TECHNOLOGY OFFER

RECOMBINANT EXTRACELLULAR VESICLES (R-EV) AS A REFERENCE MATERIAL FOR BIOFLUID PROCESSING AND INSTRUMENT CALIBRATION

BIOMARKED, a consortium of research laboratories of Ghent University, member of the Cancer Research Institute Ghent (CRIG), is seeking partners interested in licensing.

Introduction

Extracellular vesicles (EV), such as exosomes, are nanometer-sized vesicles that contain a phospholipid membrane and cell-type-specific combinations of proteins, nucleic acids and metabolites. EV transmit information between different cell types, organs and even between organisms, and have been detected in multiple body fluids. EV orchestrate physiological and pathophysiological processes
The interpretation of extracellular vesicle data remains challenging owing to the complexity of biofluids and the technical errors that are introduced during sample preparation, isolation and analysis.
To understand and mitigate these errors we present the use of recombinant extracellular vesicles (R-EV) generated by Gag self-assembling protein that harbor physical and biological traits characteristic of EV. The informed use of this reference material, ensures standardized EV measurements in various applications.

Technology

Researchers at Ghent University and VIB have developed R-EV, recombinant extracellular vesicles that harbor physical and biological traits characteristic of EV.
R-EV comprises a) a self-assembling protein that directs its own release through EV as a luminal membrane bound protein and b) a heterologous marker. A non-limiting example of such a self-assembling protein is the retroviral group specific antigen (GAG) fused to a heterologous marker such as the light-emitting enhanced green fluorescent protein (EGFP).
We analyzed and compared physical and biochemical characteristics of density gradient purified R-EV with endogenous EV at the level of density, size (nanoparticle tracking analysis), morphology (electron microscopy), composition (proteomics and lipidomics) and zeta potential (zeta sizer).
The high abundance of fluorescent fusion proteins per particle can be exploited to deduce the amount of particles, as measured with fluorescent NTA, by measuring fluorescent signals with a fluorescent plate reader, fluorescent threshold flow cytometry or by measuring the concentration of –for example the GAG polyprotein or by measuring the concentration of –for example-EGFP mRNA. The latter non-fluorescence-based measurements are more sensitive than the fluorescence measurements.
R-EV can be used as a reference material to spike in different biofluids prior to isolation and afterwards be traced, giving the possibility to calculate the recovery rate of different EV isolation methods. R-EV modifications allow to separate R-EV from sample EV for downstream analysis.

Applications

R-EV can be used:
- to quantify sample EV
- to calibrate a device for sample EV analysis, the device can be a flow cytometer, a nanoparticle tracking analysis device, a tunable resistive pulse sensing device, a plate reader, a microfluidics device, ELISA reader, a zetasizer or an electron microscope
- to estimate the isolation efficiency or recovery rate of EV in isolation methods

Use of R-EV will enable the comparison of results between different research groups, and support the use of EV measurements for the diagnosis, prognosis and therapeutic decision making of diseases such as cancer, diabetes and cardiovascular disease, improving the accuracy of an EV-based diagnosis.

Advantages

This technology offers significant advantages over other extracellular vesicle standards.
Unlike other extracellular vesicle standards, R-EV offer:

+enhanced fluorescent activity and tracebility.
+in depth analysis of physical and biochemical characteristics of endogenous, sample EV.
+ ensured stability.
+ a highly sensitive detection by multiple complementary technologies
+ production in high quantities and high purity
+ on demand separation between R-EV and sample EV

R-EV offers the users enhanced flexibility and usability and are easily adaptable to any laboratory SOPs in a wide variety of applications (Environmental, Pharmaceutical, Medical, Food and Water).

1) R-EV has common traits with sample EV allowing implementation of R-EV in whatever EV isolation, characterization method or assay/instrument calibration method:

- heterogeneous in size, similar like EV (confirmed by electron microscopy);
- similar refractive index as EV (confirmed by nanoparticle tracking analysis);
- similar zeta potential as EV (confirmed by zetasizer);
- similar density (demonstrated by density gradients);
- comparable enriched proteins such as Alix, CD9, TSG101 (demonstrated by western blot analysis and proteomics)
- comparable lipid composition (demonstrated by lipidomics)

2) Unique properties distinguish R-EV from sample EV:

- strong Gag-EGFP protein expression in R-EV detectable by easy read-out methods available in standard laboratories such as p24 ELISA, Gag-EGFP western blot analysis;
- GFP mRNA expression to be analyzed by RT-qPCR;
- High EGFP fluorescence intensities allowing fluorimetric read-outs with standard fluorimeters. In addition R-EV can be measured by high resolution flow cytometry and it can be used as a positive control for functional uptake experiments analyzed by imaging flow cytometry.

Status of development

A reproducible production method for the reference standard has been optimized.
Extensive characterization has been performed using nanoparticle tracking analysis, Western Blot, wide field and close-up Electron Microscopy analysis, size distribution analysis, proteomic and lipidomic analysis.
SOP protocols for use in plasma samples and urine samples have been optimized.
The R-EV has been evaluated as spike-in by UGent teams and is currently evaluated in a blind study by a set of third party users (external validation).

GHENT UNIVERSITY

Partnership

Ghent University is seeking a licensing partner.

Intellectual property

A Patent application EP17200743.7 “Usages of recombinant extracellular vesicles” has been filed with priority date November 9th 2017.

The inventors

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References

Not yet published.

Keywords

Exosomes, Extracellular vesicles, cancer diagnosis, liquid biopsies, EV or exosome isolation and characterization, standard, calibration

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