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

Ilaria Tanasi a,⇑, Annalisa Adamo b, Paul Takam Kamgaa, Riccardo Bazzonia a, Mauro Krampera a,⇑

a Department of Medicine, Hematology Section, University of Verona, Italy
b Department of Medicine, Immunology Section, University of Verona, Italy

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.

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

https://doi.org/10.1016/j.csbj.2020.09.027
2001-0370© 2020 The Author(s). Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
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 the detailed characterization of EVs content by identifying a variety of peptidomic, 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 a web-based tool for global analysis of EVs content, such as Gene Ontology analysis, network analysis of vesicular proteins and miRNAs, 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
EV-associated biomarkers in multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML).

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 participation of exosome-derived miRs 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 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 (''antagomiRs'')\[1,21,32,80,89,91\].

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**

| MM Methods | Source of Execs | 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-5p, miR-17-5p | Drug resistance | \[90\] |
| RNA-seq miRNA microarray analysis | Serum from MM patients and healthy donors | let7-b, miR-18a | Decreased OS and PFS | \[44\] |
| LNA miRNA microarray analysis | Plasma from MM patients and healthy donors | miR-125b-5p | Increased risk of extramedullary involvement, decreased EFS | \[36\] |
| nCounter miRNA expression assay | Primary CLL cells and cell lines | miR-21, miR-146a | Enhanced MSC proliferation, EC angiogenic activity, CLL cell survival and proliferation | \[55\] |
| Mass spectrometry miRNA microarray analysis | Primary CLL cells and cell lines | miR-202-3p | Influence on clinical outcome | \[15\] |
| AML nCounter miRNA expression assay | Plasma from CLL patients and healthy donors | miR-150, miR-155 | Drug resistance | \[87\] |
| Bioanalyser electropherogram miRNA microarray analysis | Plasma from CLL patients | S100-A9 | Tumor growth | \[59\] |
| Bioanalyser electropherogram 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 miRNA microarray analysis | BM MSCs and cells from AML patients and healthy donors | miR-155, miR-375 | Drug resistance and increased risk of relapse | \[79\] |
| Bioanalyser electropherogram miRNA microarray analysis | AML cell lines (HL60 and HL60/AR) | miR-19b, miR-20a | Drug resistance and tumor growth | \[5\] |

---

**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.
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-κB (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, RRPB1, 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 anti-apoptotic 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 [54,46,76].

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-3p, 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 management [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 Kangma: 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.

Acknowledgments

We would like to acknowledge Associazione Italiana contro Leucemie, Linfomi e Mieloma (AIL) – Sezione di Verona for donations and logistic support in managing hematological patients.

