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microRNA and exosome profiling in multiple sclerosis

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Abstract

New DNA sequencing technologies have uncovered non-coding RNA (ncRNA) as a major player in regulating cellular processes and can no longer be dismissed as “junk” or “dark” RNA. Among the ncRNA, microRNA (miRNA) is arguably the most extensively characterized category and a number of studies have implicated them in regulating critical functions that can influence autoimmune demyelination. Of specific interest to multiple sclerosis (MS), miRNA have been implicated in both regulating immune responses and myelination, thus making them an attractive candidate for both pharmacological intervention and as disease biomarkers. In addition, exosomes, small vesicles secreted by most cell types and present in all body fluids, have been also shown to play roles in immune signaling, inflammation and angiogenesis. Therefore, exosomes are also being explored as tools for therapeutic delivery and as biomarkers. This article reviews the recent advances in miRNA and exosome profiling in MS and experimental models.

Keywords

microRNA; exosome; multiple sclerosis; EAE; biomarker; immune response

Introduction:

Despite the remarkable progress in the understanding and treatment of multiple sclerosis (MS), the disease course is still highly unpredictable¹. Therefore, an urgent need exists for the discovery of biomarkers that can track and predict outcomes related to disease progression and response to therapeutic drugs. Currently, no externally validated blood immune marker has adequate sensitivity and specificity to be used for the diagnosis of MS, which probably reflects the heterogeneity of the disease. Owing to the progress in high throughput sequencing technologies and emerging data on the mechanisms of horizontal transfer of material between the cells new opportunities have arisen in this field. In this review, we discuss currently available evidence associating novel intra and extracellular

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RNAs analysis as putative biomarkers of MS as well as their potential role in autoimmune demyelination development and progress.

microRNA and other non-protein coding RNAs

Molecular biology progress over last two decades has unveiled a universe of non-protein coding RNA (ncRNA) present in our cells. It is now apparent that protein coding RNA only represents about 2% of the information encoded in genomic DNA². Furthermore recent high throughput transcriptome analyses have documented that ncRNA constitutes a major product of our genome³. Our current knowledge of the function of ncRNAs is still very limited resulting in the notion that we are just starting to understand the true value of the information encoded in our genomes.

One of the best studied groups of the ncRNA are microRNA (miRNA). miRNA are conserved, single-stranded, non-coding RNAs comprised of approximately 22 nucleotides, that play important roles in gene-regulation by targeting mRNAs for cleavage or translational repression⁴. The master role of miRNAs is regulation of gene transcription at the posttranscriptional level, which is considered as an epigenetic regulatory mechanism, through cleaving or blocking messenger RNAs (mRNAs) from further translation. A single miRNA can directly repress translation of hundreds of genes and each mRNA transcript may be regulated by multiple miRNAs. This many-to-many relationship underscores the complexity of the miRNA regulatory networks that govern cellular programs from inflammation and neurogenesis, to homeostatic maintenance. Several databases and online resources are available to lookup published miRNA sequences and annotations (e.g. miRbase, <http://mirbase.org>), including specialized software to find associations between given miRNA and its target genes (e.g. miRSystem, <http://mirsystem.cgm.ntu.edu.tw>).

The role of miRNA in autoimmune demyelination

The last decade has seen great progress in the description of the role miRNA in MS and experimental autoimmune encephalomyelitis (EAE). From unbiased transcriptomic analysis to functional dissection of specific miRNAs, the field has flourished. In one of the earliest studies, miR-155 was identified as a key regulator of these inflammatory responses, as targeted deletion of this gene in mice resulted in a decrease in Th1 and Th17 cellular differentiation both in the CNS and in peripheral lymphoid organs. Furthermore, both deletion and pharmacological targeting of miR-155 resulted in delayed course and reduced severity of EAE, which prompted authors of the study to propose its therapeutic targeting⁵. In a separate study, a rare form of this same gene (miR-155-3p) was found to drive the development of autoimmune demyelination by regulation of heat shock protein 40⁶. miR-301a was also found to be a critical regulator of myelin-reactive T-helper type 17 cells, further supporting their role as candidates for therapeutic targets for controlling of autoimmune demyelination⁷.

In addition to their role in modulating effector inflammatory T cell responses, miRNA also play a role in regulating the regulatory T cell (Treg) population, that normally counterbalances the former. Surprisingly, miR-155 has also been found to influence Treg

development and function, as recent studies showed that it is regulated by Foxp3, a transcription factor characteristic of this cell population⁸. The seemingly opposite roles of the same miRNA in different cells highlights the complexity of the regulatory networks at play in the immune system.

