Exosomes - A Potential Candidate to Combat Covid-19 and A Future Emerging Nanotechnology with Diverse Perspectives

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ABSTRACT

Corona virus causes severe contagious acute respiratory syndrome known as coronavirus disease 2019 (COVID-19). Several initiatives have been taken to tackle so far. One of them, being therapy based biological nano particles "Exosomes". Administration of exosomes loaded with immunomodulatory cargo in combination with antiviral drugs will be a promising approach. This study deals with the exosomal isolation techniques of the Bruton Tyrosine Kinase (BTK) protein from cells. It represents initial trials to establish a method to isolate exosomes from human B cells.

Key words: *Exosomes, Extracellular vesicles, Multi vesicular bodies (MVB)*

INTRODUCTION

Corona virus disease (covid -19) is contagious viral disease with symptoms of severe respiratory distress. Coronavirus had first outbreak in Wuhan, China at the end of December 2019. Due to it's rapid spread out across the world in a short duration of time, World Health Organization (WHO) announced it as a global pandemic on March 11, 2020. COVID-19 has affected many countries worldwide. Globally, the number of deaths among individuals with the novel coronavirus infections were over 6,652,393 and more than 627,940,509 have been recovered.
(https:// covid19.who.int). Still corona virus constantly infecting many people in many countries across the world[1]. Even Bangladesh has been faced the 4th wave of outbreak of this viral illness[2]. Corona virus is mostly a pulmonotrophic virus producing lung symptoms. In addition, it can affect other vital organs producing a range of life-threatening complication including death. In the first stage of infection, viral genes enter into the host cell and subsequently cause the production of viral polypeptides, which then assemble into viral proteins. These proteins are required to form the viral core and surface S protein. Then the virus matures, replicates and leaves the host cells to infect other neighboring cells[3].

Extracellular vehicles (EVs) are nano-sized particles produced by all cell types. Exosomes, belongs to EVs group, are natural vehicles measuring 40 to 100 nm in diameter cells, secreted by majority of cell types, although not all[4]. Structurally exosomes contain lipids (cholesterol, sphingomyelin, ceramide), proteins (tetraspanins, Alix, TSG 101 and HSPs) and molecules involved in antigen presentation (MHC I and MHC ll) signal transduction and targeting/adhesion[5]. (Figure 1)

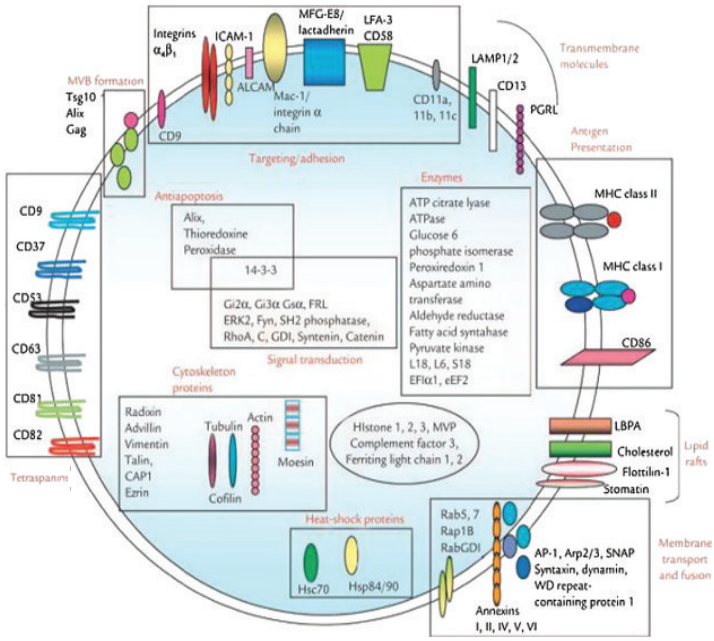


Figure-1: The structural constituents of exosomes. Adapted from "Exosome function: from tumor immunology to pathogen biology" by Schorey JS et al. 2008 Jun. Traffic.

These are responsible for a plethora of biological functions, including not only cell-to-cell communication but also signal transduction, transport of genetic material and modulation of immune responses[6]. Due to the nanometer size and inherent biological role, there are compelling evidence that indicates exosomes can be a potential delivery platform for emerging nanotechnology. The functional mechanism of exosomes are categorized into 4 groups which is on the basis of its action in the recipient cells – 1) Stimulation by surface molecule, 2) Transfer of trans-membrane receptors

through membrane fusion, 3) Delivery of intercellular contents and 4) Removal of unwanted molecules[7].

