Binding of circulating melanoma cell-derived extracellular vesicles to ultra-large von Willebrand factor induces cancer-associated thrombosis

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Background & objectives

Extracellular vesicles (EVs) released by tumor cells are being investigated as potential circulating biomarkers for cancer detection. EVs (size range 40-5000 nm) are cell-derived membrane-bound vesicles with heterogenous contents, including genetic materials, proteins, lipids, and small metabolites. Tissue factor–positive (TF)-EVs are considered as biomarkers predicting tumor progression. They were also shown to enhance cancer-associated thrombosis (CAT) and to be linked to elevated plasma levels of von Willebrand factor (vWF). VWF is a large multimeric glycoprotein promoting platelet aggregation. This study aimed to investigate whether circulating melanoma cells derived TF-EVs can bind to ultra-large vWF (ULvWF) to promote CAT.

Methods

Binding of melanoma cell-derived EVs or melanoma cells to ultra-large vWF (ULvWF) and subsequent thrombosis formation was investigated by microfluidic experiments. To this end, microfluidic channels coated with human umbilical vein endothelial cells or vWF were perfused with whole human blood supplemented with purified and fluorescently labeled EVs. EVs were characterized by nanoparticle tracking analysis, flow cytometry, and fluorescence microscopy. The expression of TF on different melanoma cells and melanoma cell-derived EVs was studied by fluorescence microscopy.

Results

We found that melanoma cell-derived EVs, but not intact cells, can bind to ULvWF. The diameter of EVs ranged from 40 nm to 5000 nm, with the majority of EVs being around 165 nm. In microfluidic experiments, mimicking a tumor-activated vascular system, we found that EVs smaller than 1000 nm were capable of binding to ULvWF. The binding rate of larger EVs was very low. ADAMTS13, the cleavage enzyme of vWF, reduced the binding of EVs significantly. Experiments with different vWF mutants identified the A1 domain as EV binding site. Accordingly, EV binding was abolished by an inhibitory peptide blocking the A1

domain. Furthermore, binding of TF-EVs to ULvWF activated platelets and induced the formation of microthrombi. Although intact melanoma cells could not bind to ULvWF directly, melanoma cells were trapped in microthrombi.

Conclusion

In conclusion, this study demonstrates that melanoma cell-derived EVs induce CAT by binding to ULvWF. While TF-EVs may serve as a promising biomarker, blocking ULvWF formation or EV binding may prevent metastasis.