Identification of tumor-specific markers on extracellular vesicles in patients with renal cell carcinoma

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Introduction
Early detection and individual prognostic evaluation could improve the outcome in cancer patients. Extracellular vesicles (EVs) secreted by tumor cells represent a new class of biomarkers from liquid biopsies. In this regard, there is a need for isolation of tumor-released EVs from blood or urine using tumor-specific markers.

Material&Methods

- **Ultracentrifugation**: Isolation of EVs from RCC cell lines (786-O, RCC53, Caki1, Caki2) and tumor tissue (by density gradient)
- **Westernblot**: Protein expression of cell-specific (GM130), exosome-specific (CD9, CD63, CD81) and tumor cell-specific markers (EpCAM, CA9, CD70, CD147)
- **Transmission electron microscopy** (TEM): EV imaging
- **Chip-based technique** was assessed to validate previous findings

EV isolation from tumor patient tissues:

AIM: to identify and to characterise tumor specific EV markers in ccRCC.

Results

- Isolation of EV with high quantity and purity from cell lines and directly from tumor tissues
- CD147, CA9 and CD70 are detectable in cells and EVs from tissue, with increased concentration in tumor derived EVs
- EpCAM is weakly expressed in cell lines and cellular as well as exosomal fractions of tissue samples (cleaved fragments in EVs.)
- Expression of CA9 in all cell lines and EVs as well as primary tumor cells and EVs.
- CD70 exhibited different expression patterns in tumor cells and their EVs depending on cell lines. It is enriched in tissue derived EVs, and its expression is increased in tumor tissue samples
- CD147 was present with higher amounts in the EVs compared to the 786-O and Caki2 parental cells and to the cell lysate of tissue samples.

Conclusion

We developed an effective technique to isolate EVs directly from human tissue samples with high purity and high concentration. The expression of tumor specific markers reflects the cellular background of EVs. In contrast to EpCAM, CA9, CD70 and CD147 could represent promising tumor specific biomarkers for EVs in RCC. Further investigations will focus on development of bead-based enrichment of tumor specific EVs from blood samples.

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