According to the statement of International Society of Extracellular Vesicles, EVs comprise three types of vesicles: Exosomes, micro vesicles (MVs) and apoptotic bodies[8]. To generate exosomes as well as identification and characterization of biological nano particles, the author have executed a project. The aim was to investigate the exosomal isolation as well as Bruton Tyrosine Kinase (BTK) protein from patients. The inter play in between exosomes and virus by mRNA or vector-based therapy could be a potential achievement for development of antiviral drug as well as for the dreadful Covid -19 disease.

METHODOLOGY

Cell culture of human B cells, (Namalwa Cells):

The experiment was performed to have adequate number of Namalwa cells to ensure a large volume of Namalwa cell-derived exosomes to isolate. The object was to grow more than 2 million cells. Cells were grown in a suitable medium.

Isolation method of exosomes:

Two types of suggested subcellular fractionation methods Ultracentrifugation and Spin filtration were tried. Isolation of exosomes from Namalwa cells were implemented. In order to confirm the presence of exosomes, western blot was executed with antibodies towards the exosome's specific protein Alix, CD9, TSG 101. Furthermore, to detect the presence of BTK in exosomes, anti BTK antibody was used in western blot. As a positive control, Namalwa cell lysate from the same cell preparation was used.

RESULT

This study represents initial trials to establish a method to isolate exosomes from human B cells. In order to determine the exosomal protein expression from Human B cell (Namalwa cell) antibodies towards the exosomal surface marker CD9 and the internal markers Alix and TSG 101 were used. Also, antibodies directed towards the B cell protein BTK, the protein of interest for theexosomal transfer in patients, were used. The results are described in Tab-1 and Fig-2.

Table-1: Outcome of the membrane incubation with different exosomes-specific antibodies.

Fig No	Exosomal Marker	Molecular weight.	1° antibody	2° antibody	Appearance of band
1	Alix	96 kDa *	Anti Alix	G ∝ M (red)	Not appeared
2	TSG 101	44 kDa *	Anti TSG 101	G ∝ R (green)	Faint band appeared
3	CD9	25 kDa *	Anti CD9	G ∝ R (green)	Not appeared
4	Btk	72 kDa *	Anti Btk	G ∝ M (red)	Not appeared

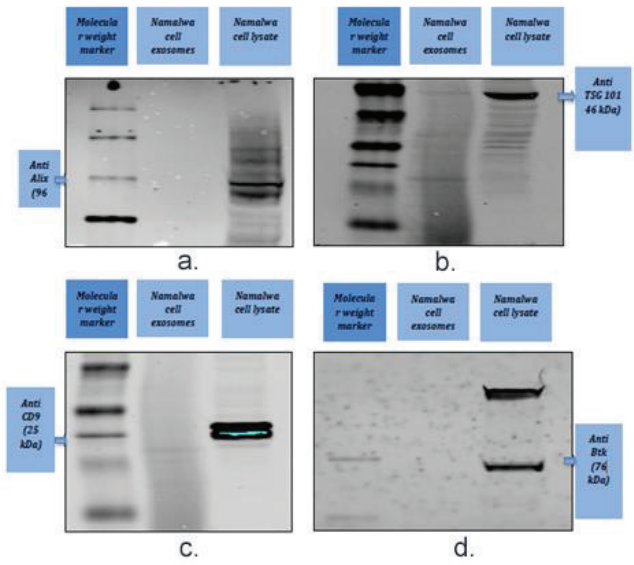


Figure-2: Membrane incubation with different markers in western blot – a. Membrane incubation with anti Alix,(96 kDa) b. Membrane incubation with anti TGS 101 (46kDa) c. Membrane incubation with anti CD9 (25kDa) d. Membrane incubation with anti BTK (76 kDa)

On analyzing the obtained result, a faint band of membrane incubation with TGS 101 antibody was noted in 46kDa location. But none of the other expected bands from the sample of Namalwa cell detected exosomal protein. As a positive control, the bands of the right molecular weight were obtained in the Namalwa cell lysate from all membranes that incubate with antibody of anti-Alix (96 kDa), TGS 101 (46 kDa), CD9 (25kDa) and BTK (76 kDa).

DISCUSSION

Exosomes are considered to be an excellent transportation media for delivering an intended drug to recipient cells. Some of inherent criteria of exosomes like capability to cross Blood Brain Barriers (BBB) as well as to protect the encapsulated material from the immune system can make exosomes more reliable in drug delivery mechanism[9]. Furthermore, exosomes that present in body fluid are less toxic, immunogenic and stable under physiological pH or in temperature and play their role in a static manner, which adds advantage in their candidacy to be considered as right candidate of drug delivery mechanism. Exosomes is excellent career that can transport specific cargo such as mRNA, non-coding RNAs, proteins and DNA from parental cells to neighboring cells. It also insists on stimulating cellular regeneration as well as plays significant roles of functional recovery under various pathological conditions.

