Title of this Session Experimental / Molecular Therapeutics, Pharmacogenomics
Type of Session Poster Session
Abstract Number:
Presentation / Posterboard number: EP-436

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Engineered exosome-mediated delivery of RNAi therapeutics towards triple negative breast cancer therapy

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Introduction

Breast cancer was the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new diagnosed cases among women. Triple negative breast cancer (TNBC) characterized by the absence of hormone receptors, lack of expression of epidermal growth factor receptor-2 and poor prognosis, represents about 10-20% of all breast cancers. Hence, an efficient targeted delivery system is urgently needed for TNBC therapy. Exosomes are natural and nanosized intraluminal vesicles, with a size range of ~30 to 150 nm, released by most cell types. They are present in almost all biological fluids and function as natural transporters of molecules between neighboring and distant cells. Thus, exosomes may serve as endogenous vehicles for drug delivery such as chemotherapeutic agents, short-interfering RNAs (siRNAs) or even microRNAs (miRNAs). Herein, we describe the development of a novel targeted cancer therapeutic platform for TNBC therapy by exosome-mediated delivery of RNAi-based therapeutics.

Material and Methods
Exosomes derived from BJ cells were isolated by differential centrifugation and further characterized by nanoparticle tracking analysis, transmission electron microscopy, flow cytometry and western blot. Moreover, *in vitro* uptake experiments and *in vivo* circulation times and biodistribuition experiments were accomplished to assess performance. These exosomes were further electroporated with MAP2K1 siRNA (exo+siMAP2K1), being the impact of down-regulation in the metastatic phenotype of highly invasive breast cancer cells evaluated by measuring migration, invasion, proliferation and metabolism. Changes in the regulation of direct and indirect genes were also assessed. Moreover, implantation of TNBC cells treated with exo+siMAP2K1 in chicken embryo chorioallantoic membrane (CAM) was also performed.

Results and Discussions
Cell-derived exosomes were efficiently uptake by different TNBC cell lines (MDA-MB-231, MDA-MB-157 and Hs 578T). In addition, such delivery system loaded with MAP2K1-targeting siRNA by electroporation, demonstrated a superior silencing effect when compared with standard transfection reagents. Moreover, implantation of TNBC cells treated with exo+siMAP2K1 in CAM led to a reduction of the tumor size and the number of blood vessels when compared with cells without any treatment.

Conclusion
Targeted exosomes led to a completely new paradigm for the therapeutic delivery of siRNAs to specific

targets, opening the door for new treatments of diseases caused by aberrant gene expression as cancer.