

Association of plasma exosomal mRNA changes with long-term durable response to ipilimumab in metastatic melanoma patients

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Introduction

Exosomes are microvesicles that are abundantly released from cells, including tumor cells, into biofluids such as urine and plasma and carry nucleic acids and proteins from the cell of origin (Fig. 1). Exosome-based liquid biopsies enable non-invasive monitoring of a patient's tumor status in real-time. Liquid biopsies provide several advantages over standard invasive tissue-based tests. However, one of the challenges of an exosome-based test for cancer monitoring is that the tumor signature must be identified against a background of exosomes originating from non-malignant cells. To reduce this background, we developed a platform to deplete exosomes from normal blood cells (Fig.2).

Here we set out to identify early changes in exosomal RNA (exoRNA) that predict long-term durable response (DR) of metastatic melanoma patients treated with ipilimumab. To facilitate the identification of gene changes related to ipilimumab treatment, we examined exoRNA from patient plasma with and without depletion of non-tumor exosomes. We hypothesized that depletion of normal blood cell-derived exosomes would enrich tumor-derived exosomes in plasma and reveal rapid exoRNA changes between baseline and week 2 or week 4 of treatment.

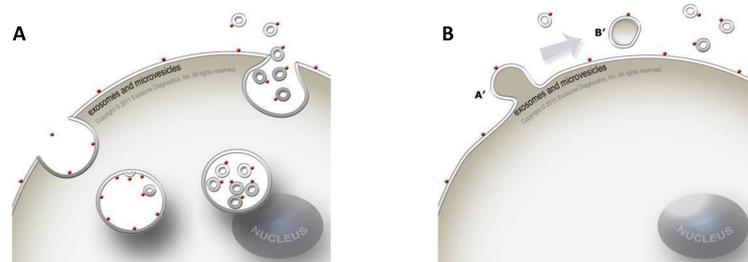


Fig.1. Exosomes and other vesicles can be released by (A) the multi-vesicular body pathway or through (B) direct budding at the plasma membrane. Exosomes and microvesicles are released from all cell types, including tumors, and can be found in biofluids such as blood and urine.

Methods

Plasma exoRNA was extracted from normal and patient samples using two extraction methods, total exoRNA isolation using exoRNeasy and exoRNA from samples depleted of exosomes derived from normal blood cells using ExosomeDx Depletion Enrichment (EDDE) platform targeting glycophorin A on reticulocyte exosomes.

Plasma was obtained from controls and 21 patients with metastatic melanoma before receiving ipilimumab (baseline) and longitudinally throughout treatment. Response to therapy was determined by RECIST. Patients who achieved at least 12 months of progression free survival (durable responders, DR) from the start of ipilimumab (n=5) were compared to patients with progressive disease (PD) on ipilimumab (n=16), (Table 1). Matched patient plasma samples at baseline and week 2 or week 4 (week 2/4) were analyzed. The plasma exoRNA signature using the glycophorin A EDDE platform was compared with total plasma exoRNA from the same patient samples. Analysis of exoRNA utilized the OpenArray® Human Inflammation Panel (Fig. 2).

Table 1. Characteristics of the 24 Patients

Patient groups	N	Duration of response (mo.) Mean ± SE	Notes
Long-term durable response (DR) ≥ 12 months	5	25.8 ± 6.8	3 patients maintain ongoing response 2 patient progressed bt 12-16 months
Progressive Disease (PD)	16	3.56 ± 0.53	7 patients progressed bt months 1-2 8 patients progressed bt months 3-6 1 patient progressed at month 9
Normal Human Plasma	3	NA	NA
Patient Total	24		

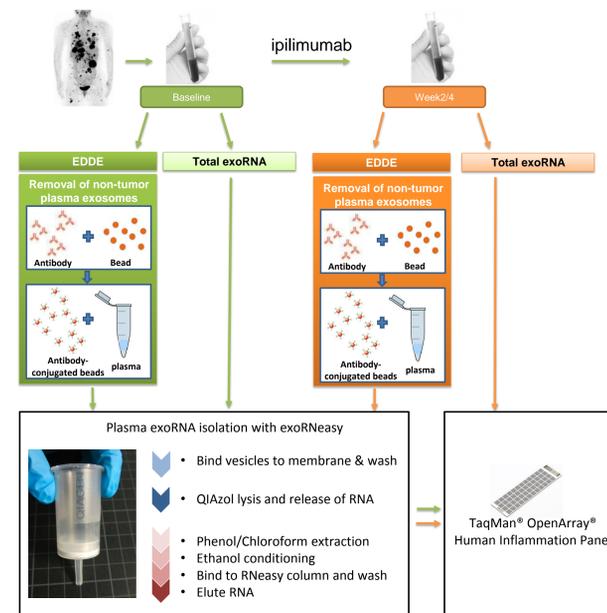


Fig. 2. Comparison of total exoRNA and EDDE workflows. The EDDE platform depletes non-relevant background for improved detection of differential exoRNA expression. The exoRNA from depleted plasma and total plasma exoRNA was reverse-transcribed and examined by TaqMan® OpenArray®.

Results

The novel EDDE platform effectively and reproducibly isolates exoRNA from plasma (Fig. 3, 4). Depletion of reticulocyte exosomes was demonstrated by the decreased expression of Let7a exoRNA in plasma (Fig. 5). Of the 607 genes examined, the EDDE platform revealed a ten-gene signature with superior ability to accurately separate patients with a long-term DR from those with PD (Fig. 6). In contrast, the gene signature identified using total exoRNA had a 12.5% false positive inclusion of patients with PD.

