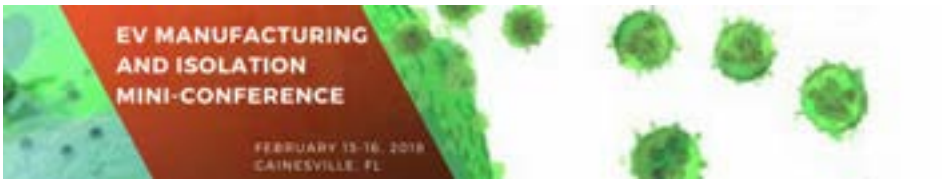
Newsletter

ERCC9, the ninth semi-annual meeting of the Extracellular RNA Communication Consortium, was open to the public and took place in Bethesda, Maryland in November. In this issue of the ERCC Newsletter, we review some of what happened there and overview progress made in the study of exRNA and extracellular vesicles (EVs).



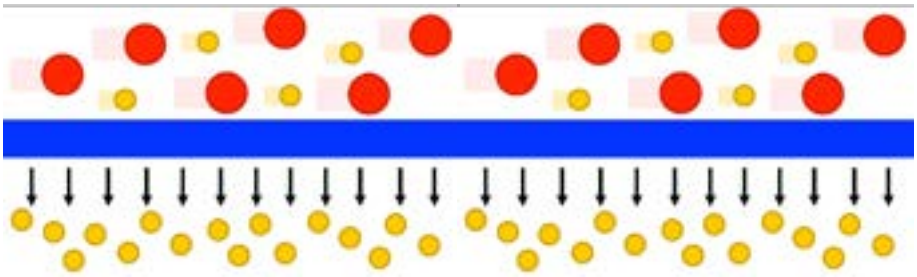
Following up on the Sunday evening workshop at ERCC9, "Vesicle Isolation & Function: Translating vesicle biology to clinical therapy," there will be a mini-conference February 15-16 in Gainesville, Florida on "EV Manufacturing and Isolation." The event will also be webcast to enable remote participation. (Contact conference organizer Thomas Schmittgen for details about the webcast.)

The goal of the conference is to find optimal separation strategies that can scale vesicle yield up to the level required for clinical production while maintaining functional activity of the vesicles. Methods to be discussed include conventional ultracentrifugation (UC), UC followed by density gradient, tangential flow filtration (TFF), and size exclusion chromatography followed by TFF.

Optimizing the production of extracellular vesicles for therapeutic applications

by Claire McCarthy

Therapeutic exosomes and Huntington's disease



Extracellular vesicles, specifically exosomes, are currently being explored as therapeutic delivery systems for disease-targeting RNA molecules. In a talk at the ERCC9 conference, Reka A. Haraszti, M.D., a researcher in Dr. Anastasia Khvorova's group at the University of Massachusetts Medical School, described how exosomes could be used to treat Huntington's disease, a progressive neurodegenerative disorder. There are currently no effective therapies for this illness, which is caused by a mutation in the Huntingtin gene. Exosomes capable of transporting molecular payloads designed to silence the defective Huntingtin gene represent a potential therapy for this fatal disease.

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ERCC Seminar Series

UCLA

Thursday March 1st 2pm ET
David Wong
UCLA

Salivary exRNA and a New Horizon in Dental, Oral and Craniofacial Biology

OLD DOMINION UNIVERSITY

Thursday April 7th 1pm ET
Sultana Hameeda
Old Dominion University

MGH 1811

Thursday May 10th 1pm ET
Mike Silverman, Das lab
Mass General

UC San Diego

Thursday June 7th 1pm ET
Louise Laurent
UCSD

Thursday July 12th 1pm ET
Subbaya Subramanian
U. Minnesota

The ERCC hosts a monthly web seminar on a variety of research topics related to extracellular RNA and extracellular vesicles. The seminars are recorded; past seminars can be viewed on the About and Presentations pages of the exRNA Portal.

Note in yellow the differences from the standard scheduled time, which is at 1pm Eastern on the first Thursday of each month.

