

Protein Profiling of the Secretome of FcεRI Activated RBL-2H3.1 Cells

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ABSTRACT

Background: Secretory proteins of IgE receptor activated mast cells and basophils play a pivotal role in the generation of immediate and long term immune responses in allergy and type I hypersensitivity. **Objective:** The present study aims to generate a 2-D map and profile of proteins secreted from a high secretory variant of the rat basophilic leukemia cell line, RBL-2H3.1, which in view of the difficulty associated with gaining adequate numbers of pure primary mast cell and basophiles, represents an accepted model system for the study and standardization of the methodology to characterize the secretome of these cell types. **Methods:** A 2-D map of secretory proteins was generated by 2-D PAGE and a shotgun mass spectrometric approach carried out for protein identification. **Results:** Study resulted into identification of 299 proteins released from resting and IgE receptor activated RBL-2H3.1 cells after 90 s, 30 min and 3 h antigen challenge. Further sequence analysis identified ~53% of total proteins as secretory proteins which could be attributed to classical and non-classical secretory pathways. Additionally, functional classification of classic secretory proteins verified the presence of proteins belonged to cytokines, receptors, membrane proteins, lysosomal proteins and proteins associated with specific sub-cellular localizations such as endoplasmic reticulum, mitochondria, nucleus, cytoplasm and ribosome. According to this data the presence of some secretory proteins such as cytokines (e.g. MCP-2, PF-4, CSF-1 and TGF-β1) are all subject to Ag challenge which may point to their importance toward pathogenesis in allergic diseases. **Conclusion:** In view of both a beneficial and adverse role of mast cell mediators in health and disease, an identification of temporal changes in the secretory pattern may form the basis for future tailor made intervention strategies that may enable us to harvest the therapeutic potential inherent in mast cell exocytosis while inhibiting/attenuating negative outcomes.

Keywords: Basophil, FcεRI, IgE, Mast Cell, RBL-2H3.1, Secretome

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INTRODUCTION

The prominent granules in the cells of mast cell/basophil (MC/B) lineage store a broad range of mediators destined for secretion. Cross-linking of high affinity receptor for IgE (FcεRI) by an immunogen results in the aggregation of FcεRI and initiates a cascade of molecular reactions inside the cell, leading to cellular responses mainly in the form of mediator release (1). Mast cell mediators have been broadly classified as i) preformed and stored mediators, such as histamine, serotonin and tryptase; and ii) newly synthesized mediators, such as interleukins and cytokines such as, IFNs, TNF-α, IL-3 and IL-4 and iii) lipid mediators such as PGD₂, PGE₂, LTB₄, LTC₄ and PAF (2).

There are mounting evidence for the importance of the role of MCs/Bs' in the steering of innate and adaptive immune responses (30). In many cases, MCs/Bs exerts their biological roles by releasing specific types of mediators. Although interaction of MC/B mediators and other cells represents an important defense mechanism to co-ordinate innate and acquired immune responses against pathogens, they also play a critical role in the pathogenesis of inflammatory diseases (4).

Apart from the functional studies of MC mediators, recently described "regulated secretory system" (RSS) in MCs and some other hematopoietic cells have increasingly attracted the attention of cell biologists (5). These studies led to the proposal that unlike conventional secretory cells such as endocrine and exocrine cells, RSS-comprising cells use lysosomes to store and release secretory proteins. Protein mediators are synthesized on ribosomes and transferred to the endoplasmic reticulum and from there to Golgi, where they are stored in the secretory granules (lysosomes) following a variety of post-translational modifications. Cell stimulation via membrane receptors is a common mechanism for the fusion of secretory granules to cytoplasmic membranes, which results in the exocytic release of mediators (6).

The aim of the current study was to employ the RBL-2H3.1 cell line as a model system for the study of MC/B exocytosis to identify their secretory protein profile following IgE receptor activation. The results of this investigation may be used to improve our understanding of the functional roles of MC/B mediators and the molecular mechanism of RSS in mast cells and basophils. They also may lead to identify potential targets for therapeutic intervention strategies in allergy, asthma and inflammatory disease, which afflict more than a quarter of the population in industrialized countries. The methods applied and developed may be employed to similar projects on primary human mast cells and basophils or other secretory cells.

MATERIALS AND METHODS

Cell Culture and Stimulation. High secretory variant cells of RBL-2H3 were cultured in DMEM (Sigma, US) with 10% FCS and 1% v/v penicillin/streptomycin (Sigma, US). Sixteen hours prior to cell stimulation, DNP-specific mouse IgE (clone SPE-1F) in a final concentration of 0.5 μg/ml was added to sensitize FcεRI receptors. Cell monolayers, at >80% confluency were washed with 37°C PBS and cell stimulation was carried out with 100 ng/ml of DNP-HSA, diluted in DMEM.

Sample Preparation for Analysis of Secretory Proteins. Cell supernatants were collected in tubes containing 100 μg/ml PMSF or a complete cocktail of protease inhibitors (Roche), and filtered through 0.20 μm membrane filters to remove cell debris. Sample

volume was decreased by ultra-filtration using Vivaspine 20, 3000 MWCO (Sartorius) to reach a minimal volume of 100 μ l. Further protein precipitation and salt removal was carried out with acetone following overnight storage at -20°C .

2-D Gel Electrophoresis. Protein pellets were re-suspended in 2-D sample buffer containing 8 M urea, 4% CHAPS, 65 mM DTT, 2% (v/v) IPG buffer and a trace amount of bromphenol blue (BPB) and loaded on DryStrips (pH=3-10 or 4-7). Strip rehydration was carried out during overnight incubation with protein samples. Isoelectric focusing (IEF) (Multiphore II, Amersham Bioescience) was started at 150 V for 1 h and continued with 300 V for 1 h and then the voltage was increased to 3500 V for 5.30 h. For the second dimension, IPG strips were equilibrated for 15 min in each of the equilibration buffers A containing 6 M urea, 50 mM Tris-HCl (pH=8.8), 30% glycerol, 2% SDS, 100 mM DTT and 0.001% BPB, and then in buffer B containing all the components of buffer A, except that it contained 270 mM iodoacetamide instead of the DTT. Strips were placed on top of a 12% sodium dodecylsulfate (SDS) polyacrylamide gel and were overlaid with 1% agarose. Electrophoresis was carried out and following protein fixation after an overnight incubation in 40% ethanol and 10% acetic acid, the gels were stained with mass spectrometry compatible silver stain (Sigma, US).

Mixed Protein Sample Digestion. The protein pellet obtained from acetone precipitation were re-suspended in 8 M urea (pH=8). The samples were reduced at a final concentration of 10 mM DTT and 50 mM ammonium bicarbonate for 1 hour at 56°C , followed by a 30 min alkylation in the dark at a final concentration of 55 mM Iodoacetamide and 50 mM ammonium bicarbonate at 37°C . Trypsin was prepared according to the manufacturer's protocol (Sigma, US) and then added to the protein mixture at a 1:50 (w/w) mass ratio. Samples were dried in a vacuum concentrator prior to MS analysis.

Nano-LC-MS/MS Analysis. A small aliquot of each sample was removed using a pipette tip and resuspended in 100 μ l 95% water/5% acetonitrile (MeCN)/0.1% formic acid (hereafter referred to as Buffer A), and resolved by LC-MS using a nanoACQUITY chromatograph (Waters MS technologies, Manchester, UK), coupled to an LTQ Orbitrap XL (ThermoFisher Scientific, Bremen, Germany), equipped with the manufacturer's dynamic nanospray source fitted with a PicoTip Emitter (New Objective, MA, USA). The sample temperature was maintained at 10°C , and 4 μ l of sample was injected to a trapping column (Waters C18 180 μ m X 20 mm), using the partial loop mode of injection, at a flow rate of 18 μ l/min 100% A1, prior to valve switching to the analytical column. The analytical column (nanoACQUITY UPLC™ BEH C18 75 μ m x 150 mm 1.7 μ m column) was maintained at a temperature of 35°C , and at a constant flow rate of 300nl/min. The gradient conditions were as follows: 0.33 min - 100% A1, 30 min - 50% B1 (where B1 consists of 100% MeCN/0.1% FA), 31 min - 85% B1, 50 min, 100% A1 (equilibration).

Mass Spectrometry Analysis. Full scan MS spectra (m/z 300-1600) were acquired in an LTQ-Orbitrap XL with the Orbitrap operating at a resolution of 30,000 (defined at m/z 400) in data-dependent mode. The top five most intense ions from the MS1 scan were selected for tandem MS by collision induced dissociation (CID) in the LTQ, at a normalized collision energy of 30%, and an activation q of 0.25. Dynamic exclusion was applied for duration of 30 seconds, with a repeat count of two and an exclude duration width of 180 seconds, and all product ion spectra were acquired in the LTQ.

Data Analysis. Peptide sequences were verified using the Mascot search engine (7) facilitated through the vendor supplied software Proteome Discoverer™ (ThermoFisher

Scientific). Tandem MS data were searched against the Swiss-PROT and NCBI nr databases, with the following parameters incorporated into the search: taxonomy restriction to 'Rattus', two miscleavages allowed, and the following dynamic modifications: Carbamidomethyl (C), Carbamidomethyl N-terminus, and Oxidation (M).

Sequence Analysis for Secretory Proteins Prediction. To identify secretory proteins within all proteins identified with LC-ESI mass spectrometry, proteins sequences were analyzed with SignalP 3.0 (<http://www.cbs.dtu.dk/services/SignalP/>) and SecretomeP 2.0 (<http://www.cbs.dtu.dk/services/SecretomeP/>) software. SignalP 3.0 software has been prepared based on a combination of several artificial neural networks and hidden Markov model and is able to identify proteins with established secretory signals. SecretomeP 2.0 analysis is based on diverse characteristics of proteins that are found to be released without having a known signal peptide.

RESULTS

To standardize the system, β -hexosaminidase release assays were carried out to ascertain the secretory potential of RBL-2H3 cells, and cells supporting >50% release were considered as high secretory variants (RBL-2H3.1) and were used in subsequent experiments for the analysis of secretory proteins. Secreted proteins were harvested from the supernatant of triggered cells 90 sec, 30 min and 3 hours after receptor activation.

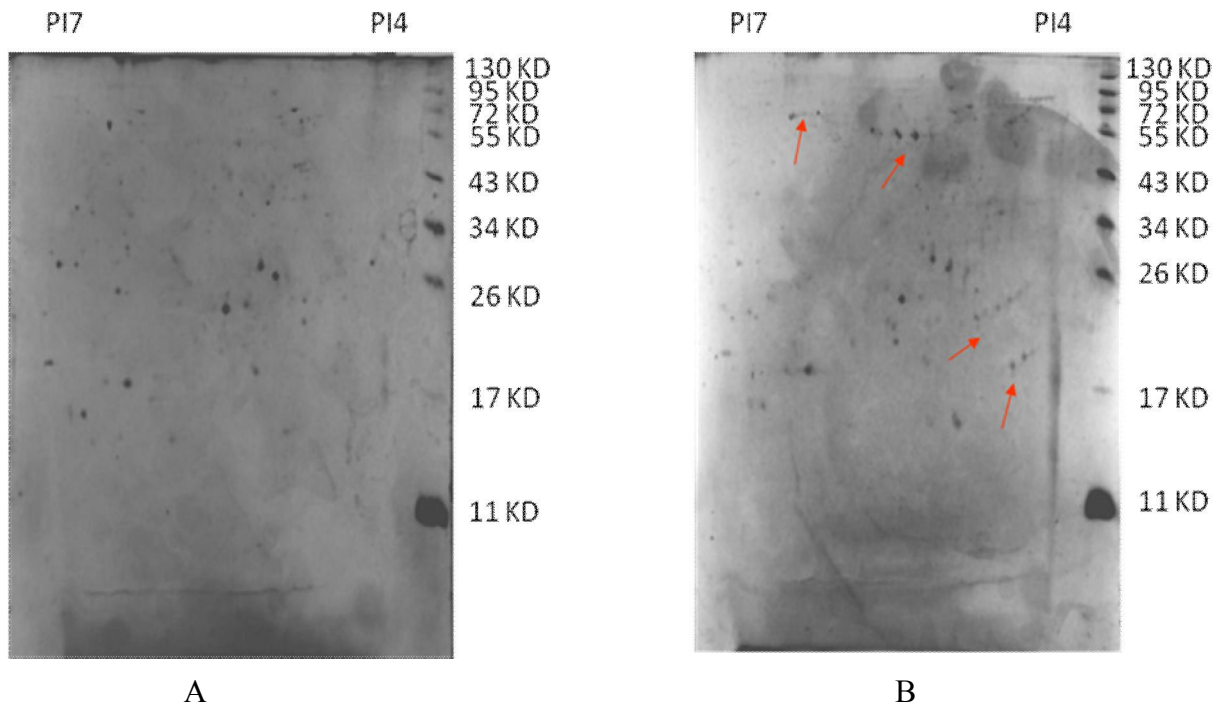


Figure 1. Representative 2-D map of secretory proteins collected from supernatant of RBL-2H3.1 cells in resting state (Sup (-)) (A), and activated cells with IgE+(DNP-HSA) for 3h (Sup3h+) (B). Horizontal movement of proteins (pointed by red arrows) on X axis (Compared to control) are showing the proteins with differential movement on PI range of 4-7, that may be representative of post translational modification on secreted proteins after cell stimulation.

