OF18.05 | Colostrum exosome-based delivery of siRNAs and inhibition of lung cancer

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Introduction: We have shown that bovine milk and colostrum provide abundant small extracellular vesicles, also referred to as exosomes, for drug delivery. We now report a novel exosome-polyethyleneimine (PEI) matrix (EPM) for delivery of siRNA targeting KRAS (siKRAS) and nuclear factor erythroid 2-related factor, NRF2 (siNRF2) to inhibit lung cancer by downregulating target protein expression. These two oncogenic targets are known to be highly expressed in lung, as well as other types of cancer.
Methods: Exosomes were isolated from bovine colostrum powder by rehydration and differential centrifugation and characterized for size, pdi and charge (Zetasizer) and hallmark proteins (Western blot). Test siRNAs were entrapped in the EPM by brief incubation of siRNA with the vector, followed by PEG precipitation. The siRNA entrapment efficiency was determined using tracer 5'-32P-labeled siRNA. The transfection efficiency was determined in vitro by measuring gene knockdown via Western blot analysis, while anti-cancer effects were assessed in vivo using lung tumor xenografts in immunocompromised mice.
Results: Colostrum exosomes exhibited similar size and charge as mature milk exosomes and carried similar surface proteins markers. Based on the presence of radioactive siRNA, we found that the EPM entrapped >90% of added siRNA (up to 20 μg). siRNA entrapped in EPM was protected from enzymatic degradation upon exposure to RNases, as detected by gel electrophoresis using radioactive tracer siRNA. EPM-siKRAS decreased mutant KRAS expression in A549 lung cancer cells dose dependently. When composed of folic acid-functionalized exosomes, EPM-siKRAS resulted in significant inhibition of orthotopic lung xenografts (>70%; $p < 0.01$) and downregulation of KRAS expression in the tumor (>50%; $p < 0.05$). Likewise, EPM-siNRF2 delivery resulted in dose-dependent decrease of NRF2 expression in the same cell line and correlated with the inhibition of cell survival. EPM-siNRF2 formulation also inhibited the growth of subcutaneous lung tumors (>57%; $p < 0.01$) in immunocompromised mice.
Summary/Conclusion: The colostrum exosome-based EPM technology provided significant gene knockdown and lung tumor inhibition by targeted delivery of siRNA and represent a simple and effective exosome-based gene therapy approach.
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OF18.06 | Matrix vesicles membrane organization and its interaction with PHOSPHO1 investigated by means of biomimetic membrane models

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Introduction: Bone biomineralization is a process mediated by osteoblasts through the release of matrix vesicles (MVs). The most accepted theory describes the MVs´ biogenesis by budding from cell membranes, secreted at specific sites in the bone extracellular matrix. The internal MVs reservoir is composed by enzymes such as PHOSPHO1, a phosphatase that hydrolyzes phosphocholine and phosphoethanolamine, and generates Pi through phospholipids degradation, that may be also involved in MVs biogenesis. In this study, we aimed to investigate the interactions of PHOSPHO1 with lipids enriched in the membrane of MVs and to compare the results with monolayers composed by native lipids extracted from MVs.
Methods: For this, we isolated MVs from the femurs of chicken embryos (16-17 days) and purified the lipids using a sequence of dispersion in organic solvents/centrifugation steps. We used DPPC, DPPS and cholesterol (Chol), and Langmuir monolayers as a mimetic membrane model. 1M of lipid chloroformic solutions were dripped at the air-liquid interface on a Tris/HCl (containing 100 mM NaCl and 2 mM MgCl2) buffer, pH 7.4. PHOSPHO1 (0.76 μg/mL) was added to the subphase, then the monolayer was compressed with the aid of a Langmuir trough.
Results: The compressional moduli of the DPPS monolayers was increased in the presence of the enzyme when compared to pure DPPS. Moreover, the area occupied per lipid molecule was increased from 56.9 Å2 to 63 Å2 in the presence of PHOSPHO1, which indicated interaction between DPPS and the enzyme. Although PHOSPHO1 also increased the compressional moduli of DPPC monolayers, its presence reduced the area occupied per lipid, revealing different interaction guided by the composition of the lipids polar head In situ fluorescence microscopy images of the monolayers revealed the formation of larger domains rich

in phospholipids induced by the presence of the enzyme. Monolayers composed by lipids extracted from native MVs were also formed in the presence of PHOSPHO1. The enzyme increased the area occupied per lipid in pH 7.4, but not in more acidic pH. The results were similar to those found in artificial monolayers composed by DPPC:DPPS:Chol, which may be an indication of the enzyme adsorption at lipid-rafts moieties.
Summary/Conclusion: The results reveal that the interaction of PHOSPHO1 with lipids in MVs can be mimicked by adjusting the composition of artificial Langmuir monolayers. The lipid organization is crucial for the interaction of PHOSPHO1 with MVs´membrane model. These results shed light in the mechanisms of biogenesis driven by this enzyme.
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OF19: EV Analysis Flow Micros

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OF19.01 | Multiplexed protein detection on single extracellular vesicles by DNA Exchange Imaging

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Introduction: There is a need for technologies that profile extracellular vesicles (EVs) on the single vesicle level to uncover functionally different subpopulations which are masked in bulk analyses. Here we report a single EV multiplexed proteomics platform named Digital Omics of Single EVs (DOSE) based on DNA exchange imaging (DEI).
Methods: We use EVs derived from HT29 and A431 cells that are purified using 220 nm filters followed by a size exclusion column step (IZON). The EVs are characterized according to MISEV2018 with TRPS, TEM and their protein content is validated by Western blot.
Results: DOSE uses a flow cell on top of a glass coverslip with antibody functionalized surface which is mounted in an inverted fluorescence microscope (Nikon Ti2). The EV capture on the surface is monitored by label-free live imaging using interferometric scattering imaging (iSCAT). We proceed to block the surface and incubate a panel of antibodies, each of which is conjugated to a target specific DNA oligo. Detection is done using complementary oligos with fluorescent labels which can be hybridized in less than five minutes and be removed after imaging by toehold mediated strand displacement. Liquid handling and imaging are fully automated so that thousands of individual vesicles can be characterized with an initial panel of twenty protein markers. The image analysis is done with a custom Matlab pipeline using the label-free images to detect vesicles, suppressing noise and non-specifically bound labels in the fluorescence channels.
We validate the label free EV detection using silica beads as size standards as well as a CD63-GFP expressing A431 cell line. Furthermore, we show proof-of-principle DEI experiments on HT29 EVs where specific capture of vesicles positive for low abundance targets results in enrichment of subpopulations compared to general capture of EVs.
Summary/Conclusion: The method overcomes several challenges in single EV techniques including spectral limitations in multiplexed fluorescence imaging and a background from non-specific interactions or imperfect EV isolation techniques. It allows for fast cycling times and high throughput at relatively low added cost making it a promising technique to be incorporated into the single EV toolbox.
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OF19.02 | Automation of clinical extracellular vesicle measurement using standardized and calibrated nanoscale flow cytometry

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