A team of speakers from the consortium will be presenting symposia in Ft. Lauderdale, Florida in March and in London in July on the same topic as Dr. David Wong's March 2st web seminar — salivaomics. See the event websites for details.

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Next issue, Spring 2018

If you need help using the resources or want to become involved in any of the research initiatives highlighted in this issue, contact us at info@exRNA.org and we will connect you with the appropriate people.

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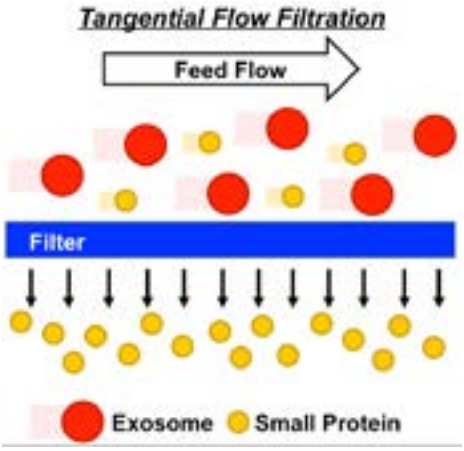
Comparison of exosome production methods

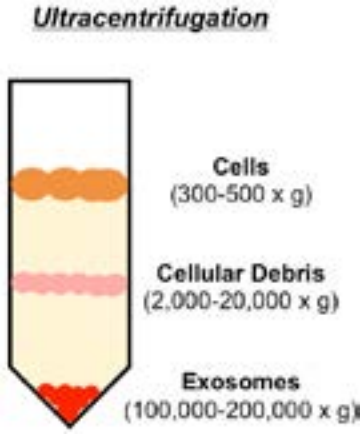
Technical challenges in the large-scale production of exosomes currently limit their utility for disease treatment. To address this issue, Dr. Haraszti and Dr. Khvorova's group teamed up with MassBiologics to develop and compare two different exosome production methods for yield and therapeutic efficacy of the exosomes. They utilized Tangential Flow Filtration (TFF) and ultracentrifugation to isolate exosomes from the conditioned media of cultured mesenchymal stem cells.



In TFF, conditioned media is continuously swept along the surface of a filter while a downward pressure is applied to force molecules through the filter. This process is like shaking a sifter to concentrate large particles blocking the holes in the filter, allowing smaller particles to pass through. In contrast, ultracentrifugation works by placing the conditioned media in a column of viscous fluid and spinning rapidly to separate extracellular vesicles in the media by their differing densities.

The researchers found that isolation by TFF resulted in 10-100 times more exosomes than ultracentrifugation. TFF-generated exosomes were also more heterogenous and contained 10 times more protein.

Exosomes produced by TFF and ultracentrifugation were also studied for their therapeutic potential in Huntington's disease. After purification, exosomes were loaded with small interfering RNA (siRNA) molecules that could silence the expression of the mutant Huntingtin gene in target cells that take up the exosomes.



In cell cultures of primary neurons, TFF-generated exosomes showed greater inhibition of Huntingtin expression than those generated by ultracentrifugation. Moreover, TFF-generated exosomes infused into the brains of mice suppressed Huntingtin gene expression *in vivo*.

Future for therapies using exosomes isolated by Tangential Flow Filtration

The findings of Dr. Khvorova's group indicate that Tangential Flow Filtration can generate a higher yield of exosomes for clinical use than older methods. Further, TFF-generated exosomes were effective gene therapy agents in experimental models of Huntington's disease, and hold promise as delivery systems for clinical treatments.

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Future directions for exRNA research from ERCC9

by Claire McCarthy

At ERCC9, speakers presented many promising new findings and advances in understanding the biology of extracellular RNA (exRNA) in health and disease. In the final session of the meeting, investigators discussed future directions and the technological challenges and opportunities that need to be addressed to make even further progress in the field.

