Determination of Primary Retinoblastoma Extracellular Vesicle Uptake Pathway via Surface Protein Inhibitors

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Retinoblastoma (Rb) is a form of rapidly developing retinal cancer, and like many cancers, metastasizes via the release of lipid-encased extracellular vesicles (EVs) which create desirable conditions for secondary tumor growth via local environment modification. Currently, there is limited understanding of specific Rb EV uptake proteins and mechanisms in target cells. My previous research has shown that using inhibitors to block surface protein activity on retinal cells allows for decreased EV uptake. However, the specific molecular mechanism that retinal cells use to internalize retinoblastoma EVs remains unknown. By finding the EV uptake pathway in Retinoblastoma, we would be able to identify drugs that can block metastasis, something which current treatments fail to address. We proposed two aims: 1) Test the novel drug MSC109438 in vitro by measuring extracellular vesicle uptake in cells; and 2) Identify specific molecular machinery of retinoblastoma EV uptake into retinoblastoma cells. MSC109438 caused a significant reduction of retinoblastoma EV uptake into retinal progenitor cells ($p=.007$). To characterize the specific molecular mechanism of EV uptake, we identified 41 important receptor proteins on the Rb EVs based on their specific endocytic pathways. We identified two inhibitors for our in vitro assay, dynasore and Methyl-β-Cyclodextrin that would inhibit the protein receptors specific for clathrin dependent (CDE) or clathrin independent endocytosis (CIE) respectively. Our results showed that CDE was the predominant uptake pathway in retinoblastoma cells, unlike most cancers. This methodology can be applied to find metastatic-inhibiting treatments in other cancers where the EV uptake pathway method is unknown.