Title	Isolation and Characterization of Liver-derived Exosomes by Liver Marker Protein Asialoglycoprotein-1 (ASGR1) and Proteomic Analysis
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Abstract	Exosomes are small lipid-bound vesicles secreted by all cell types into the extracellular space[1]. While they were originally thought to be a route of cellular "dumping" it has since been understood that these vesicles play important roles including cellular communication, cancer metastasis, and antigen presentation[1]. Exosomes contain proteins, lipids, and mRNAs that are reflective of the cells of origin and can therefore act as carriers of biomarkers in the extracellular space[1]. However, due to the complexity of the blood plasma, methods need to be developed to isolate exosomes from a specific organ of interest. Here, the ability of isolate liver-derived exosomes by immunoprecipitation with a liver-specific marker protein, ASGR-1[2], is demonstrated. Ultracentrifugation was used to isolate exosomes from cell culture media. Nanoparticle tracking analysis (NTA) was used to assess the particle size, which were found to be within the expected size range of 30 – 150 nm in diameter. The size, shape, and morphology of the isolated vesicles were further confirmed by tunneling electron microscopy (TEM). Targeted proteomic analysis and Western blot analysis of the isolated vesicles confirmed the purity of the exosomes preparation based on the enrichment of several exosomal marker proteins, HSC70, HSP90, ALIX, and CD9 and the absence of non-exosomal marker proteins GRP78, HSP60, and PHB1. Furthermore, both Western blot and targeted proteomic analyses determined the presence of ASGR-1 in the isolated exosomes. Biotinylated ASGR-1 antibody was then conjugated to streptavidin coated magnetic beads and used to "pull-down" the exosomes expressing ASGR-1. Mass spectrometry analysis was used to assess the pull-down and determined roughly a 65% recovery of the exosomal proteins while only 15% recovery using beads without antibody conjugation. Overall, this study demonstrated the feasibility of isolating liver-derived exosomes and future work will focus on isolating liver-derived exosomes from more complex matrices, such as plasma.
References	1. Raposo and Stoorvogel, *J Cell Biol* 2013 2. Conde-Vancells *et al. J Proteome Res.* 2008