UNIVERSITY OF CRETE

FORTH
INSTITUTE OF MOLECULAR BIOLOGY & BIOTECHNOLOGY

Secretome Analysis of Lymphoma cells after reactivation of wild type p53

Diploma Thesis

Submitted
by
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Abstract

The aim of this diploma thesis is the comparative study of the proteins secreted by human lymphoma cell lines, before and after the reactivation of wt-p53 with Nutlin-3a. Nutlin-3a is a small molecule that acts as an antagonist of the p53-MDM2 complex. In particular, after the collection of the protein secretome of the cultured cells in serum-free medium, techniques such as separation of the proteins by electrophoresis into a polyacrylamide gel or gel free methods take place. These techniques are followed by the enzymatic digestion of the proteins with a selective protease and their identification and quantification by high sensitivity analysis with tandem mass spectrometry coupled with nano-liquid chromatography (nanoLC - MS/MS). Final step is the investigation of their interactions and their possible involvement in the prognosis, diagnosis and treatment of lymphomagenesis, followed by bioinformatics analysis.

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Abbreviations

1DGE	1 Dimensional Gel Electrophoresis
ABS	Ammonium Bicarbonate Solution
ACN	Acetonitrile
ALCL	Anaplastic Large Cell Lymphoma
CFSs	Colony-stimulating Factors
DLBCL	Diffuse Large B cell Lymphoma
DTT	Dithiothreitol
ECM	Extracellular Matrix
ER	Endoplasmatic Reticulum
ESI	Electrospray Ion Source
EVs	Extracellular Vesicles
FA	Formic Acid
FASP	Filter Aided Sample Preparation
FBS	Fetal Bovine Serum
GC	Germinal Center
GO	Gene Ontology
HL	Hodgkin Lymphoma
HRS	Hodgkin and Reed-Sternberg
IAA	Iodoacetamide
IFNs	Interferons
LC	Liquid Chromatography
MCL	Mantle Cell Lymphoma
MS	Mass Spectrometry
N3a	Nutlin 3a

NHL	Non Hodgkin Lymphoma
SDS	Sodium dodecyl sulfate
SP	Signal Peptide
TNFs	Tumor Necrosis Factors
TFA	Trifluoroacetic acid
UA	Urea

1. Introduction

1.1 Lymphoma

Lymphoma is a generic term that refers to a group of diseases characterized by disorderly or inappropriate proliferation of lymphoid or extramedullary reticuloendothelial tissue. It is the most common hematological malignancy affecting immune system cells, the lymphocytes. Although lymphoma appears to have been observed first by Malpighi in the mid-seventeenth century, credit for directing attention to this group of diseases belongs to Thomas Hodgkin, who described seven patients in 1832 (Sturgis, 1955). Numerous classifications have been proposed for this group of diseases that involve the lymphoid tissue primarily. The most widely used at the present time, is the classification between Hodgkin and Non Hodgkin Lymphoma.

Symptoms of lymphoma typically include swelling of the lymph nodes (subcutaneously, or deep in the body) or various systemic symptoms, including fever, weight loss or drenching night sweats.

1.1.1 Hodgkin Lymphoma (HL)

Hodgkin lymphoma (HL) is characterized by a minority of neoplastic cells, the Hodgkin and Reed-Sternberg cells (HRS cells). HRS cells in nearly all cases of HL derive from B cells and more specifically from preapoptotic germinal center B cells. They are 5 times larger than a normal B cell, often multinucleated with a peculiar morphology and an unusual immunophenotype that does not resemble any normal cell in the body. Despite their rarity in HL tissues, they are the clonal tumor cells of HL.

Many studies have documented that HL is associated with disturbed cytokine production. Cytokine and chemokine production may not only promote growth of HRS cells and help to evade immune surveillance, but also cause the characteristic histology and the clinical symptoms of HL. Cross-talk between HRS cells and surrounding lymphocytes has been studied for many years, and this interaction is regarded to be important for the pathogenesis of HL.

