Emerging Role of Exosomal Secretory Pathway in Human Tumour Virus Pathogenesis

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Authors' contributions

This work was carried out in collaboration between both authors. Author NRK complied and drafted the review. Author VK proof read and approved the manuscript. Both authors read and approved the final manuscript.

Mini Review Article

ABSTRACT

Viruses are well known for their ability to hijack and manipulate the host cellular machinery to ensure immune evasion, viral survival and pathogenesis. Most animal viruses exhibit exclusive tropism and thus, infect only specific target cells. However, reports on the existence of virions and viral components in non-target cells suggest alternative mechanisms of viral spread. Studies on microvesicles and exosomes promise to provide justification for the presence of viruses at unrelated cell types. Exosomes have attracted the attention of not only cell biologists but also virologists as these vesicles can transport and deliver bioactive information (RNA, proteins, microRNA etc. including virus specific

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components from infected cells) to unrelated cell types and have the potential to regulate target cell function. Recent studies suggest that viruses can manipulate and hijack the exosome biogenesis and secretory pathway to manipulate the host microenvironment, evade immune response and increase viral accessibility. Here, we review the existing literature on viral interference and exploitation of exosome secretory mechanisms and correlate it with the increased virulence and spread of viruses in the host. Further, we discuss the prospects of exosomes as emerging biomarkers for virus induced pathology, potential of exosomes as delivery vehicles and also the new perspective to viral mediated pathogenesis.

1. INTRODUCTION

Intercellular communication is vital to the maintenance of homeostasis in metazoan. Living organisms achieve intercellular cross talk by multiple means such as soluble factors, adhesion molecules, tight junction, nano tubules etc. that allow communication between unrelated cells even at a distant location [1]. Lately, secretory vesicles have gained attention following the discovery that these were not cellular debris but small lipid-membrane bound entities containing vital bioactive substances and having the ability to modulate other cells by horizontal transfer of information [2]. Initial studies revealed that the secretory vesicles contained specific functional enzymes in the same ratio as the cell of their origin [3,4]. This helped clear the dogma that extracellular vesicles production is not just related to cell-death and cellular membrane turnover but was a well-regulated cellular mechanism. Besides, this opened up exciting new avenues to unravel the role of these tiny vesicles in intercellular transport and communication either by direct fusion, endocytosis or phagocytosis by the target cells. Specific surface receptors such as integrin may be involved for their entry in certain cases [5], but the specificity of this mode of communication is still elusive. Subsequent studies on their biogenesis, secretion and uptake by target cells have added to their pivotal role in intercellular communication between unrelated cell/tissue types which could be essential for body homeostasis under normal or pathogenic conditions [2].

1.1 Types of Secretory Vesicles

Cellular secretory vesicles can be formed by blebbing of apoptotic cells, pinching of plasma membrane of living cell or even intracellularly in the lumen of multi vesicular bodies (MVB) that eventually release their contents in the extracellular milieu. Due to their diverse modes of origin, the cellular vesicles differ in size, molecular content, physical appearance; specific marker proteins etc [6]. Accordingly, these vesicles have been named differently such as apoptotic bodies, microvesicles and exosomes that play different role under different physiological conditions. Interestingly, these vesicles are also named differently on the basis of the cell of their origin. For example, those secreted by platelets are called micro particle, while those secreted by dendritic cells are named dexosomes [6,7]. Vesicles secreted by leucocytes have been named ectosomes [8,9]. Vesicle secretion can be constitutive or induced by different stimuli such as heat shock, viral infection, chemokine exposure and hypoxia, leading to membrane remodelling and vesicle shedding [6,10]. Vesicle secretion is also perceived as an adaptive mechanism of multicellular organisms in order to adjust with the surroundings and maintain homeostasis under ever-changing extracellular environment.

The presence of secretory vesicles in most body fluids such as serum, saliva, breast milk, urine, amniotic fluid etc., make them an ideal choice for minimally invasive biomarker [8,11].

