ENGINEERING OF EXOSOMES FOR TARGETED DELIVERY OF THERAPEUTIC MICRORNAS

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Exosomes are small membrane vesicles secreted from most cell types. They contain a range of proteins, lipids, mRNAs and microRNAs (miRNA) and are naturally taken up by cells in order to deliver these contents to recipient cells. Increased understanding of this process as well as advances in bioengineering has led to investigations into the use of exosomes as targeted vehicles to transport therapeutic non-coding RNAs, proteins or drug molecules directly across cellular barriers into recipient cells. miRNAs are small non-coding RNA molecules, which play a key role in mediating biological function due to their prominent role in gene regulation. Deregulation of miRNAs is a common feature in cancer, suggesting that these molecules could serve as targets for therapeutic intervention by restoring or inhibiting their cellular function. However, various biological barriers including in vivo nuclease degradation, fibrous nature of tumors, and miRNA-induced immune response drastically hinder their bioavailability. In the context of these complex settings, exosomal delivery may display a novel strategy for targeted delivery of RNA therapeutics.

We have recently identified and characterized novel pro-apoptotic miRNAs (Kleemann et al 2016, Flum et al 2017), which are down-regulated in cancer cells suggesting promising potential for therapeutic approaches. In the current study we conducted an initial evaluation of the novel concept to enable targeted delivery of small therapeutic RNAs using exosomes as biological transport systems. CEVECs amniocyte production cells (CAP) are utile human expression hosts highly suitable for the production of glycosylated biotherapeutic proteins. However, there are currently no reports as to whether this human cell line may as well produce extracellular vesicles suitable for targeted delivery of therapeutics. In order to evaluate these cells as production hosts for exosomes, we initially cultivated parental CAP cells and were able to isolate exosomes by centrifugation. Exosomal preparations were examined for vesicle identity, size, morphology and concentration using dynamic light scattering, flow cytometry, western blotting and electron microscopy. In order to be able to visualize exosomes and track targeted delivery in subsequent experiments, we engineered CAP cells to stably overexpress a CD63-GFP-fusion protein. This overexpression of a fluorescent transmembrane protein present on exosomes enabled the tracing of produced exosomes using flow cytometry. To functionally analyze isolated exosomes regarding their potential to delivery small therapeutic agents, these fluorescently labeled exosomes were further engineered to overexpress therapeutic, pro-apoptotic and control miRNAs by stable genome integration into CAP parental cells. Resulting preparations of engineered exosomes were analyzed for modified miRNA content in comparison to parental cells using qPCR. Currently the cells are further engineered to overexpress modified surface receptors to facilitate targeted uptake by tumor cells. Fluorescently labelled exosomes carrying therapeutic miRNAs and surface receptors will finally be co-cultured with target cells to analyze the potential of engineered exosomes to crossing membrane barriers and reliably deliver therapeutic miRNAs to recipient cells.
The current study pursues the novel therapeutic concept of using exosomes as delivery vehicle for small non-coding RNAs molecules. We present data which assess for the first time the potential application of exosomes produced by a human production host for biotherapeutics. In addition to these current engineering and delivery approaches, future application of this cell therapy will heavily depend upon mass production of these delivery vectors and production issues will therefore gain increasing importance.

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