PERSPECTIVE

Curcumin-primed and curcumin-loaded exosomes: potential neural therapy

Curcumin: Curcumin is a yellow colored compound found in the rhizomes of *Curcuma longa* (turmeric), a member of the ginger family (Zingiberaceae). Structurally curcumin is a diarylheptanoid, a tautomeric compound, present in keto form in water and enolic form in the organic solvents. The therapeutic efficacy of curcumin is extensively studied against neurodegenerative diseases as it possesses anti-inflammatory, anti-lipidemic, and anti-oxidative properties (Kalani et al., 2014b, 2015b). Curcumin's neuroprotective properties led the compound to the Phase I clinical trials; however, it could not proceed further due to its limited bioavailability, poor absorption, quick metabolism and rapid systemic elimination (Kalani et al., 2015b). In order to increase its bioavailability, different investigators have proposed alternative methods for instance, encapsulation of curcumin by synthesizing microcapsule containing self-assembled nanoparticles using poly-l-lysine, trisodium citrate and silica sol (Patra and Sleem, 2013), and encapsulating curcumin in self-assembling peptide hydrogels as injectable drug delivery vehicles (Altunbas et al., 2011). Similarly, we tried to design nano-formulations of curcumin which are more soluble, more stable, less cost-effective and less labor-intensive. The two important formulations are 1) curcumin-primed exosomes (CUR-EXO) and, 2) curcumin-loaded exosomes (exocur). The given nano-formulations were prepared in exosomes and both performed extensively well in different model systems. Cells release curcumin-primed exosomes when treated with curcumin while curcumin-loaded exosomes were prepared by loading curcumin in the exosomes. The choice of exosomes for making these nano-formulations over other available options was made due to several reasons and these include, exosomes size (40–200 nm), preferred binding of curcumin with exosomes, usefulness in different administrative routes, hydrophobic and non-immunogenic nature, and superiority over other delivery agents (Kalani et al., 2013, 2014a; Kalani and Tyagi, 2015).

Curcumin-primed exosomes: The development of curcumin-primed exosomes was made in order to transfer the beneficial properties of curcumin to the exosomes. Exosomes are the mirror images of the tissue type from where they originate. In other terms, exosomes carry similar biological molecules (proteins, lipids and nucleic acid) of their source of secretion. Curcumin, a source of immense medicinal and neuro-protective properties, is believed to benefit diseases by mitigating molecular, genetic, and epigenetic factors. The preparation of curcumin-primed exosomes is the leading step in fostering the beneficial properties of curcumin that can enhance the potential therapeutic paracrine factors in exosome producing cells. As a result, the released exosomes (curcumin-primed exosomes) exert more beneficial and therapeutic effects. In our study, we prepared curcumin-primed exosomes after treating mouse brain endothelial cells with 7.5 μM curcumin for 72 hours (Kalani et al., 2014b). Curcumin-primed exosomes were further collected by ultracentrifugation of culture-conditioned media. While preparing the nano-formulation, the integrity of exosomes in curcumin-primed exosomes was ensured to be maintained. On functional aspects, curcumin-primed exosomes were found to start working as quickly as these units were introduced to the mouse brain endothelial cells. **Figure 1** shows mouse brain endothelial cells that acquire fluorescently-tagged curcumin-primed exosomes (CUR-EXO) and start providing therapeutic effects. In our study, we found the therapeutic efficacy of curcumin-primed exosomes against homocysteine (Hcy, a neurotoxicant) challenged mouse brain endothelial cells. The curcumin-primed exosomes improved the endothelial cell layer permeability when co-treated with Hcy. The study outcome suggested that the protection to the brain endothelial cell layer permeability through curcumin-primed exosomes was mediated by decreasing oxidative stress and mitigating impaired junction proteins (Kalani et al., 2014b). Tight junction and vascular junction proteins are important junction proteins that regulate endothelial cells integrity and permeability (Kalani et al., 2015a). **Figure 2** shows a typical illustration of a single endothelial cell that looses vascular junction proteins (VE-cadherin) after treatment with Hcy; however, after co-treatment with cur-

