PERSPECTIVE

Exosomes in neurological disease, neuroprotection, repair and therapeutics: problems and perspectives

Exosomes: Exosomes are a sub-population of micro-vesicles ranging from 40–100 nm that were earlier thought as artefacts under electron microscope. They recently came into attention for their storage of biological information, cell-to-cell communication, serving as biomarkers and potential use in neural protection and regeneration (Kalani et al., 2013, 2014a). Exosomes are secreted by different brain cells including neurons, microglia, astrocytes, oligodendrocytes, and neural stem cells and their presence in cerebro-spinal fluid has also been confirmed. Exosomes represent cup-shaped morphology with a buoyant density of 1.13–1.19 g/cm^2. They originate through the endocytic pathway after the fusion of multivesicular bodies (MVBs) with the plasma membrane and then release into the extracellular environment. Exosomes contain different proteins (membrane transport and fusion proteins, tetraspannins, heat-shock proteins, MVB biogenesis proteins), lipids, saccharides, and genetic materials (miRNA and other non-coding RNAs) which are collectively termed as 'cargo' contents (Kalani et al., 2014a). Recent findings suggest that exosomes contain unique molecular conservatories and therefore represent pathophysiological state of the cellular source from where they originate. On functional aspects, exosomes have been confirmed to perform roles as transporters, bridging the communication gap between cells and shuttling material from their cells of origin to the other distantly located cells. Because of their nano-size and non-complexed structure, exosomes efficiently cross blood-brain barrier (BBB) and therefore present novel ways to design therapeutic strategies against different cerebral diseases.

Characteristics of cerebral exosomes and their role in neurological disorders and neuroprotection: Exosomes derived from different cerebral cells contain specific contents, for example, 1) oligodendrocyte-derived exosomes contain key components of the myelin sheath, myelin proteolipid protein, myelin basic protein, and myelin oligodendrocyte glycoprotein, 2) neuronal exosomes carry the cell adhesion molecule L1, the glycosylphosphatidylinisotol (GPI)-anchored prion protein, and GluR2/3 subunits of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor and, 3) microglial exosomes were reported to contain surface-bound amino peptidase N, monocarboxylatetransporter 1, several SNARE proteins, and pro-inflammatory cytokines. The messages are carried from one cerebral cell to other through exosomes in a fascinating manner and that is how they regulate the complex channeling and integrity of brain functions. The presence of differential contents of exosomes derived from different cerebral cells suggests their important functions in neuronal protection, nerve regeneration, neuronal development, and synaptic plasticity. In this regard, exosomes have been observed in stabilizing genomic and synaptic plasticity and in maintaining spatial and temporal gradients which are involved in neuronal development processes. Exosomes were also involved in the removal of injury-induced toxins and releasing myelin and stress-protecting proteins. In addition to this, it has been observed that exosomes deliver regulatory elements at the injured site which help promote protein synthesis and tissue regeneration.

In contrast to exosomes' use in neuroprotection, their implication in mediating various cerebral disease were also confirmed. The cerebral pathogenic process involving increased generation of molecularly and functionally altered exosomes, augmented delivery of pathogenic foreign proteins, coding/non-coding elements through exosomes, and switching immunologically inert exosomes to active exosomes. In the pathogenic process of different cerebral diseases, exosomes are implicated for the transportation of, 1) amyloid beta 42 (Aβ42) in Alzheimer's disease, 2) Huntingtin protein in Huntington's disease, 3) α-synclein (α-syn), leucine-rich receptor kinase 2 (LRRK2), and vacuolar-sorting protein 35 (VPS35) in Parkinson's disease and, 4) Prion proteins (PrPc and PrPsc) in prion diseases. The efficient contents of cellular exosomes are transported and delivered to different cells by a process which is superior to cell-to-cell interactions. These delivered informations make significant changes by altering molecular contents or reprogramming of the target cells. It is interesting to discuss here that although there are several speculations that originate with exosomes, there are still no clear routes followed by the exosomes to transfer the biological information. Nevertheless, using electron microscope studies it is believed that by the process of internalization the exosomes bind to the cell surface and after triggering secondary messenger process, components are released to the target cells.

