Identification and Characterization of Exosomes from Cardiac Fibroblasts in Ossabaw Pigs with Metabolic Syndrome and Diabetes

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Introduction:

Cardiovascular disease (CVD) is the leading cause of death worldwide, and many patients with diabetes will develop CVD. However, there is no current method to detect CVD, and many patients are unaware of their illnesses until catastrophic events like heart attacks and strokes occur. A biomarker – a measurable substance that indicates the body's condition – for CVD in diabetic patients could aid in diagnostics and treatment of CVD before a potentially fatal event arises. This study searches for such a biomarker on vesicles (exosomes) secreted by cardiac fibroblasts. Recent research shows that healthy cells produce different exosomal RNAs and proteins than diseased cells.

Methods:

Cardiac fibroblasts of Ossabaw swine were used to produce the exosomes. Not only do Ossabaw pigs have a natural predisposition for obesity and metabolic syndrome (MetS), which leads to diabetes, but they also posses a cardiovascular physiology and pathology similar to humans. The pigs were either fed a normal diet (control) or a high calorie, high trans-fat diet (MetS) for 8-9 months. The heart tissue was minced, rinsed with saline, and underwent collagenase digestion before selective attachment to tissue culture plastic, yielding pure fibroblast culture. The cultured cells were serum-starved before exosome isolation by centrifugation, filtration, and ultracentrifugation.

Results:

Exosomes were purified from the conditioned media of primary cardiac fibroblasts of male MetS/diabetic pigs and healthy littermates. Transmission electron microscopy demonstrated that the exosomes exhibited characteristic cup-shaped morphology. Western immunoblotting was performed to confirm the presence of exosomes.

Conclusion:

Based on morphology, size, and protein composition, we defined the release of exosomes from primary cardiac fibroblasts and provide their first extensive characterization. Future studies will compare the differences of exosomal protein profiles from cardiac fibroblasts of diabetic and healthy pigs by two-dimensional gel electrophoresis (2-DGE) and mass spectrometric analysis.