Composition of exosomes derived from Atlantic salmon (*Salmo salar*) head kidney leukocytes

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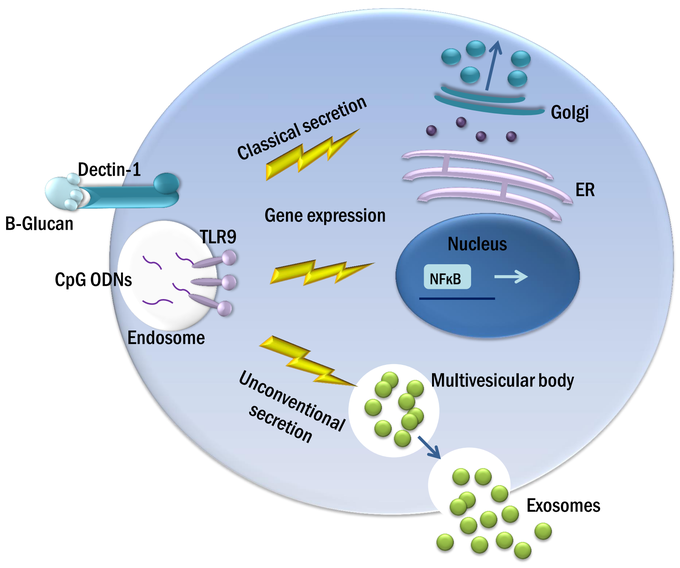
/ ABSTRACT

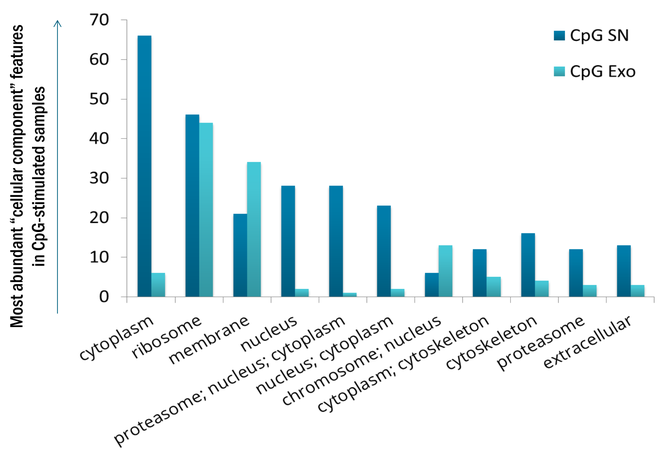
Exosomes are secreted nanosize vesicles (30−100 nm) derived from multivesicular endosomes. Exosomes are released by different immune cell types, including T- and B-lymphocytes, mast cells and antigen-presenting cells (APCs). The composition of exosomes - including protein and RNA content reflects their endosomal origin and the type of cells that produce them. Mammalian APCs produce large amounts of exosomes loaded with MHC class I and II molecules with important immunomodulatory properties. In the current study, exosomes were isolated from salmon head kidney leukocytes stimulated with CpG oligonucleotides and yeast beta-glucan (BG) and their protein composition was studied using Western blotting and LC-MS/MS analysis. Major exosome markers were detected including flotillin, CD63, CD81, CD9 and MHC-I and II molecules. Ontology analysis indicated that the exosome samples were enriched in ribosomal, membrane-associated as well as nuclear proteins the last of which reflects the abundance of histones within exosome preparations. Interestingly, label-free MS quantitation suggests that the exosomes might be derived from cross-presenting compartments as they are also enriched in proteins involved in trafficking and loading of MHC-I with exogenous antigens

