Cripto-Positive Extracellular Vesicles as a Novel Anti-Migratory Strategy for Cancer Therapy

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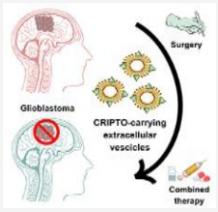
INTRODUCTION

Extracellular vesicles (EVs) are lipid bilayer structures that transport bioactive molecules and can modulate recipient cell behavior (Kalluri and LeBleu, 2020). Therefore, EVs are key modulators of cell communication in both physiological an pathological processes (Mantile et al., 2020). Tumor-derived EVs modulate cancer cell proliferation, migration, and metastasis,and are able to selectively recognize cancer cells, offering potential for targeted therapies (Liguori and Kralj-Iglič, 2023). Glioblastoma (GB) is a rare but extremely aggressive brain tumor that significantly impacts patient outcomes, affecting both duration and quality of life (Liguori, 2024). We have first isolated large EVs (lEVs) from NTERA2 teratocarcinoma cells, demostrating their ability in reducing migration of GB cells without inducing proliferation and chemoresistance. This anti-migratory effect is linked to membrane-associated CRIPTO, a key regulator in development and tumorigenesis (Mantile et al., 2022).

WORKING HYPOTHESIS



CRIPTO-carrying EVs might be used in GB therapy to contrast tumor invasiveness and improve patient prognosis (patent PCT/IB2023/053735)

EXPERIMENTAL DESIGN

1. Isolation of EVs from NTERA2 cells → **2. EV characterization** → **3. Functional Assays**

- Differential Ultracentrifugation (dUC)
- Western blotting
- Electron microscopy
- Myriade
- Cell cultures
- Ex vivo (organoids)
- In vivo (mouse models)

Figure 1. Isolation of EVs from NTERA2 cell line

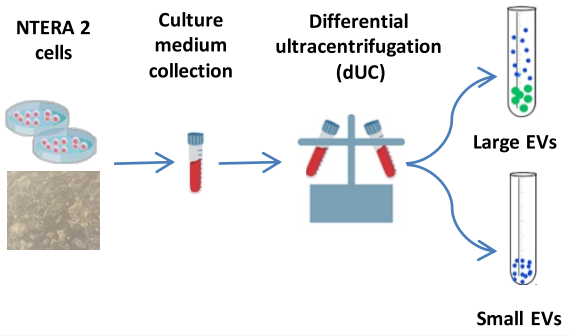
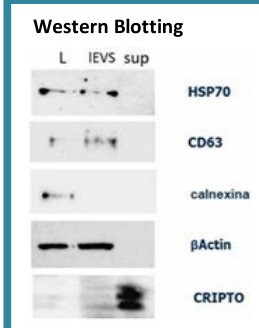
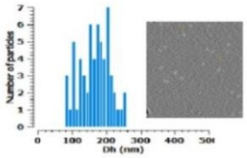


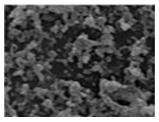
Figure 2. Large EV characterization



L: cell lysate; Sup: supernatant



Concentration	Median diameter
1,01e+11 part/ml	211,50 nm

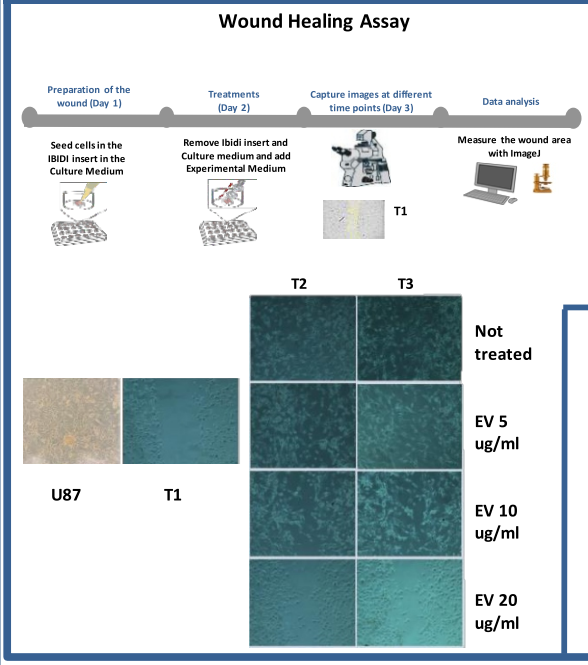


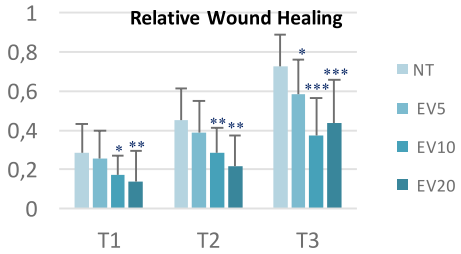
RESULTS: First, we separated lEV and sEV preparations by dUC (Fig. 1). Next, we focused on lEVs performing Western blotting with both positive and negative EV markers, Myriade analysis to assess particle concentration and size and electron microscopy (Fig. 2). Then, we performed preclinical cell culture studies to determine the effect of different EV doses on GB (U87) cell migration by wound healing assays. Finally, we investigated the effect of EVs on the viability of normal cells using XTT assays (Fig. 3).

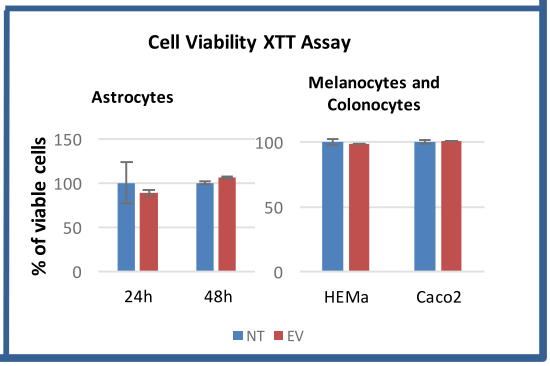
CONCLUSIONS: We identified 10ug/ml as the optimal EV dose able to cause a 50% inhibition of GB cell migration without affecting the viability of normal cells.

FUTURE PLANS: Performing preclinical assays both ex vivo on GB organoids and in vivo using GB orhotopic graft mouse models.

Figure 3. Functional assays







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Kalluri and LeBleu (2020) doi: 10.1126/science.aau6977; Liguori G.L. (2024) doi: 10.3390/cells13040336; Liguori G.L., and Kralj-Iglič V. (2023) doi: 10.3390/cancers15184425; Mantile,et al. (2020) doi: 10.1016/bs.abl.2020.05.006; Mantile et al. (2022) doi: 10.3390/cancers14153700

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