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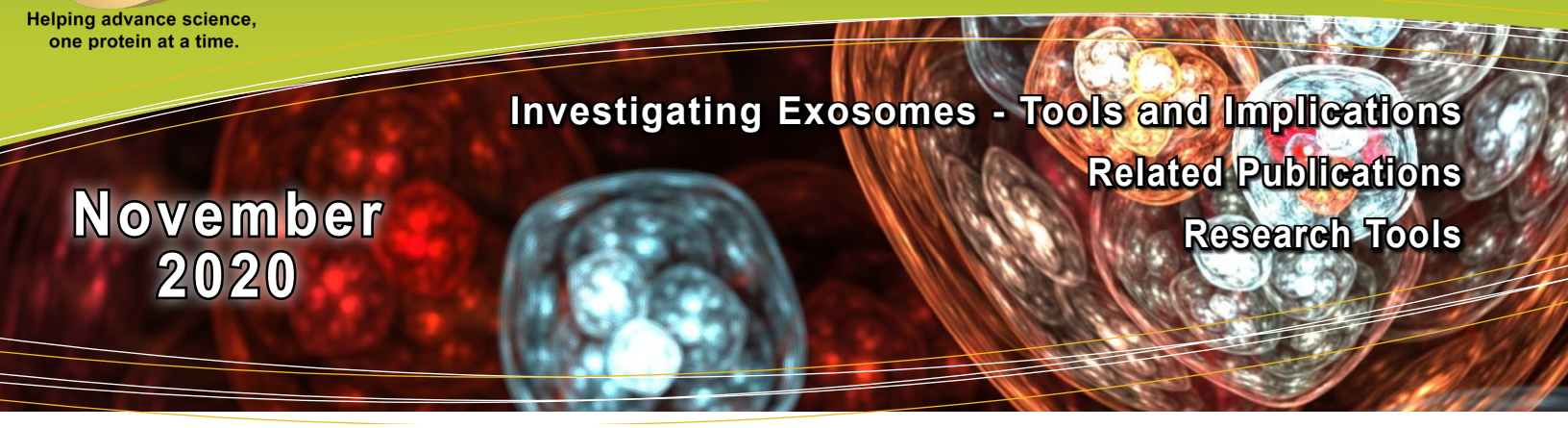

Investigating Exosomes - Tools and Implications

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Investigating Exosomes - Tools and Implications

In eukaryotic cells the plasma membrane acts as an intercellular communication hub. In addition to the classic direct contact mechanisms of cell communication such as gap junctions, cell surface-protein interactions, and secreted peptides or hormones, cells are also known to communicate via reception of lipid encapsulated cellular biomolecules known as extracellular vesicles (EVs)[1–3]. As the study of EVs has grown, so too has the delineation between the types of EVs. Apoptotic bodies are 500-2000 nm EVs that have bud off the membrane of apoptotic cells and are typically removed via macrophage phagocytosis. The 50-1000 nm microvesicles are smaller buds off the plasma membrane that deliver biological cargo to neighboring cells. Exosomes are 30-100 nm EVs that contain active biomolecule cargo but are distinguished from microvesicles based on their biogenesis.

Exosomes are constitutively produced from inward buddings of the membranes of endosomal compartments that subsequently form intraluminal vesicles (ILVs) and are typically found within multivesicular bodies (MVBs). Exosome-containing MVBs face two fates: 1) fusion with the lysosome for degradation of contents, or 2) fusion with the plasma membrane followed by expulsion of exosomes into extracellular space[4]. Exosomes are found in almost all physiological fluids and once mobilized, exosomes can facilitate cell-to-cell communication via their diverse cargo that is known to include proteins, lipids, microRNAs (miRNAs), messenger RNAs (mRNAs), and long non-coding RNAs (lncRNAs)[5,6]. Due to the bioactive content of exosomes, cellular uptake can result in a variety of cellular responses including modulating gene and/or protein expression. As such, exosomes have become an active area of investigation.

Exosomes are implicated in neuron-to-astrocyte communication

Astrocytes of the central nervous system work in concert with neurons by supplying them with neurotransmitter precursors, energy substrates, and maintaining glutamate homeostasis[7,8]. To further understand the relationship between neurons and glial cells investigators have begun to investigate how exosomes mediate intercellular communication in cell-to-cell communication within cell types (neuron-neuron), and also across cell types (glial-neuron)[9]. A recent study by Men et al. found that relative to *in vivo* astrocytes, cultured astrocytes possess diminished levels of miR-124-3p and a corresponding dearth of precursor pri-miR-12410. Injection of exogenous Cy 5-labeled exosomes into mice confirmed astroglia uptake of miR-124-3p, which was reversible by exosome secretion-inhibitor GW4869. Following GW4869 treatment, *in vivo* astrocytes exhibited reduced levels miR-124-3p, cogently supporting that miR-124-3p levels are a consequence of exosome delivery. Transfection of miR-124-3p inhibited expression of glutamate transporter 1 (GLT1)-inhibiting miR-132 and miR-218 with concomitant increased expression of GLT1 protein. These data demonstrate a previously undescribed mechanism by which exogenous miRNAs from neurons regulate astrocyte function[10].