Exosomes in therapeutics: It is notable through previous findings that stem cells mediate therapeutic benefits by paracrine mechanisms and stem cell-derived exosomes carry stem cells' paracrine factors to the site of injury and promote regeneration. The effective potential of mesenchymal stem cell derived-bone marrow derived-, and embryonic stem cell derived-exosomes have been observed in promoting neurological recovery, synaptic plasticity, immunomodulation, tissue repair and regeneration. Stem cell exosomes are typically identical to the exosomes derived from other cellular sources but known to carry more complex cargos that include nucleic acid, proteins, and lipids. By the use of mass spectrometry, microarray and antibody array, mesenchymal stem cell (MSC) exosomes showed 857 unique gene products with >157 miRNAs which can be involved in stem cell beneficiary functions (Lai et al., 2012). Alongside, treatment with therapeutic agents to the exosomes secreting cells was also found to increase the beneficiary effects of secreted exosomes by alleviating the paracrine factors. For example, our study with the use of exosomes secreted through curcumin-treated mouse brain endothelial cells (curcumin primed-exosomes) showed rescued BBB permeability after mitigating vascular junction proteins (Kalani et al., 2014b). Likewise, exosomes released by MSCs exposed to *Buyang Huanwu decoction* (BYHWD), a therapeutic Chinese herb, showed augmented angiogenetic miRNA and vascular endothelial growth factor expressions that elevated angiogenesis process in rat brain (Yang et al., 2015). They can be immensely useful for clearing aberrant protein accumulation, removal of toxins, chemicals and control of degradation. Exosomes can also come up further and overcome problems associated with current therapies for gene-related metabolic disorders by efficient targeted delivery of genes to the brain. An exciting study with engineered MSC

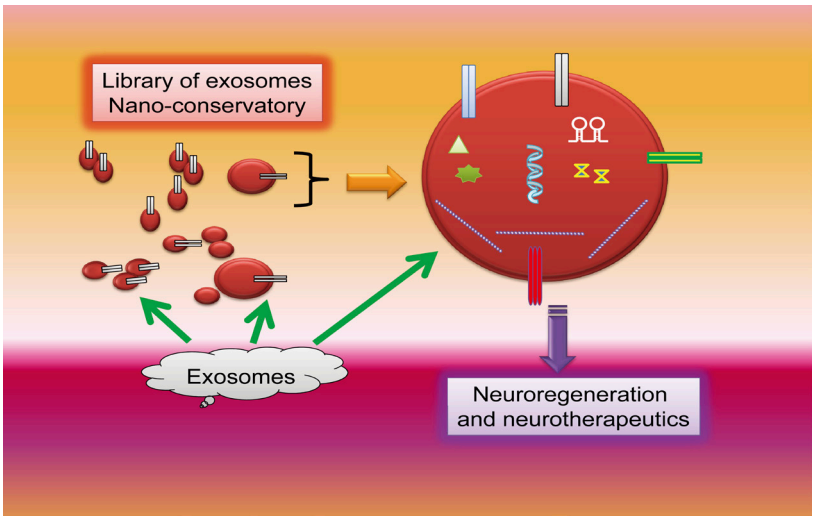

Figure 1 Construction of individual-specific exosome nano-conservatory library and their use in neuroprotection and therapeutics.

showed interesting findings of neurological recovery and axonal plasticity post-stroke in rats. The engineered MSCs were found to release exosomes laden with microRNAs (particularly miR133b) that helped in neurorestoration (Xin et al., 2013). The presence of Nedd family-interacting protein 1 (Ndfip1) and Nedd4, toxin removal proteins after injury, in exosomes suggest their role in neuro regeneration (Putz et al., 2008). In addition, their role as delivering the molecular information (mRNA, proteins, ribosomes) and promoting protein synthesis at the injured site through different neural cells also suggest their important role in neuroregeneration (Bianco et al., 2009; Kalani et al., 2014a).