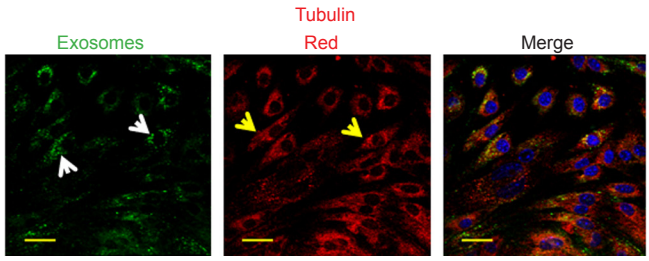

Figure 1 Acquisition of curcumin-primed exosomes through mouse brain endothelial cells.
Representative confocal images showing curcumin-primed exosomes acquisition through mouse brain endothelial cells. Green color represents curcumin-primed exosomes stained with PKH-67 (indicated with white arrows). Mouse brain endothelial cells were stained with tubulin red (indicated with yellow arrows). Scale bars: 20 μm.

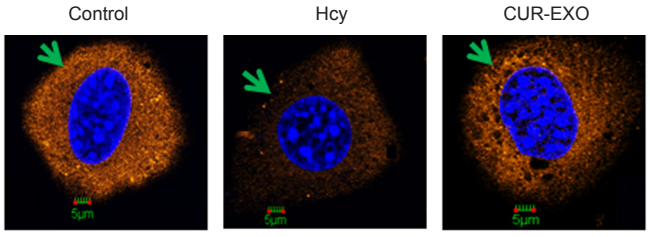

Figure 2 Effect of curcumin-primed exosomes in endothelial junction proteins.
Vascular junction protein (VE cadherin) was found to be reduced in homocysteine-treated mouse brain endothelial cells which indicate endothelial cells disruption. However, curcumin-primed exosomes alleviated VE cadherin levels in endothelial cells (indicated with green arrows). Scale bars: 5 μm. CUR-EXO: Curcumin-primed exosomes; VE: vascular endothelial.

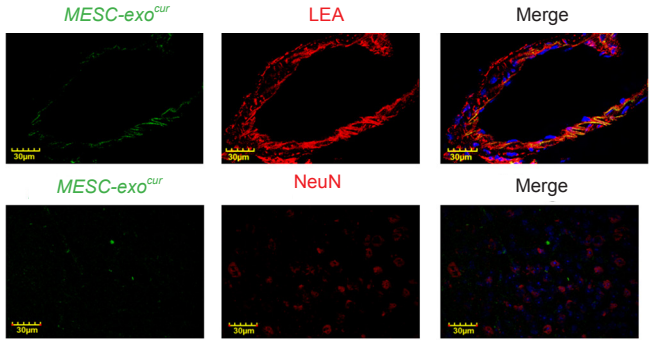

Figure 3 Acquisition of MESC-exocur (curcumin-loaded in embryonic stem cell exosomes) by neurons and brain vessels administered through intranasal route.
Immunohistochemistry images showing the presence of fluorescently labeled exosomes in brain cortical areas. Brain pial vessels were stained with Lycopersicon esculentum agglutinin (LEA; red color, upper horizontal panel) and neurons were stained with neuronal nuclei (NeuN; red color, lower horizontal panel). Cell nuclei were stained with DapI (blue) and shown in merged images (right most lanes). Scale bars: 30 μm.

cumin-primed exosomes, mouse brain endothelial cell showed significant protection against Hcy-induced loss of VE-cadherin.

Though our study results clearly indicate a potential future therapy by curcumin-primed exosomes; however, the study was limited by not describing the molecular signaling mechanisms through which curcumin-primed exosomes offered protection. Thus, exploring the pathways, molecular signaling mechanisms, and involved paracrine factors can further help in better understanding the mechanistic basis of the therapy and help in stepping ahead in development of potential therapy against neuro-degenerative disorders. Alongside, we strongly believe that the preparations of primed-exosomes can also be feasible with other therapeutic molecules that will equally be important in providing benefits as curucmin.