Exosomes may play a role in the reparatory process after insult

A study by Cheng et al. determined that acute myocardial infarcted (AMI) mice release exosomes enriched in myocardial microRNAs (myo-miRs) that can be detected and isolated from peripheral blood[11]. They further demonstrated through flow cytometry that isolation and transfer of fluorophore-labeled exosomes to non-AMI mice displayed enrichment in distant tissues, predominantly in bone marrow mononuclear cells (BM-MNCs). Naïve mice injected with exosomes purified from AMI mice exhibited a significant decrease in expression of the transmembrane chemokine receptor 4 (CXCR4). Interestingly, CXCR4 levels, co-implicated with CXCR1 in the mobilization of progenitor cells (PC) into circulation, could be reduced with injection of myo-miR loaded exosomes. Consequently, mice injected with AMI exosomes exhibited great numbers of colony-forming progenitor cells suggesting that the mobilization of bone marrow PC into circulation is partially controlled by exosome myo-miRs

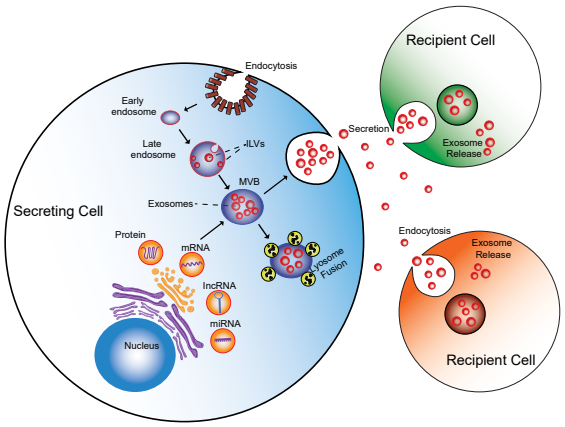

Figure 1. Schematic of exosome biogenesis, degradation, and secretion. Exosomes are produced from invaginations of late endosomes and reside within multivesicular bodies until entrance into degradation or secretion pathways. Multivesicular bodies (MVB), intraluminal vesicles (ILV).

Continued from Page 1

released from the infarcted heart and may aid recovery post-myocardial infarction[11].

Tracking fluorescently labeled exosomes reveals a role in generating pre-metastatic niches

Although investigators have characterized the biological consequence of exosome uptake, little is known about the nature of *in vivo* exosome circulation to distant organs. Hyenne et al. used intact zebrafish and MemBright dyes (MemGlow) to label and track tumor EV fate[12]. Examination of tumor EVs, isolated from zebrafish melanoma cell line Zmel1, were shown to share 65% and 40% protein similarity with human or mouse EVs, respectively. Interestingly, exosome labeling with Paul Karl Horan (PKH) dyes created problematic and emissive particle aggregates; a finding not recapitulated by MemBright. MemBright-labeled tumor EVs injected into zebrafish embryos were tracked by confocal microscopy and observed circulating from the site of injection until arrest by patrolling macrophages, endothelial cells, hematopoietic stem cells, or macrophages in the tail. Notably, injection of non-tumor EVs and inert polystyrene beads were also taken up by macrophages and endothelial cells suggesting a non-specific uptake mechanism by these cell types. Co-localization experiments using labeled exosomes and lysosomes determined that macrophage-internalized EVs are destined to late-endosome lysosomes. To investigate *in vivo* circulation and potential metastatic activity of exosomes, pre-labeled (MemBright, transgenic reporter) Zmel1 cells were grafted into zebrafish embryos. Tracking of emissive tumor EVs revealed arrest predominantly at the caudal plexus, similar to injected EVs. Assessment of the caudal plexus 7 days post-graft revealed enhanced metastatic outgrowth in embryos grafted with Zmel1, but not polystyrene beads. These data suggest that circulating tumor EVs can participate in the formation of pre-metastatic niches *in vivo*[12].

Summary

The examples above provide a very small snapshot of the growing interest in exosomes, and their role in a wide spectrum of scientific disciplines such as neuroscience, cardiology, oncology, and more. Clearly, exosomes function both physiologically in the case of intercellular communication in neural tissue, as well as pathologically, in models of AMI and cancer. Interestingly, these studies feature the use of novel research models, such as transparent zebrafish embryos, to help scientists understand the fate of exosomes and mechanisms of uptake and/or specificity. These studies also highlight the utility of cutting-edge labeling dyes, such as MemGlow probes, and high-resolution microscopy to visualize exosome fate in circulation. Future studies may wish to consider methodologies that utilize Spirochrome's new benzylguanine (BG) substrates which readily label SNAP-tag fusion proteins to specifically label critical exosome proteins to effectively track its biogenesis, localization, and uptake. Co-localization studies of SNAP-tag labeled lysosomes, endosomes, multivesicular bodies, or other organellar compartments could expand the toolset for investigators and help them effectively interrogate MemGlow-labeled exosome sorting. As exosome biology continues to be elucidated, utilizing effective models and tools will be essential for deducing their role both biologically and in disease.