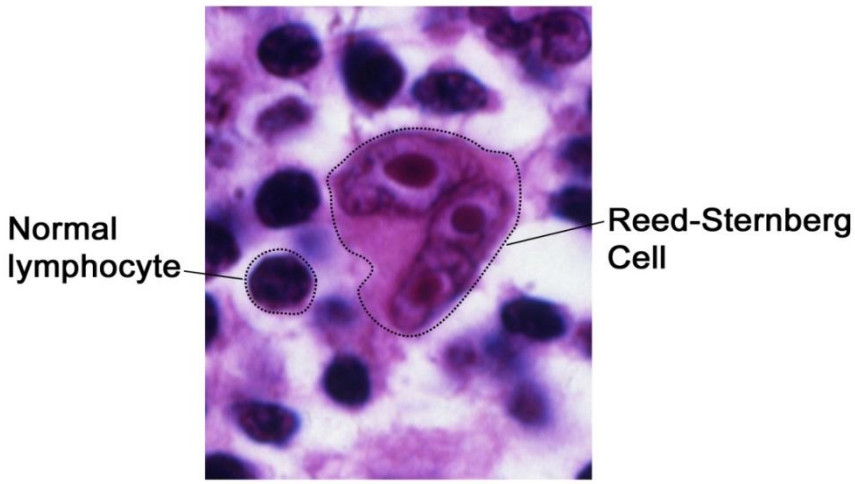


Figure 1: Reed-Sternberg Cell vs Normal Lymphocyte [23].

1.1.2 Non Hodgkin Lymphoma (NHL)

Non-Hodgkin lymphoma accounts for 90% of all the lymphoma cases and can be derived from B cells or T cells. B-cell lymphomas are the most common type of NHL, accounting for 85% of all NHL cases, according to the American Cancer Society. There are more than 60 different types of NHL. Some of them are: Mantle Cell Lymphoma (MCL), Anaplastic Large Cell Lymphoma (ALCL), Diffuse Large B Cell Lymphoma (DLBCL).

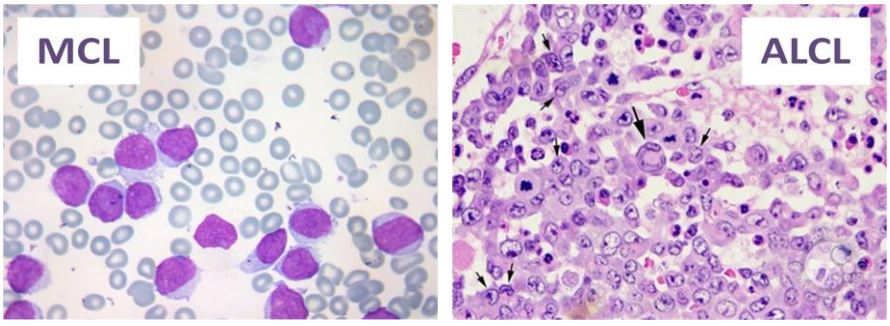


Figure 2: Non Hodgkin Lymphoma subtypes. Mantle Cell Lymphoma (left) and Anaplastic Large Cell Lymphoma (right) [24,25].

1.2 p53, a tumor suppressor protein

p53 is a potent tumor suppressor located at the nucleus and is a subject of intensive studies for more than 30 years. It is well established that p53 is a transcriptional factor activated by different types of stresses which regulates the expression of genes involved in control of cell cycle and cell death. Activated p53 can prevent the propagation of cells carrying oncogenic lesions via a multitude of pathways, i.e.: induction of growth arrest, senescence or apoptosis, autophagy, modulation of tumor stroma, angiogenesis and metabolism, as well as the block of

invasion and metastasis. This explains why loss of p53 function is selected for during tumor development, resulting in p53 inactivation in the majority of human tumors.