Proteins were collected from the supernatant fraction of non-activated (sup (-)) and post 3 hour-activated RBL-2H3.1 cells (sup3h (+)), and subjected to 2-D electrophoresis in the range of pH=3-10 (Data not presented). Since this study showed that the majority of proteins are located in the range of pH=4-7, zoom gels covering this pH range were subsequently employed for higher resolution (Figure 1).

As direct protein identification isolated from SDS gels was frequently compromised by low recovery of proteins, protein profiling was complemented by high-throughput LC-ESI mass spectrometry on the samples of mixed proteins from the supernatant of FcεRI activated and non-activated RBL-2H3.1 cells (Appendix I).

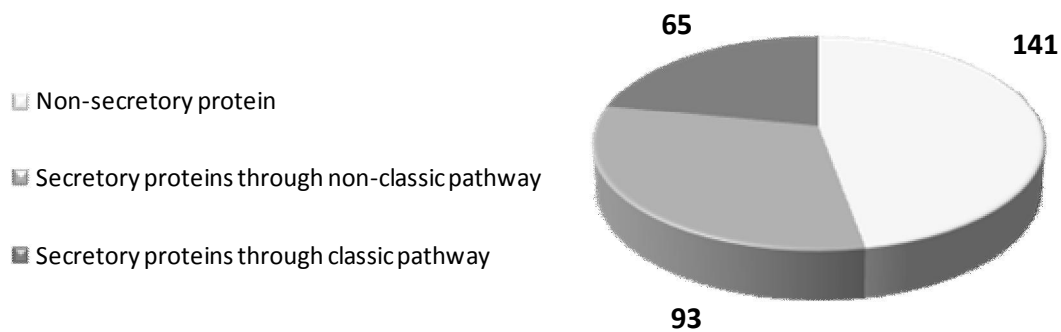


Figure 2. Pie chart for proteins identified at the secretome of RBL-2H3.1 cells. The numbers are representative of proteins found in each category.

Further analysis of identified proteins was carried out by inserting the protein sequences in SignalP 3.0 and SecretomeP 2.0 search tool and proteins were classified as “Secretory proteins of classic pathway (S)”, “Secretory proteins of non-classic pathway (NC)” and “Non-secretory proteins (NS)”. Mass spectrometric analysis resulted in identification of 299 proteins in the secretome of resting and activated cells by IgE + DNP for the time courses of 90s, 30 min and 3 h. Sequence analysis by SignalP 3.0 and SecretomeP 2.0 software packages identified ~53% (158 proteins) of total proteins as secretory proteins which could be attributed to classical (65 proteins) and non-classical (93 proteins) secretory pathways (Figure 2 and Table 1).

DISCUSSION

Recent advances in proteomic techniques assisted us toward protein profiling in the secretome of RBL-2H3.1 cells in response to IgE-receptor activation.

Comparative analysis of 2-D maps for samples of sup3h (+) and sup (-), clearly highlights similarities and differences between two maps. Attempts were made to correlate the presence of protein spots on sup3h (+) and the absence of equivalent spot on sup (-) with secretory or up-regulated proteins. However, a number of proteins in sup (-), are not matched with the protein profile of sup3h (+). At present, it remains uncertain

whether the proteins degraded by proteases, are part of the secretory proteins released from mast cells, or whether we observe down-regulation of secretory proteins in response to IgE-mediated antigenic stimulation. In addition, inclination of some proteins to horizontal line (showed by red arrows, Figure 1) on sup3h (+) map may suggest a form of post-translational modification of these proteins following cell activation.

Table 1. The list of signal/anchor sequence-consisted secretory proteins identified with SignalP 3.0 at the secretome of resting cells (R1 and R2) and cells activated with IgE+ (DNP-HSA) for 90s, 30min and 3hours. ✓ is indicative of presence of protein in each sample and attributed Mascot scores are reported in order.

| Protein name and AC number | R1 | R2 | 90s | 90s | 30m | 30m | 3h | 3h | Mascot score |
|--|----|----|-----|-----|-----|-----|----|----|---|
| Bifunctional purine biosynthesis protein PURH O35567 | ✓ | ✓ | ✓ | ✓ | | | | | 63.57-168.70-89.17-76.36 |
| Aspartate aminotransferase, mitochondrial P00507 | ✓ | ✓ | | ✓ | | | | | 37.3-117.99-69.61 |
| Mast cell protease 2 P00770 | | | | | ✓ | ✓ | | | 91.82-91.69 |
| Cathepsin B P00787 | | | | ✓ | ✓ | ✓ | | | 116.40-378.97-134.92 |
| 60S acidic ribosomal protein P2 P02401 | ✓ | | | | | | | | 22.47 |
| Serum albumin P02770 | | | | | | ✓ | | | 45.47 |
| Protein disulfide-isomerase P04785 | ✓ | | ✓ | ✓ | ✓ | | | | 424.24-259.62-448.89-149.48 |
| 78 kDa glucose-regulated protein P06761 | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | 986.74-104.65-77.25-231.10-107.30-63-88 |
| Platelet factor 4 P06765 | | | | | ✓ | ✓ | ✓ | ✓ | 98.85-67.02-66-67 |
| Beta-2-microglobulin P07151 | | ✓ | | | ✓ | ✓ | | | 58.73-80.77-46.44 |
| ATP synthase subunit beta, mitochondrial P10719 | | ✓ | | | | | | | 75.93 |
| Sulfated glycoprotein 1 P10960 | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 111.00-39.75-211.00-231.63-55-57 |
| Protein disulfide-isomerase A3 P11598 | ✓ | | | ✓ | | | | | 126.03-99.29 |
| C-C motif chemokine 2 P14844 | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | 52.99-48.45-50.3-55.82-46-37 |
| RT1 class I histocompatibility antigen, AA alpha chain P16391 | | ✓ | | | ✓ | ✓ | | | 73.81-155.79-111.79 |
| Lysosome-associated membrane glycoprotein 2 P17046 | | | | | ✓ | ✓ | | | 176.07-99.24 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|-------------|
| Transforming growth factor beta-1 P17246 | | | | | √ | √ | | | 112.56-36.31 | |
| Calreticulin P18418 | √ | | √ | √ | √ | | | | 155.22-178.53-169.08-64.53 | |
| High affinity immunoglobulin epsilon receptor subunit gamma P20411 | | | | | √ | | | | 65.14 | |
| Cathepsin D P24268 | √ | √ | √ | √ | √ | √ | √ | √ | 40.39-32.04-273.87-365.77-403.41-709.39-65-73 | |
| Urokinase-type plasminogen activator P29598 | | | | | √ | √ | | | 40.61-27.88 | |
| Glutamate [NMDA] receptor subunit zeta-1 P35439 | | | | | √ | √ | | | 31.02-0.00 | |
| Insulin-degrading enzyme P35559 | | √ | √ | | | | | | 45.79-51.21 | |
| 2-oxoisovalerate dehydrogenase subunit beta, mitochondrial P35738 | | √ | | | | | | | 30.18 | |
| Low-density lipoprotein receptor P35952 | | | | | √ | √ | | | 71.61-62.91 | |
| Sodium/calcium exchanger 2 P48768 | | | √ | √ | | √ | √ | | 0.00-0.00-0.00-47 | |
| Dipeptidyl-peptidase 1 P80067 | | | | | √ | | | | 35.92 | |
| Elongation factor Tu, mitochondrial P85834 | √ | | | | | | | | 23.06 | |
| Di-N-acetylchitobiase Q01460 | | | | | | √ | | | 55.99 | |
| Synaptic vesicular amine transporter Q01827 | | | | | √ | √ | | | 137.54-156.58 | |
| glucose transporter-3 Q07647 | | | | | | | √ | √ | 70-68 | |
| Uncharacterized protein C1orf31 homolog Q3KQW9 | | | √ | √ | | | | | 45.71-39.49 | |
| E2f8 protein Q4FZV5 | | | | | | | √ | | 54.00 | |
| UMP-CMP kinase Q4KM73 | | | | √ | | | | | 59.67 | |
| Heat shock protein 75 kDa, mitochondrial Q5XHZ0 | √ | | | | | | | | 37.75 | |
| Lysosome-associated membrane glycoprotein 3 Q5X199 | √ | √ | | | | | √ | | 0.00-25.16-0.00 | |
| Interleukin-1 receptor-like 1 Q62611 | | | | | √ | √ | | | 411.99-31.61 | |
| Glutamate receptor delta-1 subunit Q62640 | √ | | | | | | | | 33.53 | |
| Extracellular matrix protein 1 Q62894 | | | | | | | √ | √ | √ | 49.69-54-43 |

Secretory proteins in RBL-2H3.1 cells

| | | | | | | | | |
|---|---|---|---|---|---|---|---|--------------------------------|
| Protein disulfide-isomerase A6 Q63081 | √ | | | | | | | 79.90 |
| Nucleobindin-1 Q63083 | | | √ | √ | | | | 51.08-58.81 |
| Proteinase-activated receptor-2, G protein-coupled receptor 11 Q63645 | | | | | | √ | | 37.00 |
| Beta-hexosaminidase subunit alpha Q641X3 | | | √ | | | | | 35.66 |
| Alpha-N-acetylgalactosaminidase Q66H12 | | | √ | | | | | 53.93 |
| Beta-hexosaminidase subunit beta Q6AXR4 | | | √ | | | | | 76.88 |
| Out at first protein homolog Q6AYE5 | | | √ | √ | | | | 53.35-109.78 |
| protective protein for beta- galactosidase Q6AYS3 | | | | | | √ | | 46.00 |
| Protein CutA Q6MGD0 | | | √ | √ | | | | 70.59-38.58 |
| Calcium-binding atopy-related autoantigen 1 Q6P6Q9 | √ | | √ | √ | | | | 24.21-26.18-24.06 |
| Lysosomal alpha-glucosidase Q6P7A9 | | | | √ | √ | √ | √ | 135.47-126.05-87-74 |
| Transmembrane protein 132D Q76HP2 | | | √ | | | | | 28.10 |
| Apolipoprotein B-100 Q7TMA5 | √ | | | √ | √ | √ | | 69.88-0.00-183.20-232.38 |
| epididymal secretory protein E1 Q8CHN5 | | | | | | √ | √ | 47-0 |
| Macrophage colony-stimulating factor 1 Q8JZQ0 | | | √ | √ | √ | √ | | 102.36-95.97-269.42- 186.40 |
| Retinoid-inducible serine car- boxypeptidase Q920A6 | | √ | | | √ | √ | | 0.00-60.51-92.56 |
| major histocompatibility com- plex class I Q95577 | | | | | | √ | √ | 69-58 |
| Dipeptidyl-peptidase 2 Q9EPB1 | | | √ | | | √ | | 59.53-42.37 |
| Collagen alpha-1(V) chain Q9JI03 | | √ | | | | √ | | 23.74-0.00 |
| GCD-10 Q9JJ55 | | | | | | | √ | 29.00 |
| UDP-glucose:glycoprotein glu- cosyltransferase 1 Q9JLA3 | | | √ | | | | | 21.76 |
| CDK5 regulatory subunit- associated protein 1 Q9JLH6 | | √ | √ | √ | | √ | | 25.06-30.74-0.00-0.00 |
| Low-density lipoprotein recep- tor-related protein 4 Q9QYP1 | | | | √ | | | | 27.72 |

| | | | | | |
|--|---|---|---|---|---------------------------|
| Robo2 Q9QZ13 | | | | √ | 38.00 |
| Peroxiredoxin-5, mitochondrial Q9R063 | √ | √ | √ | √ | 76.6-147.76-114.54-108.30 |
| Thioredoxin-dependent peroxide reductase, mitochondrial Q9Z0V6 | | √ | | | 189.75 |

Confidence in the data obtained from LC-MS/MS is supported by i) Reproducibility of data (Ranging between 30-60%) following two runs of MS experiments and on two biological replicates and ii) Results of previous similar studies by different techniques such as 2-D PAGE (8), serial analysis of expressed genes (SAGE) (9), enzymatically based methods, Western blot (10) and RT-PCR (11).