While the previous examples highlighted the regulatory role of miRNA in proinflammatory responses during EAE, additional studies also showed their primary role in regulating myelination itself. For example, miR-23a-overexpressing mice have increased myelin thickness, providing in vivo evidence that miR-23a enhances both oligodendrocyte differentiation and myelin synthesis⁹.

miRNA as biomarkers of MS

Encouraged by the early discoveries of a handful of miRNA involved in myelination¹⁰ and inflammatory responses¹¹, several studies focused on their systematic profiling in serum and other biological fluids as disease biomarkers. In one study including 4 observational cohorts, 5 miRNAs (hsa-miR-484, hsa-miR-140-5p, hsa-miR-320a, hsa-miR-486-5p, and hsa-miR-320c) showed a significant difference between patients with MS and healthy individuals¹². A follow up study explored the potential of miRNA profiling to identify subtypes of MS. In this study, miR-92a-1 was differentially expressed in RRMS versus SPMS, and RRMS versus healthy controls. Furthermore, this miRNA showed an association with both EDSS and disease duration¹³.

In a more recent study, a large group of MS patients (n=1,088) was stratified based on brain imaging and miRNA profiling was used to identify differences across the different MRI-based phenotypes¹⁴. Interestingly, Each MRI phenotype demonstrated a miRNA signature whose underlying biology implicates blood-brain barrier pathology. Specifically, miR-22-3p, miR-361-5p, and miR-345-5p were the most valid differentiators of the MRI phenotypes.

While miRNA profiling from biofluids poses a great promise as a biomarker for MS, it must be noted that conflicting results, heterogeneity and lack of replication are ongoing challenges in the field. For example, a recent review of the literature highlighted that out of the 650 differentially expressed ncRNAs reported in MS patients, only 27.5% were found dysregulated in the same direction in at least two independent studies. If the cutoff criteria is increased to three studies, then only 9% of the reported differences remain¹⁵. This lack of replication is not foreign to biomarker projects in general and underscore the logistical difficulties behind this kind of studies. While some of these challenges (e.g. small sample size, biospecimen collection methods, profiling techniques, etc.) can be overcome with appropriate planning and funding, some are inherent to the complexity and heterogeneity of the underlying biology. The recent emergence of novel mechanisms of miRNA regulation (e.g. circular RNAs) might provide a better insight and renewed enthusiasm for the development of more robust biomarkers in autoimmune demyelination¹⁶.

Exosomes as cell-to-cell communication system

Exosomes are small extracellular vesicles released from the endocytic compartment of cells, which can be easily detected in serum and other biological fluids¹⁷. They contain material of

cell origin including proteins, lipids, DNA, mRNA, and non-coding RNA and mediate a wide array of biological functions including the modulation of immune reactions, cell differentiation and apoptosis¹⁸. Exosomes might in fact provide a mechanism for long distance intercellular communication since they can travel between different compartments of the body and across biological barriers. Due to their small size (30-120 nm) and lipophilic nature, exosomes are taken up by a number of organ tissues and cell populations¹⁹. Indeed, it was demonstrated that cells transfected with a plasmid coding for the green fluorescent protein (eGFP), when injected into mice, released exosomes carrying eGFP RNA that was found not only in the circulation but in many other cell types proving direct interaction between exosomes and target cells²⁰. The production, secretion, and transfer of exosomes arises as a crucial and still largely unexplored system of a cell-to-cell communication. Cancer research in particular provides numerous clues for the significance of exosome mediated effects²¹. An increasing amount of recently published literature confirms that cancer and cancer-associated cells use exosomes to communicate their message to malignant and non-malignant cells^{22–24}. This is done through the transfer of functional components, in order to initiate pathways that are necessary for tumor survival and propagation. Additional roles of exosomes include regulation of cell proliferation, induction of programmed cell death, metabolism, differentiation and phenotypic modification²⁵. Thus, exosome-mediated effects likely play a role in many pathological conditions and may contribute to every aspect of cellular biology and dysregulation.

Exosomes do not contain a random sampling of components of their parent cells but have a specific composition reflecting the state of cell activation and can be influenced by pathologic processes from the tissue of origin. Indeed, exosome release is considered a cellular adaptation mechanism and its biogenesis, composition and secretion are affected by the microenvironment of cells. Furthermore, extracellular RNA, RNA-binding proteins, and other cellular proteins are differentially expressed in exosomes and non-vesicle compartments¹⁸. Thanks to the recent progress in high throughput transcriptome techniques, the analysis of the low content material of total exosomes has become possible²⁶. The resulting data provide a unique insight into the wealth of the transcripts present in the extracellular vesicles^{18, 27}.

