Chapter

Role of Exosomes in Tuberculosis: Looking Towards a Future Road Map

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Abstract

Exosomes are generated by the multivesicular degradation of plasma membrane fusion, lysosomal, and extracellular release of intracellular vesicles. The exosome ranges from 30 to 150 nm in size. Exosomes are "bioactive vesicles" that promote intercellular communication. Exosomes contain a variety of biologically active substances packaged with proteins, lipids, and nucleic acids. After any microbe infection into the exosomes, the content of the exosomes changes and is released into the bloodstream. Such type of exosome content could be useful for basic research on exosome biology. Tuberculosis (TB) is a serious infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). During the *Mtb* infection, the exosomes played an important role in the body's infection and immune response by releasing several exosome components providing new ideas for diagnosis, prevention, and therapeutic treatment of *Mtb* infection. The detection of the low abundance of the *Mtb* numbers or secreted peptides in the serum of TB patients is not possible. The best way of findings for diagnosis and treatment of TB could be possible by the exploration of exosome content analysis through various useful technologies. The study and analysis of exosome content would produce a road map for the future early diagnosis, prognosis estimation, efficacy monitoring, research, and application for TB.

Keywords: exosome, TB, *Mtb*, content, roadmap, serum

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). *Mtb* is an intracellular bacteria engulfed by the macrophages through the process of phagocytosis. After invades into the host body, some of them eliminates and survives by immune escape mechanism as well but cause TB or latent tuberculosis infection (LTI). The exosomes size ranges 30–100 nm and is secreted by all living cells. Exosomes are circulating in the human body fluids rich in proteins, nucleic acids, lipids, etc. The components of the exosomes released by the body after *Mtb* infection play an important role in body's immune response and infection by providing new ideas on the diagnosis, treatment, and prevention of TB infection [1]. The vesicles were isolated through centrifugation at 10,000 *g* for

90 minutes from in vitro culture of sheep reticulocytes during the maturation of reticulocytes. These vesicles were called "exosomes" coined by Johnstone in the year 1987 [2]. Those vesicular exosomes were released during the maturation of the sheep reticulocyte and contained a few numbers of plasma membrane functions. These vesicles contained the transferrin receptor and also contain other plasma membrane activities such as nucleoside transporter and acetylcholinesterase. The formation of exosomes is a natural phenomenon with the release of the transferrin receptor [3]. Exosomes were observed in both nucleated and non-nucleated reticulocytes. The protein content of exosomes is equal to the protein content of plasma membrane. The protein content of the exosomes may vary on the origin of the species. Exosomes contain a non-transmembrane protein HSP70, a major cellular chaperone protein. The externalized proteins are the intact proteins retaining the catalytic activity and native ligand binding activity. The small exosome structures relatively contain many proteins play an important role in controlling serious human pathological problems by various pathogens. Revisiting the functions of exosomes in human pathological problems since the discovery could possibly to making a roadmap [4].

Exosomes were involved in intercellular information transmission and potential medical applications. The special insight on the biological significance of the exosome is very essential for various applications in the human biological field [4]. The characterization of exosomes is very essential during immune response for a better announcement of host-pathogen interactions. Based on exosome characterization, development of various approaches would be possible to fight infections through various pathogens. When macrophages infected with the *Mtb* release from cells small vesicles known as exosomes that contain pathogen-associated molecular patterns (PAMPs). When exosomes were exposed to the uninfected macrophages, they were stimulated with a proinflammatory response in a toll-like receptor and myeloid differentiation factor 88-dependent manner. The cell culture media along with fetal calf serum (FCS) at a centrifugal speed of 100,000 *g* for 15 h had been used to isolate contaminating exosomes [5]. The exosomes are controlling *Mtb* infection through exosome biogenesis. During *Mtb* infection, exosomes played an important role in recruiting and regulating host cells. *Mtb*-infected RAW264.7 cells secreted chemokines from C57BL/6 mouse-derived bone marrow macrophages treated with exosomes and also induced the migration of CFSE-labeled macrophages and splenocytes. Exosomes were purified using Exo Quick purification system (System Biosciences, CA) on an average of 20 μg purified exosomes from 10 million cells [6].