1.1.1 Discovery of exosomes

Studies on reticulocytes revealed the shedding of transferrin receptors in vesicles composed of limiting membranes of inclusion bodies [12,13]. These vesicles exhibited a density ranging in between plasma membrane and lysosomes [14]. These intraluminal tiny vesicles were named as "Exosomes" by Johnstone et al. [13]. Later, these were reported to be filled with bioactive molecules such as proteins, small mRNAs, microRNAs and viral factors and were identified as crucial mediators of intercellular signaling, immune response and carriers of biomolecules including foreign pathogens and viruses to uninfected cells and tissue [15] (Fig. 1).
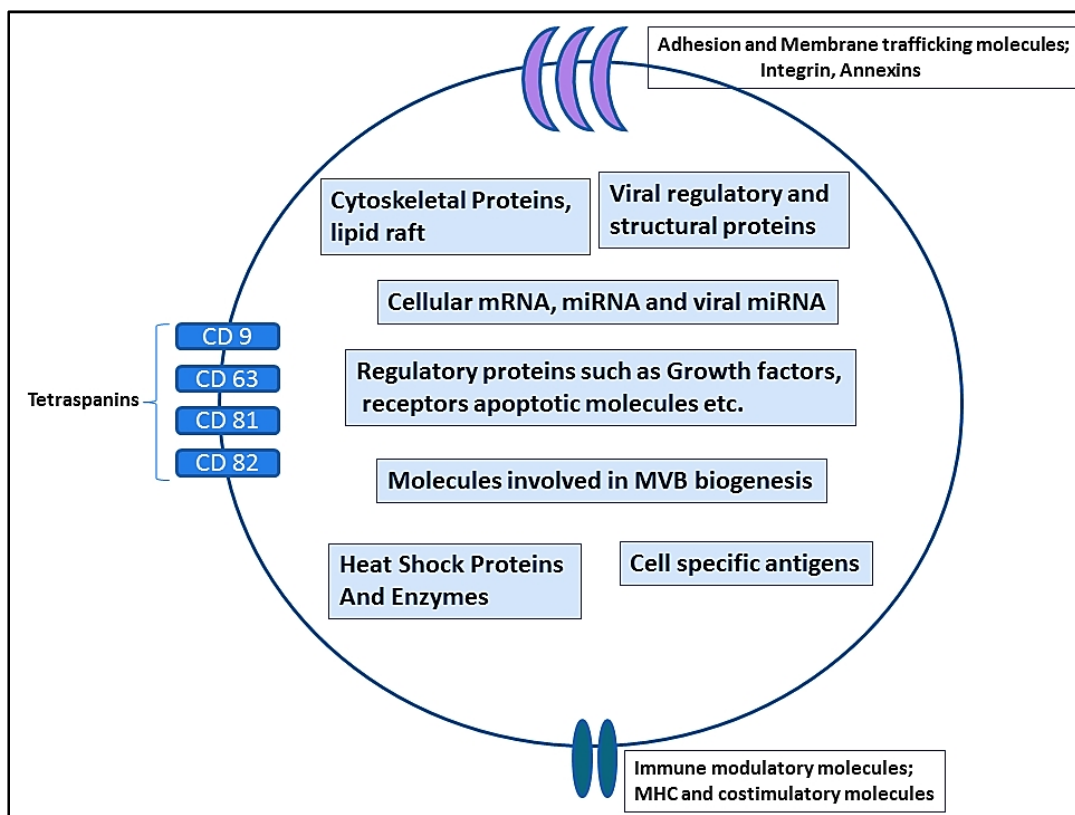
Fig. 1. Typical content of exosomes: The exosomal membrane carries adhesion molecules, tetraspanins, MHC molecules etc. enclosing a variety of cargo inside the vesicle that includes mRNA, microRNA, proteins, and cell specific antigens
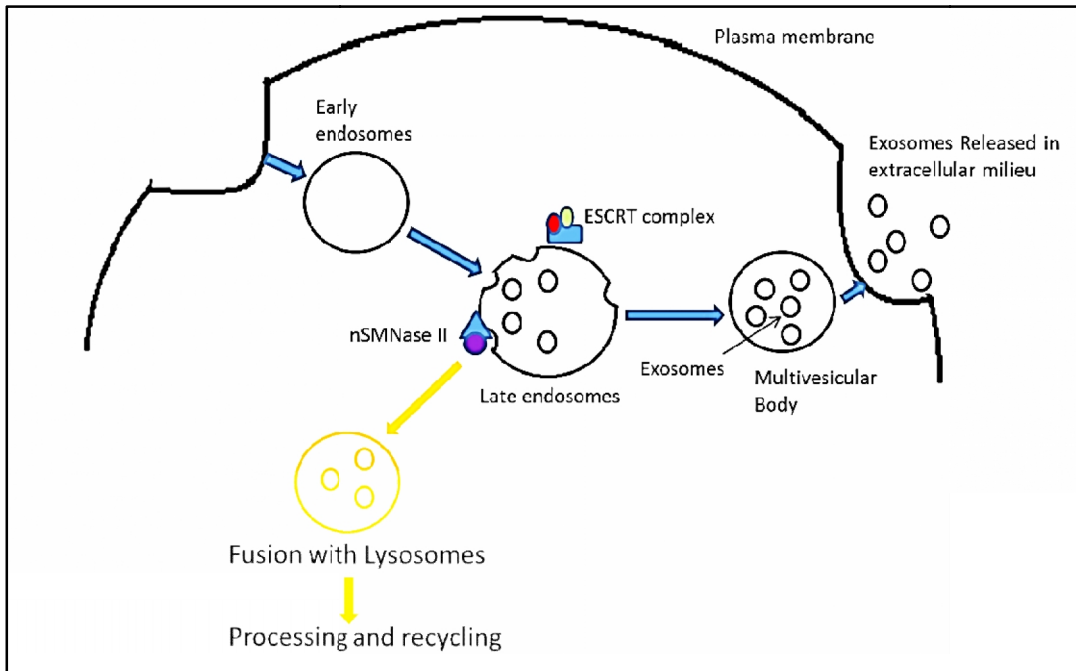
1.1.1.1 Biogenesis