Since mixing of secretory proteins and cytoplasmic proteins resulting from cell death cannot be ruled out, especially when culturing cells in serum-free media, viability of cells was ascertained to optimize the experiment for secretome collection. Cell viability test with vital staining of erythrosine on the cells cultured in FCS-free DMEM, did not show significant differences compared to cells cultured in normal media (DMEM+10% FCS), for the period of antigen challenge.

While the use of SignalP 3.0 software offers the opportunity to recognize secretory proteins comprising signal sequences which facilitate protein secretion via the classical secretory pathway, analysis with Secretome P 2.0 software was utilized to identify leaderless secretory proteins as described by Jannick Dyrlov Bendtsen et al. (12) to complement the analysis. In addition to the classical secretory proteins, a number of proteins which do not exhibit the characteristic sequences reported for secretory proteins were identified in this study. These proteins are classified as non-classic (NC) secretory proteins and non-secretory (NS) proteins (Appendix I).

We have attempted to classify the proteins identified with SignalP 3.0 in three categories of i) membrane proteins and receptors; ii) secretory proteins and iii) miscellaneous (Table 2).

Membrane proteins categorized here belong mainly to cytoplasmic or lysosome/Golgi membrane. The mechanism for translocation of membrane proteins and secretory proteins is fairly similar (13). Apart from the structural role of membrane proteins, recent observations emphasize their importance in the initiation and propagation of signal transduction in compartments called lipid rafts (14). Lipid rafts involve microenvironmental clustering of lipid molecules such as cholesterol and sphingolipids and protein molecules. The solubility of lipid rafts is mainly regulated by cholesterol homeostasis. This may point to an important role for NPC2, as a molecule involved in cholesterol delivery from lysosomes to cytoplasmic membranes (8,15).

Membrane proteins identified in this study are mostly involved in membrane transport system. For instance GLUT-3, sodium/calcium exchanger 2, NPC2, MHC I and synaptic vesicular amine transporter are involved in transport of glucose, $\text{Na}^+/\text{Ca}^{2+}$ ions, lipids, antigenic proteins and biogenic amines (e.g. dopamine, serotonin, norepinephrine and histamine), respectively. Although, most mammalian cells ubiquitously express GLUT-3 and sodium/calcium exchanger 2, the presence of NPC2 and MHC I is particularly in relation to functional characteristics of mast cells.

Expression of MHC I by mouse and human mast cells from lung, liver, uterus and skin has been reported previously (16-18).

Table 2. Functional classification of secretory proteins identified with SignalP. These proteins are classified in three categories mainly on the basis of subcellular location. Each category is divided into subgroups according to their similarity in function and subcellular location. Membrane proteins, receptors and secretory proteins comprise the actual signal/anchor sequence required for their translocation or secretion, but proteins classified as “miscellaneous” may only contain a transit peptide, which is required for their translocation to mitochondria, or may not contain of a cited signal sequence in ExpASy.

| Category | Sub-category and Proteins names |
|---------------------------------|--|
| Membrane proteins and receptors | <p>Membrane proteins: Protein disulfide-isomerase (PDI), Lysosome-associated membrane glycoprotein 2 and 3 (LAMP-2, 3), Nucleobindin-1, Protein CutA, Calcium-binding atopy-related autoantigen 1 (CBARA1), Transmembrane protein 132D, GCD-10, Epididymal secretory protein E1 (NPC2), glucose transporter-3 (GLUT-3), major histocompatibility complex class I(MHC I), Beta-2-microglobulin, RT1 class I histocompatibility antigen AA alpha chain, Sodium/calcium exchanger 2, Synaptic vesicular amine transporter (VAT2), Robo2.</p> <p>Receptors: Proteinase-activated receptor-2 (PAR-2), High affinity immunoglobulin epsilon receptor subunit gamma (FcεRI-γ), Glutamate [NMDA] receptor zeta-1 and delta-1 subunits, Low-density lipoprotein receptor (LDL-R), Low-density lipoprotein receptor-related protein 4 , Interleukin-1 receptor-like 1.</p> |
| Secretory proteins | <p>Lysosomal proteins: Cathepsin B and D, Dipeptidyl-peptidase 1 and 2, Di-N-acetylchitobiase, Beta-hexosaminidase subunits alpha and Beta, Alpha-N-acetylgalactosaminidase, Lysosomal alpha-glucosidase, Sulfated glycoprotein 1, Retinoid-inducible serine carboxypeptidase, Mast cell protease 2.</p> <p>ER Proteins: 78 kDa glucose-regulated protein, Protein disulfide-isomerase A3 and A6, Calreticulin, UDP-glucose:glycoprotein glucosyltransferase 1.</p> <p>Cytokines, chemokines and growth factors: platelet factor 4 (PF4), C-C motif chemokine 2 (MCP-1), Macrophage colony-stimulating factor (CSF-1), Transforming growth factor beta-1 (TGF-β1).</p> <p>Others: Extracellular matrix protein 1, Collagen alpha-1, Serum albumin, Urokinase-type plasminogen activator, Out at first protein homolog, Apolipoprotein B-100.</p> |
| Miscellaneous | <p>Cytoplasmic: Bifunctional purine biosynthesis protein PURH, UMP-CMP kinase, Insulin-degrading enzyme, CDK5 regulatory subunit-associated protein 1.</p> <p>Ribosomal: 60S acidic ribosomal protein P2.</p> <p>Mitochondrial: 2-oxoisovalerate dehydrogenase subunit beta, ATP synthase subunit beta, Elongation factor Tu, Uncharacterized protein C1orf31 homolog, Heat shock protein 75 kDa (HSP 75), Protective protein for beta-galactosidase, Peroxiredoxin-5, Thioredoxin-dependent peroxide reductase, Aspartate aminotransferase.</p> <p>Nucleus : E2F8</p> |

Herein, simultaneous identification of MHC I, Beta-2-microglobulin and RT1 class I histocompatibility antigen AA alpha chain suggests an active role of MCs/Bs in antigen presentation and their recruitment in antigen-specific expansion of T-cells (17,19). Biological roles of some other proteins such as protein disulfide-isomerase (PDI), lysosome-associated membrane glycoprotein 2 and 3 (LAMP-2, 3), synaptic vesicular amine transporter on MC/B membrane could be partly described by their importance in cell secretion and exocytosis. Chaperon like activity of PDI and subcellular localization of this protein may suggest a role as an in-site regulator of protein folding which may be

required for the dynamics of cytoplasmic membrane assembly/disassembly during cell secretion or induced conformational changes of secretory proteins. This interpretation is further supported by observations that show increased rate of secretory proteins following overexpression of PDI or its coexpression with secretory proteins (20,21).

LAMPs are highly glycosylated transmembrane proteins expressed on lysosomal membranes. LAMP-3 is commonly known as an activation marker for basophils, eosinophils and mast cells (22-26). Synaptic vesicular amine transporter has mostly been considered in synaptic vesicle transport of biological amines such as histamine, dopamine, serotonin and norepinephrine in neuronal systems (27). Its presence in secretory granules of MCs may imply a similar role in histamine release or storage.

This study detects the presence of 5 receptor proteins including the identification of the FcεRI-γ subunit. Furthermore, our study and that of others (28) demonstrate the presence of PAR-2 on mast cells. PAR-2 is commonly known as target receptor for mast cell tryptase on neighboring cells (29,30). However, presence of PAR-2 on MC/B may further propose a self-regulatory mechanism for autogenous tryptase. This notion is further supported by studies that show trypsin, chymotrypsin, thrombin and pro-allergenic proteases of house dust mite origin, directly induce mast cells and RBL cell secretion in the absence of IgE-mediated receptor activation (31-33). Serine proteases are involved in regulation of some important biological process such as hemostasis, fibrinolysis, tissue repair and the digestion of dietary proteins. The expression of PAR-2 on mast cells may further explain the contribution of mast cells in inflammatory responses, tissue remodeling, and injury, which is attributable to their activation via PARs (34).

Apart from the structural role of secretory proteins such as extracellular matrix protein 1 and collagen alpha-1, they may also be more considered as messenger molecules during cell-cell interactions. Lysosomal proteins identified here are mainly involved in various catalytic activities required for degradation of proteins and glycoproteins (e.g. cathepsin B and D, dipeptidyl-peptidase 1 and 2, mast cell protease 2 and di-N-acetylchitobiase), carbohydrates (e.g. glucosidase alpha and alpha-N-acetylgalactosaminidase), glycosphingolipids and gangliosides (e.g. sulfated glycoprotein 1 and beta-hexosaminidase), or possess a degradative activity towards an unknown target (e.g. retinoid-inducible serine carboxypeptidase) which is a homolog of well-described lysosomal proteins such as cathepsin A (35). Presence of lysosomal proteins in the secretome of RBL cells may point to the lysosomal origin of their secretory granules (36). Most of these proteins (except cathepsin D and retinoid-inducible serine carboxypeptidase) were detected in the secretome of activated RBL cells and not the resting cells. These findings may support the active role of lysosomal proteins in FcεRI-mediated degranulation linked to the generation of allergic responses.

Presence of ER proteins in the secretome of RBL-2H3.1 cells may further point to a linkage of ER to the cell secretion machinery. Additionally, there is increasing evidence for the presence of ER chaperon proteins (e.g. 78 kDa glucose-regulated protein) in the secretome of various cell lines (37,38). The mechanism leading to the release of heat shock proteins is unknown although secretion via exosomes (39-41) or lipid rafts (42) has been proposed. Exogenous heat shock proteins are able to act as activators of innate and adaptive immunity via interaction with a number of different receptors on immune cells (43). They also show non-immunological cytoprotective effects on a variety of cell types (44,45).

In addition to lysosomal and ER proteins, this study identified a group of cytokines, chemokines and growth factors such as platelet factor 4 (PF4), C-C motif chemokine 2

or monocyte chemotactic protein 1 (MCP-1), macrophage colony-stimulating factor 1 (CSF-1) and transforming growth factor beta-1 (TGF- β 1). The presence of these proteins only in the samples obtained from activated cells but not in non-stimulated control cells may further emphasise their significance in relation to Fc ϵ RI-activation (31,46,47). Biological roles of these proteins could be best explained by interaction of MCs/Bs with other cells of the immune system. For example, small inducible cytokine A2 or MCP-1 is able to recruit monocytes and memory T cells and dendritic cells to the site of tissue injury and infection (48). MCP-1 has previously been reported as an activator of mast cells and basophils degranulation (49,50). Chen H et al. (9) showed a significant increase (21 folds) of mRNA tag sequence of MCP-1 after IgE-mediated activation of RBL-2H3 cells. The binding of IgE to Fc ϵ RI on dermal mast cells, even in the absence of antigen, has been shown to enhance mRNA for MCP-1 (51). Identification of MCP-1 in activated mast cells and basophils strengthens the claim for a role of these cells in regulation of cellular immunity in response to foreign stimuli.

