Exosomes from Cells Harboring PTPRZ1-MET Fusion Contribute a Malignant Phenotype and Temozolomide Chemoresistance in Glioblastoma

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Abstract: Background Glioblastoma is characterized by highly infiltrative growth and invariably aggressive biological features. Despite combining surgery with radiotherapy and chemotherapy, the malignant nature and poor response to therapy of glioblastoma still presents a poor prognosis. Therefore, studies aimed at blocking glioblastoma infiltrative growth, promoting its drug sensitivity, and reducing its angiogenic potential are needed. A crucial form of cell–cell communication in tumors is the release and uptake of exosomes containing cellular proteins and RNAs. Cells take up exosomes delivering tumor-derived oncogenic factors and can a malignant phenotype in this manner. It was validated that exosomes are carriers of pro-tumorigenic factors that participate in glioblastoma progression. Fusion genes combine parts of ≥2 original genes, and can be generated from chromosomal rearrangement or abnormal transcription. Gene fusions have an important impact on the initial tumorigenesis and cancer progression steps. Many gene fusions are strong driver mutations in neoplasia and are involved in tumorigenesis. Our RNA sequencing (RNA-seq) study of 272 gliomas revealed a novel, recurrent ZM fusion transcript in secondary glioblastoma. Secondary glioblastomas or U87 cells with ZM fusion harbored more MET compared with those without ZM fusion, respectively. Endogenously expressed MET in U87 cells is not phosphorylated while exogenously expressed MET resulting from ZM fusion is phosphorylated. Clinically, survival of glioblastoma with ZM fusion is poorer than that without ZM fusion. However, whether gene fusions are transduced by exosomes is unknown. These studies reflect the need to evaluate the functional
contribution of ZM fusion to the glioblastoma phenotype and its role in exosome-associated cell interaction with the tumor microenvironment.

**Methods** We cloned a His-tagged version of CGGA_1475 ZM fusion into an adenovirus vector and stably expressed the fusion protein in a U87/ZM cell line. Anti-His tag or anti-MET antibody probe of the protein revealed stable expression. We characterized exosomes from the medium of glioblastoma cells harboring and not harboring PTPRZ1-MET fusion (ZM fusion). We also determined the effect of the ZM exosomes on pro-oncogenic secretions from the cells with ZM fusion and showed how exosomes are internalized into the recipient cells, and studied the effect of exosome-mediated intercellular communication in the glioblastoma microenvironment.

**Results** MET proto-oncogene expression was higher in exosomes from the cells with ZM fusion (ZM exosomes). Phosphorylated MET (p-MET) was detected only in ZM exosomes, and not in non-ZM exosomes. To test whether MET and p-MET can be transferred from U87/ZM to U87/SC, we cocultured U87/ZM and U87/SC. ZM exosomes transferred to non-ZM fusion glioblastoma cells and normal human astrocytes altered gene expression and induced mesenchymal–epithelial transition. The uptake of ZM exosomes induced an exosome-dependent phenotype defined by glioblastoma cell migration and invasion, neurosphere growth and angiogenesis. Additionally, ZM exosomes conferred temozolomide resistance to the glioblastoma cells. Exosomes-derived ZM fusion network proteins targeted multiple pro-oncogenic effectors in recipient cells within the glioblastoma microenvironment.

**Conclusions** The major implication of the present data is that ZM fusion in glioblastoma cells enhances the tumor cells themselves, EMT, migration, invasion, neurosphere formation, and angiogenesis in vitro and in vivo, and confers temozolomide resistance. U87/ZM exosomes caused the expected changes to the glioblastoma cell phenotype in the same sets of recipient cells. Hence, U87/ZM exosomes containing MET and p-MET not only suggest a novel approach to biomarker detection but may also provide therapeutic intervention targets in aggressive glioblastoma.

**Keywords:** Glioblastoma; Exosomes; PTPRZ1-MET Fusion; Malignant Phenotype; Chemoresistance.