In contrast to the microvesicles that are bigger in size (>100nm) and are shed directly from the plasma membrane, exosomes are rather small (~30-100nm) and produced by inward budding and pinching of the limiting membrane of endosomes giving rise to late endosomes

with tiny intraluminal vesicles collectively called the MVB. The MVBs do not fuse with the lysosomes and are directed to the plasma membrane to release their contents outside the parent cell [16,17] (Fig. 2). However, very little is known about the nature of molecules and mechanisms that regulate the biogenesis of exosomes containing MVB. Although, the Endosomal Sorting Complex Required for Transport (ESCRT) complex has a well-established role in docking of ubiquitinated cargo to the endosomes [18], its presence on exosomes and role in inward budding, biogenesis or secretion of exosomes still remains unclear. The depletion of Hepatocyte growth factor (HGF) associated tyrosine kinase, a component of ESCRT-0 complex, was reported to reduce exosome secretion in dendritic cells (DC) [19]. On the other hand ESCRT complex was reported to be dispensable for ubiquitinylation of the exosomal cargo [20]. More recently, the biogenesis and secretion of exosomes have been shown to be dependent on ceramide biosynthesis and activity of neutral sphingomyelinase II (nSMase II) [21]. Further, microRNA secretion from the cells was found to be dependent on nSMase II activity and ceramide production [22] that is also reported to enhance the secretion of exosomes leading to metastasis of tumour cells resulting in increased angiogenesis in tumour microenvironment by exosomal microRNAs [23]. Importantly, these studies demonstrated that microRNA transported by exosomes were functional after uptake, suggestive of the vital role that exosomes play in reprogramming of the target cells. However, there is limiting evidence of any specific candidate molecule or sorter associated with inclusion or exclusion of specific microRNA. Interestingly, exosomes have been reported to carry the crucial components of the RNA-induced silencing complex such as Argonaute2 and GW182 [24], which might influence the preferential packaging of specific microRNA associated with them inside the lumen of MVB [25]. Though recent studies have provided new insights into the exosome biogenesis and selective packaging, the exact pathways and mechanisms involved in the process still remains to be delineated. Here, we review the current understanding of crosstalk of viruses and viral proteins with the extracellular vesicles particularly the host exosomal biogenesis and secretory pathway that seems to play a major role in viral biogenesis, egress, spread, immune evasion and latency [26].

