Introduction

Traditionally, oncology biomarker discovery and development has required the use of material obtained from tissue biopsies. However, recent developments in the exosome field have allowed biomarker research in biofluids to evolve. Exosomes are highly stable microvesicles, approximately 30-200 nm in diameter, that are shed by cells into all biofluids, including blood, urine, and cerebrospinal fluid, carrying a rich source of intact protein and RNA (Fig.1). RNA can be efficiently isolated and addressed using technologies such as RT-qPCR and NGS. Here we demonstrate the use of RNA extracted from these vesicles to monitor transcriptional changes in response to an immunotherapy treatment for malignant melanoma.

Methods

Exosomes were isolated, using exoRNeasy columns, from plasma collected from malignant melanoma patients treated with Ipilimumab. Patients were split in two groups, based upon either a positive or negative response to the therapy (5 responders, 11 non-responders, 16 patients in total). Responders were classified as patients with at least 6 months duration of response using the RECIST1.1 criterion. Plasma was collected pre-treatment (baseline) and the first post-treatment time point (week 2 or week 4). Exosomal RNA (exoRNA) was extracted using exoRNeasy kit (Fig.2). We screened the levels of 586 mRNAs associated with inflammation and 21 mRNA controls by RT-qPCR using the OpenArray® technology. The identified potential biomarker genes subsequently were verified independently.

Results

Over 400 genes were detected across all samples and time-points. Eleven control mRNAs with robust detection in all samples were used for normalization. When compared in matched pre- and post-treatment time point patient samples, we identified 9 mRNA species with opposite expression changes. A number of mRNA species showed significant differential expression (p-value<0.05) in patients responding to the therapy compared to those that did not respond. By using high throughput PCR array screening, we have demonstrated the potential for exosome RNA profiling in longitudinal monitoring of treatment response. The signatures are being confirmed by individual assays run in 384-well format.

Conclusions

RNA expression profiling from circulating exosomes offers the ability to monitor changes in immune pathway genes serially during immunotherapy. Initial data suggest expression signature changes occur early during therapy, offering the potential for predicting response.

References


Abstract – Poster # 452

Profilng Exosomal mRNAs in Patients Undergoing Immunotherapy for Malignant Melanoma

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18th ECCO - 40th ESMO European Cancer Congress, September 25 - 29, 2015, Vienna, Austria

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