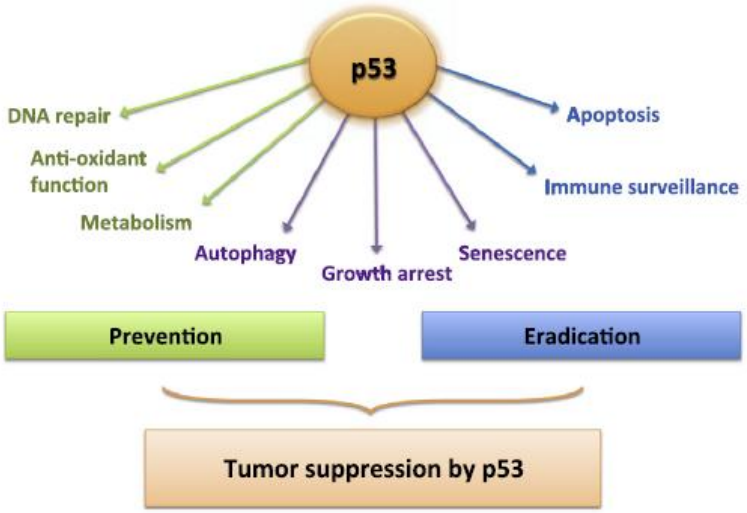


Figure 3: Different activities contribute to tumor prevention and tumor eradication by p53 [6].

1.2.1 MDM2, the negative regulator of p53

The signaling pathway regulated by the tumor suppressor gene p53 is inactivated in most cancers because of the activity of its negative regulator degrading enzymes. The most extensively studied p53 degrading enzyme is MDM2, which can inhibit p53 via a number of mechanisms. The most studied is MDM2 binding to the N-terminal transactivation domain of p53 which blocks its transcription function.

MDM2 also functions as a E3 ubiquitin ligase which promotes either monoubiquitination of p53 leading to enhanced nuclear export, or polyubiquitination of p53 that targets p53 for proteasomal degradation.

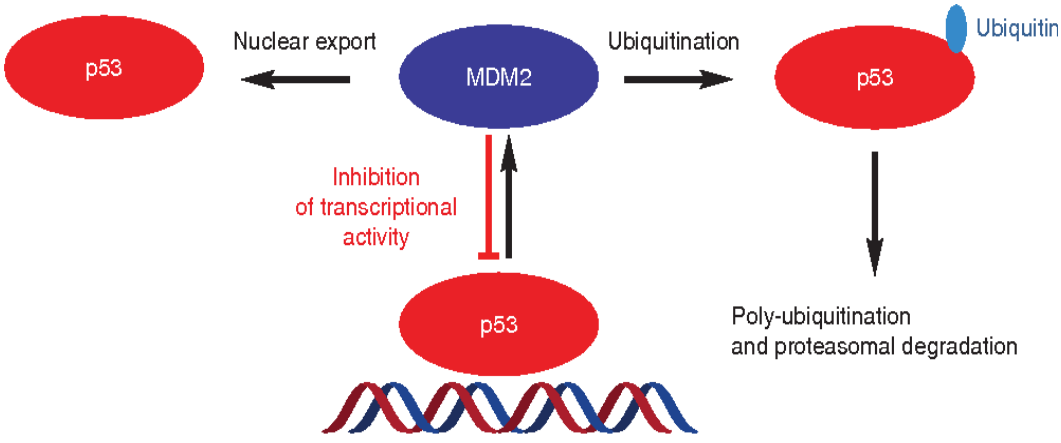


Figure 4: The MDM2 – p53 interaction [3].

Human cancers frequently have elevated levels of MDM2 leading to the inhibition of p53 function. This phenomenon has been reported in sarcomas, gliomas, hematological malignancies, melanomas, and carcinomas.

1.2.2 Nutlin 3a

In vitro and in vivo studies have shown, that reactivation of p53, either by genetic manipulation or by application of small molecules that specifically target the p53 pathway, can result in the elimination of tumors initiated by transforming events independent of p53. Accordingly, recent studies have shown that inhibition of MDM2 - p53 interaction, by using the recently developed small molecule Nutlin-3a, results in the stabilization and non-genotoxic activation of wt-p53 pathway in cancer cells, inducing apoptosis and/or cell cycle arrest.

Nutlin 3a is a cis-imidazoline analog and is known to displace p53 from MDM2 - p53 complex by blocking the MDM2 binding pocket in p53.