RNA species identified as a cargo of the cell culture released exosomes include predominantly different classes of short RNA species including miRNA, small nuclear RNA, small nucleolar RNA, Piwi-interacting RNA, vault RNA, Y-RNA, small conditional RNA, signal recognition particle RNA, 7SK-RNA²⁸. Furthermore a ribosomal RNA, transfer RNA, messenger RNA, long non-coding RNAs, and various intergenic repeats have also been found within the exosomes. So far only few studies have addressed the human biofluid derived exosomes full transcriptome content^{27, 29–31}. All of them demonstrated a wealth of sequences present in the circulating exosomes. Majority of the RNAs were ncRNA, including miRNA. These studies provided evidence that large part of the cellular transcriptome is present in exosomes and therefore could potentially be transferred to other cells. This complexity of the exosomal RNA cargo combined with their seclusion from the circulating RNAs provides an exciting platform for a biomarker discovery research.

Circulating exosomes as a biomarkers

Exosomes provide a means of cell-to-cell communication by transporting their cargo and delivering it to target cells. The content of these microvesicles representing a molecular bioprint of their parental cells. Therefore exosomes present in blood and other biofluids are potentially noninvasive biomarkers for early diagnosis and prognosis of various types of diseases³². Exosome profiling is often called a “liquid biopsy” as it may provide an insight about the content of the hard to access organs without the need of their direct sampling³³. Exosome release is considered a cellular adaptation mechanism and its biogenesis, composition and secretion are affected by the microenvironment of cells.

Currently clinicaltrials.gov registry enlists 35 active trials aiming at the observation of the exosomes biomarkers analysis. The majority of these trials is dedicated to the cancer and tumor research, and only one study, is dedicated to a neurological condition, (i.e. dementia)³⁴. Interestingly, blood circulating exosomes show presence of the CNS markers resulting from the brain origin^{35, 36}. The analysis of these exosomes should provide a unique insight into the status of the CNS bypassing the need for a biopsy³⁷. Furthermore exosomes can provide a source of the long sought CNS antigens trafficking through the BBB³⁶. These arguments should prompt for more trials involving exosomes analysis in the CNS conditions including MS.

Exosomes in MS monitoring

Emerging data on the use of miRNA and other molecular cargo carried by circulating exosomes as biomarkers of various conditions has renewed enthusiasm for further exploring the role of these microvesicles as a putative indicators of MS status and for disease monitoring³⁸. Current search of the PubMed database, using MeSH terms “exosomes” and “multiple sclerosis”, following an individual curation revealed 16 original publications relating to the biomarker search in MS (Table). The majority (9 articles) analyzed serum or plasma derived exosomes, whereas 3 addressed CSF microvesicles, 3 urine, 2 described cell cultures. Most frequently analyzed exosomes content was miRNA (9 papers), whereas 3 papers investigated lipids and only 2 proteins. Only two publications aimed at global profiling of exosomes cargo, either RNA²⁷ or protein³⁹. These reports document technical feasibility of exosomes extraction and subsequent analysis of MS patients derived material. Promising MS biomarkers (e.g. levels of blood circulating exosomal miR-122-5p) are starting to emerge^{27, 40}. Although the number of publications on the significance of exosomes analysis as biomarkers in MS is still limited the current results are encouraging and warrant further research.

Conclusion and further perspectives

Large-scale profiling of circulating miRNA and exosome content in EAE and MS has prompted early enthusiasm for their potential to emerge as the long-sought biomarker that can characterize patient status using a minimally invasive approach, yet with high sensitivity and specificity. However, while promising results have arisen, limited replication and high heterogeneity remain unsolved challenges. Further research is warranted to firmly establish

these relatively newly discovered biomolecules as firm contenders in the quest for the MS biomarker.

