A step toward precision medicine using extracellular vesicles derived from different temozolomide-treated glioblastoma cells

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Recently, extracellular vesicles (EVs) have been suggested as potential biomarkers for glioblastoma (GBM) multiforme and used as "liquid biopsy" for diagnostic purposes, monitoring disease progression, and responding to treatment. In this study, temozolomide (TMZ), a chemotherapeutic agent for GBM treatment, was evaluated for effects on EV release, function, and protein fingerprints. Atomic force microscopy (AFM) was used to determine the topographical distribution and the size of budding vesicles directly on cell surface of four GBM cell lines (U87MG, T98G, U373MG, U251MG), with or without 200 µm TMZ treatment. Furthermore, EVs from the same four GBM cell lines, with or without 200 µm TMZ treatment, were isolated using ultrafiltration combined with size exclusion chromatography (SEC) and analysed by AFM and scanning transmission electron microscopy (STEM) for morpho-quantitative characteristics and size distribution. In parallel, EV-associated markers and protein profile were identified by Western Blotting and shotgun liquid chromatography-tandem mass spectrometry proteomic analysis. The amount and the size of EVs produced under normal conditions varies between the four GBM cell lines and is further affected following treatment with TMZ. A significant difference was found between the four GBM cells in term of distribution and number of budding vesicles and, influenced by TMZ treatment. Proteomic analysis showed different drug sensitivities of GBM cell lines, especially for U373MG and U87MG, which exhibited significant increase and differential expression of EV-associated protein profile after treatment with TMZ. Moreover, proteomic analysis of GBM-derived EVs revealed differences in protein expression after TMZ treatment, mainly those involved in cell proliferation, migration, invasion, and cell death. As a result of this knowledge, new diagnostic and therapeutic approaches tailored for precision medicine can be developed to understand the aggressive behavior and chemoresistance observed in GBM.