Exosomal NGFR witnesses the dynamics of inflammation-induced dedifferentiation in TNF-α-treated melanoma cells

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Background & objectives

In metastatic melanoma, immune checkpoint inhibition (ICI) allows a long-lasting tumor control in nearly half of patients. However, high number of non-responders, acquired resistance and immune-related adverse events (irAEs) limit the benefit of ICI. For early and easily accessible identification of therapy-resistant subclones, non-invasive biomarkers indicating resistance are urgently needed. Therefore, we are approaching to translate insights about phenotype switches of melanoma cells as ICI resistance mechanism into a liquid biopsy "immune resistant" signature for clinical routine.

Recent reports have emphasized the role of reversible dedifferentiation of melanoma cells to resist T-cells, initiated by tumor necrosis factor (TNF)-α (inflammation-induced dedifferentiation). Thereby, tumor cells acquire characteristics of their progenitor cell, the neural crest stem cell and upregulate neural growth factor receptor (NGFR). Interestingly, dedifferentiation requires neither mutational alterations nor cell division, hampering the application of circulating tumor DNA (ctDNA) to monitor this process.

We hypothesized that tumor-secreted small extracellular vesicles (sEVs) are a suitable liquid biopsy target to capture the dynamic phenotype switch by alterations in the receptor tyrosine kinase (RTK) profile.

Methods

We analyzed the RTK and tetraspanin (CD9, CD63, CD81) profile of human melanoma cell lines, their secreted sEVs and plasma-derived sEVs from 5 high-tumor load patients and 5 heathy donors. We stimulated melanoma cells with TNF-α and measured NGFR expression on sEVs by western blot.

Results

We identified the melanocytic antigen Melan-A on sEVs from pigmented melanoma cells and NGFR and AXL RTK on sEVs from dedifferentiated melanoma cells. TNF-α treatment could significantly increase NGFR on sEVs. Additionally, western blot analysis revealed a post-translational modification of CD63 on sEVs from differentiated melanoma cells that was present on melanoma patient-derived sEVs, but not in healthy donors.

Conclusion

Melanoma cell-derived sEVs carry RTKs and represent the dynamics of dedifferentiation after TNF-α stimulation, as measured by an increase in NGFR protein content. In future, we aim to establish T-cell and NK-cell resistant melanoma cell lines to explore their sEV proteomic profile and analyze sEVs as early indicator for resistance to ICI in melanoma patients.