/ CPGS AND BETA GLUCAN – INNATE IMMUNE STIMULI KNOWN TO INDUCE SECRETION OF EXOSOMES



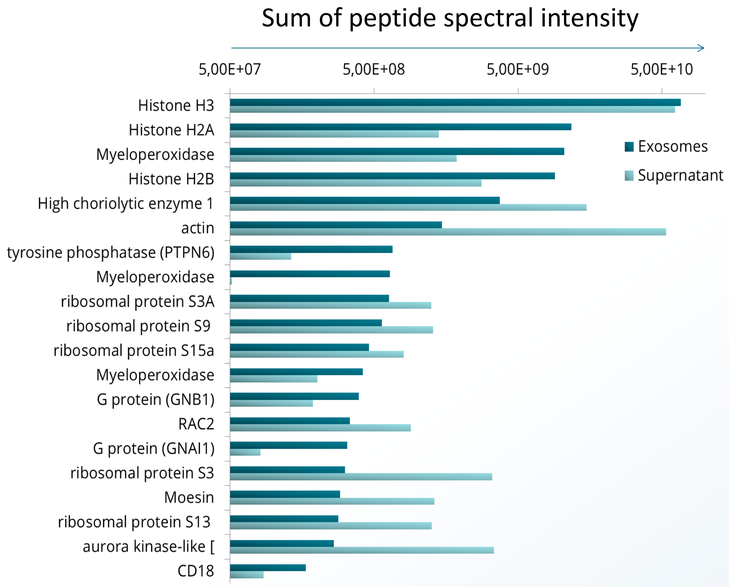
- CpG ODNs activate immune signaling through TLR9 (mammals) and TLR21 (chicken) and have been found to induce secretion of exosomes containing MHCII from salmon head kidney leukocytes (Iliev et al. Dev Comp Immunol. 2010 Jan;34(1):29-41)
- Yeast BG induces exosome secretion from macrophages through a dectin-1/autophagy-dependent pathway (Öhman et al. Immunol. 2014 Jun 15;192(12):5952-62)

/ PROTEOMIC DATA – EXOSOMES ARE ENRICHED IN MEMBRANE AND NUCLEAR PROTEINS – GENE ONTOLOGY ANALYSIS



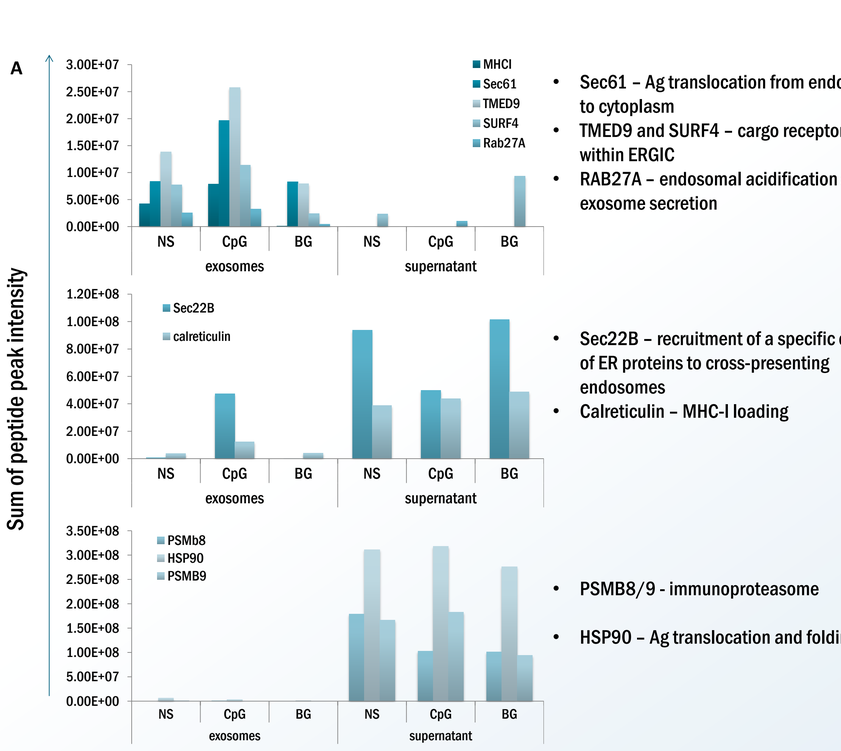
Protein composition of exosomes and supernatants (SN) was analyzed using Orbitrap Q Exactive instrument. The histogram displays the most abundant «Cellular component» features associated with proteins identified in exosomes and supernatants from CPG-stimulated cells

/ MOST ABUNDANT PROTEINS IDENTIFIED IN SALMON EXOSOMES – LABEL-FREE MS QUANTITATION



The quantitation was carried out using MaxQuant software. The values represent the mean of the sum of the spectral intensity of the all of the peptides matching each of the identified proteins identified in samples from 2 individuals