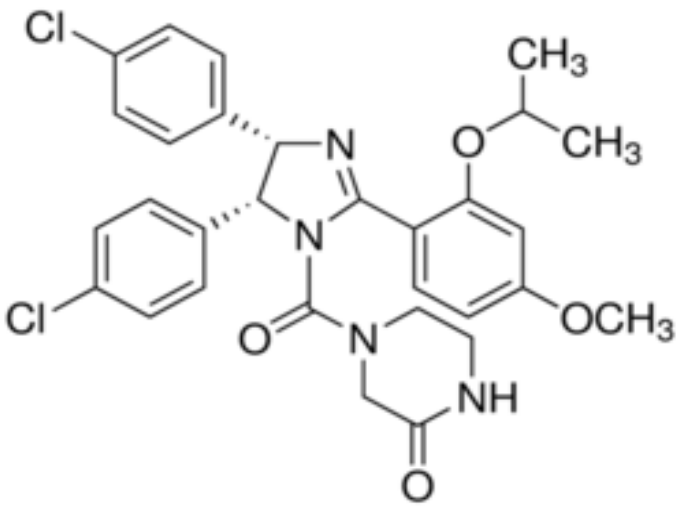


Figure 5: Nutlin-3a [26].

1.3 Cell Secretome

The term secretome refers to a set of proteins that includes extracellular matrix (ECM) proteins, proteins shed from the cell membrane, as well as intracellular proteins released into the supernatant as the result of vesiculation (e.g., from exosomes and microsomal vesicles) , cell lysis, apoptosis, and necrosis. Secreted proteins account for approximately 10% of the total proteins encoded by a genome. These proteins play important roles in homeostasis, immune response,

development, proteolysis, adhesion, extracellular matrix organization, cell migration, cell signaling and communication. Some examples of secretory proteins are:

- hormones
- digestive enzymes
- cytokines
- chemokines
- interferons (IFNs)
- colony-stimulating factors (CSFs)
- growth factors
- tumor necrosis factors (TNFs)

Cancer cell lines secretome has been considered fundamental for the identification of hallmarks of cancer such as uncontrolled proliferation, reduced apoptosis, invasion and metastasis, alteration in energy metabolism or resistance against anti-cancer therapy.

1.3.1 Secretory Pathways

Proteins can be released from cells into the extracellular space through two main kinds of mechanisms: the classical and the non-classical secretory pathways.

1.3.1.1 Classical Secretory Pathway

The classical secretory pathways, also known as conventional secretion, implicate the activation of intracellular signaling pathways that target proteins characterized by a signal peptide located at the N-terminus, to the endoplasmatic reticulum (ER) and subsequently to the Golgi complex, from which vesicles or storage granules, incorporate those proteins for the release in the extracellular space.