1.2 Viruses and Exosome

Constantly evolving nature of viruses allows them to design and modify strategies to overcome host immune responses, evade clearance by host system and hijack host cellular machinery to derive maximal benefit for their own survival and spread [26]. Exosome biogenesis and secretion pathways have also been exploited by viruses to transport virionand viral components to far unreached body compartments as an effective cover from the surveillance of the host immune system. Studies done on animal viruses have provided useful information for better understanding of exosome biogenesis and mechanism of cargo upload [27]. These secretory pathways seem to be easily hijacked by viruses for their spread and immune suppression making it difficult to track them by the immune system [28,29] (Table 1). Exosomes secreted by virus-infected and tumour cells have been shown to carry functional biomolecules such as microRNAs, proteins etc., justifying the potential of exosomes as a source of biomarkers [30]. Most data relating to virus-exosome interaction has emerged from studies on pathophysiology of retroviruses like Human Immunodeficiency Virus-1 (HIV-1) and members of Herpes virus family such as Human Herpes virus (HHV-6), Herpes Simplex Virus (HSV-1) and Epstein Barr viruses (EBV).

Fig. 2. Model of Exosome Biogenesis: Endosomes formed by inward invagination of plasma membrane are the site of exosome biogenesis after which they either fuse with lysosomes or exit out of the cell by fusion with plasma membrane thus releasing their content (Exosomes) outside the cell

Table 1. Evidence of modulation of host exosomal machinery by viruses

Virus	Modulation of exosomal pathway	Reference
HIV-1	• Enhanced Exosome biogenesis	• [40,41]
	• Trans-infection from mDC to CD4$^+$T cells leading to Immune suppression	• [35]
	• Envelope independent budding from host cell	• [30,36,37]
	• Transfer to non-target cells	• [38]
HHV-6	• Increases MVB biogenesis and exosome production	• [49,50]
EBV	• Immune suppression	• [52]
	• enhanced exosome biogenesis	• [57,58]
	• Virus induced tumorigenesis by modulation of exosomal microRNA	• [62]
HSV-1	• Immune suppression and Exosome mediated enhanced infectivity	• [66]
CMV	• Immune suppression	• [67]
HCV	• Virion budding	• [68,70]

