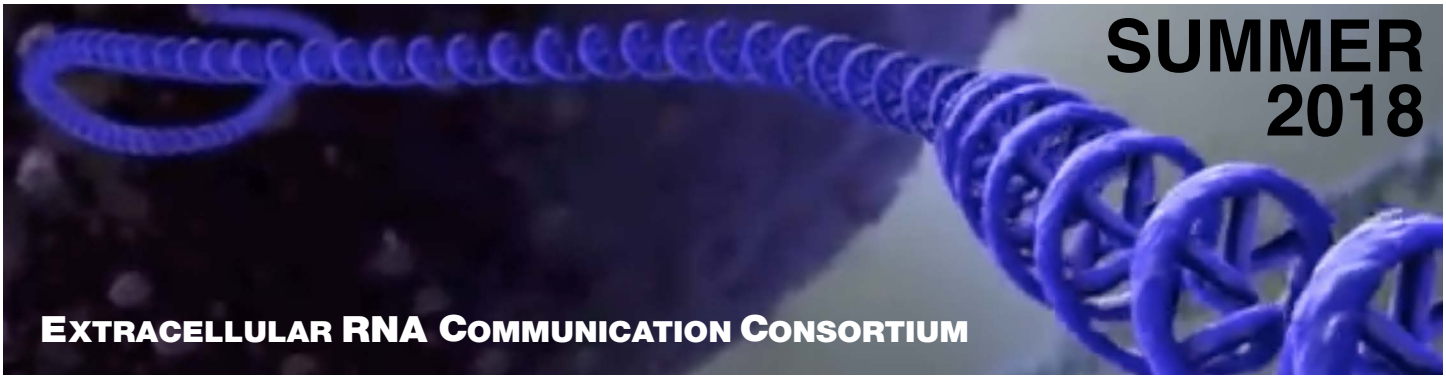
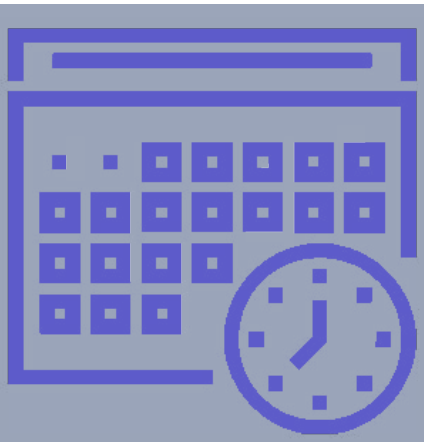


Newsletter

Advancing Extracellular RNA Communication Research

Stage 2 Funding Opportunities

The NIH Common Fund has released funding opportunity announcements (FOAs) for a second stage of research into extracellular RNA communication.



Important Dates

Aug. 24th 2:00-3:30pm EST
Informational webinar
(See page 2 for details.)

September 10th
Letters of Intent due

October 23rd
Applications due

RFA-RM-18-027

Improved Isolation and Analysis of exRNA Carrier Subclasses

ExRNAs are transported in body fluids in association with a variety of carrier vehicles of varying complexity including extracellular vesicles (EVs), ribonucleoproteins (RNPs), and lipoproteins (LPPs). While these carriers (EVs, RBPs, and LPPs) share the functional attribute of protecting exRNAs from degradation by ubiquitous extracellular RNAses, their respective contributions to biodistribution, uptake, and function in target cells of their respective exRNA cargo is less clear.

See CARRIERS on page 2

RFA-RM-18-028

Towards Single Extracellular Vesicle Sorting, Isolation, and Analysis of Cargo

The ability to isolate and analyze single EVs and their cargoes from human biofluids would provide a unique opportunity to understand the cell or tissue from which their respective exRNAs originate (heterogeneity) and, importantly, add significant depth to our understanding of exRNA communication. The overarching goal of this FOA is to develop and demonstrate innovative technologies and reagents towards isolating single EVs and to characterize the exRNA cargos associated with specific EV subpopulations based on cell of origin and their intended target cell. Shedding light on the diversity of exRNAs

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EXTRACELLULAR RNA COMMUNICATION CONSORTIUM
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The overarching goal of this FOA is to develop and evaluate innovative separation tools, technologies, and approaches that will enable the scientific community to rapidly and reproducibly sort complex biofluids into homogenous carrier populations of EVs (including EV subsets), RNPs, and LPPs, and that also support high-throughput isolation and analysis of their extracellular RNA content and associated molecular cargo.

While significant strides have been made in the field of exRNA biology, specific technological challenges remain. For example, RNA concentrations in biofluids are significantly lower than in tissues, necessitating the development of improved methods for their isolation and analysis. Currently available separation approaches are time-consuming and do not adequately discriminate exRNAs encapsulated in EVs from exRNAs associated with non-vesicular RNPs or LPPs. Ultimately, this can lead to unintended biases in exRNA population profiling based upon the specific isolation method used. Further, little is known about the normal physiology of exRNAs, the functional consequences of exRNA uptake, or deficiency of uptake, by recipient cells, and the level of variation existing at the subpopulation level in both normal and disease states. These technological impediments limit the utility and impact of current efforts to catalog exRNAs and assess their contribution(s) to human health and disease.