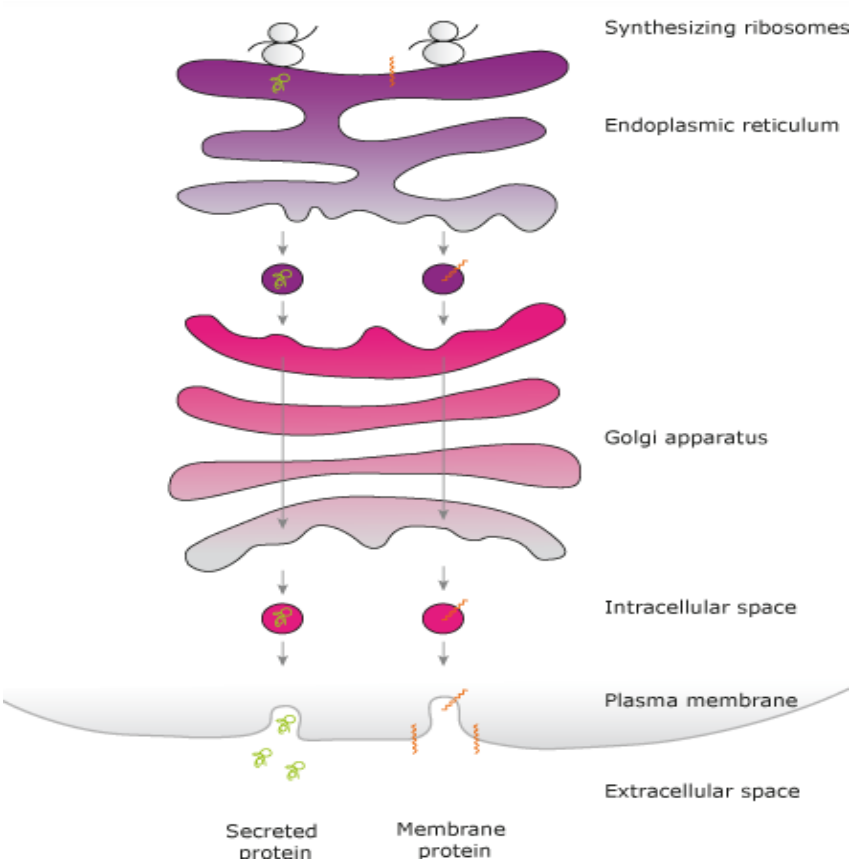


Figure 6: The classical secretory pathway [27].

The N-terminal cleavable signal peptide (SP) is typically 15–30 amino acids long. Signal peptides do not show high sequence homology, but they share the same structural composition: a hydrophobic core flanked by hydrophilic N- and C-terminal regions, with conserved amino acids at the −3 and −1 positions relative to the cleavage site.

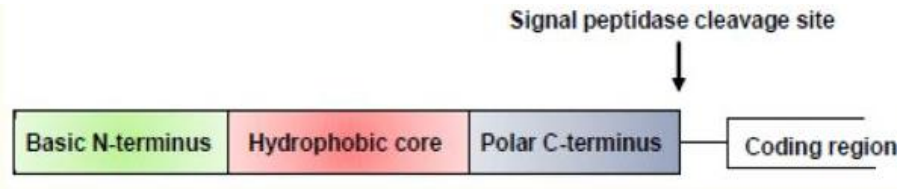


Figure 7: Structural composition of the signal peptide [7].

1.3.1.2 Non Classical Secretory Pathway

In the non-classical secretory pathways (unconventional secretion pathways), proteins lack the N-terminal signal peptide, and are secreted into the extra-cellular space by ER to Golgi-independent ways. These types of secretion include the non-vesicular and the vesicular transport. The first one implicates the direct

translocation of cytoplasmic proteins across the plasma membrane or the ABC transporter-based secretion. Instead, vesicular transport comprises, the autophagic-based secretion mediated by autophagosome-like vesicles (i.e. lysosome, microvesicles, multivesicular bodies), and Golgi Bypass.

Among the non-classical secretion modes also fall the release by cells of membranous vesicles defined as extracellular vesicles (EVs). EVs are spherical particles enclosed by a phospholipid layer classified as microvesicles (ectosome), exosomes and apoptotic bodies based on their size (ranging from 30 nm to 1000 nm in diameter), lipid and protein composition, and biogenesis morphology. Exosomes are EV having a size of 40–100 nm in diameter, present in many biological fluids such as urine, saliva, blood, amniotic fluid as well as conditioned media of cells.