Critical research questions about exRNA and extracellular vesicles

Presentations at ERCC9 illustrated recent progress in exRNA research, such as how viruses produce and utilize exRNAs during viral infection, potential exRNA biomarkers for neurological diseases, and the use of exRNA for cancer therapies. At the end of the meeting, Alissa Weaver, M.D., Ph.D., a clinician scientist at Vanderbilt University, led a discussion of important research questions about RNA biology that still need to be answered: 1) What are the differences between vesicular and non-vesicular exRNA? 2) What are the mechanisms of exRNA biogenesis and sorting into various exRNA carriers? 3) What is the functional impact of exRNA on recipient cells? 4) How are different types of exRNAs transported, and how much of the exRNA is active or degraded? She described the importance of addressing these questions by saying, "The better we understand the biology of these (exRNA) particles and their cargo, the better we can formulate hypotheses and questions to test." There are still many aspects of exRNA that need to be studied to improve our knowledge of its role in physiological and pathological processes.

Technical challenges

The discussion also touched on technical barriers associated with exRNA studies, such as identification and labeling of vesicular carriers of exRNAs. Although techniques like Nano Fluorescence-Activated Cell Sorting (NanoFACS) have contributed to recent progress in exRNA research, Jennifer Jones, M.D., Ph.D., a researcher at the National Cancer Institute, said that it still is not ready for "prime-time" clinical studies. Thus, she advocated for the development of a technology readiness levels framework that would help the exRNA community identify and overcome barriers in the field. This approach, which was developed by NASA, starts with a hypothesis, moves to testing in a simple model, and ends with the final goal, such as the validation of an exRNA clinical test.

Mark Ansel, Ph.D., an Associate Professor at UCSF School of Medicine, talked about how to assess the heterogeneity of exRNA carriers using advanced new methods to sort and concentrate them at the single-particle level. He specifically described the use of flow cytometry to measure fluorescently-labeled extracellular vesicles (EVs) in the lung lavage fluid of mT/mG reporter mice. Further, he compared the desired qualities of EV purifications, including sample throughput, homogeneity, and yield, for different types of studies. Dr. Ansel also discussed the visualization of source cells and uptake of exRNA carriers by target cells using novel imaging technologies, such as super-resolution microscopy. According to him, the best evidence for exRNA transfer between cells would be real-time observations.

In an open discussion at the end of the session, ERCC9 attendees brought up the need for exRNA measurement tools with greater sensitivity and specificity than the current gold standard of quantitative PCR. Additionally, researchers would like to be able to examine the native state of exRNAs in biological tissues. Clearly, single molecule detection approaches will need to be developed to overcome current limitations in both detection and specificity. There was also

discussion about developing integrated tools that could be applied to multiple exRNA research questions, since no single technique is perfect for different types of studies.

Future clinical applications of exRNA

Saumya Das, M.D., Ph.D., an Associate Professor at Massachusetts General Hospital - Harvard Medical School, talked about exRNA biomarkers as a "novel niche" that can be used to address unmet clinical needs in diagnostics, prognostics, and precision medicine. He shared challenges in utilizing exRNAs as clinical biomarkers, including the large variability in technical measurements of exRNAs, issues of normalization across experiments, and uncertainties regarding the fluctuation in measurements of exRNAs over time. Moreover, at the end of the session, ERCC scientists discussed standardization problems in biofluid and biospecimen collection for exRNA biomarker research.

In addition to biomarkers, exRNA can potentially be used in clinical therapy. Tushar Patel, M.B., Ch.B., a faculty member at the Mayo Clinic, shared in vitro and in vivo data indicating that exRNA enhances cancer immunotherapy and acts as an anti-cancer agent. These research findings have not yet been followed up by clinical trials investigating the safety and efficacy of exRNAs as therapeutics. More preclinical and clinical studies are needed before the potential of exRNAs to track and treat diseases can be realized.

