AN AUDIENCE WITH…

Dan Tagle

Extracellular RNA (exRNA) — a broad set of ribonucleotides that circulate outside cells and that may have important roles in intercellular communication and cell signalling — have caught the eye of the US National Institutes of Health (NIH). In August, the biomedical funding body awarded US$17 million to 24 projects that will explore basic exRNA biology and investigate whether these molecules and the vesicles that transport them can be used as therapeutics and as biomarkers. The NIH's Dan Tagle, an architect of the funding programme, discussed the emerging promise of exRNA with **Asher Mullard**.

Q *What is the history of the exRNA field?*
Back in the 1960s researchers recognized that certain cellular particulates float around in cell preparations, and these were called cellular debris at that time. With the advent of high-throughput screening and various analytical tools, we have been able to work out that these circulating particulates are made up of lipids, proteins and nucleotides, including various categories of regulatory RNA molecules. Once we started identifying these molecules, we needed to find out what they were doing.

As far as we know these vesicles — and their RNA contents — are secreted in such a way that they can home in on specific target cells. For the most part it seems that different donor cells produce different and specific vesicles, and these can perhaps transform the phenotype of target cells to mimic and resemble the donor cells.

One of the earliest discoveries in this field is that this may be a mechanism by which mesenchymal stem cells can repair damaged tissues from distant sites. That is, the stem cells may not actually migrate but rather induce other cells using exRNA to become more like stem cells that can then repair and regenerate damaged tissues.

The effects of exRNA have been shown both *in vitro* and *in vivo*. In one controversial *Cell Research* paper that came out in 2012, a Chinese group showed that microRNA from rice actually survives digestion and enters the circulatory system of animals, binding low-density lipoprotein receptor mRNA and potentially elevating low-density lipoprotein levels.

But, it is only within the past couple of years that we have been able to recognize these recent developments. So we set up funding opportunities to address the key issues.

Q *One of these issues is exRNA biogenesis. What are the key questions here?*
We awarded five grants to researchers who will look at the origins of exRNA, because we still don't know how these vesicles or exRNA are produced by different cell types.

Different cell types produce and secrete different populations of RNA, they each package and decorate the vesicles in a unique way, and glycoprotein decorations can target vesicles to specific populations of cells. Researchers have also observed that diseased cells produce different signatures of exRNA than do normal cells. So, how do cells decide what types of RNA to produce? Is the differential expression part of the disease process? These are some of the biogenesis issues that we want to start thinking about, along with vesicle loading, docking and release and targeting.

Q *What are the aims with the biomarker grants?*
We awarded 10 grants for biomarker development, based on the observation that diseased and normal cells produce different populations of exRNA, and that exRNA is readily available in body fluids including saliva, plasma, urine, cerebrospinal fluid and breast milk.

The grants cover a wide range of diseases, including cancer, immune deficiencies, neurodegenerative disorders, cardiovascular diseases and placental dysfunction. The basic premise is that investigators have a collection of biological fluids from which they can extract exRNA, look at the various categories of regulatory RNA, and then look for correlations and associations between normal and disease states.

We have challenged the recipients of these grants to look at exRNA in existing collections in the first 2-year phase of the award. They will then do larger validation studies in the second phase, in which prospective biospecimen collection can be done even in the context of a clinical trial. We have designed the programme so that we can see whether these biomarkers can be used as clinical end points, as surrogates for determining efficacy of treatment.

I think the work on cancer will mature quickest in terms of development for clinical use.

Q *And how about the focus on therapeutics?*
Eight awards will look at developing this science for therapeutics. exRNA circulates in endogenous vesicles that our body produces normally, and these can evade the immune system and can even cross the blood–brain barrier. The projects that we funded aim to take advantage of those features in a range of indications.

For example, can we harvest or synthesize these microvesicles and then load them up with therapeutics — whether these be small molecules or therapeutic nucleotides — and take advantage of the observation that vesicles can be targeted to specific cell types. We also have projects that are aimed at identifying exRNA molecules that can mitigate disease processes. The indications we are focusing on include Huntington's disease, breast cancer and kidney failure.

The therapeutics projects are also designed in two phases. During the first 1–3 years, researchers will do proof-of-concept studies, showing that the therapeutic molecule actually has the desired effect in an *in vitro* or *in vivo* system. The second expansion phase, in disease models, is geared towards enabling investigators to file an investigational new drug application at the end of 5 years of funding.

Q *What else do you hope will come out this programme?*
A data management award that we funded will take the information that is produced by these projects and make it publicly available. We will also award grants for exRNA profiling, in which recipients will look to see what types of exRNA each different cell type produces — essentially developing an exRNA catalogue.