In the treatment of COVID-19 disease, exosomes can be a promising applicant. Due to the canonical properties of exosomes, it can be engineered with various types of therapeutics agents i.e. loading immune modulatory cargoes as well as anti-viral drugs which could be useful therapeutic application. On the other hand, by inhibiting exosomes uptake to neighbor cells can be another initiative to limit virus spread out[10]. Due to the high rate of mutation of SARS-CoV-2, virus can directly suppress host T cell function, which makes various therapies ineffective[11]. Several strategies have been adopted to develop suitable treatments for COVID-19, including Mesenchymal stem cells and MSC-derived exosomes labeled with desire drug or drug conjugates. For instance, immune cells including T cells, B cells, dendritic cells, macrophages, and natural killer cells directly interact with MSCs can produce various cytokines and paracrine factors. (Figure - 3).

Due to similar structural and physicochemical properties of exosomes and viruses, exosomes can take extra facilities to communicate with cells and biogenesis. In addition, Soluble molecules proteins and nucleic acids such as microRNA (miRNA) and messenger RNA (mRNA) also contained in this particle[8]. Thus, drugs can be encapsulated into exosomes. (Figure 4). Polak et al. engineered EV-based vaccine platforms displaying native viral envelope proteins embedded in EVs and stimulating a robust anti-SARS-CoV-2 response in mice[12].The success rate of exosomes treatments resulted in 71% of the patients recovering from COVID-19[13].

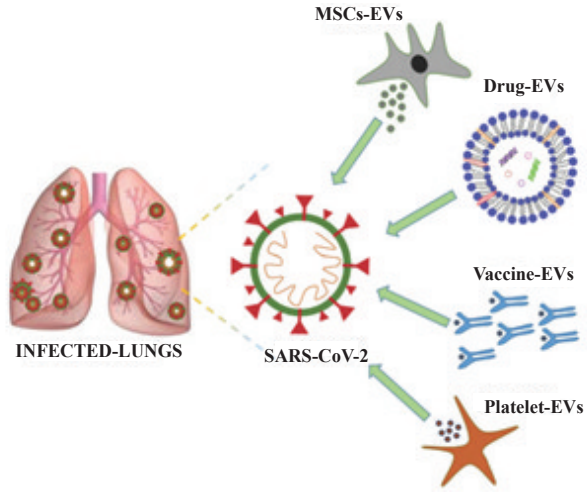
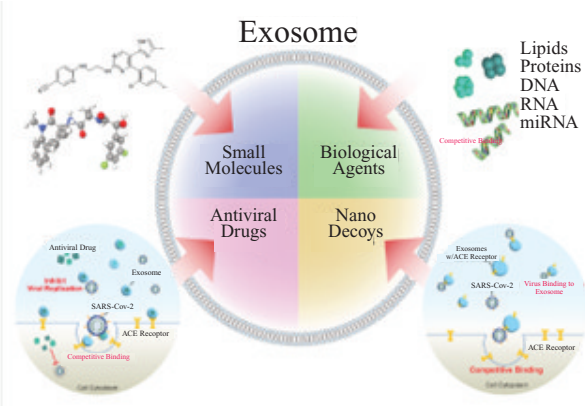


Figure 3: Various extracellular vesicle based therapeutic plan against COVID-19. Adapted from "COVID-19 and Extracellular Vesicles: An Intriguing Interplay" by Pocsfalvi G,et al. 2020. Sep 21;1–10. Kidney Blood Press Res.



This study represents initial trials to establish a method to isolate exosomes from human B cells. It also comprised of different techniques with different markers. Among these groups, one marker, TGS 101 could be detected by TGS 101 antibody in 46kDa. (Molecular weight).

Yet, large scale of research and of clinical trials are necessary to determine the safety, specificity, proficiency, and delivery mechanisms of drugs to target tissues. Exosomes have received significant attention in both academic and industrial sectors due to their beneficial concerns and are now being immense subject of investigation to be used as biomarkers, immunomodulators, therapeutics and vaccines.

CONCLUSION

In conclusion, role of exosomes in transmission, infections, diagnosis, treatment, therapeutic and drug delivery represent future era with potentiality of their use for combating COVID-19. But to have a positive role of exosomes, the first step would be to properly isolate exosomes with canonical marker. This initial study has shown a possible way of isolation of exosomes. Further studies are recommended to establish a proper working method for exosomes isolation.

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