Research Objectives

Determining which exRNAs are transported by each specific carrier vehicle (EV, RNP, or LPP) is essential for elucidating exRNA sorting pathways, identifying associated cargo that may confer targeting or functionality, leading to a better understanding of the role of

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Informational Webinar about ERCC Stage 2 Funding Opportunities

August 24, 2018 from 2:00-3:30 pm EST

NIH staff will host a teleconference to provide technical assistance to potential applicants and answer questions related to the Advancing exRNA Communication Research funding opportunity announcements (FOAs).

Please submit questions ahead of time to exRNAcommunication@mail.nih.gov.

Join the conference via WebEx using this link:
https://nih.webex.com/nih/onstage/g.php?MTID=ef29317fb2d940f2f6f0c3c410e5df5ff

Call-in toll number (US/Canada): 1-650-479-3208
Event number: 629 822 206
Event password: exRNA

ERCC Quarterly Newsletter - Summer 2018
Next issue, Fall 2018

Editor
Roger P. Alexander, ERCC Scientific Outreach Coordinator

Newsletter

ERCC Seminar Series

The ERCC hosts a monthly web seminar on a variety of research topics related to extracellular RNA and extracellular vesicles. With permission from the presenter, seminars are recorded and posted for later viewing on the About and Presentations pages of the exRNA Portal.

The standard scheduled time is at 1pm Eastern on the first Thursday of each month. The upcoming seminar schedule is below. Clicking the institution links to the speaker's website.

October

M	T	W	T	F	S	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

Thursday October 4th 1pm ET
Leonora Balaj
Mass. General Hospital
Blood based assays for the diagnosis and stratification of glioma patients

November

M	T	W	T	F	S	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

Thursday Nov. 1st 1pm ET
Kendall Van Keuren-Jensen
Translational Genomics Research Institute (TGen)

December

M	T	W	T	F	S	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24/31	25	26	27	28	29	30

Thursday Dec. 6th 1pm ET
TBA

January

M	T	W	T	F	S	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

Thursday January 3rd 1pm ET
Jane Freedman
U. Mass. Medical School

SINGLE-EV continued from page 1

carried by EVs will allow for a better understanding of the precise role of exRNAs as signaling molecules for both physiological and pathophysiological processes, ultimately accelerating development of exRNAs as therapeutics and diagnostics.

EVs are heterogeneous in size, origin, and molecular cargo (including proteins, RNA, DNAs, and lipids). Additional complexity exists due to the heterogeneous exRNAs (mRNA, miRNA, lncRNA, and other RNA species) within EVs. These exRNAs can be transported to distal sites and may play an important role in intercellular communication by regulating physiological and pathological processes.

Current methods for isolating EVs from complex biofluids are limited by the inability to sort EVs by cell or tissue of origin, creating significant technological barriers to analytical comparison of datasets generated across studies.

Currently, it is not clear which RNA species (if any) are packaged and transported by different EV sub-populations, if the exRNA carrying capacity of different EV populations differs, or whether the exRNA content of EVs differs under homeostatic or pathologic conditions.

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Newsletter

CARRIERS continued from page 2

exRNA intercellular communication in human health and disease.

Ideally, protocols for separating exRNA-carrier complexes should

(1) be rapid and reproducible,

(2) yield highly enriched carrier-specific exRNAs with no cross-contamination, and

(3) have the capacity for high-throughput isolation and characterization of carrier-specific genomic, proteomic, and lipidomic signatures.

None of the separation technologies currently employed to isolate exRNAs from culture media or biofluid (i.e., precipitation, ultracentrifugation, sequential filtration, or immunoaffinity capture) meet all these performance standard. Thus, there is a critical need to develop and validate novel tools, technologies, and approaches to segregate exRNA carriers based on their unique biophysical characteristics and to rapidly and accurately sort homogeneous carrier-specific exRNAs from complex biofluids. Further, these approaches should incorporate high-throughput capacity for downstream analysis of their respective exRNAs and associated molecular cargo.

To be responsive to this FOA, applicants are expected to:

• Propose separation strategy/strategies for all three of the known exRNA carriers - EVs (and EV subsets), RBPs and LPPs.

• Outline strategies that allow for high-throughput separation and analysis of carrier-specific molecular cargo including genomics, proteomics, and lipidomics.

• Justify how the proposed separation strategies will improve the state of the art, either by enabling purification of carrier types in a way that has previously not been possible, or dramatically improving (by at least 5-fold) our ability to purify carrier types with respect to increased purity, increased speed, or decreased cost.

• Propose using human biofluids in the second (UH3) Phase.

• Incorporate approaches to share the strategies and tools developed with the broader scientific community.

