Extracellular RNA Communication

NIH Common Fund Program
Extracellular RNA Communication Program

New Paradigm: RNAs are released from cells and (may) go on to influence cells that receive them. RNAs are found in the environment: food, bacteria (think microbiome).

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Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells

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An opportunity to explore new paradigms of cell-to-cell communication based on release, transport, uptake, and regulatory role of exRNAs
Extracellular RNA Communication Program

Focus of this program:

- How do cells use exRNAs to send messages to cells in distant organs?
- Do diseased cells produce different exRNAs than healthy cells, and what is the impact of these exRNAs? Can diseases like cancer spread through release of exRNAs?
- Can researchers harness the communication powers of exRNAs to turn a diseased cell into a healthy cell? (Therapeutic potential.)
- Can exRNAs be used as biomarkers to diagnose disease, monitor progression, and measure response to therapy?
The ExRNA Communication Program is a 
Trans-NIH Effort

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Program Initiatives

- **Defining a Comprehensive Reference Profile of Circulating Human Extracellular RNAs (U01)**
  - Goal: To develop reference profiles for non-coding regulatory exRNAs from healthy human body fluids.

- **Extracellular RNA Biogenesis, Biodistribution, Uptake, and Effector Function (U19)**
  - Goal: To determine the biological principles guiding exRNA biogenesis, biodistribution, uptake, and effector function.

- **Clinical Utility of Extracellular RNA for Biomarker Development (UH2/UH3)**
  - Goal: To identify and quantify exRNA-based biomarkers derived from human body fluids in order to diagnose diseases, monitor disease progression, and measure response to therapy.

- **Clinical Utility of Extracellular RNA for Therapy Development (UH2/UH3)**
  - Goal: To develop and demonstrate the potential for clinical utility of exRNAs as therapeutic agents.

- **Data Management and Resource Repository (DMRR)**
  - Goal: To integrate the efforts of all ExRNA Communications program components and serve as a community-wide resource for exRNA standards, protocols, and data.
Defining a Comprehensive Reference Profile of Circulating Human Extracellular RNAs (U01)

- Defining RNA profiles in Bile, CFS, Serum, Plasma, etc (751 profiles available)
- This represents “normal” individuals for comparison to experimental/disease/affected cohort.
- Appreciation for complexity and diversity in all sources, including plasma\(^1\):
  - Extracellular small RNAs are widely detected in the circulation in large populations
  - identified over a thousand human extracellular RNAs including microRNAs, piwi-interacting RNA (piRNA), and small nucleolar RNAs
  - Non-human RNAs detected

Cancer exosomes use Dicer, TRBP, and AGO2 to process pre-miRNAs to generate mature tumor inducing miRNAs.

Cancer exosomes mediate efficient and rapid silencing of mRNAs to reprogram a target cell transcriptome.

KRAS-MEK-ERK signaling promotes Ago2 phosphorylation which inhibits Ago2-endosome association and sorting to exosomes.

Ago2 levels and phosphorylation status control secretion of candidate miRNAs in exosomes.

Indication that RNPs (like Ago2) likely very involved in chaperoning RNAs to final destinations.

First demonstration that cell surface receptor signaling can determine loading of a specific miRNA into vesicles.

Cha et al., eLife (2015)
McKenzie et al., Cell Reports (2016)

Calin (University of Texas)
Coffey, Weaver, Patton (Vanderbilt)
Dr. Richard Kraig (University of Chicago) has shown microRNA-containing exosomes promote the formation of myelin in animal models of multiple sclerosis.

- Exosomes promoting myelin formation are released from immune cells stimulated with IFNγ, and are also produced by young animals and animals experiencing environmental enrichment.
- These exosomes contain microRNAs that promote oligodendrocyte differentiation and protect against inflammation.
- MicroRNA-containing exosomes promote myelin formation in the brain when administered nasally.

Pusic et al. (2014) J. Immunology, 266(1-2); Pusic et al. (2014) Glia, 62(2)
Verified biomarkers for validation: 9 Long RNAs and 7 small RNAs (100 GC and 100 non-GC).

Final cohort: Saliva from 750 GC patients and 750 non-GC controls
Individual biomarker validation (500 GC/ 500 non-GC)

Salivary exRNA biomarker panel configuration

Next: Identification of bacterial exRNA biomarkers for gastric cancer in salivary

David Wong (UCLA)

Genboree Workbench Long RNA-Seq analysis pipeline
exceRpt - Genboree Workbench Small RNA-seq analysis pipeline
Some Consortium Wide Accomplishments

- **197 total publications in 50 different scientific journals**, demonstrating broad reach of the program into various biomedical specialties.

- Generation of **highly standardized protocols** for vesicle isolation and RNA purification from body fluids, body fluid collection and processing, etc.

- Revision of Gene Ontology terms (**GO terms**) relevant for exRNA-containing particles.

- Developed **standards** and standard formats, tools and applications, for RNA-Seq data and metadata standards, which is essential to data harmonization.

- PIs are leading a new Gordon Conference and a Keystone Meeting.

- Developing **imaging tools** to visualize and track vesicles.

- Developing a set of cell lines with mutations in all the known components of vesicle biogenesis.
Remaining Challenges

- ExRNA communication mechanisms are in early discovery stage
- The consortium has gelled and is working together to address some of the cross-cutting fundamental discovery goals of the program.
- All therapy (8) and biomarker (10) projects are moving forward
  - oligodendrocyte differentiation
  - siRNA targeting of mutant Huntingtin mRNA
  - edible plant-derived nano-vector delivery
  - biomarkers for various cancers, cardiovascular risk, placental dysfunction, CNS disease
- However, successful therapeutics should have a foundation of defined biological principles behind them. Some therapeutic goals may be dependent on determining underlying biology – which takes more time.
- Much of this foundational work is hypothesis-generating and broadly enabling, but are there are opportunities for additional focused investments to reveal new paradigms within another 4-5 years?