Mtb peptides were detected in serum extracellular vesicles with latent tuberculosis-infected (LTBI) individuals. The identification of biomarkers from a serum source of latent *Mtb*-infected patients could be a better target for preventive therapy. Multiple reaction monitoring mass spectrometry (MRM-MS) assays detected 40 *Mtb* peptides from 19 LTBI patients. Mtb peptide detection in serum extracellular vesicles is a useful technique in diagnosis of LTBI [7]. Exosomes containing highly antigenic proteins could be an alternative approach for the development of a TB vaccine [8]. Extracellular vesicles (EVs) delivered Mycobacterium RNA into the host to promote host immunity by killing the bacteria. This technology is a novel approach to treat drug-resistant TB [9]. Exosomes were used as a tool for rapid diagnosis of TB. The detection of *Mtb* lipoarabinomannan and CFP-10 from the urinary EVs of pulmonary tuberculosis (PTB) and extrapulmonary tuberculosis (EPTB) patients would be helpful in the rapid diagnosis of TB [10].

Mtb-infected exosome contains a lot of proteins, nucleic acids for the rapid or slow manner detection and diagnosis of TB whether PTB or LTBI or drug-resistant (DR-TB). The collection of various *Mtb*-infected exosome materials from various research papers could give a better road map on the diagnosis of TB in a better way and plan out the future for rapid diagnosis on the development, detection, and cure of TB in the world.

2. Exosomes response to the *Mtb*

The host interactions with the pathogens are always a challenge in chronic diseases and to understand the mechanism, complexities, and sequential events. TB is a major worldwide disease and the understanding of TB immunology become a major refined since the identification of *Mtb.* Understanding the mechanism of how the immune cells are recognizing *Mtb* can be an important issue for development of therapeutic strategies and vaccine development. Several classes of pattern recognition receptors (PRRS) including toll-like receptors (TLRs), C-type lectin receptors (CLRs), and nod-like receptors (NLRs) were involved in the recognition of *Mtb.* TLRs family such as TLR1, TLR2, TLR4, TLR9, IL-1β, and IL-18 played an important role in the pathogenesis of TB [11].

Exosomes are the potential mediator of T cell activation. The released exosomes from mouse *Mtb* infection contribute significantly to T cell response. Rab27a played an important role in exosome biogenesis. The Rab27a deficiency mice showed diminishing of the protein components to exosomes and *Mtb* strains. Exosomes function to promote T cell immunity during *Mtb* infection and an important source of extracellular antigen [12]. Exfoliated vesicles with 5′-nucleotidase activity was reflected from the culture of various normal and neoplastic cell lines. Exfoliated membrane vesicles were served in physiologic function and referred to as exosomes. It was observed by electron microscopy that the shredded vesicles were a constituted part of plasma membrane [13].

EVs were packed with proteins, nucleic acids, and lipids released from the mammalian and bacterial cells. EVs played an important role through the intercellular transduction acts like a messenger. The *Mtb*-infected EVs released cells played an important regulatory role in the anti-*Mtb* immune response. EVs regulate innate and acquired immune responses of the body against *Mtb* and for this key immune response, EVs were considered an important factor in the development of *Mtb* vaccine [14]. The microbial and host interaction components were spread through exosomes either activate or suppress the immune system of the host. Exosomes were involved in multiple infection processes including formation or modification of the infection, T or B cells activation, and interaction with nonimmune cells such as fibroblasts and endothelial cells (**Figures 1–3**). When the bacteria exposure to the exosome, the release of cellular components begins with the activation/submersion of the immune response of the host [15].

Proteins secreted from the Mycobacterium species were identified those were contributed to the protective immunity. Mycobacterial surface proteins were analyzed from infected macrophages. The fibronectin and 85 kDa protein complexes were identified among the mycobacterial proteins released by the infected macrophages [16].

The exosomes promoted the macrophages for the release of chemotactic factors by activating immune cells in vivo and in vitro [6]. The microvesicles and exosomes