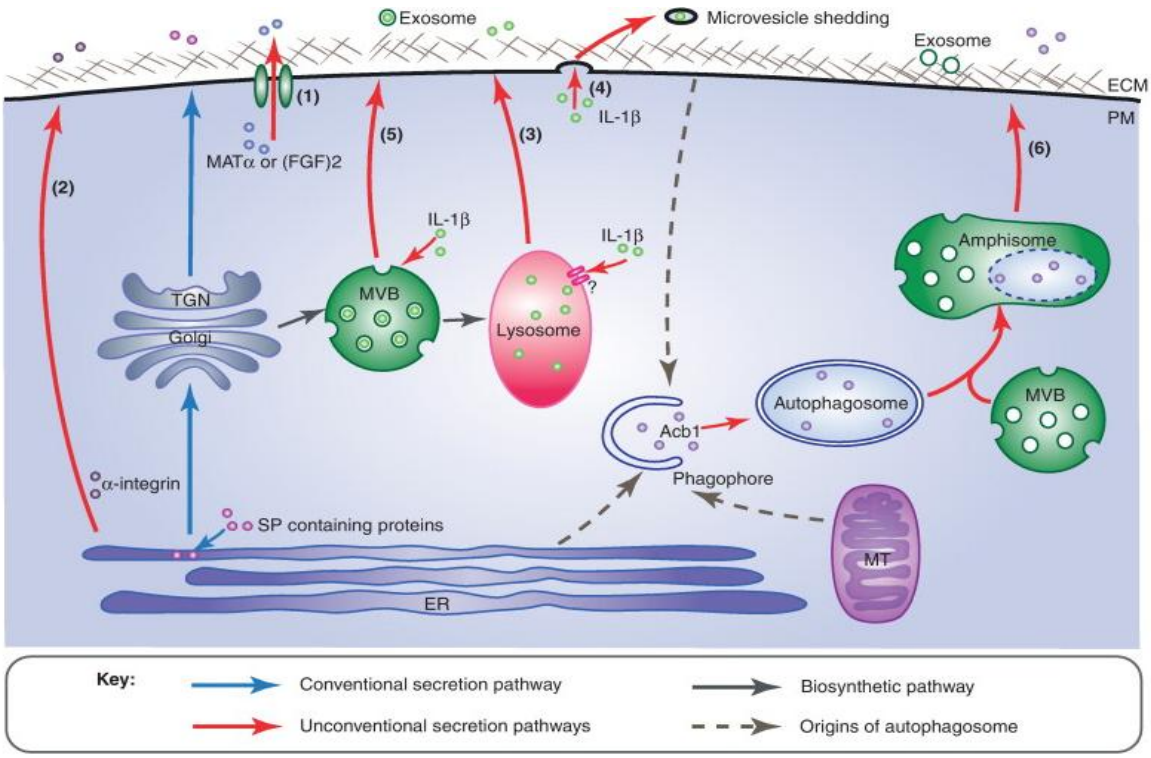


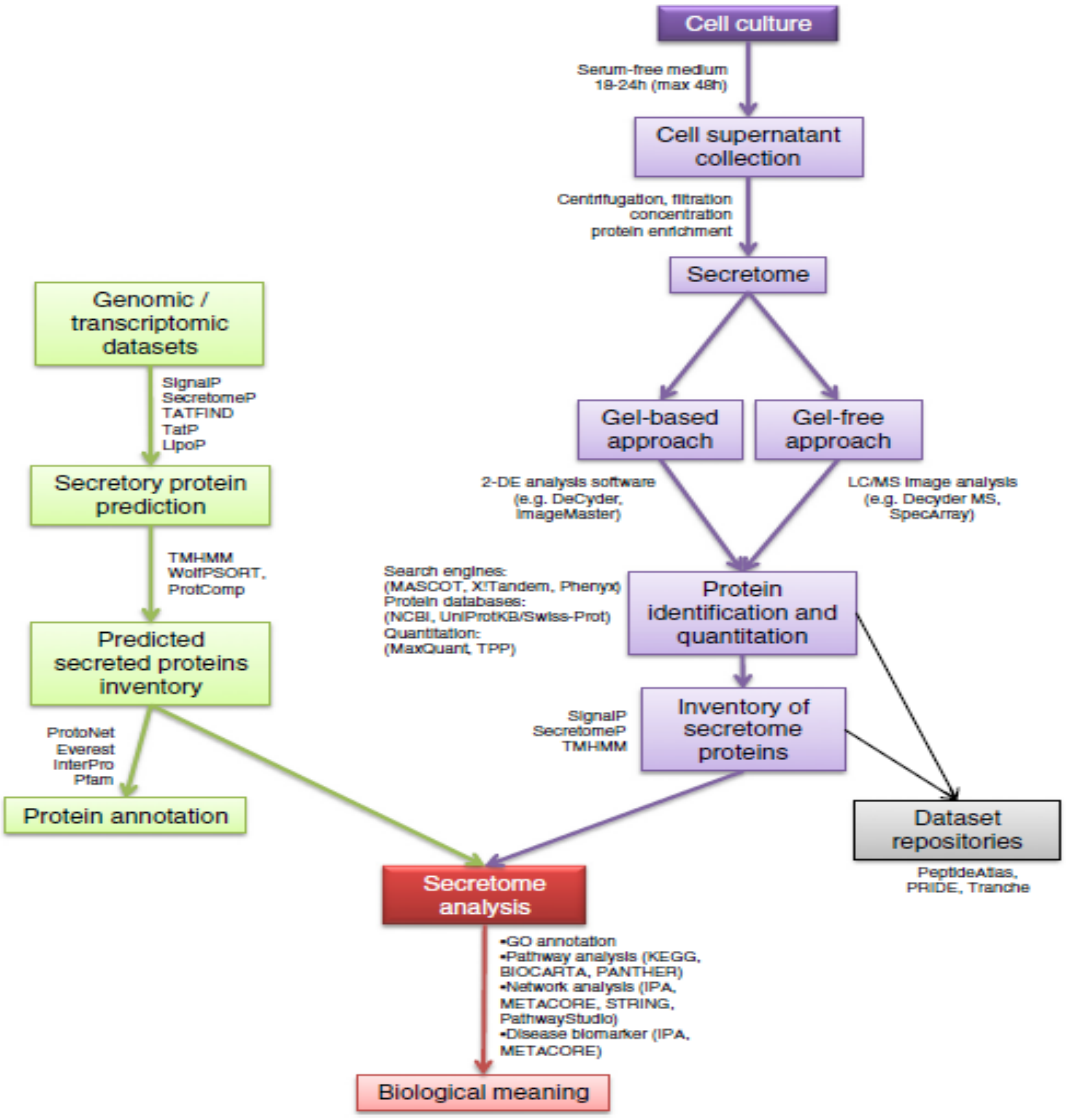
Figure 8: The Non Classical Secretory Pathway [10].

