

Tetraspanin immunoassay for the detection of extracellular vesicles and renal cell carcinoma

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Abstract

Half of patients with renal cell carcinoma (RCC) will develop metastases. The disease is likely to be curable at early stages but incurable when metastatic. New and non-invasive biomarkers are needed for the diagnosis of RCC. Extracellular vesicles (EVs) are considered promising new biomarker targets for the diagnosis of various diseases. Our study aimed to develop an EV-based assay for the detection of RCC using a highly sensitive nanoparticle-aided time-resolved fluorescence immunoassay (NP-TRFIA). To confirm that the tetraspanins were located on EVs, we used size exclusion chromatography to separate EV- and PE (protein-enriched)-fractions from RCC4 and 786-O RCC cancer cell lines and HEK293. EV- and PE-fractions were quantified using NP-TRFIA assays established for tetraspanins CD9, CD63, CD81, and CD151. Tetraspanin biomarkers were further measured from RCC cell culture medium as well as serum samples of RCC (n=14), benign (n=17), and healthy (n=9) individuals. Among the tetraspanins, CD63 showed 3-5-fold higher expression on EVs derived from RCC4 and 786-O cell lines compared to those from the HEK293. A sandwich CD63-CD63 assay demonstrated significant discrimination of RCC patients from benign (p=0.0003), and healthy (p= 0.005) individuals, respectively. Similarly, the CD81-CD81 assay also enabled significant separation of RCC patients compared to benign (p=0.014), and healthy (p= 0.003) controls, respectively. This result suggests that RCC cell lines and serum of RCC patients show higher amounts of CD63- and CD81-enriched EVs compared to controls. Detection of these tetraspanin-enriched EVs using our NP-TRFIA approach may play a vital role in the detection of RCC.

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