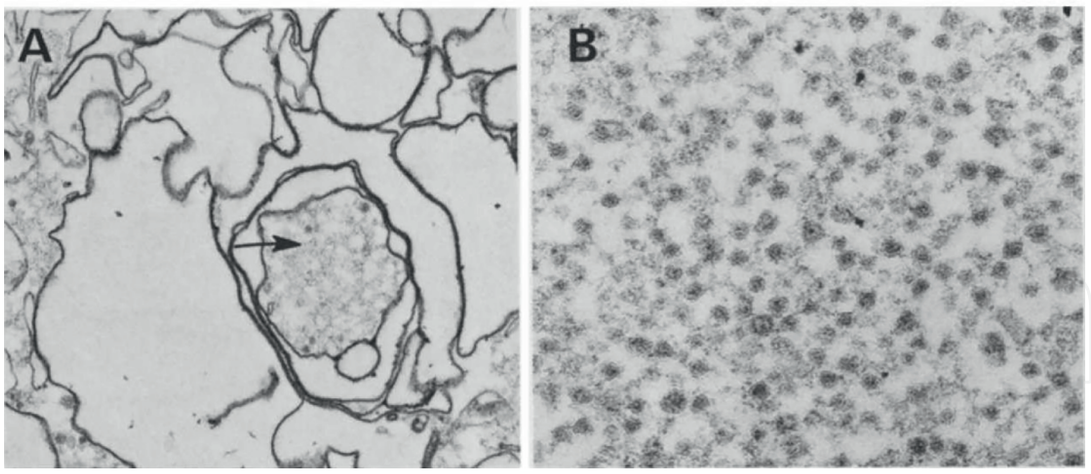

Figure 1.
(A) Electron photomicrograph of vesicular particles sedimented from superfusate of C-6 rat glioma monolayer cultures. Particles in conditioned medium. (magnification X 33 600). Note smaller vesicles contained within the larger vesicles (arrow). (B) Small vesicle population at greater magnification (glutaraldehyde fixed, magnification X 78 400) [13].

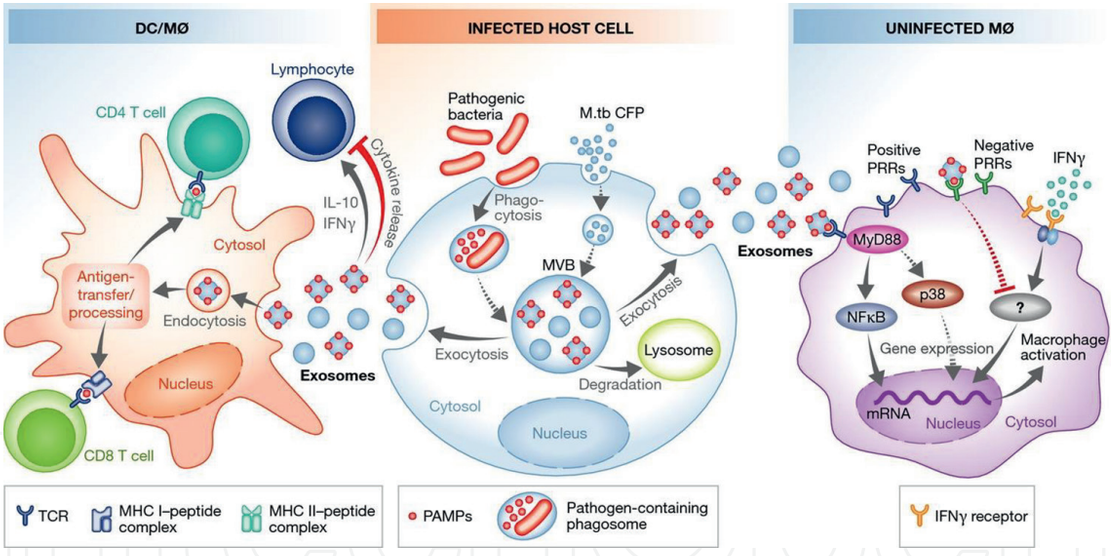

Figure 2.
Exosomes from bacteria-infected macrophages release exosomes containing antigens that induce cross-priming to activate antigen-specific $CD4^+$ and $CD8^+$ T cells. Some exosomes released from infected cells inhibit cytokine production by T cells. Exosomes from infected cells also contain PAMPs that stimulate macrophage production of proinflammatory mediators like TNF-α or limit the macrophage response to IFN-Y. Dashed line indicates unknown mechanism [15].

from the *Mtb* macrophages could activate T cells in response to antigen presentation. Adenosine triphosphate (ATP) induced exosomes were generated very rapidly and yielded much higher allowing significant time and cost advantages. *Mtb* interacted with ATP to induce the release of exosomes. These induced exosomes contained the major histocompatibility complex class-II (MHC-II) molecules for antigen presentation. ATP-induced exosomes could be used for a therapeutic purpose as an alternative to conventional exosomes [17].