1.3.2 Methods for Secretome Analysis

The majority of secretome studies are conducted in vitro using cell culture methods in which secreted proteins are obtained from conditioned media of serum-starved cultured cell lines after 16 - 24 hours of incubation. An obstacle in the study of actively secreted proteins in conditioned medium is the passive release of highly abundant serum proteins such as albumin, transferrin and proteins secreted by dead cells into the media. These proteins may mask and dilute the secretome

making its isolation difficult. For that reason, cells are often incubated in serum-free media for only a small period of time, such as 24 hours. A disadvantage of culturing cells in medium without serum is the reduced viability and proliferation potential.

After pre-processing manipulations (centrifugation, filtration, etc.), a protein-enriched secretome can be fractionated by gel-based or gel-free approaches. These studies routinely employ high-resolution separation techniques, such as one or two-dimensional gel electrophoresis and/or liquid chromatography, in combination with advanced mass spectrometric methods for the unequivocal identification and quantification of peptides and proteins in samples. These secretome protein lists are analyzed then with several bioinformatic tools in the context of the biological meaning. (Fig. 9)



<u>Figure 9:</u> Workflow of secretome analysis with indications of the major bioinformatics tools available for each step. In silico secretome analysis (green), experimental secretome analysis (purple) [8].

2. Materials and Methods

2.1 Cell lines and culture

This study included three human cell lines from three different subtypes of Hodgkin and Non-Hodgkin Lymphoma:

Cell Line	Lymphoma Classification	Lymphoma Subtype	Origin	Genotype
MDA-V	Hodgkin Lymphoma	-	preapoptotic GC B cells	B-cell
JMP-1	Non-Hodgkin Lymphoma	Mantle Cell Lymphoma (MCL)	blastoid variant of MCL, pre-GC B cells of the mantle zone	B-cell
SUP-M2	Non-Hodgkin Lymphoma	Anaplastic Large Cell Lymphoma (ALCL)	T- or null-cell lineage	T-cell

Table 1: Cell lines used for this study and their lymphoma classification, subtype and cell origin.

The cells cultured in RPMI 1640 without serum for 24 hours and then stored at -80^{o}C until further processing with 1 dimensional gel electrophoresis or FASP (Filter Aided Sample Preparation). Protein concentration was measured by Bradford assay.