1.2.1 Human Immunodeficiency Virus-1 (HIV-1)

1.2.1.1 Exosomes in biogenesis and transport of HIV-1

Retroviruses such as HIV-1 exhibit a striking similarity in their particle formation and secretion with the cellular exosome biogenesis [31]. Studies on HIV-1 have suggested the involvement of cellular exosome biogenesis pathways of macrophages and dendritic cells in HIV-1 budding [32,33]. HIV-1 enters mature dendritc cells (mDC) via exosomes that exhibit greater ability to capture incoming virions and maintain them in infectious form [33,34]. mDC, in turn transfer the virions to CD4^{+} T cells in the lymph nodes with the help of exosomes resulting in the depletion of CD4^{+} T cells and consequent immune suppression and chronic viral persistence [35]. This strategy of HIV-1 presents an interesting example of the use of intrinsic property of mDC to disseminate viral antigens via exosomes, without involving viral specific cellular receptors. This role of exosomes in HIV-1 budding has endorsed the Trojan horse hypothesis suggesting the viral exploitation of pre-existing cellular machinery for intercellular transport [30,36,37]. In fact, the presence of HIV-1 virion in non-target organs such as central nervous system (CNS) is also suggestive of a non-canonical mode of viral transmission that is independent of viral specific receptors and co-receptors. This could be mediated by HIV-1 carrying monocytes and macrophages capable of crossing the blood brain barrier [38]. Although, these reports support an exosome-dependent and envelope-independent mode of HIV-1 transfer, they fail to explain how the viral and host factors regulate differential assembly of the virions on plasma membrane and on MVB.

1.2.1.2 Modulation of exosomal pathway by HIV-1 proteins

Gag, a major structural proteins as well as Nef, an accessory protein of HIV-1 have been shown to be secreted into exosomes. Nef utilises this pathway to suppress the host immune response by depleting CD4^{+} T cells - a hall mark of AIDS [39]. Further, Nefenhances its own export by increasing the production of exosomes in the infected cells which could contribute to egress of viral particles from the cells [40,41]. More recently, the release of Nef in exosomes has shown to be dependent on a cellular protein called Mortalin [42]. The HIV-1 Gag protein, which is capable of forming empty viral particles without viral RNA and other viral proteins, is also dependent on exosomal machinery for budding as indicated by co-localisation of Gag with exosomal markers such as CD81 and CD63 [43]. Further, Gag protein which has an endosomal localisation, co-purifies with exosomal preparations supporting the view that both HIV-1 packaging and release could be dependent on the exosome biogenesis pathway [44,45]. Interestingly, exosome-mediated transfer of the two most important chemokine co-receptors CCR5 and CXCR4 is crucial for HIV-1 infection to non-target cells such as kidney parenchymal cells or CNS [46,47] and may explain the mechanism of HIV-1-related kidney diseases [48]. Thus, exosomes seem to add further complexity to HIV-1-associated disease as facilitators of both non canonical mode of HIV-1 biogenesis and its transport to non-target cells.

1.2.2 Human Herpes Virus (HHV)

Different members of this DNA virus family have been studied in detail for their interference with the exosomal pathways. Proteomics based studies have identified alteration of protein content, inclusion of viral specific proteins and biomolecules in exosomes from HHV infected cells, that are potent enough to modulate host system thus contributing to disease pathogenesis. The members of this family are discussed in subsections below.

1.2.2.1 Human herpes virus 6 (HHV-6)

HHV-6 has been reported to increase the size of infected cells due to increased biogenesis of MVB. The viral envelope glycoproteins co-localize with the late endosomal marker CD63 and the HHV-6 virions are reported to bud out of the cell via exosomal pathway [49]. Further, HHV-6 infection in T cell lines is associated with increased production of exosome like particles [50]. Thus, association of virions and their components in MVB/exosomal biogenesis has provided fresh evidence supporting the hijacking of cellular machinery by viruses. Besides, this gives survival advantage through viral spread and also allows viral persistence in the lumen of vesicles insulated from lysosomal degradation.