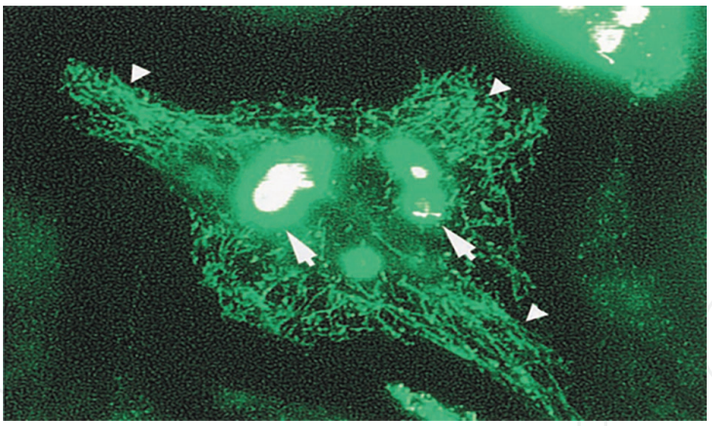

Figure 3.
The release of mycobacterial proteins from the phagosome in infected macrophages. Release of labeled mycobacterial proteins from the phagosome in infected macrophages. Live BMMf infected for 24 h with fluorescein succinimidyl ester-labeled BCG were analyzed by fluorescence microscopy. Labeled bacterial proteins were released from the mycobacterial phagosome into subcellular compartments of the infected macrophage (small arrowheads). The labeled bacteria are intensely fluorescent and are indicated by the large arrows [16].

3. Exosome contents and proteomic profiles of exosome proteins with TB

Exosomes are nanovesicles secreted by most but not all cells and specifically mediate intercellular communication through the transfer of genetic information of coding and noncoding RNA to recipient cells. The exosomes played an important biological role in the regulation of normal physiological and pathological processes through altered gene regulatory networks. Exosomes were targeted for the delivery of human genetic therapies through exogenous genetic cargoes such as siRNA [18]. *Mtb* is always in a dormant state for many years in the host system which is the cause of latent tuberculosis (LTB). Exosome contains a lot of *Mtb* antigens may be used as an alternative approach to develop the TB vaccine. A study was reported through the LC–MS/MS technique identified 41 *Mtb* proteins such as antigen 85-C, PckA, GabD1, β-1,3-Glucanase precursor, DnaK, LpdC, LprA, EST-6, etc. These were presented in exosomes released from *Mtb*-infected J774 cells and 29 *Mtb* proteins such as antigen 85-C, PckA, Fba, PepN, SahH, GroES, etc. Many of the released exosome proteins were highly immunogenic [8]. Flow cytometry analysis is a suitable method to characterize the surface markers of the extracellular vesicle's subpopulations in cells. The surface marker proteins were detected those were unique to exosomes naïve and *Mtb* infected THP-1 macrophages. The most similar findings of the surface protein markers such as CD63, CD9, CD81, and CD29 were detected in the exosomes of THP-1 cell culture supernatants by flow cytometry method. The purpose of characterization of the exosome surface proteins from the cell culture supernatants. Thus, the establishment of more sensitive methods enables the researcher to characterize the *Mtb* proteins in exosomes [19]. The main function of exosomes is interaction between cells through contact and exchange of soluble materials.

In TB patients, the exosomes were released from the *Mtb*-infected cells. The plasma of active TB patients generally contains the lipids and proteins derived from the exosome. Exosomes of all TB patients contains a lot of proteins such as sphingomyelins (SM), phosphatidylcholines, phosphatidylcholine inositol, free fatty acids, triglycerols, cholesteryl esters, etc. *Mtb* infections to the host proteins changed the

host protein composition of a total of 37 proteins. Proteomic study indicates the expression of low levels of proteins such as apolipoproteins, antibacterial proteins cathelicidin, scavenger receptor cystine rich family member, ficolin3, etc. were observed in TB patients but the adhesion proteins (integrins, intercellular adhesion molecule2 (ICAM2), CD151, proteoglycan4 were highly prevalent in PTB patients. Analysis of these exosome proteins in TB patients is a new achievement in the host-pathogen interaction and helps the development of new vaccines and therapies in TB research [20].

Exosomes were loaded with the microbial proteins after *Mtb* infection. After *Mtb* infection into the exosome, there must be changes in the composition of exosomal proteins and the study of the exosomal proteins could contribute to the understanding of the progression of TB after *Mtb* infection and open the way to understand the development of a specific biomarker for diagnosis of the TB. An experimental analysis of the *Mtb*-infected cells by the tandem mass spectrometry analysis specifically showed that the 41 proteins were significantly more abundant in exosomes. Some of the proteins were identified through the novel biotinylation strategy to confirm the protein localization in the exosomal membrane. The *Mtb* influenced the changes in the protein composition of exosomes released from the *Mtb*-infected cells [21]. These proteins are given in **Tables 1–3**.