References

[1] Ahmad N, Haider S, Jagannathan S, Anaisie E, Driscoll JJ. MicroRNA therapeutics for the clinical management of multiple myeloma. Leukemia 2014;28:732–8. https://doi.org/10.1038/leu.2013.252
[2] Arendt BK, Walters DK, Wu X, Tschumper RC, Jelinek DF. Multiple myeloma- derived microvesicles are enriched in CD147 expression and enhance tumor cell proliferation. Oncotarget 2014;5:5686–99. https://doi.org/10.18632/oncotarget.2155
[3] Barrera-Ramirez J, Lavoie JR, Magnani 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 ST. Exosomes from 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] Boursy C, Wannez A, Laloy J, Chatelan 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.lucres.2017.09.014
[6] Brunetti 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] Caiano A, La Rocca F, Laurenza I, Trino S, D’Auria F, Traficante A, Maiteri M, Izzo T, D’Arena G, Mansueto G, Pietrantuono G, Laurenza I, Trino S. Extracellular vesicles in hematological malignancies: breast cancer to therapy. Int. J. Mol. Sci. 2017;18. https://doi.org/10.3390/ijms18061183
[8] Caiano A, Laurenza I, De Luca L, La Rocca F, Simeone V, Trino S, D’Auria F, Traficante A, Maiteri M, Izzo T, D’Arena G, Mansueto G, Pietrantuono G, Laurenza I, Trino S. Extracellular vesicles in hematological malignancies: breast cancer to therapy. Int. J. Mol. Sci. 2017;18. https://doi.org/10.3390/ijms18061183
[9] Chen Y, Jacamo R, Konopleva M, Gao Z, Aveller-Perez M, Croce CM. Circulating exosomal microRNAs in acquired aplastic anemia and myelodysplastic syndromes. Tumour Biol. 2015;36:9739–52. https://doi.org/10.1007/s13277-015-3741-3
[10] Colmone A, Amorini M, Pontier AL, Wang S, Jablonski E, Sipkins DA. Leukemic cells: role of extracellular vesicles in immunomodulation. Immunol. Lett. 2014;2014:28:732–8. https://doi.org/10.1038/leu.2013.262
[11] Corcoran C, Rani S, O’Brien K, O’Neill A, Prencipe M, Sheikh R, Webb G, Martin D, Merkel A, Knowles DG, Lagarde J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JR, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Skehelhatar G, Gingeras TR, Hubbard TJ. Notredame C, Harrow J, Guigo 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.121591.111
[12] Derrien T, Johnson R, Bussotti G, Tanzer A, Djebali S, Tilgner H, Guernec G, Martin D, Merkel A, Knowles DG, Ladage J, Veeravalli L, Ruan X, Ruan Y, Lassmann T, Carninci P, Brown JR, Lipovich L, Gonzalez JM, Thomas M, Davis CA, Skehelhar G, Gingeras TR, Hubbard TJ. Notredame C, Harrow J, Guigo 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.121591.111
[13] Di Martino MT, Campani V, Misso G, Gallo Cantafio ME, Gullà A, Foresta U, Maffeis L, Tagliaferri P, De Rosa G, Tassone P, Caraglia M. In vivo activity of miR-34a 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
[14] Di Martino MT, Campani V, Misso G, Gallo Cantafio ME, Gullà A, Foresta U, Maffeis L, Tagliaferri P, De Rosa G, Tassone P, Caraglia M. In vivo activity of miR-34a 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
[15] Farooqi AA, Desai NN, Qureshi MZ, Librelotto DRN, Gasparri ML, Bishayee A, Morais Ribeiro LN, de Paula E, Saad ST. Exosomes from 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
[16] 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
[17] Giudice V, Banaszak LG, Gutierrez-Rodrigues F, Kajigaya S, Panjwani R, Ibanez I. Clinical applications of circulating exosomal microRNAs in adult acute myeloid leukemia: implications for disease progression and drug resistance. Int. J. Mol. Sci. 2020;21. https://doi.org/10.3390/ijms21093064
[18] Giudice V, Banaszak LG, Gutierrez-Rodrigues F, Kajigaya S, Panjwani R, Ibanez I. Clinical applications of circulating exosomal microRNAs in adult acute myeloid leukemia: implications for disease progression and drug resistance. Int. J. Mol. Sci. 2020;21. https://doi.org/10.3390/ijms21093064
[19] I. Tanasi et al. Computational and Structural Biotechnology Journal 18 (2020) 2670–2677
[20] Griessinger E, Moschri R, Biondani G, Peyron J-F. Mitochondrial transfer in the leukemia microenvironment. Trends Cancer. 2017;3:382–9. https://doi.org/10.1016/j.trecan.2017.10.010.

[21] Guilla U, Di Martino MT, Gallo Cantaviejo 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. 2018;24:222–33. https://doi.org/10.1158/1078-0432.CCR-17-0489.

[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;38:655–75. https://doi.org/10.3892/or.2017.5714.

[23] Harshman SW, Canella A, Ciarlariello PD, Rocci A, Agarwal K, Smith EM, Harshman SW, Canella A, Ciarlariello PD, Rocci A, Agarwal K, Smith EM, Talabere T, Efebera YA, Hofmeister CC, Benson DM, Paulatis 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.201300141.

[24] Harshman SW, Canella A, Ciarlariello PD, Agarwal K, Smith EM, Talabere T, Efebera YA, Hofmeister CC, Benson DM, Paulatis 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.201300141.

[25] Henderson-Maclennan NK, Papp JC, Talbot CC, McCabe ERB, Presson AP.