Exosomes as delivery vehicles: Exosomes carry enormous potential to deliver therapeutic drugs, nucleic acids and proteins to the distal targets. There are several advantages that make exosomal nano-units superior to other delivery vehicles, such as, liposomes and nano-particles. ***Particular size:*** Exosomes are advantageous in terms of their sizes (40–100 nm) that help stabilize them in cerebrovascular circulation. Micro-vesicles that are bigger than exosome size (100–1,000 nm) are rapidly removed from systemic circulation while smallest micro-vesicles (1–40 nm) rapidly cross the vessel walls. Because of their persistent presence in circulation and maintaining the integrity of their cargos from various proteases and nucleases, exosomes are now becoming the prime target to study the novel biomarker discoveries. ***Maintenance of therapeutic agents:*** Recent evidences suggest that exosomes can be delivery vehicles for potential therapeutic agents. The major specialty of choosing exosomes as delivery vehicles is their efficient drug delivery after traveling a long distance. Encapsulation of therapeutic molecules in exosomes increases their stability and bioavailability by inhibiting their rapid systemic elimination. The incorporation of the therapeutic agents in the exosomes was found to increase their stability, circulation time, and drug therapeutic activity. For example, curcumin encapsulated in exosomes was shown to raise plasma curcumin concentration to 1,250 ng/mL administered within 30 minutes which was 5–10 times higher than the curcumin concentration when administered alone. Interestingly, curcumin encapsulated in exosomes showed high plasma concentration for 12 hours (Sun et al., 2010). In addition to therapeutic drugs, exosomes were also found to efficiently deliver small non-coding RNAs (miRNA, siRNA) and thus represent an important delivery system for RNAi therapy (Shtam et al., 2013). Efficient process of packing therapeutic entities in exosomes is currently being standardized using various methods that include simple incubation, freeze-thaw cycles, chemical methods, sonication, extrusion, and electroporation. However, to facilitate targeted molecular delivery, several studies are currently exploring the ways to introduce tissue specific ligands in exosomes so that these formatted units can be attracted by the specific tissue types. ***Non-invasive BBB crossing:*** BBB constitutes selective barrier that restricts entry of harmful particles to the brain and is formed by specialized endothelial cells tightly molded with unique tight and adherent junctions. Defective BBB has been reported in, 1) Alzheimer's disease, where compromised BBB allowed entry of circulatory Aβ to the brain and, 2) Parkinson's disease, where faulty BBB allowed increased peripheral immune invasion that contributed to the severity of the disease. Exosomes are reported to cross BBB and help in therapeutics (Zhuang et al., 2011; Kalani et al., 2014a). ***Effective administrative routes:*** Delivery of nano-units to the brain was confirmed through intravenous and intracerebral routes. To overcome the over-spreading of exosomal therapeutic units to different organs through intravenous route, and discover alternate administration strategy which is not as invasive as intracerebral route, effective non-invasive intranasal delivery was studied and experimentally proven (Zhuang et al., 2011). Although the exact route of exosome targeting to the brain through intranasal administration is not yet clear, it is speculated that the exosomes can reach the brain through 1) systemic pathway, where the drugs are absorbed through nasal cavity and reach the systemic circulation and, 2) olfactory pathway where drugs are absorbed through olfactory epithelial cells and cross the cell membrane by endocytosis after moving through cellular interstitial space. However, efficient delivery of curcumin, catalase, miRNAs and other therapeutic moieties to the brain potentially validated the intranasal route to be used for effective drug deliveries in future. ***Hydrophobic and non-immunogenic nature:*** Exosomes contain lipid-bilayer hydrophobic membranes and this feature can be important for ligating hydrophobicity-sensitive therapeutic molecules, for example, curcumin (Kalani et al., 2015). The other important feature of exosomes to be used as effective brain delivery agents is their non-cytotoxic

and non-immunogenic nature (Kalani et al., 2014a). ***Superior choice to other delivery agents:*** Exosomes are superior choice to other delivery agents, for example 1) nano-particles that remain in the circulation for longer time and sometime show toxicity, 2) liposomes that show less affinity for packaging therapeutic molecules and less attracted to the cellular targets and 3) adeno-, and lenti-virus that show limitation due to inadequate efficiency in gene transduction and remaining functional at injury site.

Problems and future directions: There are several studies that significantly contribute a clear picture of exosome biology in terms of cellular and molecular contents, biogenesis, communication strategies, regulatory functions, potential as biomarkers and effective ways to use them as therapy either alone, primed, or packed with therapeutic agents. However, directional research is still needed to explore hidden aspects of exosomes. Alongside, there are certain issues which are needed to be explored under conventional research for example: 1) size variability, since the exosomes are heterogeneous in size; 2) biogenesis, as the exact way of exosomes synthesis is still not well understood; 3) scalable production that needs a lot of studies to produce good yield of exosomes; 4) packing the therapeutic molecules, as there are no stringent methodological comparison studies that were performed in order to check the efficient way of packing; 5) factors controlling exosomes generation, as the information is needed that explains the factors that guide exosome generation and the issues that up-regulate or down-regulate exosome synthesis under certain conditions (Chaturvedi et al., 2015); 6) encapsulated molecular agents, since the ligation of packed ncRNA and other agents to exosomes need clear picture and; 7) targeted delivery, exploring the factors and external agents that help selective homing to the targeted tissue. It will be profitable to perform *in vivo* studies with different genetic animal models to explore the uniqueness and importance of exosomes. In addition, profiling of exosomes derived from different sources will be conducted in view of addressing toxicity and immunogenicity. These parameters are very important to explore exosomes' existence, role in spreading cerebral and other diseases and also in novel therapies. Furthermore, well-organized and effective designing of exosomes, exosomal contents and exosomal conjugates can improve targeting and therapy. Exciting results with exosomes can be carried further to validate and translate into clinics, although different standardizations will be required to set up optimum novel formulations, administration routes, doses and scalable productions to treat different diseases.