PF4 is described in alpha granules of platelets with high affinity binding to heparin. Presence of heparin in secretome of mast cells, and its role in mast cell pathogenesis, has been established (52). Therefore, PF4 activity in mast cells could be explained as relating to the roles of heparin. The major physiological role of PF4 is to neutralize the heparin-like molecules on the endothelial surface of blood vessels and to locally inhibit antithrombin III activity to promote local coagulation. In addition, PF4 acts as strong chemoattractant for a variety of cells involved in wound repair and inflammation (53).

Although sequence analysis of proteins grouped as "miscellaneous" by SignalP 3.0 identified them as secretory proteins, currently these proteins are not classified as signal sequence/anchor-consisted proteins in ExPASy database. However, the existence of transit peptide at the sequence of some proteins such as mitochondrial proteins is required for their sub-cellular translocation. Presence of these proteins in cell secretome could imply their erroneous classification or errors in signal sequence identification as a result of data analysis with SignalP (54).

The sequence analysis of proteins identified for secretome of RBL-2H3.1 cells with SecretomeP 2.0 software, revealed the identity of 93 proteins which are released through the non-classical pathway. The list of these proteins (shown by NC) is provided as complementary data in appendix I. The reason to classify these proteins as non-classical secretory proteins is the absence of known signal peptides at their N-terminal, as defined for other secretory proteins (55,56). Regardless of the mechanism underlying the secretion of these proteins, they may play a significant role in the biological interactions of mast cells and other cells and also contribute to the generation of symptoms in mast cell-related diseases.

A straightforward explanation for presence of NS proteins (Appendix I) in the secretome of RBL-2H3.1 cells would be the contamination with the intracellular proteins from the source of cell lysis. However, other unknown secretory mechanisms should also be considered as a possibility.

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REFERENCES

- 1 Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in anaphylaxis. *J Allergy Clin Immunol.* 2009; 124:639-46.
- 2 Prussin C, Metcalfe DD. 5. IgE, mast cells, basophils, and eosinophils. *Journal of allergy and clinical immunology.* 2006; 117:S450-6.
- 3 Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. *Curr opin immunol.* 2000; 12:624-31.
- 4 Theoharides TC, Kempurja D, Tagen M, Conti P, Kalogeromitos D. Differential release of mast cell mediators and the pathogenesis of inflammation. *Immunol Rev.* 2007; 217:65-78.
- 5 Puri N, Roche PA. Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms. *Proc Natl Acad Sci U S A.* 2008; 105: 2580-5.
- 6 Blank U, Rivera J. The ins and outs of IgE-dependent mast-cell exocytosis. *Trends immunol.* 2004; 25:266-73.
- 7 Perkins DN, Pappin DJ, Creasy DM, Cottrell JS. Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis.* 1999; 20:3551-67.
- 8 Pathak A, Helm BA. Proteomic identification and characterisation of secreted N-glycosylated NPC2 following crosslinking of the high affinity receptor for IgE on mast cells. *Cell Biol Int.* 2010; 34:353-9.
- 9 Chen H, Centola M, Altschul SF, Metzger H. Characterization of gene expression in resting and activated mast cells. *J Exp Med.* 1998; 188:1657-68.
- 10 Dragonetti A, Baldassarre M, Castino R, Demoz M, Luini A, Buccione R, et al. The lysosomal protease cathepsin D is efficiently sorted to and secreted from regulated secretory compartments in the rat basophilic/mast cell line RBL. *J Cell Sci.* 2000; 113:3289-98.
- 11 Stenton GR, Nohara O, Dery RE, Vliagoftis H, Gilchrist M, Johri A, et al. Proteinase-activated receptor (PAR)-1 and -2 agonists induce mediator release from mast cells by pathways distinct from PAR-1 and PAR-2. *J Pharmacol Exp Ther.* 2002; 302:466-74.
- 12 Bendtsen JD, Nielsen H, von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol.* 2004; 340:783-95.
- 13 Matlack KE, Mothes W, Rapoport TA. Protein Translocation: Review Tunnel Vision. *Cell.* 1998; 92:381-90.
- 14 Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol.* 2000; 1:31-9.
- 15 Cheruku SR, Xu Z, Dutia R, Lobel P, Storch J. Mechanism of cholesterol transfer from the Niemann-Pick type C2 protein to model membranes supports a role in lysosomal cholesterol transport. *J Biol Chem.* 2006; 281:31594-604.
- 16 Lipski S, Grabbe J, Henz BM. Absence of MHC class II antigen on mast cells at sites of inflammation in human skin. *Exp Dermatol.* 1996; 5:120-4.
- 17 Henz BM, Maurer M, Lippert U, Worm M, Babina M. Mast cells as initiators of immunity and host defense. *Exp Dermatol.* 2001; 10:1-10.
- 18 Frandji P, Oskeritzian C, Cacaraci F, Lapeyre J, Peronet R, David B, et al. Antigen-dependent stimulation by bone marrow-derived mast cells of MHC class II-restricted T cell hybridoma. *J Immunol.* 1993; 151:6318-28.
- 19 Wynn TA. Basophils trump dendritic cells as APCs for T(H)2 responses. *Nat Immunol.* 2009; 10:679-81.
- 20 Hayano T, Hirose M, Kikuchi M. Protein disulfide isomerase mutant lacking its isomerase activity accelerates protein folding in the cell. *FEBS Lett.* 1995; 377:505-11.
- 21 Robinson AS, Hines V, Wittrup KD. Protein disulfide isomerase overexpression increases secretion of foreign proteins in *Saccharomyces cerevisiae*. *Biotechnology. (NY).* 1994; 12:381-4.
- 22 Knol EF, Mul FP, Jansen H, Calafat J, Roos D. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol.* 1991; 88:328-38.
- 23 Monneret G, Benoit Y, Debard AL, Gutowski MC, Topenot I, Bienvenu J. Monitoring of basophil activation using CD63 and CCR3 in allergy to muscle relaxant drugs. *Clin Immunol.* 2002; 102:192-9.
- 24 Moneret-Vautrin DA, Sainte-Laudy J, Kanny G, Fremont S. Human basophil activation measured by CD63 expression and LTC4 release in IgE-mediated food allergy. *Ann Allergy Asthma Immunol.* 1999; 82:33-40.
- 25 Mahmudi-Azer S, Downey GP, Moqbel R. Translocation of the tetraspanin CD63 in association with human eosinophil mediator release. *Blood.* 2002; 99:4039-47.
- 26 Monneret G, Gutowski MC, Bienvenu J. Detection of allergen-induced basophil activation by expression of CD63 antigen using a tricolour flow cytometric method. *Clin Exp Immunol.* 1999; 115:393-6.
- 27 Liu Y, Edwards RH. The role of vesicular transport proteins in synaptic transmission and neural degeneration. *Ann Rev Neurosci.* 1997; 20:125-56.
- 28 Liu Y, Edwards RH. The role of vesicular transport proteins in synaptic transmission and neural degeneration. *Annual review of Neuroscience.* 1997; 20:125-56.
- 29 Akers IA, Parsons M, Hill MR, Hollenberg MD, Sanjar S, Laurent GJ, et al. Mast cell tryptase stimulates human lung fibroblast proliferation via protease-activated receptor-2. *Am J Physiol Lung Cell Mol Physiol.* 2000; 278:193-201.
- 30 Corvera CU, Dery O, McConalogue K, Bohm SK, Khitin LM, Caughey GH, et al. Mast cell tryptase regulates rat colonic myocytes through proteinase-activated receptor 2. *J Clin Investigation.* 1997; 100:1383-93.
- 31 Machado DC, Horton D, Harrop R, Peachell PT, Helm BA. Potential allergens stimulate the release of mediators of the allergic response from cells of mast cell lineage in the absence of sensitization with antigen-specific IgE. *Eur J Immunol.* 1996; 26:2972-80.
- 32 Dugina TN, Kiseleva EV, Glusa E, Strukova SM. Activation of mast cells induced by agonists of proteinase-activated receptors under normal conditions and during acute inflammation in rats. *Eur J pharmacol.* 2003; 471:141-7.
- 33 Reed CE, Kita H. The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol.* 2004; 114:997-1008.
- 34 Steinhoff M, Buddenkotte J, Shpacovitch V, Rattenholl A, Moormann C, Vergnolle N, et al. Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. *Endocr Rev.* 2005; 26:1-43.
- 35 Kollmann K, Damme M, Deuschl F, Kahle J, D'Hooghe R, Lüllmann_Rauch R, et al. Molecular characterization and gene disruption of mouse lysosomal putative serine carboxypeptidase 1. *FEBS J.* 2009; 276:1356-69.

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- 36 Stinchcombe JC, Griffiths GM. Regulated secretion from hemopoietic cells. *J Cell Biol.* 1999; 147:1-6.
- 37 Delpino A, Castelli M. The 78 kDa glucose-regulated protein (GRP78/BIP) is expressed on the cell membrane, is released into cell culture medium and is also present in human peripheral circulation. *Biosci Rep.* 2002; 22:407-20.
- 38 Coppinger JA, Cagney G, Toomey S, Kislinger T, Belton O, McRedmond JP, et al. Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood.* 2004; 103:2096-104.
- 39 Mathew A, Bell A, Johnstone RM. Hsp-70 is closely associated with the transferrin receptor in exosomes from maturing reticulocytes. *Biochem J.* 1995; 308:823-30.
- 40 Théry C, Duban L, Segura E, Veron P, Lantz O, Amigorena S. Indirect activation of naïve CD4+ T cells by dendritic cell-derived exosomes. *Nat Immunol.* 2002; 3:1156-62.
- 41 Hegmans JP, Bard MP, Hemmes A, Luijckx TM, Kleijmeer MJ, Prins JB, et al. Proteomic analysis of exosomes secreted by human mesothelioma cells. *Am J Pathol.* 2004; 164:1807-15.
- 42 Broquet AH, Thomas G, Masliah J, Trugnan G, Bachelet M. Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. *J Biol Chem.* 2003; 278:21601-6.
- 43 Pockley AG. Heat shock proteins as regulators of the immune response. *Lancet.* 2003; 362:469-76.
- 44 Johnson AD, Berberian PA, Bond MG. Effect of heat shock proteins on survival of isolated aortic cells from normal and atherosclerotic cynomolgus macaques. *Atherosclerosis.* 1990; 84:111-9.
- 45 Houenou LJ, Li L, Lei M, Kent CR, Tytell M. Exogenous heat shock cognate protein Hsc70 prevents axotomy-induced death of spinal sensory neurons. *Cell Stress Chaperones.* 1996; 1:161-6.
- 46 Smyth LJ, et al. Assessment of the molecular basis of the proallergic effects of cigarette smoke. *Environmental Science and Technology.* 2000; 34:1370-4.
- 47 Dudler T, Machado DC, Kolbe L, Annand RR, Rhodes N, Gelb MH, et al. A link between catalytic activity, IgE-independent mast cell activation, and allergenicity of bee venom phospholipase A2. *J Immunol.* 1995; 155:2605-13.
- 48 Leonard EJ, Yoshimura T. Human monocyte chemoattractant protein-1 (MCP-1). *Immunol Today.* 1990; 11:97-101.
- 49 Conti P, Pang X, Boucher W, Letourneau R, Reale M, Barbacane RC, et al. Impact of Rantes and MCP-1 chemokines on in vivo basophilic cell recruitment in rat skin injection model and their role in modifying the protein and mRNA levels for histidine decarboxylase. *Blood.* 1997; 89:4120-7.
- 50 Bischoff SC, Krieger M, Brunner T, Dahinden CA. Monocyte chemoattractant protein 1 is a potent activator of human basophils. *J Exp Med.* 1992; 175:1271-5.
- 51 Bryce PJ, Miller ML, Miyajima I, Tsai M, Galli SJ, Oettgen HC. Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity.* 2004; 20:381-92.
- 52 Azizkhan RG, Azizkhan JC, Zetter BR, Folkman J. Mast cell heparin stimulates migration of capillary endothelial cells in vitro. *J Exp Med.* 1980; 152:931-44.
- 53 Deuel TF, Senior RM, Chang D, Griffin GL, Henrikson RL, Kaiser ET. Platelet factor 4 is chemotactic for neutrophils and monocytes. *Proc Natl Acad Sci.* 1981; 78:4584-7.
- 54 Bendtsen JD, Nielsen H, Von Heijne G, Brunak S. Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol.* 2004; 340:783-95.
- 55 Muesch A, Hartmann E, Rohde K, Rubartelli A, Sitia R, Rapoport TA. A novel pathway for secretory proteins? *Trends Biochem Sci.* 1990; 15:86-8.
- 56 Kuchler K, Thorner J. Membrane translocation of proteins without hydrophobic signal peptides. *Curr Opin Cell Biol.* 1990; 2:617-24.