1.2.2.1 Epstein - Barr virus

A member of the gamma herpes family, EBV has been studied in great details for its role in MVB biogenesis, viral egress, infection to neighbouring uninfected cells and modulation of immune response via exosomes [51]. Initial report on the immune suppression by this DNA tumour virus suggested that its major oncoprotein, latent membrane protein-1 (LMP-1) is secreted by the infected cells and is involved in T cell anergy of the infiltrating lymphocytes [52]. Later, LMP-1 secretion was shown to be mediated by exosomes [53]. Incidentally, LMP-1 is abundantly detected in blood of nasophyrangeal carcinoma (NPC) patients [54]. The association of LMP-1 with exosomal marker CD63 and its accumulation and secretion via the intraluminal vesicles is reported to interfere with NF-κB signalling [55,56] and stimulate the secretion of more exosomes [57,58]. The exosomes from EBV-infected cells carry enhanced levels of fibroblast growth factor-2 [59]. LMP-1 is associated with up-regulation of proliferative, invasive and metastatic factors such as epidermal growth factor (EGF), EGF receptor (EGFR), Cox-2, hypoxia inducible factor 1 alpha (HIFα), Matrix metallo-proteinases -9. This indicates its possible role in selective sorting of cargo into exosomes, immune evasion and intercellular communication [58,60,61]. Interestingly, the exosomes secreted by EBV infected B cells are also rich in viral microRNAs also known as BART microRNAs and other signalling molecules [62]. The functional transfer of BART microRNA to the Monocyte-derived dendritic cells (MoDC) via exosomes leads to down regulation of CXCL11/ITAC, an immune-regulatory gene leading to immune suppression in EBV-associated lymphomas [63].

1.2.2.3 Herpes Simplex virus (HSV)

HSV-1 infected cells are also reported to secrete vesicles known as L particles [64]. The L particles carry viral tegument protein, viral glycoprotein and other cellular components but without viral DNA. These particles play a key role in enhancing the infectivity/replication of HSV-1 via transfer of the tegument protein to naïve cells [65]. HSV-1 glycoprotein (gB) has been documented to hijack the transport of HLA-DR molecules to plasma membrane and instead en routes them to be secreted in membrane enclosed exosomes helping the virus escapes the host immune response [66]. Similarly, the cytomegalovirus (CMV)-infected endothelial cells are also shown to release the CMV antigens to antigen presenting cells via exosomes, pointing to the involvement of host secretory machinery in transporting viral antigens to the host immune system [67].

1.2.3 Hepatitis C virus (HCV)

Indications of a crosstalk between exosomal biogenesis and HCV secretion by the infected cells emerged from the findings that the envelope proteins E1 and E2 of HCV could

associate with tetraspanin CD81 and released via exosomal pathway [68]. This is further supported by the observation that the HCV assembly and virion secretion was dependent on the hepatocyte growth factor associated tyrosine kinase-dependent exosome pathway, a component of the ESCRT complex. The HCV structural proteins such as core and envelope are also reported to associate with MVB, consolidating previous reports [69]. However, very recently, a ground-breaking study by Ramakrishnaiah et al., has established that HCV virions take up the exosomal path for their spread to uninfected hepatocytes leading to a productive infection in naïve cells [70]. Altogether, existing reports highlight the potential role of exosomal pathways in transfer of HCV virions to the uninfected as well as non-target cells, resulting in spread of infection in the presence of neutralising antibodies and in absence of specific cell surface receptors.

1.2.4 Hepatitis B virus

Although, Hepatitis B virus (HBV) has been shown to release viral antigen, surface glycoprotein and hepatocellular carcinoma-associated microRNAs in the blood of patients in huge amounts and proposed to be a strategy to tick the host immune system [71]. A recent study indicated modulation of microRNA packaging in exosomes present in the sera of chronic HBV and relatedHCC which could affect cellular apoptosis, proliferation and molecular signalling pathways [72] however, the molecular mechanism of the same remains to be elucidated [73]. To give an insight into exosomal modulation by HBV, our preliminary results have also documented a concentration of growth factors and their receptors, viz., HGF α and EGFR in exosomes secreted from HBV infected hepatocytes which may have some viral specific implications on the neighbouring cells.

2. CONCLUSION

The present review highlights the exploitation of exosome biogenesis and secretion pathways by viruses and speculates on possible use of exosome as source of candidate biomarkers during viral infections. Viruses are well known for utilizing the host packaging and secretory pathway for their own packaging and release.

