O19 Biomarker exploration of extracellular vesicles in renal cell carcinoma

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Introduction & Objectives: Exosomes are a subpopulation of Extracellular vesicles (EVs) secreted by normal and tumor tissues. They mediate cellular communication and represent a new class of biomarkers from liquid biopsies. From this point of view, isolation of tumor-released EVs from blood or urine using tumor-specific markers covers potential to discover prognostic and therapeutic markers. Consequently, the aim of this study is to identify and to prove specific EV markers in clear cell renal carcinoma (ccRCC).

Materials & Methods: EVs from RCC cell lines (786-O, RCC53, RCC26, Caki1, Caki2) were isolated using ultracentrifugation (UC). Isolation of EVs from tumor tissue was done by ultracentrifugation with three-layer-gradient (0.6 M, 1.3 M, 2.5 M sucrose. Protein expression was analyzed via Westernblot using cell-specific (GM130), exosome-specific (CD9, CD63, CD81) and tumor cell-specific markers (EpCAM, CA9, CD70, CD147). EV imaging was carried out by transmission electron microscopy (TEM). A chip-based technique as well as nano scaled flow cytometry (nanoFCM) were assessed to validate previous findings.

Results: EVs from tumor tissues and cell cultures have been isolated with high quantity and purity, although specific markers have demonstrated diverse expression patterns. EpCAM was found to be weakly expressed in cells and EVs from cell culture, these findings were validated by chip-based techniques. In addition, EpCAM was expressed in cellular and exosomal fraction of tissue samples showing increased occurrence of cleaved fragments in EVs. The expression of CA9 was observed in all cell lines and EVs as well as in primary tumor cells and EVs. CD70 exhibited different expression patterns in tumor cells and their EVs depending on cell lines. Higher amounts of CD147 were present in the EVs compared to the 786-O and Caki2 parental cells and to the cell lysate of tissue samples.

Conclusions: Here we have shown that ultracentrifugation represents an outstanding enrichment method to analyze tumor-derived exosomes from different sources. The Expression of tumor specific markers reflects the cellular background of exosomes. In contrast to EpCAM, CA9 and CD147 could represent promising tumor specific biomarkers in RCC. Our findings suggest specific sorting mechanisms and functions of these proteins depending on the tumor cell origin. The use of ultracentrifugation combined with classical techniques for proteomics, together with novel high-resolution techniques, are potential tools for EV biomarker discovery.