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References

1. Thompson AJ, Baranzini SE, Geurts J, Hemmer B and Ciccarelli O. Multiple sclerosis. *Lancet*. 2018; 391: 1622–36. [PubMed: 29576504]
2. Consortium EP. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012; 489: 57–74. [PubMed: 22955616]
3. Sarropoulos I, Marin R, Cardoso-Moreira M and Kaessmann H. Developmental dynamics of lncRNAs across mammalian organs and species. *Nature*. 2019; 571: 510–4. [PubMed: 31243368]
4. Bartel DP. Metazoan MicroRNAs. *Cell*. 2018; 173: 20–51. [PubMed: 29570994]
5. Murugaiyan G, Beynon V, Mittal A, Joller N and Weiner HL. Silencing microRNA-155 ameliorates experimental autoimmune encephalomyelitis. *J Immunol*. 2011; 187: 2213–21. [PubMed: 21788439]
6. Mycko MP, Cichalewska M, Cwiklinska H and Selmaj KW. miR-155-3p Drives the Development of Autoimmune Demyelination by Regulation of Heat Shock Protein 40. *J Neurosci*. 2015; 35: 16504–15. [PubMed: 26674874]
7. Mycko MP, Cichalewska M, Machlanska A, Cwiklinska H, Mariasiewicz M and Selmaj KW. MicroRNA-301a regulation of a T-helper 17 immune response controls autoimmune demyelination. *Proc Natl Acad Sci U S A*. 2012; 109: E1248–57.
8. Lu LF, Thai TH, Calado DP, et al. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity*. 2009; 30: 80–91. [PubMed: 19144316]
9. Lin ST, Huang Y, Zhang L, Heng MY, Ptacek LJ and Fu YH. MicroRNA-23a promotes myelination in the central nervous system. *Proc Natl Acad Sci U S A*. 2013; 110: 17468–73. [PubMed: 24101522]
10. Emery B Regulation of oligodendrocyte differentiation and myelination. *Science*. 2010; 330: 779–82. [PubMed: 21051629]
11. Xiao C and Rajewsky K. MicroRNA control in the immune system: basic principles. *Cell*. 2009; 136: 26–36. [PubMed: 19135886]
12. Regev K, Healy BC, Paul A, et al. Identification of MS-specific serum miRNAs in an international multicenter study. *Neurol Neuroimmunol Neuroinflamm*. 2018; 5: e491. [PubMed: 30175165]
13. Gandhi R, Healy B, Gholipour T, et al. Circulating microRNAs as biomarkers for disease staging in multiple sclerosis. *Annals of neurology*. 2013; 73: 729–40. [PubMed: 23494648]
14. Hemond CC, Healy BC, Tauhid S, et al. MRI phenotypes in MS: Longitudinal changes and miRNA signatures. *Neurol Neuroimmunol Neuroinflamm*. 2019; 6: e530. [PubMed: 30800720]
15. Piket E, Zheleznyakova GY, Kular L and Jagodic M. Small non-coding RNAs as important players, biomarkers and therapeutic targets in multiple sclerosis: A comprehensive overview. *J Autoimmun*. 2019; 101: 17–25. [PubMed: 31014917]
16. Zurawska A, Mycko MP and Selmaj KW. Circular RNAs as a novel layer of regulatory mechanism in multiple sclerosis. *J Neuroimmunol*. 2019; 334: 576971. [PubMed: 31163273]

