M, Herling M, von Strandmann EP. Extracellular vesicles released from chronic lymphocytic leukemia cells exhibit a disease relevant mRNA signature and transfer mRNA to bystander cells. Haematologica 2017;102:e100–3. https://doi.org/10.3324/haematol.2016.153197.

[63] Robbins PD, Dorronsoro A, Booker CN. Regulation of chronic inflammatory and immune processes by extracellular vesicles. J. Clin. Invest. 2016;126:1173–80. https://doi.org/10.1172/JCI81131.

[64] Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat. Rev. Immunol. 2014;14:195–208. https://doi.org/10.1038/nri3622.

[65] Roccaro AM, Sacco A, Maiso P, Azab AK, Tai Y-T, Reagan M, Azab F, Flores LM, Campigotto F, Weller E, Anderson KC, Scadden DT, Ghobrial IM. BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. J. Clin. Invest. 2013;123:1542–55. https://doi.org/10.1172/JCI66517.

[66] Rocco GD, Baldari S, Toietta G. Exosomes and other extracellular vesicles-mediated microRNA delivery for cancer therapy. Transl. Cancer Res. 2017;6. S1321–S1330–S1330.

[67] Rutherford SC, Fachel AA, Li S, Sawh S, Muley A, Ishii J, Saxena A, Dominguez PM, Caldas Lopes E, Agirre X, Chambwe N, Correa F, Jiang Y, Richards KL, Betel D, Shaknovich R. Extracellular vesicles in DLBCL provide abundant clues to aberrant transcriptional programming and genomic alterations. Blood 2018;132:e13–23. https://doi.org/10.1182/blood-2017-12-821843.

[68] Saito R, Smoot ME, Ono K, Ruscheinski J, Wang P-L, Lotia S, Pico AR, Bader GD, Ideker T. A travel guide to Cytoscape plugins. Nat. Methods 2012;9:1069–76. https://doi.org/10.1038/nmeth.2212.

[69] Sethi S, Ali S, Philip PA, Sarkar FH. Clinical advances in molecular biomarkers for cancer diagnosis and therapy. Int. J. Mol. Sci. 2013;14:14771–84. https://doi.org/10.3390/ijms140714771.

[70] Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Curry WT, Carter BS, Krichevsky AM, Breakefield XO. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat. Cell Biol. 2008;10:1470–6. https://doi.org/10.1038/ncb1800.

[71] Spoo AC, Lübbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in acute myelogenous leukemia. Blood 2007;109:786–91. https://doi.org/10.1182/blood-2006-05-024844.

[72] Sullivan LB. Taking metabolism on the road. Nat. Chem. Biol. 2017;13:924–5. https://doi.org/10.1038/nchembio.2455.

[73] Tannetta D, Dragovic R, Alyahyaei Z, Southcombe J. Extracellular vesicles and reproduction-promotion of successful pregnancy. Cell. Mol. Immunol. 2014;11:548–63. https://doi.org/10.1038/cmi.2014.42.

[74] Umezu T, Ohyashiki K, Kuroda M, Ohyashiki JH. Leukemia cell to endothelial cell communication via exosomal miRNAs. Oncogene 2013;32:2747–55. https://doi.org/10.1038/onc.2012.295.

[75] Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 2007;9:654–9. https://doi.org/10.1038/ncb1596.

[76] van Haaften G, Agami R. Tumorigenicity of the miR-17-92 cluster distilled. Genes Dev 2010;24:1–4. https://doi.org/10.1101/gad.1887110.

[77] van Stijn A, Feller N, Kok A, van der Pol MA, Ossenkoppele GJ, Schuurhuis GJ. Minimal residual disease in acute myeloid leukemia is predicted by an apoptosis-resistant protein profile at diagnosis. Clin. Cancer Res. 2005;11:2540–6. https://doi.org/10.1158/1078-0432.CCR-04-1973.

[78] van Stijn A, Kok A, van der Pol MA, Feller N, Roemen GMJM, Westra AH, Ossenkoppele GJ, Schuurhuis GJ. A flow cytometric method to detect apoptosis-related protein expression in minimal residual disease in acute myeloid leukemia. Leukemia 2003;17:780–6. https://doi.org/10.1038/sj.leu.2402885.

[79] Viola S, Traer E, Huan J, Hornick NI, Tyner JW, Agarwal A, Loriaux M, Johnstone B, Kurre P. Alterations in acute myeloid leukaemia bone marrow stromal cell exosome content coincide with gains in tyrosine kinase inhibitor resistance. Br. J. Haematol. 2016;172:983–6. https://doi.org/10.1111/bjh.13551.

[80] Wang X, Li C, Ju S, Wang Y, Wang H, Zhong R. Myeloma cell adhesion to bone marrow stromal cells confers drug resistance by microRNA-21 up-regulation. Leuk. Lymphoma 2011;52:1991–8. https://doi.org/10.3109/10428194.2011.591004.