Appendix I. The table is representative of proteins identified at two biological replicates of secretory proteins collected from supernatant of non-activated RBL-2H3.1 cells, as control (C2 and C2), and activated cells for different time courses (90s, 30m and 3h). From total of 299 proteins identified with ESI-MS, sequence analysis by SignalP 3.0 recognized 65 of them as proteins consisting of signal sequences or signal anchor (C). Further analysis by SecretomeP 2.0 recognized 93 proteins as secretory proteins through non-classic pathway (NC). √ is representative of presence of each protein in the samples collected at different time courses and sequence coverage and Mascot scores are reported separately for each individual experiment in order.

Cat, Category; NS, Non-secretory.

| AC No | Protein name | Cat | C1 | C2 | 90s | 90s | 30m | 30m | 3h | 3h | Sequence coverage | Mascot score |
|--------|--|-----|----|----|-----|-----|-----|-----|----|----|----------------------------|---------------------------------|
| O08623 | Sequestosome-1 | NC | | | | | √ | | | | 2.51 | 47.49 |
| O08651 | D-3-phosphoglycerate dehydrogenase | NC | √ | | | | | | | | 3.00 | 74.27 |
| O35179 | Endophilin | NC | | | | | √ | | | | 3.69 | 32.31 |
| O35244 | Peroxiredoxin | NS | | √ | √ | | | | | | 13.39-4.46 | 74.04-34.99 |
| O35543 | Glutathione-requiring prostaglandin D synthase | NS | | | √ | √ | | | | | 6.53-7.54 | 43.74-80.96 |
| O35567 | Bifunctional purine biosynthesis protein PURH | C | √ | √ | √ | √ | | | | | 5.57-10.47-6.93-6.76 | 63.57-168.70-89.17-76.36 |
| O35760 | Isopentenyl-diphosphate Delta-isomerase 1 | NS | | | √ | | | | | | 5.29 | 75.38 |
| O35763 | Moesin | NC | √ | | √ | √ | √ | √ | | | 5.89-17.85-22.53-4.16-3.29 | 123.22-258.55-407.62-0.00-36.98 |
| O35787 | Kinesin-like protein KIF1C | NS | | √ | √ | √ | √ | | | | 0.73-2.01-0.73-0.73 | 0.00-0.00-34.33-31.36 |
| O35814 | Stress-induced-phosphoprotein 1 | NS | √ | | | | | | | | 1.84 | 42.52 |
| O54753 | Hydroxysteroid 17-beta dehydrogenase 6 | NC | √ | | | √ | | | | | 3.79-12.62 | 0.00-31.80 |
| O54975 | Xaa-Pro aminopeptidase 1 | NC | | √ | √ | | | | | | 2.57-4.82 | 0.00-62.13 |
| O70467 | Protein arginine N-methyltransferase 3 | NS | | | | | √ | √ | | | 1.52-3.79 | 27.24-31.88 |

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| | | | | | | | | | | | |
|--------|--|----|---|---|---|---|---|---|---|-------------------------------------|---|
| O70608 | Synaptonemal complex protein 2 | NS | | | | | √ | | | 1.59 | 29.62 |
| O88480 | Calcineurin-binding protein cabin-1 | NS | √ | √ | | √ | √ | | | 0.96-1.51-0.64-1.65 | 41.79-40.82-0.00-29.0 |
| O88600 | Heat shock 70 kDa protein 4 | NS | √ | | √ | | | | | 1.55-5.0 | 21.62-49.77 |
| O88761 | 26S proteasome subunit p112 | NS | | | | | | | √ | | 51.00 |
| O88767 | Protein DJ-1 | NC | | | √ | √ | | | | 14.29-14.29 | 81.44-113.87 |
| O88989 | Malate dehydrogenase, cytoplasmic | NS | √ | √ | √ | √ | | | | 19.16-23.65-17.96-12.87 | 79.21-250.42-174.09-126.23 |
| P00507 | Aspartate aminotransferase, mitochondrial | C | √ | √ | | √ | | | | 2.09-6.74-3.95 | 37.3-117.99-69.61 |
| P00770 | Mast cell protease 2 | C | | | | | √ | √ | | 8.91-4.86 | 91.82-91.69 |
| P00787 | Cathepsin B | C | | | | √ | √ | √ | | 5.31-13.86-13.86 | 116.40-378.97-134.92 |
| P01041 | Cystatin-B | NC | | | √ | √ | | | | 17.35-20.41 | 46.65-42.55 |
| P02401 | 60S acidic ribosomal protein P2 | C | √ | | | | | | | 16.52 | 22.47 |
| P02770 | Serum albumin | C | | | | | | √ | | 5.26 | 45.47 |
| P02793 | Ferritin light chain 1 | NS | | | | | | √ | √ | | 123-97 |
| P04256 | Heterogeneous nuclear ribonucleoprotein A1 | NS | √ | | | | | | | 8.13 | 65.75 |
| P04636 | Malate dehydrogenase, mitochondrial | NS | √ | √ | √ | √ | | √ | | 12.43-34.32-18.34-16.27-6.21 | 91.71-349.56-119.12-47.41-52.03 |
| P04642 | L-lactate dehydrogenase A chain | NC | √ | √ | √ | √ | √ | √ | | 38.25-31.02-32.83-34.34-13.86-24.10 | 774.99-685.81-382.12-456.01-199.27-400.62 |
| P04764 | Alpha-enolase | NC | √ | √ | √ | √ | | √ | √ | 44.24-46.31-9.22 | 691.15-906.52-165.99-73-60 |
| P04785 | Protein disulfide-isomerase | C | √ | | √ | √ | √ | | | 32.81-19.65-27.90-9.82 | 424.24-259.62-448.89-149.48 |
| P04797 | Glyceraldehyde-3-phosphate dehydrogenase | NC | √ | √ | √ | √ | | | | 13.81-8.71-12.01-12.01 | 125.32-50.84-92.01-105.45 |

| | | | | | | | | | | | | |
|--------|--|----|---|---|---|---|---|---|---|---|--|---|
| P04905 | Glutathione S-transferase Mu 1 | NS | √ | √ | √ | √ | | √ | | 7.34-13.76- 18.35-17.89-7.34 | 74.22-79.71-135.27- 135.80-54.48 | |
| P05065 | Fructose-bisphosphate aldolase A | NS | √ | √ | √ | √ | √ | √ | | 28.3-31.04- 18.96-24.18- 6.04-12.36 | 255.59-379.93- 270.88-239.48- 51.63-120.20 | |
| P05197 | Elongation factor 2 | NS | √ | √ | √ | √ | √ | √ | | 13.64-3.73-5.01- 15.85-4.20-1.52 | 221.38-107.84- 109.40-403.15- 44.60-48.64 | |
| P05369 | Farnesyl pyrophosphate synthetase | NC | √ | √ | √ | √ | √ | √ | | 21.53-21.53- 18.98-18.98- 4.25-4.25 | 216.46-341.46- 224.38-249.87- 24.23-37.79 | |
| P05370 | Glucose-6-phosphate 1-dehydrogenase | NS | | | √ | √ | | | | 3.11-7.77 | 41.44-36.13 | |
| P05765 | 40S ribosomal protein S21 | NC | √ | | | | | | | 10.84 | 28.59 | |
| P05982 | NAD(P)H dehydrogenase 1 | NS | √ | √ | √ | √ | | | | 22.99-24.82- 32.12-39.42 | 141.15-275.75- 371.98-402.31 | |
| P06761 | 78 kDa glucose-regulated protein | C | √ | √ | √ | √ | √ | | √ | √ | 39.6-6.12-7.19- 6.57-7.03 | 986.74-104.65- 77.25-231.10- 107.30-63-88 |
| P06765 | Platelet factor 4 | C | | | | | √ | √ | √ | √ | 22.86-22.86 | 98.85-67.02-66-67 |
| P07150 | Annexin A1 | NS | √ | √ | √ | √ | √ | √ | | | 2.6-2.6-2.60- 7.23-2.6-2.6 | 30.83-30.86-30.51- 93.57-0.00-33.01 |
| P07151 | Beta-2-microglobulin | C | | √ | | | √ | √ | | | 7.56-7.56-7.56 | 58.73-80.77-46.44 |
| P07632 | Superoxide dismutase | NC | √ | √ | √ | √ | √ | √ | √ | √ | 32.47-38.96- 28.57-36.36- 7.14-32.47 | 139.35-271.42- 148.90-267.24- 36.08-91.10-73-82 |
| P07824 | Arginase-1 | NC | √ | √ | | | √ | | | | 2.48-6.81-2.48 | 0.00-28.15-0.00 |
| P07896 | enoyl-Coenzyme A, hydratase/3-hydroxyacyl Coenzyme A dehydrogenase | NC | | | | | | | √ | | | 50.00 |
| P07943 | Aldose reductase | NS | | √ | √ | √ | | | | | 4.43-7.28-7.28 | 46.11-57.13-70.88 |
| P08699 | Galectin-3 | NC | | | | √ | | | | | 4.20 | 48.02 |
| P09117 | Fructose-bisphosphate aldolase C | NS | | | | √ | | | | | 4.13 | 58.84 |

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| | | | | | | | | | | | | |
|--------|---|----|---|---|---|---|---|---|---|---|-------------------------------------|---|
| P09456 | cAMP-dependent protein kinase type I-alpha regulatory subunit | NS | √ | √ | √ | | | | | | 2.62-10.24-8.66 | 0.00-49.79-50.74 |
| P09495 | Tropomyosin alpha-4 chain | NS | | | √ | | | | | | 18.15 | 84.08 |
| P0C5Y8 | Alsin | NS | | | | √ | | | | | 1.94 | 31.11 |
| P10111 | Peptidyl-prolyl cis-trans isomerase A | NS | √ | √ | √ | √ | √ | √ | √ | √ | 53.05-42.07-49.39-57.32-21.95-33.54 | 481.60-393.15-392.04-466.26-162.64-247.22-59-46 |
| P10719 | ATP synthase subunit beta, mitochondrial | C | | √ | | | | | | | 6.05 | 75.93 |
| P10760 | Adenosylhomocysteinase | NC | √ | | √ | √ | | | | | 6.94-2.31-10.88 | 34.62-34.78-121.26 |
| P10867 | L-gulono-gamma-lactone oxidase precursor | NC | | | | | | | √ | | | 40.00 |
| P10960 | Sulfated glycoprotein 1 | C | | | √ | √ | √ | √ | √ | √ | 8.66-3.79-15.88-14.98 | 111.00-39.75-211.00-231.63-55-57 |
| P11030 | Acyl-CoA-binding protein | NS | √ | √ | √ | √ | | | | | 11.49-41.38-42.53-3.79 | 35.57-119.50 |
| P11232 | Thioredoxin | NC | √ | √ | √ | √ | | | √ | √ | 22.86-22.86 | 108.99-124.66-125.16-39.75-76-66 |
| P11348 | Dihydropteridine reductase | NS | | | | √ | | | | | 5.39 | 49.69 |
| P11506 | Plasma membrane calcium-transporting ATPase 2 | NS | | | √ | √ | | | | | 1.05-2.49 | 0.00-34.05 |
| P11598 | Protein disulfide-isomerase A3 | C | √ | | | √ | | | | | 13.66-7.13 | 126.03-99.29 |
| P11762 | Galectin-1 | NS | | | √ | √ | | | | | 8.89-8.89 | 43.77-46.76 |
| P11980 | Pyruvate kinase isozymes M1/M2 | NS | √ | √ | √ | √ | | | √ | | 12.05-13.94-18.08-23.16-2.45 | 312.19-327.55-268.53-352.70-79.46 |
| P12785 | Fatty acid synthase | NS | √ | | √ | √ | | | | | 1.2-1.96-1.88 | 159.53-141.46-183.61 |
| P13221 | Aspartate aminotransferase, cytoplasmic | NS | | √ | √ | √ | | | | | 11.14-13.56-11.14 | 191.78-150.25-97.42 |

