Exosomes/Microvesicles: Novel Mechanisms of Cell-Cell Communication

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1001 Plasma extracellular vesicles in chronic stress and functional link to epithelial cell migration and wound repair

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Background: Repair after injury or wound and cellular stress response are related processes with poorly understood mechanisms and limited therapies. We hypothesized that circulating extracellular vesicles (EVs) from patients with chronic stress-associated gastrointestinal dysfunction contain stress response signals with functional roles in cell repair.
Methods: Plasma EVs from Irritable Bowel Syndrome patients and controls were isolated (size exclusion chromatography). EV-associated CD9, lysozyme, and mucins were characterized (immunogold electron microscopy). Patients versus controls differential blood transcriptomes were used to generate interaction networks. Migration capacity of colon epithelial cells CRL-1790 in response to wound and serum starvation, with or without lysozyme and CXCR4 ligand (CXCL12), was examined (quantitative fluorometry; scratch wound). Immune response-related mRNAs and proteins in cells which survived post injury were quantitated (NanoString).
Results: Plasma EVs from patients and controls contained CD9, Mucin1, Mucin4, and lysozyme. Differential blood transcriptomes analyses revealed pyridoxal 5-phosphate salvage, cellular movement, assembly and organization as top networks driven by membrane associated-CXCR4 and CD9. Lysozyme connected to the network via Ubiquitin and Akt. Lysozyme and CXCL12 induced CRL-1790 cell migration following injury and starvation. NanoString analysis of immune mRNAs (770) and proteins (30) from cells which survived post-injury with or without lysozyme revealed links to cell repair and regeneration through mRNAs (IL8, IL6, L1, ANXA1) and proteins (CD9, NT5E, PD-L1).
Conclusion: Signals for repair and migration in response to injury are associated with plasma EVs from patients with chronic stress.

1002 Mitochondrial Regulation of exosomal micro RNA cargo in Vascular Smooth Muscle Cell Proliferation

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[illegible]
VSM cell exosomes.
[illegible]
VSM cell proliferation.

References
[illegible] (2008). [illegible] Proteomics [illegible]
[illegible] (2015). [illegible] Circulation Research 117:[illegible]
[illegible] Vascular Smooth Muscle Cell Proliferation [illegible]

1003 Adenocarcinomas and their derived lymphovascular emboli contain dual compartmental populations of tumor microvesicles which differ in size and possibly content and function.

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Adenocarcinomas and their associated lymphovascular emboli metastasize as tight aggregates of tumor cells. Using a xenograft model of human inflammatory breast cancer (Mary-X), which exhibited florid lymphovascular emboli in mice and high density tumor aggregates (spheroids) in vitro, we previously demonstrated that both emboli and spheroids are mediated by an intact overexpressed E-cadherin axis which regulates homotypic tumor cell adhesion. We now report that Mary-X spheroids secrete 5–10 fold higher levels of microvesicles (MVs) than most other carcinoma cell lines. Despite the high density of tumor cells and the strong juxtaposition of tumor cells to each other in the spheroids, intercellular spaces exist which contain numerous MVs. These intraspheroidal (IS) MVs entrapped between the E-cadherin adherens junctions can be isolated by dissociating the spheroids in Ca++-free media with EDTA. These IS MVs compared to the extraspheroidal (ES) MVs found in the conditioned media (CM) comprise a significant (approximately 25%) subpopulation of MVs which persisted for several days in culture (day 3: 0.48 v 2.0; day 7: 1.1 v 3.9; day 14: 0.64 v 3.7 (# x 10E10)). The IS MVs differed in size compared to the ES MVs. By nanoparticle tracking analysis (Nanosight), IS MV mean volume was 79 nm compared with the ES MV mean volume of 152 nm. These size differences were also confirmed by transmission electron microscopy (TEM), though both were smaller (50 nm v 110 nm). The proteome profile of the IS MVs can be readily compared with that of the ES MVs found in CM. Cellular target differences between the IS MVs and the ES MVs can also be studied. It would be attractive to hypothesize that IS MVs preferentially function in autocrine signaling whereas ES MVs signal in a paracrine manner.

1004 Bioactivity of Exosomal microRNAs in High Immune Responder Cows' Colostrum and Milk

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The expression of bioactive host defense proteins in bovine colostrum and milk is regulated by miRNAs. The packaging of miRNAs within milk-derived exosomes ensures their stability and resistance to degradative conditions, and allows for shuttling from cell to cell. Notably, immune related miRNA are highly expressed in milk, particularly in colostrum, with the potential to regulate gut immunity of newborn calves and humans. While high immune responder (HIR) cows have superior colostrum quality and concentrations of bioactive proteins compared to average (A) and low (L) responders, the expression profiles of colostrum and milk miRNAs and their biological significance remain to be elucidated. The objectives of the study are to test the hypotheses that milk exosomes containing miRNAs are: (1) differentially expressed in HIR cows compared A and L responders, (2) functionally active at the intestinal epithelial interface, and (3) regulators of other cell functions. Colostrum and milk exosomes from A, L and HIR were isolated by differential ultracentrifugation and their presence was confirmed by immunogold labeling electron microscopy, ELISA ExoELISA kit, and Western blot. The bioactivity of milk exosomes on healthy and cancerous intestinal epithelial cells is being assessed. Similarly, the role of exosomes containing miRNA released by conditioned intestinal epithelial cells on hepatocytes is being evaluated. Six different methods were compared for optimal isolation of total exosomal RNA containing miRNA. Out of 10 samples, four passed the library generation quality control, sequenced using the Illumina miRNA-HiSeq2500 platform and subjected to bioinformatics analysis. This study is expected to provide new insight into the immunoregulatory role of bovine colostrum and milk miRNAs in humans.