

Methods for systematic identification of membrane proteins for specific capture of cancer-derived extracellular vesicles

ABSTRACT

Analysis of cancer-derived extracellular vesicles (EVs) in biofluids potentially provides a source of disease biomarkers. At present there is no procedure to systematically identify which antigens should be targeted to differentiate cancer-derived from normal host cell-derived EVs. Here, we propose a computational framework that integrates information about membrane proteins in tumors and normal tissues from databases: UniProt, The Cancer Genome Atlas, the Genotype-Tissue Expression Project, and the Human Protein Atlas. We developed two methods to assess capture of EVs from specific cell types. (1) We used palmitoylated fluorescent protein (palmtmTomato) to label tumor-derived EVs. Beads displaying antibodies of interest were incubated with conditioned medium from palmtmTomato-expressing cells. Bound EVs were quantified using flow cytometry. (2) We also showed that membrane-bound Gaussia luciferase allows the detection of cancer-derived EVs in blood of tumor-bearing animals. Our analytical and validation platform should be applicable to identify antigens on EVs from any tumor type.

Keyword: Extracellular vesicles; Membrane proteins; Biomarker; The Cancer Genome Atlas; Genotype-Tissue Expression Project; Human Protein Atlas; Palmitoylated fluorescent protein; Membrane-bound Gaussia luciferase