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| P13383 | Nucleolin | NS | √ | | | | | | | 4.35 | 97.75 |
| P13444 | S-adenosylmethionine synthetase isoform type-1 | NS | | | | | √ | √ | | 3.53-3.53 | 30.5-0.00 |
| P13668 | Stathmin | NC | √ | √ | √ | | | | | 8.72-8.72-8.72 | 30.9-52.61-43.45 |
| P13697 | NADP-dependent malic enzyme | NS | | | √ | | | | | 3.15 | 39.30 |
| P14844 | C-C motif chemokine 2 | C | | √ | √ | √ | √ | √ | √ | 8.78-8.78-8.78-8.78 | 52.99-48.45-50.3-55.82-46-37 |
| P15429 | Beta-enolase | NS | | √ | | | | √ | √ | 17.97-6.22 | 345.52-153.20-68-46 |
| P15865 | Histone H1.2 | NS | √ | √ | √ | √ | | | | 15.53-15.53-19.63-15.53 | 72.75-171.44-147.03-131.71 |
| P16391 | RT1 class I histocompatibility antigen, AA alpha chain | C | | √ | | | √ | √ | | 3.5-9.97-8.89 | 73.81-155.79-111.79 |
| P16617 | Phosphoglycerate kinase 1 | NS | √ | √ | √ | √ | | | | 7.67-21.58-31.65-33.57 | 64.32-327.74-492.91-586.21 |
| P16638 | ATP-citrate synthase | NC | √ | √ | √ | √ | | √ | | 2.82-3.45-5.27-8.18-3.73 | 51.69-43.22-178.47-208.88-66.34 |
| P16884 | Neurofilament heavy polypeptide | NS | √ | | | √ | | | | 1.77-1.77 | 25.84-32.92 |
| P17046 | Lysosome-associated membrane glycoprotein 2 | C | | | | | √ | √ | | 4.87-4.87 | 176.07-99.24 |
| P17246 | Transforming growth factor beta-1 | C | | | | | √ | √ | | 5.13-5.64 | 112.56-36.31 |
| P17425 | Hydroxymethylglutaryl-CoA synthase, cytoplasmic | NC | | √ | | √ | | | | 2.31-4.23 | 54.85-69.96 |
| P18265 | glycogen synthase kinase 3 alpha | NS | | | | | | | √ | | 43.00 |
| P18418 | Calreticulin | C | √ | | √ | √ | √ | | | 12.26-10.82-10.82-3.61 | 155.22-178.53-169.08-64.53 |
| P19527 | Neurofilament light polypeptide | NS | √ | | | | √ | | | 2.77-1.85 | 25.84-0.00 |
| P19804 | Nucleoside diphosphate kinase B | NS | √ | √ | √ | √ | √ | √ | √ | 56.58-62.50-42.11-42.11-14.47-39.47 | 214.93-263.12-152.36-147.48-50.15-91.30-40-42 |
| P19944 | 60S acidic ribosomal protein P1 | NS | √ | | | | | | | 14.04 | 72.42 |

Secretory proteins in RBL-2H3.1 cells

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| P20411 | High affinity immunoglobulin epsilon receptor subunit gamma | C | | | | | √ | | | | 15.12 | 65.14 |
| P22985 | Alpha-internexin | NS | √ | | | | | | | | 3.76 | 25.84 |
| P23565 | Granulins | NS | | | | | √ | √ | | | 2.72-7.65 | 108.31-122.53 |
| P24155 | Thimet oligopeptidase | NS | | √ | √ | | | | | | 2.04-3.49 | 98.29-122.13 |
| P24268 | Cathepsin D | C | √ | √ | √ | √ | √ | √ | √ | √ | 7.13-4.67-28.01- 23.83-26.04- 37.10 | 40.39-32.04-273.87- 365.77-403.41- 709.39-65-73 |
| P24623 | Alpha-crystallin A chain | NC | | | | | √ | | | | 4.08 | 38.97 |
| P26772 | 10 kDa heat shock protein, mitochondrial | NC | √ | √ | √ | √ | | | √ | | 32.35-43.14- 25.49-35.29- 11.76 | 138.78-250.79- 160.85-155.31- 63.35 |
| P27139 | Carbonic anhydrase 2 | NC | √ | √ | √ | √ | | | | | 9.62-9.62-6.15- 6.15 | 0.00-64.31-69.74- 88.84 |
| P29315 | Ribonuclease inhibitor | NC | | | √ | √ | | | | | 9.65-16.23 | 113.18-130.19 |
| P29598 | Urokinase-type plasminogen activator | C | | | | | √ | √ | | | 1.85-1.85 | 40.61-27.88 |
| P29994 | Inositol 1,4,5-trisphosphate receptor type 1 | NS | | | | | √ | √ | | | 1.2-0.47 | 37.22-36.56 |
| P30337 | N-chimaerin | NS | √ | | | | | | | | 3.59 | 20.22 |
| P30904 | Macrophage migration inhibitory factor | NC | √ | √ | √ | √ | | | | | 7.83-17.39- 17.39-17.39 | 78.06-89.12-119.20- 120.65 |
| P31000 | Vimentin | NC | √ | √ | √ | √ | √ | √ | √ | √ | 37.98-25.97- 19.53-33.05-3.0- 12.45 | 621.97-524.82- 374.26-583.57- 42.98-183.95-39-28 |
| P31044 | Phosphatidylethanolamine-binding protein 1 | NC | √ | √ | √ | √ | | | | √ | 18.18-30.48- 41.71-29.95 | 103.60-342.98- 221.68-197.33-35 |
| P31977 | Ezrin | NC | √ | √ | | | | | | | 4.27-2.90 | 57.01-54.37 |
| P34058 | Heat shock protein HSP 90-beta | NS | √ | | | | √ | √ | | | 14.92-1.38-2.90 | 211.41-0.00-0.00 |
| P34926 | microtubule-associated protein 1 A | NS | | | | | | | | √ | | 39.00 |

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| P35439 | Glutamate [NMDA] receptor subunit zeta-1 | C | | | | √ | √ | 1.71-2.67 | 31.02-0.00 |
| P35559 | Insulin-degrading enzyme | C | √ | √ | | | | 2.65-1.57 | 45.79-51.21 |
| P35704 | Peroxiredoxin-2 | NC | | √ | | | | 9.09 | 91.20 |
| P35738 | 2-oxoisovalerate dehydrogenase subunit beta, mitochondrial | C | √ | | | | | 3.33 | 30.18 |
| P35952 | Low-density lipoprotein receptor | C | | | | √ | √ | 1.82-1.82 | 71.61-62.91 |
| P36972 | Adenine phosphoribosyltransferase | NS | √ | √ | √ | √ | | 18.33-7.22- 23.89-42.22 | 131.76-77.38- 125.04-309.08 |
| P38983 | 40S ribosomal protein SA | NC | √ | √ | √ | √ | √ | 31.19-9.49-4.41- 5.76-4.07 | 158.29-180.94- 56.55-68.32-0.00 |
| P42123 | L-lactate dehydrogenase B chain | NC | | | | √ | | 6.29 | 76.37 |
| P45592 | Cofilin-1 | NC | √ | √ | √ | √ | | 38.55-6.63- 39.16-49.40 | 149.36-38.48- 129.11-363.28 |
| P46462 | Transitional endoplasmic reticulum AT-Pase | NS | √ | | √ | √ | √ | 14.14-2.98- 49.40-3.23-3.72 | 356.60-79.34- 363.28-0.00-91.16 |
| P47942 | Dihydropyrimidinase-related protein 2 | NS | | | √ | √ | | 4.72-7.87 | 108.43-207.44 |
| P48500 | Triosephosphate isomerase - | NS | √ | √ | √ | √ | | 36.55-36.14- 49.40-46.18 | 466.96-532.95- 384.34-428.07 |
| P48675 | Desmin | NC | √ | √ | | | | 7.25-5.33 | 54.14-47.77 |
| P48768 | Sodium/calcium exchanger 2 | C | | | √ | √ | √ | 1.09-1.09-1.09- | 0.00-0.00-0.00-47 |
| P49088 | Asparagine synthetase | NS | | | √ | | | 1.60 | 44.97 |
| P50137 | Transketolase | NS | √ | √ | √ | √ | √ | 18.14-20.87- 16.85-24.56 | 254.51-380.44- 452.44-382.75-52- 44 |
| P50398 | Rab GDP dissociation inhibitor alpha | NS | | | | √ | | 10.07 | 112.92 |
| P50878 | 60S ribosomal protein L4 | NS | √ | | | | √ | 2.85-2.85 | 28.22-0.00 |
| P51156 | Ras-related protein Rab-26 | NC | √ | | | | | 7.78 | 18.38 |

Secretory proteins in RBL-2H3.1 cells

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| P51635 | Alcohol dehydrogenase [NADP+] | NS | √ | √ | √ | √ | | | 5.85-10.46-9.54-18.15 | 76.05-120.54-113.58-96.86 |
| P52296 | Importin subunit beta-1 | NC | √ | | √ | √ | √ | | 1.83-3.43-4.34-1.83 | 41.33-59.56-76.00-25.00 |
| P52953 | Homeobox protein MSX-2 (Fragment) | NC | √ | | √ | √ | | | 7.19-7.19-7.19 | 30.83-0.00-0.00 |
| P55063 | heat shock protein 70 | NS | | | | | | √ | √ | 74-96 |
| P58771 | Tropomyosin alpha-1 chain | NC | | | √ | | √ | | | 8.10-2.11 32.60-0.00 |
| P60712 | Actin, cytoplasmic 1 | NS | √ | √ | √ | √ | √ | √ | | 50.4-45.33-48.00-52.00-20.0-20.0 950.52-638.24-787.69-801.45-224.76-342.03 |
| P61589 | Transforming protein RhoA | NC | | | | √ | | | | 12.44 70.25 |
| P61955 | Small ubiquitin-related modifier 2 | NC | √ | √ | √ | √ | | | | 12.63-12.63-12.63-12.63 71.15-47.37-67.50-67.54 |
| P61978 | Heterogeneous nuclear ribonucleoprotein K | NS | √ | | | | | | | 2.59 68.24 |
| P62144 | Calmodulin | NC | √ | √ | √ | √ | | | | 16.11-11.41-11.41-11.41 55.27-34.56-71.52-48.73 |
| P62261 | 14-3-3 protein epsilon | NS | √ | √ | √ | √ | | | | 11.76-4.71-9.41-11.41 91.64-66.61-92.26-48.73 |
| P62326 | Thymosin beta-4 | NC | √ | √ | √ | √ | √ | √ | | 43.18-43.18-54.55-54.55-29.55-29.55 63.51-104.37-125.91-134.21-59.45-48.46 |
| P62629 | Elongation factor 1-alpha 1 | NS | √ | √ | √ | √ | √ | √ | | 24.24-4.11-8.44-8.23-8.44-8.87 371.93-99.39-106.64-67.09-192.07-119.13 |
| P62630 | EF-1-alpha | NS | | | | | | | √ | √ 73-73 |
| P62631 | Elongation factor 1-alpha 2 | NS | | | | √ | | | | 5.40 68.42 |
| P62634 | Cellular nucleic acid-binding protein | NC | | | | √ | | | | 8.47 54.09 |
| P62755 | 40S ribosomal protein S6 | NS | | | | | √ | | | 11.24 23.89 |
| P62803 | Histone H4 | NS | √ | | | | √ | | | 11.65-20.39 53.51-49.88 |