A study investigated the regulation of protein N-glycosylation in human macrophages and their secreted microparticles (MPs) after *Mtb* infection. Upon *Mtb* infection, the protein N-glycosylation of macrophages rapidly and significantly occurred following *Mtb* infection [22]. Always searching for a rapid and sensitive biomarker is useful for the diagnosis of TB. Exosome markers were stable within the double membrane of the exosome. Heat shock protein HSP16.3 was an important capsule protein produced by *Mtb*. The HSP16.3 protein was secreted in excess amount in exosomes from the U937 cells infected with *Mtb* and an amount of HSP16.3 proteins was detected in blood exosomes of ATB patients. Thus, the HSP16.3 protein act as an exosome-based TB biomarker for *Mtb* diagnosis [23]. Blood-secreted exosome-based "biosignature" through the multiple reactions monitoring mass spectrometry assay (MRM-MS) could be used as a diagnostic biomarker for active TB [24]. The details of the peptides are given in **Tables 4** and **5**.

Exosome RNA sequencing analysis were derived from the clinical samples of ATB, LTB revealed the gene expression profiles. The selective packaging of RNA cargoes into exosomes in different stages of *Mtb* infection would facilitate the potential targets for prevention, treatment, and diagnosis of TB. The gene enrichment analysis of the *Mtb* RNA in exosomes identified a lot of functions in active and LTB patients [25]. The details of total function of *Mtb* exosome are given in **Figure 4**.

These gene-enrichment analysis of the *Mtb*-infected exosome gives an idea of the future roadmap of the TB diagnosis in active population level. Generally, TB diagnosis was performed through microscopy, PCR amplification, or culture of *Mtb* DNA from sputum or the biopsy of infected tissue from human beings. The current improvement of detection methods for diagnosis of TB in serum samples could possible by advanced methods. Sometimes the detection of Mycobacterial products in serum is not possible due to the low abundance number of *Mtb.* The exploration of the exosome enrichment advance assay would require to improve the sensitivity of the assay.

No. of proteins	Identified proteins
1.	60S acidic ribosomal protein P0
2.	Coronin-1 C
3.	Lupus La Protein
4.	Heterogenous nuclear ribonucleoprotein K
5.	Heat shock 70KDa protein 4
6.	Alanine -tRNA ligase, cytoplasmic
7.	Calreticulin
8.	Protein disulphide isomerase A3
9.	L-amino acid oxidase
10.	Moesin
11.	Nucleolin
12.	Vimentin
13.	Protein disulfide-isomerase A6
14.	Spliceosome RNA helicase DDX39B
15.	Fermitin family homolog 3
16.	Programmed cell death -6 interacting protein
17.	S-adenosylmethionine synthase isoform type -2
18.	Glyceral dehyde -3 phosphate dehydrogenase
19.	ATP dependent RNA helicase A
20.	60 kDa heatshock protein, mitochondrial
21.	Cytosol aminopeptidase
22.	Ubiquitin like modifier activating enzyme-1
23.	ITIH4 protein
24.	Serine/threonine protein phosphatase 2A 65kDa regulatory subunit A alpha isoform
25.	Tryptophan t-RNA ligase cytoplasmic
26.	Transketolase
27.	Zyxin (fragment)
28.	Heat shock protein HSP90-beta
29.	Tyrosine-tRNA ligase, cytoplasmic
30.	6-Phosphogluconate dehydronase, decarboxylating
31.	X-ray repair cross complementing protein-6
32.	78kDa glucose regulated protein
33.	Eukaryotic initiation factor 4A-I
34.	Thrombospondin -4
35.	Bifunctional purine biosynthesis protein PURH
36.	Staphylococcal nuclease domain containing protein-1
37.	Heat shock cognate 71kDa protein
38.	Integrin beta-1

No. of proteins	Identified proteins
39.	UDP glucose 6-dehydronase
40.	Purine nucleoside phosphorylase
41.	Lamin B-1
42.	Transforming growth factor beta induced protein ig-h3
43.	Palmitoyl protein thioesterase -1
44.	Complement C4- A

Table 1.
Proteins significantly different between exosomes from Mtb-infected and control macrophages.