SINGLE-EV continued from page 3

Further, current technologies do not permit single EV isolation and analysis, thereby limiting our understanding of the normal variation (heterogeneity) of EVs produced, even using reductionist (i.e., cell culture) models.

The diagnostic or therapeutic functionality of exRNAs can only be truly realized once the range of EV subpopulations from a given cell source are fully described and characterized for complete analysis of molecular cargo. Determining the origin of EVs from various tissues or cell types is essential for understanding where their cargo exRNAs originate and how they affect their target cells.

Furthermore, being able to isolate and analyze single EVs from human biofluids would provide a unique opportunity to understand the cell or tissue from which their respective exRNAs originate and, importantly, would add significant depth to our understanding of exRNA communication.

Ideally, protocols for isolating single vesicles should

(1) be rapid and reproducible,

(2) yield highly enriched EV subpopulations from a single source cell or tissue with no cross-contamination, and

(3) have the capacity to analyze and catalog exRNAs present in the single EVs from various cell and tissue types.

None of the separation technologies currently employed for EVs (i.e., differential ultracentrifugation, density gradients,

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Newsletter

SINGLE-EV continued from page 4

polymer-based precipitation, microfiltration and size-exclusion-based methods) meet all these performance standards. Further, the diagnostic or therapeutic functionality of EVs can only be truly realized once the range of EV subpopulations from a given cell source are fully described and isolated for complete analysis of their molecular cargo.

A number of approaches have been developed to characterize EVs in the clinical setting, including sandwich ELISA-based microarray chip, immunomagnetic exosome RNA (iMER) analysis, miniaturized micro–nuclear magnetic resonance (μNMR) microfluidic chip system, Exochip, and label-free high-throughput nano-plasmonic exosome assay (nPLEX) using surface plasmon resonance (SPR).

However, none of these current methodologies can address EV heterogeneity or clearly define cell of origin for exRNA cargo. Thus, there is a critical need to develop and validate novel tools, technologies, and approaches to characterize and sort EV subpopulations based on "cell of origin" from complex biofluids for downstream analysis and cataloging of their respective exRNAs and associated molecular cargo, with the capacity to do so at scale.

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Upcoming Events

For more detail about the upcoming events listed below related to research into exRNA and extracellular vesicles, visit **exRNA.org/Events.**

August 19 – 24, 2018

Gordon Research Conference on Extracellular Vesicles: *From Basic Research to Clinical Diagnostic and Therapeutic Applications of Extracellular Vesicles*

Grand Summit Hotel at Sunday River, Newry, Maine

August 29 – 31, 2018

BBEV 2018: Biomarkers and Biogenesis of EVs

University of Padua, Italy

October 20 – 24, 2018

ASEMV 2018: Annual meeting of the American Society for Exosomes and Microvesicles

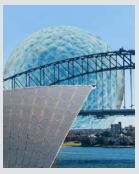
Marriott Waterfront Conference Center
Baltimore, Maryland

October 24 – 26, 2018

Exosomes and Liquid Biopsies Europe 2018

Rotterdam Marriott
Rotterdam, The Netherlands

November 14 – 16, 2018



Australian Extracellular Vesicles Conference 2018

University of Technology Sydney (UTS), Australia

More EVENTS on page 6

Newsletter

SINGLE-EV continued from page 5

To be responsive to this FOA, applicants are expected to:

• Propose strategies for isolating and tracking EV subpopulations, cataloging EV-associated exRNAs based on cell or tissue of origin and intended target cell. Applications aiming to characterize exRNAs associated with other carrier vehicles (RBPs or LPPs) should apply to RFA-RM18-027.

• Outline strategies that allow for high-throughput separation and analysis of EV subpopulation-specific molecular cargo including genomics, proteomics, and lipidomics.

• Justify how the proposed EV isolation strategies will improve the state of the art either by enabling purification of single EVs in a way that has previously not been possible, or dramatically improving (by at least 5-fold) our ability to purify EV subpopulations with respect to increased purity, increased speed, or yield.

• Demonstrate isolation effectiveness using a variety of human biofluids in the second (UH3) phase.

• Incorporate approaches to share with the broader scientific community the strategies and tools developed.

Upcoming Events

For more detail about the upcoming events listed below related to research into exRNA and extracellular vesicles, visit **exRNA.org/Events.**

EVENTS cotinued from page 5

November 18 – 20, 2018

ISEV China Workshop 2018
EV-based Clinical Theranostics
Southern Medical University, Guangzhou, China

January 13 – 18, 2019

Gordon Research Conference on RNA Nanotechnology: *Nucleic Acid Architectures for Therapeutics, Diagnostics, Devices and Materials*
Four Points Sheraton, Ventura, California

April 24 – 28, 2019

ISEV 2019: Annual meeting of the International Society for Extracellular Vesicles
Miyako Messe, Kyoto, Japan

Please contact info@exRNA.org to add an event to the exRNA Portal.