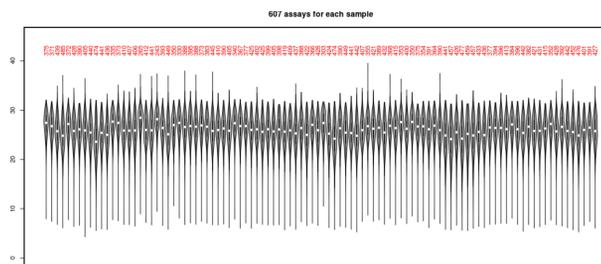


Fig. 3. exoRNA is abundantly isolated from plasma using EDDE platform and exoRNeasy. Each violin plot represents an individual patient plasma sample. The number in red represents the genes detected (out of 607 examined) for each sample on the OpenArray®. The cycle threshold (Crt) is plotted on the y-axis, and the width of the violin plot represents the number of genes.

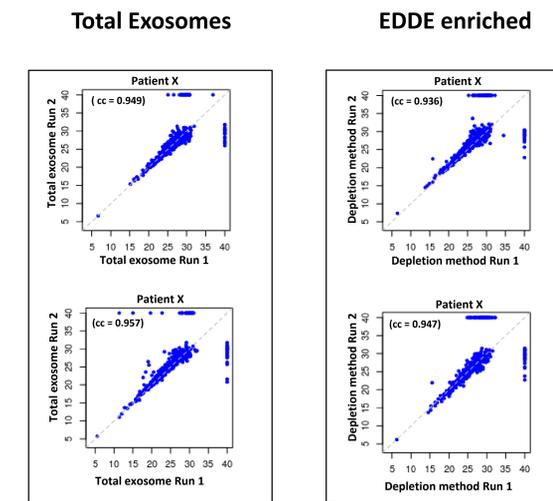


Fig. 4. Reproducibility of the EDDE platform. Scatter plot of gene expression profiles (raw Crt values) between two independent runs of the same plasma sample isolated using either total exosome isolation or the EDDE platform are shown. For each sample examined, a Pearson pairwise correlation coefficient (cc) is listed in top left corner.

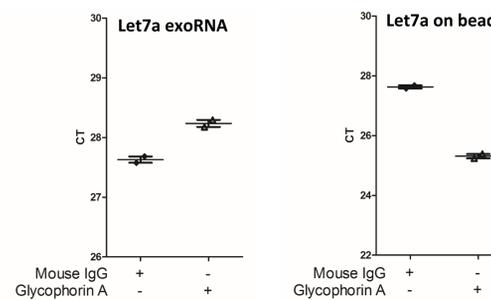


Fig. 5. The EDDE platform leads to removal of reticulocyte exoRNA. The amount of exoRNA removed depends on the gene expression level in reticulocyte exosomes. For Let7a exoRNA, 90% is removed by targeting and depleting plasma of Glycophorin-A positive exosomes, compared to control (IgG). The cycle threshold (CT) is plotted on the y-axis.

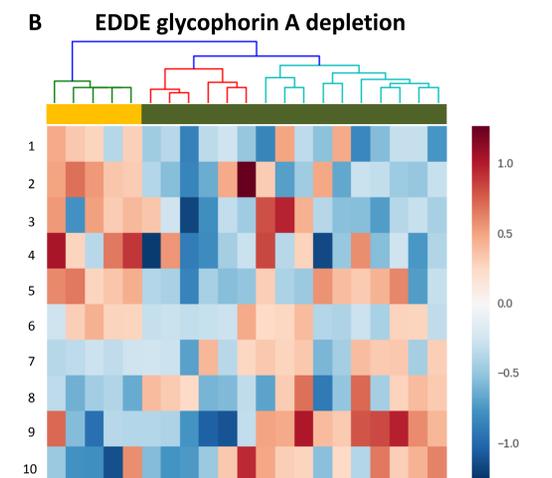
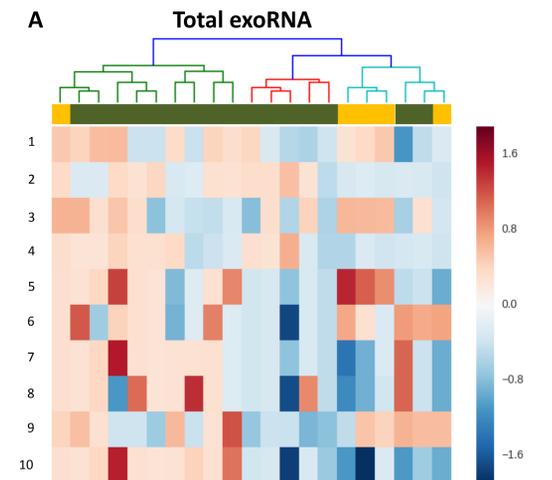


Figure 6: The gene signature identified by the EDDE platform accurately separates DR from PD. In the heatmap displayed, each block represents a single gene and the color (blue to red) corresponds to the change in plasma exoRNA expression (Δ Crt) at week 2/4 relative to baseline. Each orange bar represents an individual durable responder (DR). Dark green bars represent patients with progressive disease (PD). (A) The gene signature identified from total plasma exoRNA only partially separates DR from PD. The dendrogram from hierarchical clustering shows a 12.5% false positive inclusion of non-responders. (B) The gene-signature identified using the glycophorin A EDDE platform accurately segregates all responders into a well separated cluster in the hierarchical clustering dendrogram.

Conclusions

We have developed a platform to deplete normal exosomes from plasma which increases the power to detect early exoRNA changes associated with improved progression free survival in metastatic melanoma patients in response to ipilimumab.