No. of proteins	Membrane associated proteins
1.	60S acidic ribosomal protein P0
2.	Coronin-1 C
3.	Heterogenous nuclear ribonucleoprotein K
4.	Alanine -tRNA ligase, cytoplasmic
5.	Calreticulin
6.	Protein disulphide isomerase A3
7.	Moesin
8.	Nucleolin
9.	Vimentin
10.	Protein disulfide-isomerase A6
11.	Fermitin family homolog 3
12.	Programmed cell death -6 interacting protein
13.	Glyceral dehyde -3 phosphate dehydrogenase
14.	ATP dependent RNA helicase A
15.	60 kDa heatshock protein, mitochondrial
16.	Cytosol aminopeptidase
17.	Serine/threonine protein phosphatase 2A 65kDa regulatory subunit A alpha isoform
18.	Transketolase
19.	Heat shock protein HSP90-beta
20.	78kDa glucose regulated protein
21.	Eukaryotic initiation factor 4A-I
22.	Bifunctional purine biosynthesis protein PURH
23.	Staphylococcal nuclease domain containing protein-1
24.	Heat shock cognate 71 kDa protein
25.	Integrin beta-1
26.	Lamin-B1

Table 2.
Membrane-associated proteins significantly more abundant in exosomes from Mtb infected cells and their biotinylation pattern.

No. of proteins	Protein name and sequences
1.	Eukaryotic initiation factor 4AI and EVQkLQMEAPHIIVGTPGRVF
2.	Glyceral dehyde 3 phosphate dehydrogenase and DNFGIVEGLMTTVHAITATQkTV
3.	Heat shock cognate 71 kDa protein and DPVEkALR
4.	Heat shock protein HSP90-beta and ERIMkAQALR
5.	Moesin and EFAkEALLQASR
6.	Nucleoside diphosphate kinase and ERTFIAIkP
7.	Vimentin and DVRQQYESVAAkNLQEA

Table 3.
List of proteins and specific peptides labeled with LC-LC biotin.

An enhanced MRM-MS is a method to detect ultra-low abundance of ultra-*Mtb* peptides in human serum exosomes. This MRM-MS technology could be useful for the detection and diagnosis of low-abundance *Mtb* peptides in the circulating serum exosome for the search of biomarkers [26]. As TB is a chronic infectious disease, attention to be paid to the non-coding RNA of exosome content of *Mtb* patients. Research on progress reported by Shu-hui et al. [27] on exosome non-coding RNA of *Mtb* patients could be useful as a potential biomarker on TB. A comprehensive proteomic analysis of the serum exosome proteins was analyzed in active TB (ATB) patients. A total of 123 differential proteins were identified in the serum exosome of ATB patient's. The characterization and identification of proteins in exosome of serum-active patients could consider a potential biomarker for TB [28]. The details of upregulated and downregulated proteins are given in **Tables 6** and 7.

The study and analysis of exosome contents are suitable for the development of a suitable biomarker for the diagnosis and treatment of TB. The exosome protein components were identified.

Sequence	Protein
a. PTB patient	
FALNAANAR	GlcB
VYQNAGGTHPTTTYK	Mpt64
AFDWDQAYR	Mpt64
EAPYELNITSATYQSAIPPR	Mpt64
b. EPTB patient	
PGLPVEYLQVPSPSMGR	Ag85
FLEGLTLR	Ag85c
LYASAEATDSK	Mpt32
ATIEQLLTIPLAK	GlcB
DGQLTIK	HspX
SEFAYGSFVR	HspX

Table 4.
Peptides that distinguish (a) pulmonary tuberculosis (PTB) or (b) extra-PTB patients from non-Tb patients.

Sequence	Protein
FALNAANAR	GlcB
DGQLTIK	HspX
SEFAYGSFVR	HspX
WHDPWVHASLLAQNNTR	Mpt51
GSVTPAVSQFNAR	Mpt63
VYQNAGGTHPTTTYK	Mpt64
EAPYELNITSATYQSAIPPR	Mpt64
IPDEDLAGLR	AcpM
ATIEQLLTIPLAK	GlcB

Table 5.
Peptides specifically detected in active Tb patients.