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| P62805 | histone H4/o | NS | | | | | | √ | √ | | 63-68 |
| P62822 | Ras-related protein Rab-1A | NS | √ | | | | | | | 7.80 | 20.44 |
| P62839 | Ubiquitin-conjugating enzyme E2 D2 | NC | | | | √ | | | | 16.33 | 49.07 |
| P62960 | Nuclease-sensitive element-binding protein 1 | NC | | √ | √ | √ | | | | 5.90-5.90-5.90 | 146.6-84.59-58.83 |
| P62962 | Profilin-1 | NC | √ | √ | √ | √ | | | | 25.71-30.0- 12.86-31.43 44.74-44.74- | 81.90-145.07- 153.33-101.94 85.75-137.08-119- |
| P62988 | Ubiquitin | NC | √ | √ | √ | √ | √ | √ | | 44.74-44.74- 44.74-61.84 | 104.14-67.74- 115.95 |
| P62993 | Growth factor receptor-bound protein 2 | NS | | | | | | √ | | 6.45 | 25.61 |
| P63017 | Heat shock cognate 71 kDa protein | NS | √ | √ | √ | √ | √ | √ | | 28.79-21.05- 24.92-32.97- 5.73-21.52 | 691.19-623.50- 529.81-809.65- 115.25-471.89 |
| P63028 | Translationally-controlled tumor protein | NC | √ | √ | √ | √ | | | | 8.14-8.14-8.14- 15.70 | 91.63-64.70-90.79- 113.75 |
| P63037 | DnaJ homolog subfamily A member 1 | NS | √ | | √ | | | | | 2.02-2.02 | 25.17-0.00 |
| P63101 | 14-3-3 protein zeta/delta | NS | √ | √ | √ | √ | √ | √ | | 35.51-18.78- 20.41-35.51- 14.29-9.39 | 327.22-157.23- 181.38-247.50- 78.22-140.09 |
| P63243 | Guanine nucleotide-binding protein subunit beta-2-like 1 | NS | √ | | | | | √ | | 3.47-2.21 | 49.81-0.00 |
| P63267 | actin, gamma 2 propeptide | NC | | | | | | | √ | | 117.00 |
| P63323 | 40S ribosomal protein S12 | NC | √ | √ | | | | | | 7.58-7.58 | 29.52-30.15 |
| P68254 | 14-3-3 protein theta | NS | √ | | √ | √ | | | | 22.45-14.69- 10.20 | 77.44-74.50-129.06 |
| P68361 | Tubulin alpha-1B chain | NS | √ | | √ | √ | | | | 33.7-26.39-27.94 | 522.01-401.83- 449.49 |
| P68509 | 14-3-3 protein eta | NS | √ | | √ | √ | | | | 19.11-6.10-13.41 | 51.20-63.17-129.34 |
| P80067 | Dipeptidyl-peptidase 1 | C | | | | | | √ | | 4.98 | 35.92 |

Secretory proteins in RBL-2H3.1 cells

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| P80254 | D-dopachrome decarboxylase | NS | | √ | √ | | | | 51.69-32.20 | 96.13-119.64 | |
| P85834 | Elongation factor Tu, mitochondrial | C | √ | | | | | | 2.65 | 23.06 | |
| P97526 | Neurofibromin | NS | √ | | | | | | 0.25 | 30.56 | |
| Q00715 | Histone H2B type 1 | NC | √ | | | | | √ | √ | 7.20 | 26.62-49-47 |
| Q01460 | Di-N-acetylchitobiase | C | | | | | | √ | | 4.09 | 55.99 |
| Q01827 | Synaptic vesicular amine transporter | C | | | | √ | √ | | | 3.3-3.30 | 137.54-156.58 |
| Q03555 | Chain B, Structural Basis Of Dynamic Glycine Receptor Clustering | NS | | | | | | √ | | | 46.00 |
| Q04462 | Valyl-tRNA synthetase | NS | √ | | | | | | | 2.14 | 69.26 |
| Q07647 | glucose transporter-3 | C | | | | | | √ | √ | | 70-68 |
| Q08163 | Adenylyl cyclase-associated protein 1 | NS | √ | √ | √ | √ | | | | 10.97-4.64-12.03-4.01 | 166.9-83.32-164.19-71.61 |
| Q09429 | ATP-binding cassette transporter sub-family C member 8 | NC | | | | √ | | | | 1.20 | 37.02 |
| Q13748 | Tubulin alpha-3C/D chain | NS | | √ | | | | | | 20.67 | 243.81 |
| Q13885 | Tubulin beta-2A chain | NC | | | | | | √ | | 6.74 | 49.37 |
| Q2KJ93 | Cell division control protein 42 homolog | NC | | √ | √ | √ | | | | 8.9-8.90-8.90 | 26.6-21.21-33.51 |
| Q2KJD0 | Tubulin beta-5 chain | NS | √ | √ | √ | √ | √ | | | 36.26-3.38-15.99-13.51-4.05 | 494.75-0.00-196.82-246.20-37.48 |
| Q2YDE4 | Proteasome subunit alpha type-6 | NS | √ | | | | | | | 5.28 | 74.44 |
| Q38PG0 | AHNAK 1 | NS | | | | | | √ | | | 46.00 |
| Q3B7T9 | Rab11 family-interacting protein 1 | NS | √ | | | | | | | 1.54 | 28.33 |
| Q3KQW9 | Uncharacterized protein C1orf31 homolog | C | | | √ | √ | | | | 10.4-10.40 | 45.71-39.49 |
| Q3MHU3 | Putative ATP-dependent RNA helicase TDRD9 | NC | √ | √ | √ | √ | | | | 0.57-0.57-0.57-0.57 | 0.00-0.00-28.90-33.86 |

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| Q3SZ65 | Eukaryotic initiation factor 4A-II | NS | √ | | √ | √ | | 6.39-5.90-5.90 | 72.69-66.23-78.96 |
| Q3T054 | GTP-binding nuclear protein Ran | NC | √ | √ | √ | √ | | 22.22-6.48- 22.22-19.44 | 99.67-50.74-125.10- 155.45 |
| Q3T0R1 | 40S ribosomal protein S18 | NS | √ | | | | √ | 5.26-5.26 | 47.77-0.00 |
| Q3T1H8 | Transmembrane protein 79 | NC | | | | | √ | 4.09 | 32.66 |
| Q3T1J1 | Eukaryotic translation initiation factor 5A-1 | NS | | | √ | | | 7.79 | 58.78 |
| Q3ZC07 | Actin, alpha cardiac muscle 1 | NC | √ | | | √ | | 29.97-30.50 | 367.34-382.40 |
| Q4AEF8 | Coatomer subunit gamma | NS | √ | | | | | 1.95 | 37.16 |
| Q4FZV5 | E2f8 protein | C | | | | | √ | | 54.00 |
| Q4G075 | Leukocyte elastase inhibitor A | NC | √ | √ | √ | √ | | 8.18-18.47- 27.18-32.19 | 64.01-172.30- 402.43-412.13 |
| Q4KM49 | Tyrosyl-tRNA synthetase, cytoplasmic | NS | √ | | | | | 3.98 | 57.95 |
| Q4KM73 | UMP-CMP kinase | C | | | | √ | | 6.63 | 59.67 |
| Q56JX6 | 40S ribosomal protein S28 | NC | √ | √ | √ | √ | | 17.39-17.39- 17.39-17.39 | 61.04-60.45-67.35- 47.53 |
| Q5BJP3 | Ubiquitin-fold modifier 1 | NS | | | | √ | | 17.65 | 20.62 |
| Q5E9B5 | Actin, gamma-enteric smooth muscle | NC | | √ | | | | 18.88 | 330.81 |
| Q5FVC7 | Centaurin, beta 2 | NS | | | | | √ √ | | 40-0 |
| Q5I0D1 | Glyoxalase domain-containing protein 4 | NC | | √ | | | | 5.70 | 62.95 |
| Q5KR61 | GPI mannosyltransferase 2 | NS | √ | √ | √ | √ | | 2.03-2.03-2.03- 2.03 | 0.00-46.45-0.00- 0.00 |
| Q5M819 | Phosphoserine phosphatase | NS | | | | √ | | 6.22 | 45.99 |
| Q5PQL7 | Integral membrane protein 2C | NS | | | | | √ √ | 12.27-15.99 | 181.7-173.61 |
| Q5PQS3 | Ventricular zone-expressed PH domain-containing protein homolog 1 | NS | √ | √ | | | √ √ | 2.52-2.16-0.84- 0.84 | 25.3-0.00-32.49- 0.00 |

Secretory proteins in RBL-2H3.1 cells

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| Q5RK10 | WD repeat-containing protein 1 | NC | √ | √ | √ | | 1.65-7.26-14.10 | 0.00-55.67-119.62 |
| Q5U1X1 | Oligoribonuclease, mitochondrial | NC | √ | | √ | | 7.59-7.59 | 85.81-67.90 |
| Q5U216 | ATP-dependent RNA helicase DDX39 | NC | √ | | | | 2.34 | 56.58 |
| Q5XHZ0 | Heat shock protein 75 kDa, mitochondrial | C | √ | | | | 3.40 | 37.75 |
| Q5XI22 | Acetyl-CoA acetyltransferase, cytosolic | NC | √ | | √ | | 7.81-2.77 | 48.94-41.58 |
| Q5XI32 | F-actin-capping protein subunit beta | NC | | | √ | | 9.19 | 57.05 |
| Q5XI57 | Probable tubulin polyglutamylase TTL10 | NS | | √ | | | 3.83 | 31.96 |
| Q5XI99 | Lysosome-associated membrane glycoprotein 3 | C | √ | √ | | √ | 3.43-3.43-0.00 | 0.00-25.16-0.00 |
| Q5XIN6 | LETM1 and EF-hand domain-containing protein 1, mitochondrial | NS | √ | √ | | √ | 1.62-1.62-3.25-6.36 | 30.72-32.74-37.68-36.47 |
| Q5XIS8 | Smith-Magenis syndrome chromosome region candidate gene 7 protein-like | NC | √ | | | | 4.10 | 24.03 |
| Q5XIT1 | Microtubule-associated protein RP/EB family member 3 | NS | √ | | | | 2.85 | 29.33 |
| Q61206 | Platelet-activating factor acetylhydrolase IB subunit beta | NS | √ | | | | 3.93 | 49.39 |
| Q61598 | Rab GDP dissociation inhibitor beta | NS | √ | √ | √ | √ | 6.74-2.47-11.46-8.99 | 164.87-35.03-134.05-149.71 |
| Q62611 | Interleukin-1 receptor-like 1 | C | | | | √ | 17.67-6.89 | 411.99-31.61 |
| Q62640 | Glutamate receptor delta-1 subunit | C | √ | | | | 0.89 | 33.53 |
| Q62658 | Peptidyl-prolyl cis-trans isomerase FKBP1A | NS | | √ | | | 12.04 | 66.87 |
| Q62736 | Peptidyl-prolyl cis-trans isomerase FKBP1A | NS | | √ | | | 12.04 | 66.87 |
| Q62826 | Heterogeneous nuclear ribonucleoprotein M | NS | √ | √ | √ | | 3.62-1.16-1.16 | 0.00-32.19-0.00 |
| Q62875 | Mast cell antigen 32 | NS | | | | √ | 5.24-5.24 | 69.55-60.10 |