References

1. Mathieu, M., Martin-Jaular, L., Lavieu, G. & Théry, C. Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. Nature Cell Biology vol. 21 9–17 (2019).
2. Ciardiello, C. et al. Focus on Extracellular Vesicles: New Frontiers of Cell-to-Cell Communication in Cancer. Int. J. Mol. Sci. 17, 175 (2016).
3. Tetta, C., Ghigo, E., Silengo, L., Deregibus, M. C. & Camussi, G. Extracellular vesicles as an emerging mechanism of cell-to-cell communication. Endocrine vol. 44 11–19 (2013).
4. Meldolesi, J. Exosomes and Ectosomes in Intercellular Communication. Curr. Biol. 28, R435–R444 (2018).
5. D'Asti, E. et al. Oncogenic extracellular vesicles in brain tumor progression. Front. Physiol. 3 JUL, 1–15 (2012).
6. Kelemen, E., Danis, J., Göblös, A., Bata-Csörgő, Z. & Széll, M. Exosomal long non-coding RNAs as biomarkers in human diseases. Electron. J. Int. Fed. Clin. Chem. Lab. Med. 30, 224–236 (2019).
7. Mahmoud, S., Gharagozloo, M., Simard, C. & Gris, D. Astrocytes Maintain Glutamate Homeostasis in the CNS by Controlling the Balance between Glutamate Uptake and Release. Cells 8, 184 (2019).
8. Farhy-Tselnicker, I. & Allen, N. J. Astrocytes, neurons, synapses: A tripartite view on cortical circuit development. Neural Dev. 13, 1–12 (2018).
9. Frühbeis, C. et al. Neurotransmitter-Triggered Transfer of Exosomes Mediates Oligodendrocyte-Neuron Communication. PLoS Biol. 11, (2013).
10. Men, Y. et al. Exosome reporter mice reveal the involvement of exosomes in mediating neuron to astroglia communication in the CNS. Nat. Commun. 10, (2019).
11. Cheng, M. et al. Circulating myocardial microRNAs from infarcted hearts are carried in exosomes and mobilise bone marrow progenitor cells. Nat. Commun. 10, 959 (2019).
12. Hyenne, V. et al. Studying the Fate of Tumor Extracellular Vesicles at High Spatiotemporal Resolution Using the Zebrafish Embryo. Dev. Cell 48, 554-572.e7 (2019).

Plasma Membrane Probes

Product	Ex / Em	Amount	Cat. #
MemGlow™ 488 Fluorogenic Membrane Probe	499 / 507 nm	2 nmol 10 nmol	MG01-02 MG01-10
MemGlow™ 560 Fluorogenic Membrane Probe	555 / 570 nm	2 nmol 10 nmol	MG02-02 MG02-10
MemGlow™ 590 Fluorogenic Membrane Probe	595 / 613 nm	2 nmol 10 nmol	MG03-02 MG03-10
MemGlow™ 640 Fluorogenic Membrane Probe	650 / 672 nm	2 nmol 10 nmol	MG04-02 MG04-10
MemGlow™ 700 Fluorogenic Membrane Probe	689 / 713 nm	2 nmol 10 nmol	MG05-02 MG05-10

BG Substrates

Product	Ex / Em	Amount	Cat #
SPY555-BG Substrate	555 / 580 nm	35 nmol	CY-SC204
SPY620-BG Substrate	619 / 635 nm	35 nmol	CY-SC404
SiR650-BG Substrate	652 / 674 nm	35 nmol	CY-SC504
SiR700-BG Substrate	696 / 718 nm	35 nmol	CY-SC604

Live Cell Imaging Products

Product	Ex / Em	Amount	Cat #
SiR-Actin™ Kit	630 / 680 nm	50 nmol	CY-SC001
SiR-Tubulin™ Kit	630 / 680 nm	50 nmol	CY-SC002
Cytoskeleton Kit	630 / 680 nm	50 nmol each	CY-SC006
SiR-DNA™ Kit	630 / 680 nm	50 nmol	CY-SC007
SiR-Lysosome Kit	650 / 680 nm	50 nmol	CY-SC012
SiR700-Actin Kit	690 / 720 nm	35 nmol	CY-SC013
SiR700-Tubulin Kit	690 / 720 nm	35 nmol	CY-SC014
SiR700-DNA Kit	690 / 720 nm	35 nmol	CY-SC015
SPY555-Actin	555 / 580 nm	100 stains	CY-SC202
SPY505-DNA	512 / 531 nm	100 stains	CY-SC101
SPY555-DNA	555 / 580 nm	100 stains	CY-SC201
SPY555-Tubulin	555 / 580 nm	100 stains	CY-SC203
SPY595-DNA	599 / 615 nm	100 stains	CY-SC301
SPY650-DNA	652 / 674 nm	100 stains	CY-SC501
SPY650-Tubulin	652 / 674 nm	100 stains	CY-SC503
SPY700-DNA	696 / 718 nm	100 stains	CY-SC601