It is established beyond doubt that many human viruses have evolved strategies to utilize micro vesicular secretory pathway not only for their own packaging but also for sending out signals that will change the host environment to support viral persistence. The unique capacity of viruses to transfer their genetic material independent of the cognate viral envelope or capsid not only allows them to escape the host immune surveillance but also exposes non-target tissues and organs to viral infection in absence of the tissue specificity conferred by the viral envelope-cell surface receptor interactions. This may be serving a great survival advantage to the viruses to lie latent in non-target cells as well as to escape clearance from the host system by targeted therapy and may explain the failure to completely cure certain infections. If such a mechanism operates for most of pathogenic viruses, a new thinking is required for developing interventional strategies against such evasive pathogens. Additionally, identification of the exosomal receptors involved in transfer of viruses to non-target cell should be the prime focus for intervention therapies in order to curtail viral spread via this mode.

Engineering exosomes with specific membrane receptors for gene therapy or targeted drug delivery is another direction in which the scientists are likely to focus in the near future. Five members of the BART family of EBV encoded microRNAs have been identified in exosomes both in cell culture and in plasma of NPC patients [62] that can be used as important

biomarkers. In this regard potential of exosomes to give intracellular information needs to be tapped and decoded for other pathogens as well (Fig. 3). However, more clinical studies are needed in this direction to elucidate the signature contents of exosomes under various physiological and pathological conditions to establish their usefulness as they pose immense potential for the discovery of new minimally invasive, more effective and accurate biomarkers.
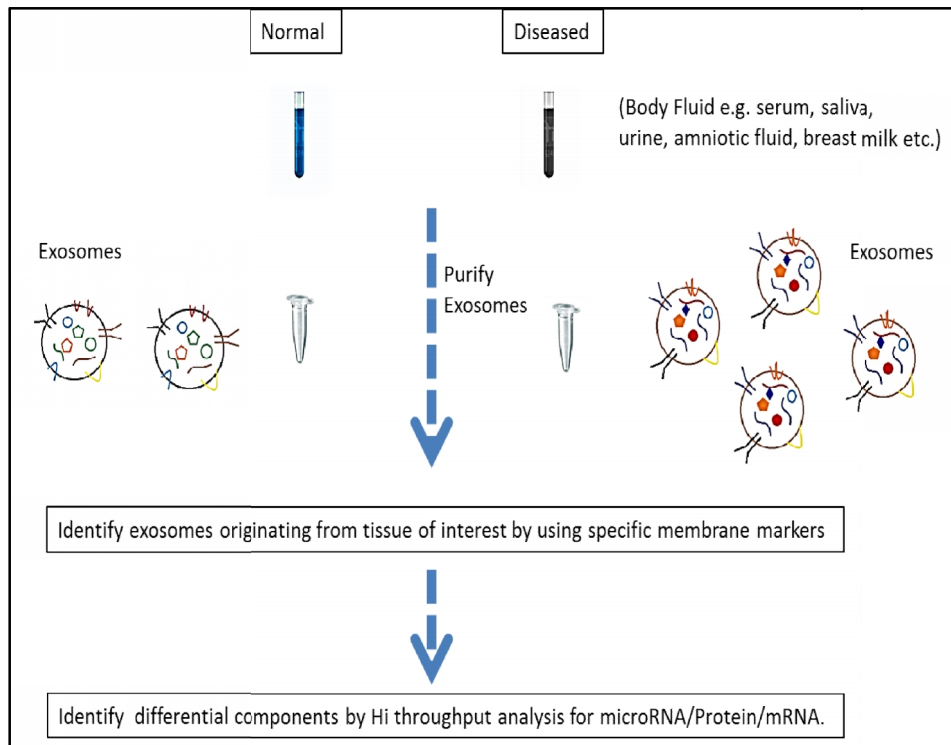
Fig. 3. Exosomes as Biomarker and Diagnostics: Serum derived exosomes can be of diagnostic importance in high throughput analysis

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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