17. van Niel G, D'Angelo G and Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol.* 2018; 19: 213–28. [PubMed: 29339798]
18. Jeppesen DK, Fenix AM, Franklin JL, et al. Reassessment of Exosome Composition. *Cell.* 2019; 177: 428–45 e18. [PubMed: 30951670]
19. Tkach M and Thery C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell.* 2016; 164: 1226–32. [PubMed: 26967288]
20. Jiang H, Li Z, Li X and Xia J. Intercellular transfer of messenger RNAs in multiorgan tumorigenesis by tumor cell-derived exosomes. *Mol Med Rep.* 2015; 11: 4657–63. [PubMed: 25673258]
21. Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW and Simpson RJ. Extracellular vesicles in cancer - implications for future improvements in cancer care. *Nat Rev Clin Oncol.* 2018; 15: 617–38. [PubMed: 29795272]
22. Aghebati-Maleki A, Nami S, Baghbanzadeh A, et al. Implications of exosomes as diagnostic and therapeutic strategies in cancer. *J Cell Physiol.* 2019; 234: 21694–706. [PubMed: 31161617]
23. Alyamani H, Obeid MA, Tate RJ and Ferro VA. Exosomes: fighting cancer with cancer. *Ther Deliv.* 2019; 10: 37–61. [PubMed: 30730823]
24. Bu H, He D, He X and Wang K. Exosomes: Isolation, Analysis, and Applications in Cancer Detection and Therapy. *Chembiochem.* 2019; 20: 451–61. [PubMed: 30371016]
25. Broekman ML, Maas SLN, Abels ER, Mempel TR, Krichevsky AM and Breakefield XO. Multidimensional communication in the microenvirons of glioblastoma. *Nat Rev Neurol.* 2018; 14: 482–95. [PubMed: 29985475]
26. Das S, Extracellular RNACC, Ansel KM, et al. The Extracellular RNA Communication Consortium: Establishing Foundational Knowledge and Technologies for Extracellular RNA Research. *Cell.* 2019; 177: 231–42. [PubMed: 30951667]
27. Selmaj I, Cichalewska M, Namiecinska M, et al. Global exosome transcriptome profiling reveals biomarkers for multiple sclerosis. *Ann Neurol.* 2017; 81: 703–17. [PubMed: 28411393]
28. Turchinovich A, Drapkina O and Tonevitsky A. Transcriptome of Extracellular Vesicles: State-of-the-Art. *Front Immunol.* 2019; 10: 202. [PubMed: 30873152]
29. Huang X, Yuan T, Tschannen M, et al. Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics.* 2013; 14: 319. [PubMed: 23663360]
30. Cheng L, Sun X, Scicluna BJ, Coleman BM and Hill AF. Characterization and deep sequencing analysis of exosomal and non-exosomal miRNA in human urine. *Kidney Int.* 2014; 86: 433–44. [PubMed: 24352158]
31. Ogawa Y, Taketomi Y, Murakami M, Tsujimoto M and Yanoshita R. Small RNA transcriptomes of two types of exosomes in human whole saliva determined by next generation sequencing. *Biol Pharm Bull.* 2013; 36: 66–75. [PubMed: 23302638]
32. Graner MW, Schnell S and Olin MR. Tumor-derived exosomes, microRNAs, and cancer immune suppression. *Semin Immunopathol.* 2018; 40: 505–15. [PubMed: 29869058]
33. Poudineh M, Sargent EH, Pantel K and Kelley SO. Profiling circulating tumour cells and other biomarkers of invasive cancers. *Nat Biomed Eng.* 2018; 2: 72–84. [PubMed: 31015625]
34. Raine CS, Bonetti B and Cannella B. Multiple sclerosis: expression of molecules of the tumor necrosis factor ligand and receptor families in relationship to the demyelinated plaque. *Rev Neurol (Paris).* 1998; 154: 577–85. [PubMed: 9809372]
35. Katsu M, Hama Y, Utsumi J, et al. MicroRNA expression profiles of neuron-derived extracellular vesicles in plasma from patients with amyotrophic lateral sclerosis. *Neurosci Lett.* 2019; 708: 134176. [PubMed: 31173847]
36. Galazka G, Mycko MP, Selmaj I, Raine CS and Selmaj KW. Multiple sclerosis: Serum-derived exosomes express myelin proteins. *Mult Scler.* 2018; 24: 449–58. [PubMed: 28273783]
37. Ramirez SH, Andrews AM, Paul D and Pachter JS. Extracellular vesicles: mediators and biomarkers of pathology along CNS barriers. *Fluids Barriers CNS.* 2018; 15: 19. [PubMed: 29960602]
38. Saenz-Cuesta M, Irizar H, Castillo-Trivino T, et al. Circulating microparticles reflect treatment effects and clinical status in multiple sclerosis. *Biomark Med.* 2014; 8: 653–61. [PubMed: 25123034]