[81] Willimott S, Wagner SD. Stromal cells and CD40 ligand (CD154) alter the miRNome and induce miRNA clusters including, miR-125b/miR-99a/let-7c and miR-17-92 in chronic lymphocytic leukaemia. Leukemia 2012;26:1113–6. https://doi.org/10.1038/leu.2011.299.

[82] Wojtuszkiewicz A, Schuurhuis GJ, Kessler FL, Piersma SR, Knol JC, Pham TV, Jansen G, Musters RJP, van Meerloo J, Assaraf YG, Kaspers GJL, Zweegman S, Cloos J, Jimenez CR. Exosomes secreted by apoptosis-resistant acute myeloid leukemia (AML) blasts harbor regulatory network proteins potentially involved in antagonism of apoptosis. Mol. Cell Proteomics 2016;15:1281–98. https://doi.org/10.1074/mcp.M115.052944.

[83] Xia H-F, He T-Z, Liu C-M, Cui Y, Song P-P, Jin X-H, Ma X. MiR-125b expression affects the proliferation and apoptosis of human glioma cells by targeting Bmf. Cell. Physiol. Biochem. 2009;23:347–58. https://doi.org/10.1159/000218181.

[84] Yáñez-Mó M, Siljander PR-M, Andreu Z, Zavec AB, Borràs FE, Buzas EI, Buzas K, Casal E, Cappello F, Carvalho J, Colás E, Cordeiro-da Silva A, Fais S, Falcon-Perez JM, Ghobrial IM, Giebel B, Gimona M, Graner M, Gursel I, Gursel M, Heegaard NHH, Hendrix A, Kierulf P, Kokubun K, Kosanovic M, Kralj-Iglic V, Krämer-Albers E-M, Laitinen S, Lässer C, Lener T, Ligeti E, Linē A, Lipps G, Llorente A, Lötvall J, Manček-Keber M, Marcilla A, Mittelbrunn M, Nazarenko I, Nolte-'t Hoen ENM, Nyman TA, O'Driscoll L, Olivan M, Oliveira C, Pállinger É, Del Portillo HA, Reventós J, Rigau M, Rohde E, Sammar M, Sánchez-Madrid F, Santarém N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Grein SG, Vasconcelos MH, Wauben MHM, De Wever O. Biological properties of extracellular vesicles and their physiological functions. J. Extracell. Vesicles 2015;4:27066. https://doi.org/10.3402/jev.v4.27066.

[85] Yang C, Yang H, Liu J, Zhu L, Yu S, Zhang X, Gao L. Focus on exosomes: novel pathogenic components of leukemia. Am. J. Cancer Res. 2019;9:1815–29.

[86] Yarmishyn AA, Kurochkin IV. Long noncoding RNAs: a potential novel class of cancer biomarkers. Front. Genet. 2015;6:145. https://doi.org/10.3389/fgene.2015.00145.

[87] Yeh Y-Y, Ozer HG, Lehman AM, Maddocks K, Yu L, Johnson AJ, Byrd JC. Characterization of CLL exosomes reveals a distinct microRNA signature and enhanced secretion by activation of BCR signaling. Blood 2015;125:3297–305. https://doi.org/10.1182/blood-2014-12-618470.

[88] Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: composition, biological relevance, and methods of study. Bioscience 2015;65:783–97. https://doi.org/10.1093/biosci/biv084.

[89] Zarone MR, Misso G, Grimaldi A, Zappavigna S, Russo M, Amler E, Di Martino MT, Amodio N, Tagliaferri P, Tassone P, Caraglia M. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Sci. Rep. 2017;7:17949. https://doi.org/10.1038/s41598-017-18186-0.

[90] Zhang L, Pan L, Xiang B, Zhu H, Wu Y, Chen M, Guan P, Zou X, Valencia CA, Dong B, Li J, Xie L, Ma H, Wang F, Dong T, Shuai X, Niu T, Liu T. Potential role of exosome-associated microRNA panels and in vivo environment to predict drug resistance for patients with multiple myeloma. Oncotarget 2016;7:30876–91. https://doi.org/10.18632/oncotarget.9021.

[91] Zhao J-J, Chu Z-B, Hu Y, Lin J, Wang Z, Jiang M, Chen M, Wang X, Kang Y, Zhou Y, Ni Chonghaile T, Johncilla ME, Tai Y-T, Cheng JQ, Letai A, Munshi NC, Anderson KC, Carrasco RD. Targeting the miR-221-222/PUMA/BAK/BAX pathway abrogates dexamethasone resistance in multiple myeloma. Cancer Res. 2015;75:4384–97. https://doi.org/10.1158/0008-5472.CAN-15-0457.