Curcumin-loaded exosomes: The preparation of curcumin-loaded exosomes was made in view of achieving powerful and targeted therapy against stroke (Kalani et al., 2016). In our study, we derived exosomes from mouse embryonic stem cells. Stem cells contain enormous rejuvenating capacities that can reprogram the target cells and enhance the repair/regeneration processes. Furthermore, the potential therapeutics of embryonic stem-cells exosomes is believed to be due to the paracrine factors of the stem cells that not only work similar to as of stem cells but also overcome the limitations of cell therapy associated with the stem cells. Earlier studies have also represented the therapeutic aptitudes of mesenchymal stem cell derived exosomes that possess immense beneficiary effects against stroke by promoting functional recovery, neurovascular plasticity, neuroprotection, neuroregeneration and modulating peripheral post-stroke immune responses (Xin et al., 2013). Loading curcumin in exosomes, derived from stem cells, increases its solubility, stability, bioavailability, and therapeutic activity (Kalani and Tyagi, 2015; Kalani et al., 2016). Interestingly, we loaded curcumin in exosomes in a manner that the integrity of the stem cell exosomes was not affected and that we confirmed through nano-tracking analysis, western blotting with exosomes specific antibody, and acetylcholinesterase activity (Kalani et al., 2013, 2016). In a previous report, Zhuang et al. (2011) have described that curcumin-loaded exosomes were detectable till 12 hours in olfactory bulb and maintained curcumin concentration at an average of 2.6 ± 0.4 nmol/g of brain tissue, when administered intranasaly. We also administered curcumin-loaded embryonic stem cell exosomes (MESC-exo^{cur}) through the nasal route in ischemia-injured mice and found improvements in the neurological score after 3 days of treatment. The distribution of MESC-exo^{cur} was observed in all parts of the brains. In that regard, **Figure 3** shows the presence of MESC-exo^{cur} in the brain vessels and neurons. We next found improvements in the stroke volume, ischemia-reperfusion injured neurons, and brain vasculature (Kalani et al., 2016) with treatment of MESC-exo^{cur}. The improvements in vascular junction proteins were also confirmed. The considerable improvements in the behavior and neuro-vascular unit were the outcomes of decrease in inflammation, reactive oxygen species generation, and suppression in glutamate receptor activation (Kalani et al., 2016). The striking results of MESC-exo^{cur} through improvements in neuro-glia-vascular loss after ischemia-reperfusion injury presented an innovative direction for the future non-invasive therapy. These nano-formulations can also be tried in different experimental settings to test their efficacy in different model systems.

Future direction: Although the clear molecular mechanisms have not been addressed in our studies for curcumin-primed and curcumin-loaded exosomes; however, the similar thing that we found with both formulations was that these two units provide anti-oxidative and anti-inflammatory properties. Curcumin also possesses the same properties but with the use of exosomes, the limitations of curcumin can be greatly minimized. In future, these curcumin's nano-formulations can be tested in different model systems and if successful, their results can be further be validated and can translate into clinics. However, much work is needed to optimize more efficient loading, molecular characterization, optimization of the dose, comparison of efficient delivery routes, achievement of more targeted delivery, and recovery of the structural and functional outcomes. Considering the therapy to a larger extent, the idea of molecular nano-conservatory (Kalani and Tyagi, 2015), can also be tried with these formulations that will also provide helps in a concept of personalized medicine. Interestingly, the loading of therapeutic molecules in individual-specific exosomes, or preparation of primed-exosomes after treating the individual-specific cells ex-situ with therapeutic molecules, can generate different choices of potential future therapeutics. The personalized molecular nano-conservatory will not only be effective and safe but will also be immunogenetically effective. Concluding, the therapy of curcumin-primed and curcumin-loaded exosomes can be used as a potential neural therapy for different neurodegenerative diseases.

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