[26] Hornick NI, Huan J, Doron B, Goloviznina NA, Lapidus J, Chang BH, Kurre P.

[27] Hrustincova A, Krejcik Z, Kundrat D, Szikszai K, Belickova M, Pecherkova P, Hrustincova A, Krejcik Z, Kundrat D, Szikszai K, Belickova M, Pecherkova P, Dostalova Merkerova M. Circulating small noncoding RNAs have specific potential marker for poor prognosis in intermediate-risk acute myeloid leukemia. PLoS ONE 2014;9.

[28] Hui SK, Carlesso N, Kuo Y-H, Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[29] Jiang L, Deng T, Wang D, Xiao Y. Elevated serum exosomal miR-125b level as a novel marker for poor prognosis in intermediate-risk acute myeloid leukemia. Leukemia 2013;27:1552–6. https://doi.org/10.1038/leu.2013.259.

[30] Jiang L, Deng T, Wang D, Xiao Y. Elevated serum exosomal miR-125b level as a novel marker for poor prognosis in intermediate-risk acute myeloid leukemia. Leukemia 2013;27:1552–6. https://doi.org/10.1038/leu.2013.259.

[31] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[32] Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D, Kaplan RN, Brady MS, Wolchok JD, Chapman PB, Kang Y, Bromberg J, Lyden D.

[33] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[34] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[35] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[36] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[37] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[38] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[39] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[40] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[41] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[42] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[43] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.

[44] Kassambara A, Jourdan M, Bruyer A, Robert N, Pantesco V, Elemento O, Klein B, LeBleu VS, Kalluri R. Exosomes as a multicomponent biomarker platform in solid tumors. Trends Cancer 2018;4:416–24. https://doi.org/10.1016/j.trecan.2018.02.004.
Rocco GD, Baldari S, Toietta G. Exosomes and other extracellular vesicles.

Yang C, Yang H, Liu J, Zhu L, Yu S, Zhang X, Gao L. Focus on exosomes: novel approaches to investigate exosomes.

I. Tanasi et al. Computational and Structural Biotechnology Journal 18 (2020) 2670–2677

[63] Robbins PD, Dorrornoso A, Booken CR. Regulation of chronic inflammatory and immune processes by extracellular vesicles. J. Clin. Invest. 2016;126:1173–80.

[64] Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat. Rev. Immunol. 2014;14:195–208. https://doi.org/10.1038/nri3822

[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/JCI65137

[66] Rocco GD, Baldari S, Toietta G. Exosomes and other extracellular vesicles-mediated microRNA delivery for cancer therapy. Transl. Cancer Res. 2017;6:5121–5130.

[67] Rutherford SC, Fachel AA, Li S, Sawh S, Muley A, Ishii J, Saxena A, Dominguez JM, Kurre P. Alterations in acute myeloid leukaemia bone marrow stromal cell leu.2402885

[68] Santarém N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Pol MA, Ossenkoppele GJ, Schuurhuis GJ. A flow cytometric method to detect exosome-associated microRNA panels and in vivo environment to predict drug resistance. J. Extracell. Vesicles 2015;4:27066. https://doi.org/10.3402/ev.v4.27066

[69] Spoo AC, Lübbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in chronic lymphocytic leukemia. Blood 2008;10:1470–6. https://doi.org/10.1182/blood-2006-05-024844

[70] Sullivan LB. Taking metabolism on the road. Nat. Chem. Biol. 2017;13:924–5.

[71] Spoo AC, Lübbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in chronic lymphocytic leukemia. Blood 2008;10:1470–6. https://doi.org/10.1182/blood-2006-05-024844

[72] Sullivan LB. Taking metabolism on the road. Nat. Chem. Biol. 2017;13:924–5.