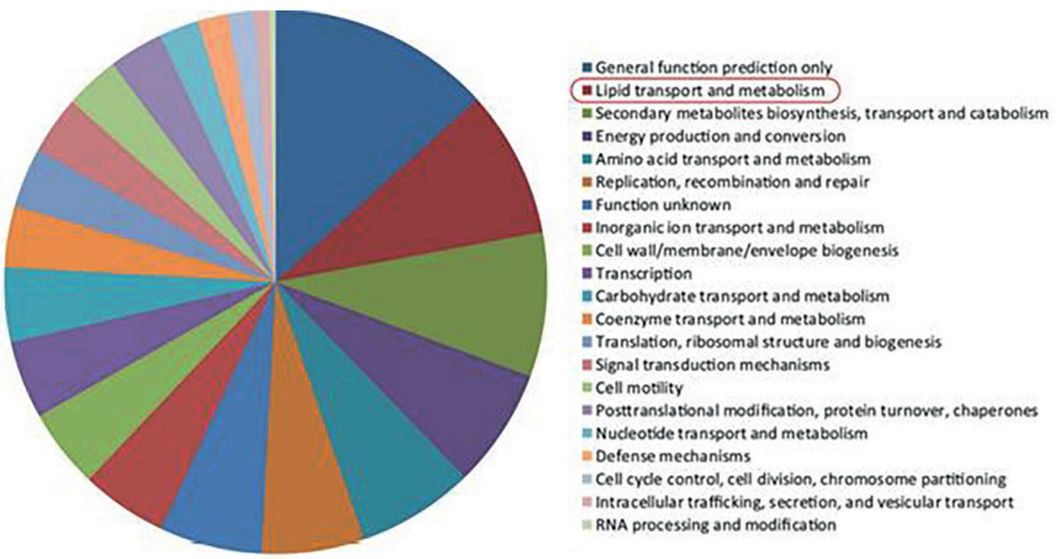

Figure 4.
Total function of Mtb exosome.

4. Exosome miRNA as a biomarker source for diagnosis and treatment of TB

Serum exosomes expressed CD81, the exosome marker protein. When these exosomes were infected with the *Mtb,* contains the increased level of miRNA such as miR484, miR 425, and miR96 in TB patients compared with the healthy control. As these markers were associated with active PTB, the expression of these miR could possibly increase the diagnostic power for diagnosis of TB patients as a biomarker [29]. Selection of biomarkers for diagnosis and treatment of TB is the most important issue. Analysis of blood samples from TB patients showed that the upregulation of miR-106b-5p was increased in exosomes. miR106b-5p promoted lipid droplet accumulation through the regulation of Creb5-SOAT1-CIDEC and suppressed macrophage apoptosis via Creb5-CASP9-CASP3 pathway leads to survival of *Mtb* in the host. The miR-106b-5p could be used as a biomarker for diagnosis of TB patients [30].

Now a days, TB is a threat to human health problem has an accuracy to the current TB diagnosis. Circulating exosome could be used as a diagnostic biomarker in TB. The study was examined the expression of the biomarkers for the diagnosis of TB infection. The miR-484, miR425, and miR96 were significantly increased in TB patients as compared with the healthy control and was examined the expression of miRNA as biomarker candidates for diagnosis of TB infection [31]. miRNA and electronic health records (EHRs) could be used to develop diagnostic models for

Number	Protein	Protein name	Gene
1	P02671	Fibrinogen alpha chain	FGA
2	G3V1N2	HCG1745306, isoform CRA_a	HBA2
3	A0N071	Delta globin	HBD
4	A0A024R035	Complement component C9	C9
5	V9HWE3	Carbonic anhydrase	HEL-S-11
6	Q9Y6C2	EMILIN-1	EMILIN-1
7	A0A024RC61	Aminopeptidase	ANPEP
8	Q8TCF0 L	Lipopolysaccharide-binding protein	LBP
9	P02786	Transferrin receptor protein 1	TFRC
10	P01023	Alpha-2-macroglobulin;α-2	A2M
11	C9JB55	Serotransferrin	TF
12	P19652	Alpha-1-acid glycoprotein 2	ORM2
13	Q1L857	Ceruloplasmin	N/A

Table 6.
Upregulated proteins with significant interesting in exosomes from ATB patients.

Number	Protein	Protein name	Gene
1	A3KPE2	Apolipoprotein C-III	APOC3
2	V9HVZ4	Glyceraldehyde-3-phosphate dehydrogenase	HEL-S-162eP
3	B0UXD8	HLA-DRA	HLA-DRA
4	P04899	Guanine nucleotide-binding protein G(i) subunit alpha-2	GNAI2
5	E7EU05	Glycoprotein IIIb	CD36
6	P23229	Integrin alpha-6	ITGA6
7	A0A024R4F1	2-phospho-D-glycerate hydro-lyase	HEL-S-17
8	G8GBV0	MHC class I antigen	HLA-A
9	P07737	Profilin-1	PFN1
10	L7UUZ7	Integrin beta	ITGB3
11	Q5JP53	Tubulin beta chain	TUBB
12	V9HWF0	Integrin-linked protein kinase	HEL-S-28