Collaborations and communication between exRNA researchers to drive progress

In closing, experts in the exRNA field deliberated on the future directions and technological developments needed to fulfill the promise of exRNA research for improving human health.

v According to Louise Laurent, M.D., Ph.D., the Director of Perinatal Research for the UCSD Department of Reproductive Medicine, and one of the chairs of this session, "One of the reasons that we had this meeting is to set up communications and collaborations both within, outside, and among all of the people here to bring together everyone's ideas and expertise." Researchers in the field need to work together to overcome technical challenges and make substantial progress in developing biomedical and clinical applications of exRNA.

New developments in extracellular RNA standards and resources from ERCC9

by Claire McCarthy

While extracellular RNAs are currently being pursued as novel biomarkers of health and disease, exRNA research has been hampered by a lack of standardization across the field and limited experimental approaches for exRNA quantification. At the ERCC9 meeting, researchers shared exciting new technologies, procedures, and resources under development to overcome these limitations.

Breaking barriers in measuring exRNA in blood

Researchers Klaus Max, Ph.D., of Rockefeller University and Vasily Aushev, Ph.D., of the Icahn School of Medicine at Mt. Sinai described new experimental approaches to measure miRNA in serum and plasma. These biofluids are ideal for exRNA biomarker studies because blood is routinely collected for clinical tests. However, it is difficult to isolate and profile exRNA from blood products due to low circulating concentrations of exRNA, rapid and differential RNA degradation, a lack of standardized quality controls for measuring exRNA, and an incomplete understanding

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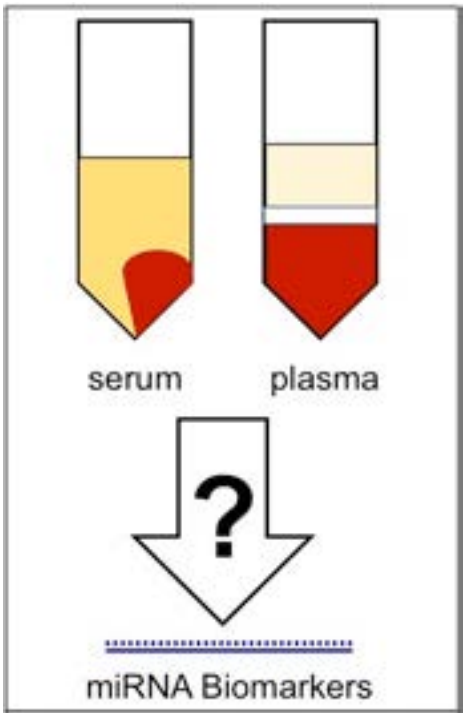
of factors that influence the high variability in the expression level of exRNAs observed between and within individuals. Some known contributors to this variability are gender differences and time of day.

To improve the recovery of exRNA



How can we isolate and measure miRNA biomarkers from blood?

from plasma and serum, Dr. Max's group added spike-in calibrator RNA as a control and decreased variability in exRNA yield by adding silica column purifications to their RNA isolation protocol. Dr. Aushev's group compared different technical platforms for measurement of extracellular miRNA to determine the best approach for profiling exRNA in blood. They found that RNA sequencing was better than hybridization-based NanoString platforms, Exiqon miRNA quantitative PCR panels, and EdgeSeq technology for measuring miRNAs in plasma.

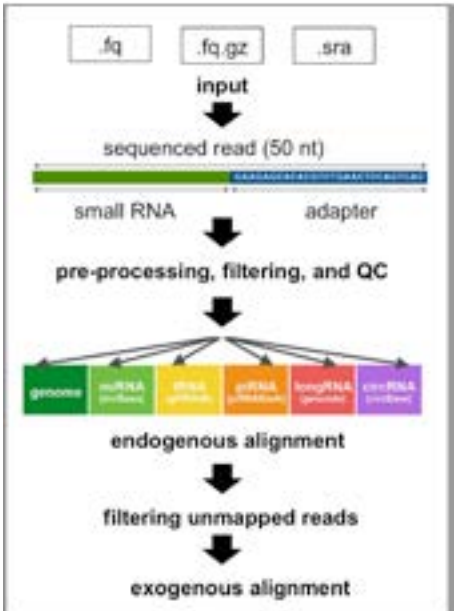
Overall, these talks presented new experimental approaches that could improve biomedical analyses of exRNA and identify potential extracellular miRNA biomarkers in blood.