[73] Tanetta D, Dragovic R, Aliyayev Z, Southombe J. Extracellular vesicles and their physiological functions. J. Extracell. Vesicles 2015;4:27066. https://doi.org/10.3402/ev.v4.27066

[74] Zarone MR, Misso G, Grimaldi A, Zappavigna S, Russo M, Amler E, Di Martino S, Tassone P, Caraglia M. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Sci. Rep. 2015;5:16845. https://doi.org/10.1038/srep16845

[75] Zarone MR, Misso G, Grimaldi A, Zappavigna S, Russo M, Amler E, Di Martino S, Tassone P, Caraglia M. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Sci. Rep. 2015;5:16845. https://doi.org/10.1038/srep16845

[76] van Haaften G, Agami R. Tumorigenicity of the miR-17-92 cluster distilled. Nat. Rev. Cancer 2013;13:924–5.

[77] van Stijn A, Feller N, Piersma SR, Knol JC, Pham TV, Jansen G, Musters RJP, van Meerloo J, Assaraf YG, Kaspers GJL, Zweegman S, Jansen S, Croes J, Iljina J. 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

[78] Xie 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/10.1155/2010/471811

[79] Yáñez-Mó M, Siljander PR-M, Andrews Z, Zawie AB, Borrás FE, Buzas EÍ, 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, Kubokub K, Kozacevic M, Kralj-Iglic V, Kramer-Albers E-M, Latiniun S, Lassier C, Lerner T, Lijeti E, Line A, Lippis C, Lloroote A, Lótvall J, Manček-Keber M, Marcilla A, Mellbrunn M, Nazarenko I, Nolte’t Hoen ENM, Nyman TA, O’Driscoll L, Olivan M, Oliveira C, Pällinger E, Del Portillo HA, Reventós J, Rigau M, Rohde E, Sammar M, Sánchez-Madrid F, Santarem N, Schallmoser K, Ostenfeld MS, Stoorvogel W, Stukelj R, Van der Grein SG, Vasconcelos MH, Waeben MHM, De Wever O. Biological properties of extracellular vesicles and their physiological functions. J. Extracell. Vesicles 2015;4:27066. https://doi.org/10.3402/ev.v4.27066

[80] 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.

[81] 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

[82] Yeh Y-Y, Özer 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.

[83] Zaborowski MP, Balaj L, Breakefield XO, Lai CP. Extracellular vesicles: composition, biological relevance, and methods of study. Bioscience 2017;7:17949. https://doi.org/10.1038/s41598-017-18186-0

[84] Zarone MR, Misso G, Grimaldi A, Zappavigna S, Russo M, Amler E, Di Martino S, Tassone P, Caraglia M. Evidence of novel miR-34a-based therapeutic approaches for multiple myeloma treatment. Sci. Rep. 2015;7:17949. https://doi.org/10.1038/srep17949

[85] Zhang L, Pan X, Li Y, Zhao J, Chen Y, Li X, Zhou X, Xu M, Gao L, Zhao Y, Zhang J, Yin J. MiR-191a-3p modulates the expression of hypoxia inducible factor-1α and miR-34a to regulate glioma cell viability. PLoS One 2015;10:e0127983. https://doi.org/10.1371/journal.pone.0127983

[86] Zhang L, Pan X, Li Y, Zhao J, Chen Y, Li X, Zhou X, Xu M, Gao L, Zhao Y, Zhang J, Yin J. MiR-191a-3p modulates the expression of hypoxia inducible factor-1α and miR-34a to regulate glioma cell viability. PLoS One 2015;10:e0127983. https://doi.org/10.1371/journal.pone.0127983

[87] Zhao J-J, Chu Z-B, Hu Y, Lin J, Wang Z, Jiang M, Wang X, Kang Y, Zhou Y, Qin H, Fang B, Li J, Wang Y, Wang H, Zhong R. Myeloma cell adhesion to bone marrow stromal cell clustered differentiation 42 and miR-125b via-3a and miR-17-92 in chronic lymphocytic leukemia. Leukemia 2012;26:1113–6. https://doi.org/10.1038/leu.2011.250

[88] Zhou J, Chu Z-B, Yu H, 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, Carasco RD. Targeting the miR-221-222/PI3K/AKT/BAX pathway abrogates chemoresistance in multiple myeloma. Cancer Res. 2015;75:4384–97. https://doi.org/10.1158/0008-5472.CAN-15-0457.

I. Tanasi et al. Computational and Structural Biotechnology Journal 18 (2020) 2670–2677

2677