TNC positive extracellular vesicles as clinical biomarkers in glioblastoma

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Background & objectives

Extracellular vesicles (EVs) have been established in the liquid biopsies field as they carry biological information from tumors in the bloodstream, enabling non-invasive detection of tumor material and disease monitoring. This clinical utility is of particular interest for tumors that are difficult to access, as glioblastoma (GBM). However, the bulk of non-tumor EVs in plasma still complicates the specific tumor-derived EVs (tEVs) detection with sufficient sensitivity for translational purposes. Here, we phenotyped glioma-related markers in plasma EVs and investigated if they could be useful for enrichment of tEVs.

Methods

EVs were isolated by differential ultracentrifugation (dUC) from plasma of 40 primary GBM IV patients (*IDH1* wild-type; matched pre- and post-OP), 11 matched relapses and 12 healthy donors (HD). EVs were characterized by NTA and electron microscopy (EM). EV concentrations per mL of plasma were analyzed by Imaging Flow Cytometry (IFCM) using EV markers (CD9, CD63 and CD81) and 8 glioma-related antigens (TNC, ITGB1, CD44, CD133, SPARC, GPNMB, PFN1 and HLA-II), which were selected according to a prior proteomic screen on glioma cell-derived EVs. In order to investigate if TNC could serve to enrich tEVs in plasma, magnetic sortings were performed for TNC+ EVs in dUC plasma from GBM. TNC enrichment was confirmed by FACS. The detection of tumor-derived *TERT*C228T* mutation was investigated in DNA obtained from TNC+ and TNC- EV fractions by digital droplet PCR (ddPCR).

Results

We found TNC as a marker with the most significant differences between the groups, especially in the subpopulations: TNC+/CD63+ EVs, which dropped in GBM patients after tumor removal (FC = -12, $p < 0.001$); and TNC+/CD9+ EVs, which had the highest levels in primary (FC = 17.4, AUC = 0.86, $p < 0.001$) and recurrent GBM (FC = 10.9, AUC = 0.94, $p < 0.01$) when compared to HD. Moreover, the frequency of *TERT*C228T* tumor-derived mutation was 20 times higher in TNC+ EVs than in the TNC negative fraction ($p = 0.02$).

Conclusion

These results not only confirm TNC as a potential clinical glioma EV biomarker, but also suggest an utility for tEV enrichment, which could optimize downstream analyses in GBM plasma samples for translational purposes.