Measuring the landscape of exRNA

Along with miRNA, other classes of noncoding exRNA could play a role in disease processes and act as prognostic markers. In her ERCC9 talk, Anna Krichevsky, Ph.D., an investigator at Brigham and Women's Hospital and Harvard Medical School, described novel methods to assess the entire set of exRNA and exRNA carriers secreted from cells. (See this related blog post for more details.) Her experimental system included a size-based filtration method to differentiate exRNA carriers, an end-modifying procedure to sequence exRNA content with minimal bias, spike-in RNA for normalization, and baseline measurements of exRNA in cell culture media alone. This system can be used to examine the whole landscape of exRNA classes during homeostatic and disease conditions.

exRNA data repositories

An important goal of the exRNA Communication Consortium, in addition to developing exRNA measurement techniques, is data management and sharing. To improve the understanding of exRNA biology, it is important to systematize exRNA data analysis and compare findings across the exRNA research community. Therefore, the ERCC has developed a data repository to standardize the analysis and comparison of exRNA data.



Simplified flowchart of the exceRpt small RNA-seq analysis pipeline.

For more detail, see http://exrna.org/resources/software/excerpt-flowchart/

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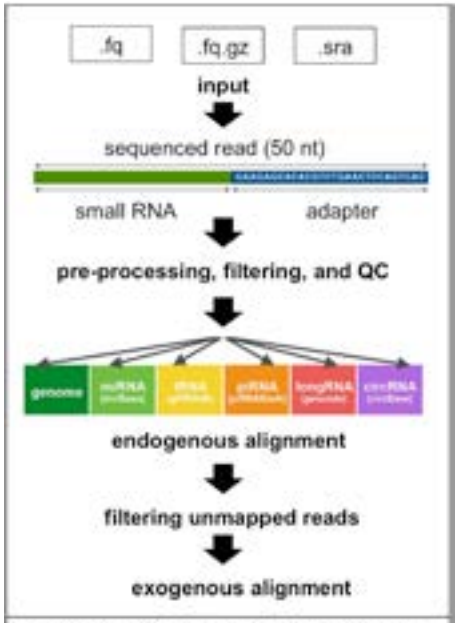
At ERCC9, Joel Rozowsky, Ph.D., a research scientist at Yale University, presented exceRpt, the extracellular RNA processing toolkit. This data management system performs quality control measures on exRNA sequencing reads, which are often of poor quality due to small input samples and the presence of contaminants. This software system is also able to align and normalize exRNA data and can be used to quantify exRNA profiles.



Simplified flowchart of the exceRpt small RNA-seq analysis pipeline.

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Moreover, exRNA sequencing data sets from exceRpt can be stored in the exRNA Atlas, which is the first large repository for exRNA profiles from human biological samples. Aleksander Milosavljevic, Ph.D., a professor at Baylor College of Medicine, illustrated how computational tools available at the Atlas can be used to deconvolute exRNA data and compare exRNA presence and expression levels across different types of biofluid. Using this resource, his group was able to identify carrier-specific exRNA profiles across different biological samples. Additionally, he showed that comparisons between carrier-specific groups can differentiate exRNA expression related to diseases.

Both exceRpt and the exRNA Atlas are important data analysis tools for exRNA researchers and can help scientists discover important exRNA pathways for human physiology.

Summary

Talks at ERCC9 presented innovative experimental methods, technologies, and exRNA research resources that address standardization issues and current limitations in exRNA studies. Researchers described new methods to isolate and measure exRNA from blood, technical approaches to assess the entire landscape of exRNA in biological samples, and the exRNA Atlas, a data management system available to all scientists interested in studying extracellular RNA. These protocols and databases can be used to expand our knowledge of exRNA biology.

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