/ EXOSOMES ACCUMULATE MHC-I, PROTEINS IMPLICATED IN CROSS-PRESENTATION AND APOPTOTIC BODY ANTIGENS



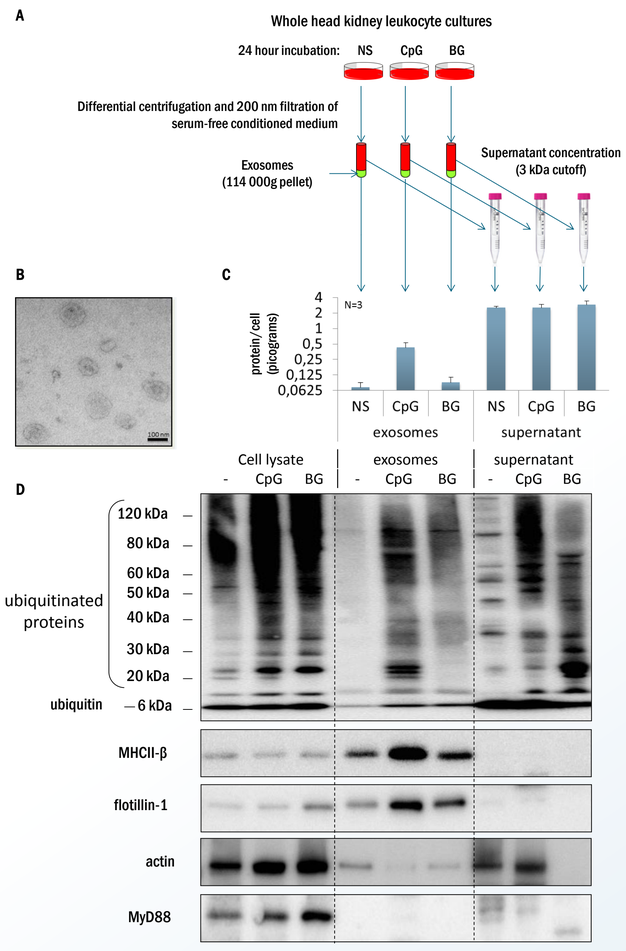
- Sec61 – Ag translocation from endosome to cytoplasm
- TMED9 and SURF4 – cargo receptors within ERGIC
- RAB27A – endosomal acidification and exosome secretion
- Sec22B – recruitment of a specific cargo of ER proteins to cross-presenting endosomes
- Calreticulin – MHC-I loading
- PSMB8/9 - immunoproteasome
- HSP90 – Ag translocation and folding