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| Q62894 | Extracellular matrix protein 1 | C | | | | √ | √ | √ | 4.27 | 49.69-54.43 | |
| Q62967 | Diphosphomevalonate decarboxylase | NS | | √ | √ | | | | 2.99-3.49 | 0.00-52.51 | |
| Q62968 | Sodium channel protein type 10 subunit alpha | NS | | √ | √ | | | | 2.45-1.43 | 0.00-31.72 | |
| Q63016 | Large neutral amino acids transporter small subunit 1 | NC | | | | √ | | | 2.73 | 29.99 | |
| Q63081 | Protein disulfide-isomerase A6 | C | √ | | | | | | 3.18 | 79.90 | |
| Q63083 | Nucleobindin-1 | C | | | | √ | √ | | 4.79-2.83 | 51.08-58.81 | |
| Q63569 | 26S protease regulatory subunit 6A | NS | √ | | | | | | 2.28 | 24.20 | |
| Q63610 | Tropomyosin alpha-3 chain | NS | √ | √ | √ | | | √ | √ | 29.03-15.73-30.24 | 302.14-41.15-141.52-59.62 |
| Q63624 | serine arginine-rich pre-mRNA splicing factor SR-A1 | NS | | | | | | √ | | | 40.00 |
| Q63645 | Proteinase-activated receptor-2, G protein-coupled receptor 11 | C | | | | | | √ | | | 37.00 |
| Q63654 | polyubiquitin | NC | | | | | | √ | √ | | 139-157 |
| Q63679 | JmjC domain-containing histone demethylation protein 2A | NS | | | | | √ | | | 1.65 | 44.81 |
| Q63690 | Apoptosis regulator BAX | NC | | | √ | | | | | 6.77 | 47.71 |
| Q63716 | Peroxiredoxin-1 | NC | √ | √ | √ | √ | | | | 29.15-10.05-15.58-32.66 | 149.68-52.85-83.80-125.03 |
| Q63797 | Proteasome activator complex subunit 1 | NS | | | √ | √ | | | | 4.82-4.82 | 50.93-70.39 |
| Q641X3 | Beta-hexosaminidase subunit alpha | C | | | | | √ | | | 3.60 | 35.66 |
| Q64361 | Latexin | NS | | | √ | | | | | 6.73 | 60.47 |
| Q64559 | Cytosolic acyl coenzyme A thioester hydrolase | NS | | | √ | √ | | | | 5.77-2.89 | 34.29-39.86 |
| Q64598 | H2A histone | NC | | | | | | | √ | | 60.00 |
| Q66H12 | Alpha-N-acetylgalactosaminidase | C | | | | √ | | | | 2.65 | 53.93 |

Secretory proteins in RBL-2H3.1 cells

| | | | | | | | | | | | | | |
|--------|---|----|---|---|---|---|---|---|---|-----------------|-------------------|---------|---------------------|
| Q66HA4 | Tax1-binding protein 1 homolog | NS | | | | √ | | | | 5.17 | 83.61 | | |
| Q66HE5 | NUAK family SNF1-like kinase 2 | NC | √ | | | √ | | | | 1.59-1.59 | 0.00-27.55 | | |
| Q675B9 | Taste receptor type 2 member 109 | NC | | | | | | | √ | 5.00 | 44.67 | | |
| Q68FQ7 | RNA polymerase II-associated protein 3 | NS | | | | | | √ | √ | 1.82 | 26.13-43 | | |
| Q68FR6 | Elongation factor 1-gamma | NS | √ | | | | | | | 13.50 | 136.19 | | |
| Q68FS4 | Cytosol aminopeptidase | NS | | √ | √ | √ | | | | 3.08-10.60-3.08 | 35.08-47.82-34.14 | | |
| Q6AXR4 | Beta-hexosaminidase subunit beta | C | | | | | | | √ | 11.55 | 76.88 | | |
| Q6AY56 | Tubulin alpha-8 chain | NC | | | √ | | | | | 15.81 | 176.24 | | |
| Q6AYE5 | Out at first protein homolog | C | | | | | | √ | √ | 4.61-17.02 | 53.35-109.78 | | |
| Q6AYS3 | protective protein for beta-galactosidase | C | | | | | | | √ | | 46.00 | | |
| Q6AYZ1 | Tubulin alpha-1C chain | NS | | √ | | √ | √ | | | 3.34-6.46 | 78.97-122.37 | | |
| Q6F596 | Geranylgeranyl pyrophosphate synthetase | NS | | | | | | | √ | 6.67 | 20.92 | | |
| Q6MGD0 | Protein CutA | C | | | √ | √ | | | | 7.91-7.91 | 70.59-38.58 | | |
| Q6P6Q9 | Calcium-binding atopy-related autoantigen 1 | C | √ | | √ | √ | | | | 4.61-4.61-4.61 | 24.21-26.18-24.06 | | |
| Q6P799 | Seryl-tRNA synthetase, cytoplasmic | NS | √ | | | | | | | 4.10 | 49.62 | | |
| Q6P7A9 | Lysosomal alpha-glucosidase | C | | | | | | √ | √ | √ | √ | 6.4-6.4 | 135.47-126.05-87-74 |
| Q6PEC1 | Tubulin-specific chaperone A | NS | | | √ | √ | | | | 9.26-9.26 | 41.06-41.40 | | |
| Q6RI86 | NF-kappa B essential modulator | NS | | | | | | | √ | √ | | 44-0 | |
| Q6UPE1 | Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial | NC | | | | | | | √ | 2.60 | 23.12 | | |
| Q76HP2 | Transmembrane protein 132D | C | | | √ | | | | | 2.01 | 28.10 | | |

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|--------|--|----|---|---|---|---|---|---|---|------|-------------------------------|------------------------------------|
| Q76N24 | 40S ribosomal protein S4, X isoform | NC | √ | | | | | | | 3.80 | 28.80 | |
| Q794E4 | Heterogeneous nuclear ribonucleoprotein F | NS | | | | | | √ | | 4.10 | 28.06 | |
| Q794F9 | 4F2 cell-surface antigen heavy chain | NC | | | | | | | √ | √ | 15.37-4.17 | 306.63-67.55 |
| Q7TMA5 | Apolipoprotein B-100 | C | √ | | | √ | √ | √ | | | 0.55-0.53-1.86-2.34 | 69.88-0.00-183.20-232.38 |
| Q7TNB2 | Troponin T, slow skeletal muscle | NC | | √ | | | | | | | 3.07 | 31.69 |
| Q7TP47 | Heterogeneous nuclear ribonucleoprotein Q | NS | | | √ | | | | | | 4.50 | 55.17 |
| Q7TPK6 | Serine/threonine-protein kinase WNK4 | NS | √ | √ | √ | √ | | | | | 2.21-1.15-2.54-1.47 | 29.2-32.85-30.98-35.15 |
| Q80U96 | Exportin-1 | NC | √ | √ | √ | | | | | √ | 1.87-1.87-1.12-0.93 | 0.00-28.84-56.15-0.00 |
| Q8CHN5 | epididymal secretory protein E1 | C | | | | | | | √ | √ | | 47-0 |
| Q8CJB9 | E3 ubiquitin-protein ligase BRE1B | NC | √ | √ | √ | √ | √ | √ | | | 1.7-2.89-2.79-1.70-1.70-1.0 | 36.46-31.72-37.56-42.02-0.00-31.21 |
| Q8JZQ0 | Macrophage colony-stimulating factor 1 | C | | | √ | √ | √ | √ | | | 4.59-4.95-7.6-7.24 | 102.36-95.97-269.42-186.40 |
| Q8K586 | GTP-binding nuclear protein Ran, testis-specific isoform | NS | √ | | | | | | | | 18.98 | 55.29 |
| Q8R1M2 | Histone H2A.J | NC | √ | √ | √ | √ | √ | | | | 30.23-14.73-14.73-14.73-21.71 | 36.91-93.98-63.94-67.89-139.45 |
| Q91Y78 | Ubiquitin carboxyl-terminal hydrolase isozyme L3 | NC | | | √ | | | | | | 6.96 | 59.16 |
| Q91Z79 | Liprin-alpha-3 | NS | √ | √ | | | | | | √ | 0.92-0.92-2.94 | 0.00-27.03-0.00 |
| Q91ZW1 | Transcription factor A, mitochondrial | NC | √ | √ | | √ | | | | | 3.28-3.28-6.56 | 35.85-37.64-0.00 |
| Q920A6 | Retinoid-inducible serine carboxypeptidase | C | | √ | | | | | √ | √ | 2.43-3.98-6.42 | 0.00-60.51-92.56 |
| Q95577 | major histocompatibility complex class I | C | | | | | | | | √ | √ | 69-58 |
| Q99376 | Transferrin receptor protein 1 (Fragment) | NC | | | | | | | √ | √ | 1.45-1.45 | 39.44-45.12 |

Secretory proteins in RBL-2H3.1 cells

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|--------|--|----|---|---|---|---|---|---------------------------------|--|-------------|
| Q99MI7 | NEDD8-activating enzyme E1 catalytic subunit | NC | | | √ | | | 3.25 | 60.58 | |
| Q9DBJ1 | Phosphoglycerate mutase 1 | NS | √ | √ | √ | √ | √ | 18.9-22.05- 22.73-23.23-5.51 | 92.17-190.50- 165.44-193.94- 25.33 | |
| Q9EPB1 | Dipeptidyl-peptidase 2 | C | | | √ | | √ | 2.0-3.8 | 59.53-42.37 | |
| Q9EQS0 | Transaldolase | NS | √ | | √ | √ | | 8.61-3.86-9.50 | 26.31-77.44-108.89 | |
| Q9JHE5 | amino acid system A transporter | NC | | | | | √ | √ | 69-53 | |
| Q9JHU0 | Dihydropyrimidinase-related protein 5 | NS | | | | | √ | 1.42 | 29.99 | |
| Q9JI03 | Collagen alpha-1(V) chain | C | | √ | | | √ | 3.1-0.38 | 23.74-0.00 | |
| Q9JJ55 | GCD-10 | C | | | | | | √ | 29.00 | |
| Q9JLA3 | UDP-glucose:glycoprotein glucosyltransferase 1 | C | | | √ | | | 1.44 | 21.76 | |
| Q9JLH6 | CDK5 regulatory subunit-associated protein 1 | C | | √ | √ | √ | √ | 3.41-1.71-1.71- 1.71 | 25.06-30.74-0.00- 0.00 | |
| Q9JLJ3 | 4-trimethylaminobutyraldehyde dehydrogenase | NS | | | | √ | | 5.87 | 23.81 | |
| Q9JM53 | Apoptosis-inducing factor 1, mitochondrial | NS | | | | | √ | √ | 5.23-6.05 | 26.56-26.76 |
| Q9QYM0 | Multidrug resistance-associated protein 5 | NC | | | | | √ | √ | 2.02-0.56 | 30.65-30.97 |
| Q9QYP1 | Low-density lipoprotein receptor-related protein 4 | C | | | | √ | | 0.42 | 27.72 | |
| Q9QZI3 | Robo2 | C | | | | | | √ | 38.00 | |
| Q9R063 | Peroxiredoxin-5, mitochondrial | C | √ | √ | √ | √ | | 7.98-24.88- 33.80-24.88 | 76.6-147.76-114.54- 108.30 | |
| Q9VIF7 | Selenium binding protein 2 | NS | | | | | | √ | 41.00 | |
| Q9WV48 | synaptic SAPAP-interacting | NS | | | | | √ | √ | 47-0 | |
| Q9WV63 | Kinesin-like protein KIF2A | NC | | | | √ | | 1.99 | 22.48 | |

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|--------|--|----|---|---|-----------|-------------|
| Q9WVA1 | Mitochondrial import inner membrane translocase subunit Tim8 A | NC | √ | | 11.34 | 60.60 |
| Q9WVC7 | A-kinase anchor protein 6 | NS | | √ | | 38.00 |
| Q9Z0V6 | Thioredoxin-dependent peroxide reductase, mitochondrial | C | √ | | 9.73 | 189.75 |
| Q9Z1P2 | Alpha-actinin-1 | NS | √ | √ | 2.47-2.80 | 99.84-69.82 |