First proteomic profiling of exosomes in rodent intestinal lymph

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Introduction

Exosomes are released by many cell types and can be taken up by other cells. They may play an important role in cell-to-cell communication and disease pathogenesis. Exosomes derived from plasma or urine have been extensively studied but that of intestinal lymph has not been reported due to the difficulty in obtaining these samples.

Intestinal lymph is continuously draining from the intestine and enters the veins just before the heart and lungs. Exosomes carried in intestinal lymph may therefore directly influence these organs and may be implicated in pathogenesis of multiple organ dysfunction syndrome in critical illness.

Aim

Profile the proteome of exosomes isolated from rodent intestinal lymph and determine the changes of protein profile in critical illness.

Methods

Rodent experimental models of critical illness
(sham, sepsis, gut ischemia/reperfusion)

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Collection of intestinal lymph over 5 hours

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Isolation of "exosomes"
using exosome precipitation solution (Macherey Nagel) → Particle analysis by NanoSight NS300

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Exosomal protein lysis buffer (101Bio)

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Methanol-chloroform precipitation and delipidation

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Peptide preparation

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iTRAQ labelling (115, 116, 117, 118, 119, 121)

↓

LC-MS/MS proteomics analysis
(2 hr gradient on 40 cm PicoFrit column and TripleTOF 6600)

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Protein identification using ProteinPilot v5.0

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Data analysis

Results

1. Particle analysis on lymph exosomes

No significant differences in diameter (82.5 ± 2.31 nm; median ± SE) and concentration (3.15×10^{12} ± 0.39 particles/ml) of "exosomes" between rodent experimental groups ($P > 0.05$ for all pairwise comparisons)

2. Number of proteins identified from proteomics analyses

Comparison	Precipitation /delipidation step	iTRAQ labelling	Run #	# Proteins identified	# Proteins identified in both runs #1 and #2
Effect of precipitation /delipidation step	-	-	1	249	204
	+	-	2	378	
Comparison of 3 rodent groups, N=4 each	+	6-plex	1	205	166 (81 quantifiable)
	+	6-plex	2	197	

NOTE: ProteinPilot unused score >1.3 (confidence 95%)

3. 166 proteins identified from two 6-plex iTRAQ runs of lymph exosomes

(A) Gene Ontology cellular component enrichment analysis

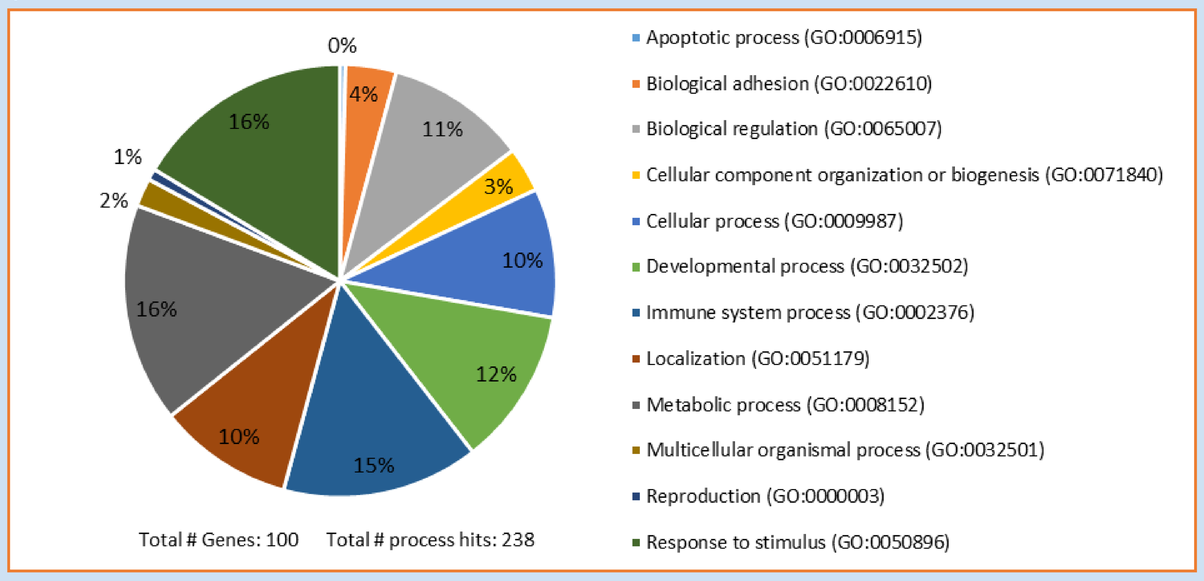
GO cellular component complete	Fold enrichment	+/-	Bonferroni adjusted *P*-value
Spherical high-density lipoprotein particle (GO:0034366)	> 100	+	1.04E-09
Fibrinogen complex (GO:0005577)	> 100	+	2.19E-07
Chylomicron (GO:0042627)	> 100	+	2.07E-06
Very-low-density lipoprotein particle (GO:0034361)	96.17	+	2.00E-09
Blood microparticle (GO:0072562)	61.11	+	3.45E-65
Immunoglobulin complex, circulating (GO:0042571)	46.71	+	1.96E-16
External side of plasma membrane (GO:0009897)	11.75	+	1.35E-10
Extracellular exosome (GO:0070062)	4.63	+	1.09E-20