Exosomal molecular nano-conservatories: The success of stem cell banks for future clinical therapeutics provides the hope that similar, safe and potential approaches can also be used for human benefits. Exosomes have special cellular functions and neovascularization properties, angiogenetic potential and rejuvenating powers as stem cells do. Hence, devastating cerebral diseases can be challenged with exosomes in an individual-specific manner. Considering the enormous intriguing paracrine properties of exosomes, there is future probability of constructing an individual specific nano-vesicle banks that could help as a therapy in lifetime of humans. **Figure 1** shows exosome unit containing proteins, RNAs and other molecules and exosomal nano-conservatory (library) formulations that can be potentially used for neuroregeneration and neurotherapeutics. Hence, the directional studies are needed which can assist looking at the aspects of using individual specific exosomes for constructing nano-exosomal conservatories similar to stem-cell libraries that could be more effective in treating all cerebral diseases in future.

Anuradha Kalani*, Neetu Tyagi

Department of Physiology and Biophysics, School of Medicine, University of Louisville, Louisville, KY, USA

***Correspondence to:** Anuradha Kalani, Ph.D., anukalani@gmail.com, a0kala02@louisville.edu.*
***Accepted:** 2015-08-17*
***orcid:** 0000-0003-0856-7637 (Anuradha Kalani)*
***doi:** 10.4103/1673-5374.165305* ***http**://www.nrronline.org/*
Kalani A, Tyagi N (2015) Exosomes in neurological disease, neuroprotection, repair and therapeutics: problems and perspectives. Neural Regen Res 10(10):1565-1567.

References

Bianco F, Perrotta C, Novellino L, Francolini M, Riganti L, Menna E, Saglietti L, Schuchman EH, Furlan R, Clementi E, Matteoli M, Verderio C (2009) Acid sphingomyelinase activity triggers microparticle release from glial cells. EMBO J 28:1043-1054.

Chaturvedi P, Kalani A, Medina I, Familtseva A, Tyagi SC (2015) Cardiosome mediated regulation of MMP9 in diabetic heart: Role of mir29b and mir455 in exercise. J Cell Mol Med doi: 10.1111/jcmm.12589.

Kalani A, Mohan A, Godbole MM, Bhatia E, Gupta A, Sharma RK, Tiwari S (2013) Wilm's tumor-1 protein levels in urinary exosomes from diabetic patients with or without proteinuria. PLoS One 8:e60177.

Kalani A, Tyagi A, Tyagi N (2014a) Exosomes: mediators of neurodegeneration, neuroprotection and therapeutics. Mol Neurobiol 49:590-600.

Kalani A, Kamat PK, Chaturvedi P, Tyagi SC, Tyagi N (2014b) Curcumin-primed exosomes mitigate endothelial cell dysfunction during hyperhomocysteinemia. Life Sci 107:1-7.

Kalani A, Kamat PK, Kalani K, Tyagi N (2015) Epigenetic impact of curcumin on stroke prevention. Metab Brain Dis 30:427-435.

Lai RC, Tan SS, Teh BJ, Sze SK, Arslan F, de Kleijn DP, Choo A, Lim SK (2012) Proteolytic potential of the MSC exosome proteome: implications for an exosome-mediated delivery of therapeutic proteasome. Int J Proteomics 2012:971907.

Putz U, Howitt J, Lackovic J, Foot N, Kumar S, Silke J, Tan SS (2008) Nedd4 family-interacting protein 1 (Ndfip1) is required for the exosomal secretion of Nedd4 family proteins. J Biol Chem 283:32621-32627.

Shtam TA, Kovalev RA, Varfolomeeva EY, Makarov EM, Kil YV, Filatov MV (2013) Exosomes are natural carriers of exogenous siRNA to human cells in vitro. Cell Commun Signal 11:88.

Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang HG (2010) A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther 18:1606-1614.

Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, Zhang ZG, Chopp M (2013) MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. Stem Cells 31:2737-2746.

Yang J, Gao F, Zhang Y, Liu Y, Zhang D (2015) Buyang Huanwu Decoction (BYHWD) enhances angiogenic effect of mesenchymal stem cell by up-regulating vegf expression after focal cerebral ischemia. J Mol Neurosci 56:898-906

Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, Zhang L, Steinman L, Miller D, Zhang HG (2011) Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther 19:1769-1779.