39. Lee J, McKinney KQ, Pavlopoulos AJ, et al. Exosomal proteome analysis of cerebrospinal fluid detects biosignatures of neuromyelitis optica and multiple sclerosis. *Clin Chim Acta*. 2016; 462: 118–26. [PubMed: 27609124]
40. Manna I, Iaccino E, Dattilo V, et al. Exosome-associated miRNA profile as a prognostic tool for therapy response monitoring in multiple sclerosis patients. *FASEB J*. 2018; 32: 4241–6. [PubMed: 29505299]
41. Azimi M, Ghabaee M, Naser Moghadasi A and Izad M. Altered Expression of miR-326 in T Cell-derived Exosomes of Patients with Relapsing-remitting Multiple Sclerosis. *Iran J Allergy Asthma Immunol*. 2019; 18: 108–13. [PubMed: 30848579]
42. Saenz-Cuesta M, Alberro A, Munoz-Culla M, et al. The First Dose of Fingolimod Affects Circulating Extracellular Vesicles in Multiple Sclerosis Patients. *Int J Mol Sci*. 2018; 19.
43. Pieragostino D, Cicalini I, Lanuti P, et al. Enhanced release of acid sphingomyelinase-enriched exosomes generates a lipidomics signature in CSF of Multiple Sclerosis patients. *Sci Rep*. 2018; 8: 3071. [PubMed: 29449691]
44. Martelli F, Macera L, Spezia PG, et al. Torquetenovirus detection in exosomes enriched vesicles circulating in human plasma samples. *Virology*. 2018; 15: 145. [PubMed: 30236130]
45. Mallardi A, Nuzziello N, Liguori M, Avolio C and Palazzo G. Counting of peripheral extracellular vesicles in Multiple Sclerosis patients by an improved nanoplasmonic assay and dynamic light scattering. *Colloids Surf B Biointerfaces*. 2018; 168: 134–42. [PubMed: 29428682]
46. Kimura K, Hohjoh H, Fukuoka M, et al. Circulating exosomes suppress the induction of regulatory T cells via let-7i in multiple sclerosis. *Nat Commun*. 2018; 9: 17. [PubMed: 29295981]
47. Soundara Rajan T, Giacoppo S, Diomede F, Bramanti P, Trubiani O and Mazzon E. Human periodontal ligament stem cells secretome from multiple sclerosis patients suppresses NALP3 inflammasome activation in experimental autoimmune encephalomyelitis. *Int J Immunopathol Pharmacol*. 2017; 30: 238–52. [PubMed: 28764573]
48. Ebrahimkhani S, Vafae F, Young PE, et al. Exosomal microRNA signatures in multiple sclerosis reflect disease status. *Sci Rep*. 2017; 7: 14293. [PubMed: 29084979]
49. Singh J, Deshpande M, Suhail H, Rattan R and Giri S. Targeted Stage-Specific Inflammatory microRNA Profiling in Urine During Disease Progression in Experimental Autoimmune Encephalomyelitis: Markers of Disease Progression and Drug Response. *J Neuroimmune Pharmacol*. 2016; 11: 84–97. [PubMed: 26277791]
50. Moyano AL, Li G, Boullerne AI, et al. Sulfatides in extracellular vesicles isolated from plasma of multiple sclerosis patients. *J Neurosci Res*. 2016; 94: 1579–87. [PubMed: 27557608]
51. Giovannelli I, Martelli F, Repice A, Massacesi L, Azzi A and Giannecchini S. Detection of JCPyV microRNA in blood and urine samples of multiple sclerosis patients under natalizumab therapy. *J Neurovirol*. 2015; 21: 666–70. [PubMed: 25678142]

Table 1:

List of original publication describing analyzing exosomes as biomarkers in MS

	Publication	Journal	Source of exosomes	Cargo analyzed
1	Azimi et al., 2019 ⁴¹	Iran J Allergy Asthma Immunol	T cell cultures	miRNA
2	Saenz-Cuesta et al., 2018 ⁴²	Viruses	plasma	miRNA
3	Pieragostino et al., 2018 ⁴³	Sci Rep	CSF	lipids
4	Olascoaga et al., 2018 ⁴²	Int J Mol Sci	T cell cultures	functional assays
5	Martelli et al., 2018 ⁴⁴	Viruses	urine	miRNA
6	Manna et al., 2018 ⁴⁰	Faseb J	serum	miRNA
7	Mallardi et al., 2018 ⁴⁵	Sci Rep	serum	equivalent vesicle radius, number of EV, lipids, total RNA concentration
8	Kimura et al., 2018 ⁴⁶	Nat Commun	plasma	miRNA
9	Galazka et al., 2018 ³⁶	MS J	serum, CSF	proteins
10	Soundara Rajan et al., 2017 ⁴⁷	Int J Immunopathol Pharmacol	human periodontal ligament stem cells cultures	functional assays
11	Selmaj et al., 2017 ²⁷	Ann Neurol	serum	total transcriptome, miRNA
12	Ebrahimkhani et al., 2017 ⁴⁸	Sci Rep	serum	miRNA
13	Singh et al., 2016 ⁴⁹	J Neuroimmune Pharmacol	urine	miRNA
14	Moyano et al., 2016 ⁵⁰	J Neurosci Res	plasma	lipids
15	Lee et al., 2016 ³⁹	Clin Chim Acta	CSF	proteomic profile
16	Giovannelli et al., 2015 ⁵¹	J Neurovirol	plasma, urine	miRNA