NOTE: Results from PANTHER (Protein ANalysis THrough Evolutionary Relationships) overrepresentation test; +/- indicates over- and under-representations; displayed only results with $P < 0.005$.

(B) Top 10 most abundant proteins in exosomes of intestinal lymph

N	Name	Gene symbol	Accession
1	Complement C3	C3	tr|M0RBF1|M0RBF1_RAT
2	Apolipoprotein B-100	Apob	tr|F1M6Z1|F1M6Z1_RAT
3	Alpha-1-macroglobulin	A1m	sp|Q63041|A1M_RAT
4	Fibronectin	Fn1	sp|P04937|FINC_RAT
5	Complement component 4, gene 1	C4a	tr|Q6MG79|Q6MG79_RAT
6	Protein Cfh	Cfh	tr|G3V9R2|G3V9R2_RAT
7	Alpha-1-inhibitor 3	A1i3	sp|P14046|A1I3_RAT
8	Serum albumin	Alb	sp|P02770|ALBU_RAT
9	Complement C5	C5	tr|A0A096P6L9|A0A096P6L9_RAT
10	Plasminogen	Plg	sp|Q01177|PLMN_RAT

(C) Protein classification of lymph exosomes by PANTHER GO-Slim biological process



4. Protein profile changes in critical illness

- Using linear mixed models for fitting and two-sample t-tests for pairwise comparisons
- Our initial analysis identified 10 proteins with FDR-adjusted *P*-value < 0.05:
  - Three Apolipoproteins
  - Two proteins involved in blood coagulation
  - Two highly abundant plasma proteins (complement C3, serum albumin)
  - Three proteins that are not well characterized

Conclusion

- This present study provides the first attempt at a proteomic profile of exosome preparation from intestinal lymph.
- Our exosome preparations were enriched with proteins previously identified in microparticles or exosomes (e.g. inter-alpha trypsin inhibitor), but also contained the highly abundant plasma proteins. Exosomal markers such as TSG101 and CD63 were not detected. Instead, substantial amounts of Apo B and A-I were found, indicating presumed co-isolation of chylomirons.
- Ten proteins in lymph exosomes were found altered in critical illness. Proteins that are enriched in exosomes of intestinal lymph during critical illness may have a key role in the development of multiple organ dysfunction syndrome, thus warrant further investigation.
- Lymph chylomicrons are similar to size of "exosomes", and produced in high concentration from the intestine. Our study indicates the unexpected difficulty in isolating pure exosomes using a commercial kit in the intestinal lymph. New purification methods will be needed to study pure isolates of each particle type in this unique fluid.

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