Focusing on a quantification and characterization of extracellular vesicles from blood in brain tumors

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Molecular chaperones are required to maintain the proteome in a folded and functional state. Chaperones are structurally and functionally normal but participate in pathways that favor disease, as a tumorigenesis (1). The incidence of brain tumors is increasing rapidly and surgery is the first therapeutic intervention to safeguard the patient's life. The prognosis is poor even after surgical resection, followed by post-operatory chemo- and radio-therapy (2). Tumor-secreted extracellular vesicles (EVs) are critical mediators of intercellular communication between tumor cells and stromal cells in local and distant microenvironments. EVs play an essential role in both primary tumor growth and metastatic evolution. This concept suggests new diagnostic strategies in which the primary targets are chaperones. The purpose of this study is to search for particular chaperones, in terms of presence and / or absence, level of expression and distribution in the tumor. The presence and level of HSP60, HSP27 and HSP10 in exosomes isolated by blood samples obtained from patients with cancer before and after ablative surgery were investigated. For each patient, blood samples were collected after one week, after one month, and after three months of surgery, and processed for plasma isolation. EVs were isolated by size-exclusion chromatography (SEC), with most particles being present in fractions 7–10, while the bulk of the plasma proteins were present in fractions 15–20. Vesicle markers peaked in fractions 7–10. Exosome characterization included morphological analyses, determination of particle concentration, stability and exosome preparations' purity, using different approaches such as Nanoparticle Tracking Analysis (NTA), and Transmission Electron Microscopy (TEM). NTA analysis was used to determine the concentration of particles in each fraction and vesicle markers peaked in fractions 7–9. Electron microscopy focused on various criteria, including size ranging from 30 to 150 nm, confirmation of spherical morphology. Western Blotting analysis was also performed to verify the presence and the levels of those HSPs. Data regarding exosomal fractions assessment by standard methods (TEM) and WB analysis (for Alix) confirmed their identity. Hsp60 levels showed no significant changes in EVs from the same patients after surgery. Our work provided evidences about presence and levels of the main chaperonins involved in regulation of brain tumors, which could be useful in detecting the disease and monitoring its progression. For this reason, we hypothesize that chaperonins could be good candidates as biomarkers for brain tumors.

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References

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