/ SELECTED IMMUNE PROTEINS IDENTIFIED IN EXOSOMES RELEASED FROM SALMON HEAD KIDNEY LEUKOCYTES AND OTHER SPECIES

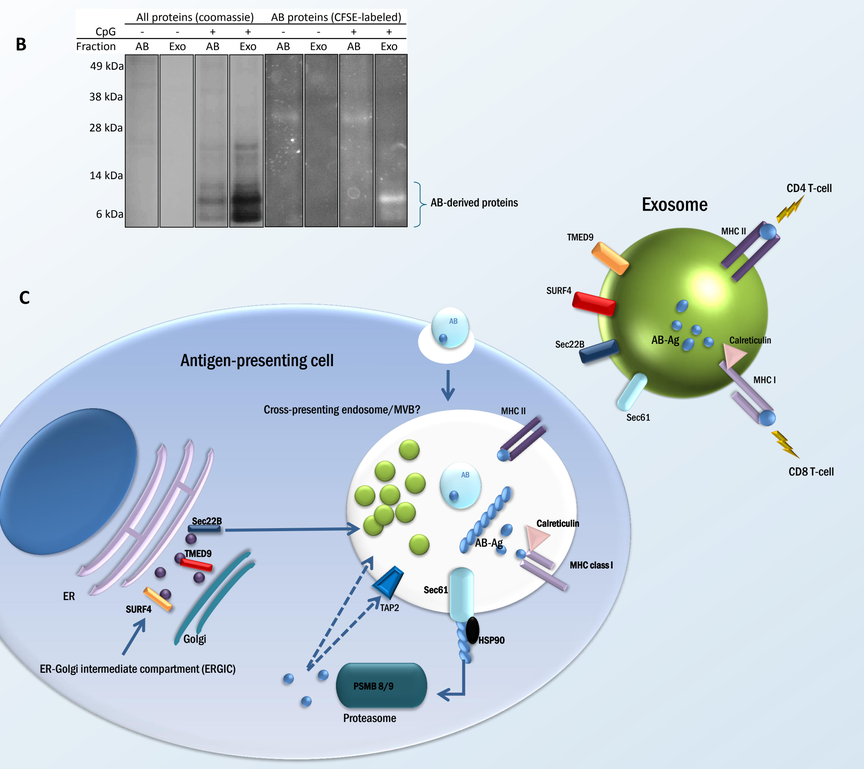
	Identified in other species/cells*	
Salmon homologs	Species	Cell types
CD63, LAMP3 (CD63)	Human	B-cells, DCs, endothelial cells, mast cells
CD81	Human	B-cells, T-cells, dendritic cells
CD9 molecule (CD9)	Human	B-cells, DCs, endothelial cells, mast cells
MHC class I (Sasa-UBA)	Human	B-cells, endothelial cells, epithelial cells
MHC class II beta (MHCIIDBB)	Human	B-cells
myeloperoxidase (MPO)	Human	Endothelial cells
C type lectin receptor (CD209-like)	None	
CD169-like (SIGLEC1)	None	
CD26-like T-cell activation (DPP4)	Human	B-cells
CD29 (ITGB1)	Human	B-cells
chemokine (C-C motif) ligand 4 (CCL4)	None	
CD2 family, SLAMF (CD48/CD84-like)	Human	B-cells
CD206, mannose receptor (MRC1)	None	
CD68, LAMP4 (CD68)	None	
MHC class II antigen alpha (MHC2DAA)	Human	B-cells
NK-lysin, granulysin-like (NKL)	None	
plastin-2 (LCP1)	Human	B-cells
CD11A (ITGAL)	Human	B-cells
arachidonate 5-lipoxygenase (ALOX5)	Rat	Reticulocyte
CD2 molecule (CD2)	Human	T-cells
matrix metalloproteinase 9 (MMP9)	None	
complement factor D, adipsin (CFD)	Human	Urine
CD208 (LAMP3)	Rat	Dendritic cells
CD11B (ITGAM)	Rat	Reticulocytes
CD2 family, LFA-3 CD58-like (CD58)	Human	B-cells
CD45 (PTPRC)	Human	B-cells
perforin-1 (PRF1)	Human	NK-cells
CD87 (PLAUR)	None	
Ig heavy constant mu (IGHM)	Human	
CD18 (ITGB2)	Human	B-cells, T-cells

*according to vesiclepedia.org (June 2015)

/ OVERVIEW OF THE EXPERIMENTAL SETUP AND WESTERN BLOT ANALYSIS OF EXOSOME PELLETS AND SUPERNATANTS FROM CONDITIONED MEDIUM



- A, Schematic representation of the experiment. Stimulations: 2 µM CpG-B and 50 µg/ml of Saccharomyces cerevisiae BG for 24 hours
- B, The electron micrograph of isolated salmon exosomes
- C, Protein concentration measured with micro BCA and presented as picograms of secreted protein per cell
- D, Western blot data showing that CpGs, and to a lesser extent BG, induce secretion of exosomes enriched in ubiquitinated proteins, MHCII and flotillin. Secreted proteins derived from equal numbers of cells (~20x10^6) were loaded in each lane



- A, Label-free MS quantitation - mean values from samples isolated from two individuals
- B, exosomes and apoptotic bodies (AB) were isolated from supernatant of cells pre-incubated with fluorescently labelled AB and analyzed using SDS-PAGE
- C, A model showing the intracellular distribution of the components implicated in ER-endosome trafficking and MHC-I cross-presentation and the putative association of exosomes with cross-presenting endosomes. AB - apoptotic body

/ SUMMARY

- CpGs and to a lesser extent yeast beta-glucan, induced exosome secretion from salmon leukocytes
- Exosomes released from salmon leukocytes contain large amounts of core histones, myeloperoxidase and ribosomal proteins along with other exosome markers, immune receptors and mediators
- Exosomes released by salmon leukocytes also contain MHC-I and proteins involved ER-endosome trafficking and cross-presentation along with Ags derived from apoptotic cells
- The results indicate that exosomes isolated from salmon leukocyte cultures might originate from cross-presenting organelles in APCs and might be involved in cross-presentation of cellular antigens

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