Number	Protein	Protein name	Gene
13	A0A024R611	Coronin	CORO1A
14	V9HWN7	Fructose-bisphosphate aldolase	HEL-S-87p
15	G9FP35	Guanine nucleotide binding protein	GNAQ
16	D3DVF0	Junctional adhesion molecule 1	F11R
17	Q9NZN3	EH domain-containing protein 3	EHD3
18	A0A024R3Q0	ADP-ribosylation factor 1, isoform CRA_a	ARF1
19	V9HWF5	Peptidyl-prolyl cis-trans isomerase	HEL-S-69p
20	B0V023	C6orf25	C6orf25
21	X6RJP6	Transgelin-2	TAGLN2
22	Q12913	Receptor-type tyrosine-protein phosphatase eta	PTPRJ
23	P08567	Pleckstrin	PLEK
24	P48059	LIM and senescent cell antigen-like-containing domain protein 1	LIMS1
25	Q86UX7	Fermitin family homolog 3	FERMT3
26	Q9Y490	Talin-1	TLN1
27	P21333	Filamin-A	FLNA
28	V9HWI5	Cofilin, non-muscle isoform	HEL-S-15
29	P61160	Actin-related protein 2	ACTR2
30	A8K0T9	F-actin-capping protein subunit alpha	N/A

Table 7.
Down-expressed proteins with significant interesting in exosomes from ATB patients.

PTB and tuberculosis meningitis (TBM) in a selective cohort study with the support vector machine (SVM) algorithm. Exosomal miRNAs (miR 20b, miR191 and miR486) along with EHR data through a machine learning algorithm could suggest for the diagnosis of the PTB and TBM [32]. The development of potential molecular targets for the detection and diagnosis of latent and active TB is possible by the miRNAs and repetitive region-derived small RNAs in exosomes. The most possible specifically expressed miRNA in LTBI patients were (hsa-let-7e-5p, hsa-let-7d-5p, hsa-miR-450a-5p, and hsa-miR-140-5p) and in TB patients were (hsa-miR-1246, hsa-miR-2110, hsa-miR-370-3P, hsa-miR-28-3P, and hsa-miR-193b-5p). Over all findings on miRNA, indicates the presence of biomarkers on the detection and diagnosis of the LTBI and TB patients [33].

The emerging role of functional and diagnostic potential of the several exosomal miRNA was studied by the several investigators and could explore as a possible diagnostic and therapeutic biomarker in *Mtb* infection [34]. TB biomarkers are generally predicting the treatment efficacy, cure of active tuberculosis, induction of protein immune responses by vaccination and reactivation of LTI. The suitable efforts are needed for development of suitable biomarker to meet the future challenges to cure the TB.

5. Exosomal DNA as a novel diagnostic biomarker for TB

Exosome is suitable for the detection of pathogen-derived nucleic acids. A novel approach was adopted for diagnosis of TB using exosomal DNA (exoDNA) through the droplet digital PCR (ddPCR). The ddPCR platform was used for detection of *Mtb* DNA in suspected PTB cases. The exosomal DNA was the primary method for the detection of the *Mtb* DNA in the ddPCR. The ddPCR is more sensitive than the real-time PCR. Therefore, the detection of exoDNA would be a sensitive and accurate method for diagnosis of *Mtb* infection [35].

6. Basic needs of exosomes as a biomarker content in the diagnosis and treatment of TB

Mtb causes the high morbidity and mortality for human TB. The pathogenesis of *Mtb* is very complex and is difficult to explained the mechanism of infection into human beings. The current TB diagnosis tools is inadequate and had several shortcomings on *Mtb* pathobiology. The study of the genetic diversity, pathogenesis of the *Mtb* through multi-omics approach leads to development of host biomarker in early diagnosis of TB. The discovery of new biomarkers has a great challenge on TB prevention and treatment. The search of a suitable biomarker for early diagnosis of TB is a great achievement in clinical context. TB remains a worldwide problem of human health. In order to prevent the TB infection, we must need the accurate vaccine development and reliable diagnostic tools.

Exosomes were isolated from human body fluids and considered for early detection of *Mtb* for diagnosis. From the above descriptive research papers, the research on the *Mtb*-derived exosomes (Mtbexo) is still at the preliminary stage and miRNA, protein, or DNA content of the *Mtb*-derived exosome from TB patients could possible for making a road map for biomarker discovery for the early diagnosis, treatment, and prevention of TB.

7. Conclusion

Exosome emerged as a potent genetic information for therapeutic potential through transfer agents corroborating a range of biological processes. Exosomes were used as a research tool for diagnosis and treatment of TB because the exosomes were released from cells packaged with biochemical materials. The characterization and detection of various packaged biochemical materials in exosome could make a future roadmap for the diagnosis and treatment of TB in human population level.

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