2.2 Sample Preparation

2.2.1 Gel-based Method: One Dimensional SDS-PAGE and Tryptic Digestion

Electrophoresis is used to separate complex mixtures of proteins (e.g., from cells, subcellular fractions), to identify proteins and to verify homogeneity of protein samples. It can also serve to purify proteins for use in further applications. In polyacrylamide gel electrophoresis, proteins migrate in response to an electrical field through pores in a polyacrylamide gel matrix in the presence of sodium dodecyl sulfate (SDS). The anionic detergent binds tightly to most proteins, imparting a negative charge to the resultant complexes. Most electrophoresis is done in vertical chambers in gel slabs formed between two glass plates. Visualization of the bands was performed overnight by Coomassie Brilliant Blue G-

250 based staining. The protein bands were excised and cut into 23 to 24 small pieces. Then they stored at the freezer in 1,5 ml LoBind Eppendorf vials.

An example of an SDS-PAGE gel 10% from one of the three cell lines is presented below. 35 µL of the samples loaded in each well after their redilution with sample buffer.

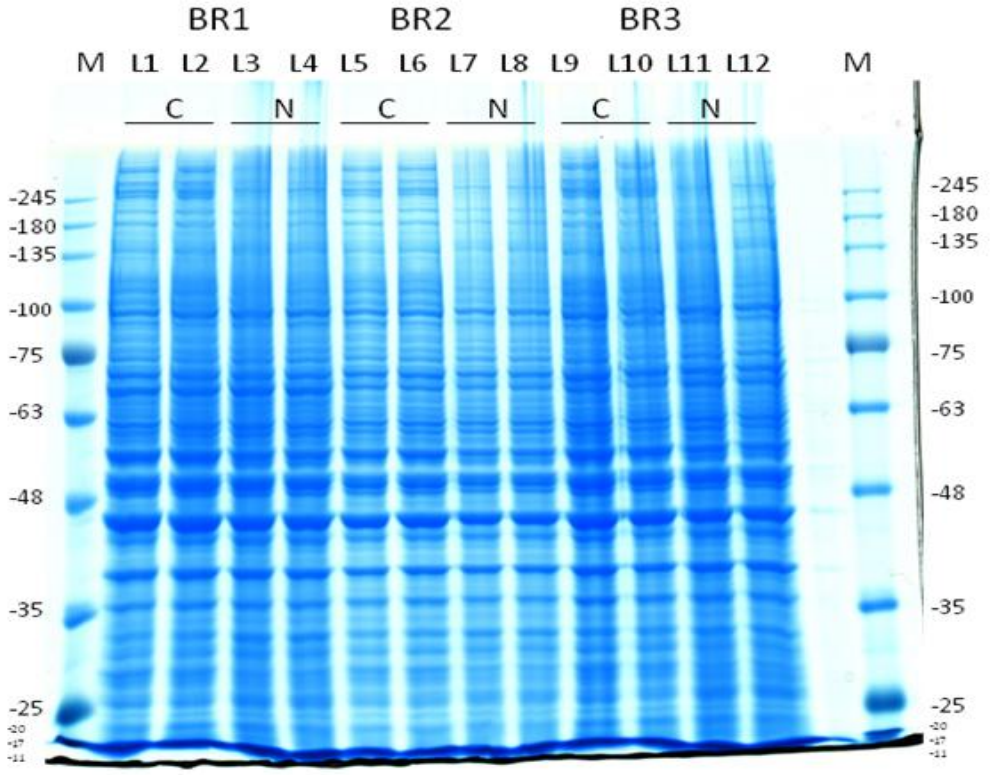


Figure 10: SDS-PAGE gel of JMP-1 cell line (PRO118_1DG006) after 24 h incubation in media free of FBS. L3, L4, L7, L8, L11, L12 were treated with N3a. BR: Biological Replicate, M: Marker

Before the analysis with mass spectrometry, the enzymatic degradation of proteins to peptides is an important step. The most common enzyme for this job is Trypsin. Trypsin is a serine protease that cleaves peptide chains mainly at the carboxyl side of the amino acids lysine or arginine, except when either is followed by proline. This makes the fragmentation by tandem mass spectrometry (MS/MS) more effective as it can place the highly basic residues at the C termini of the peptides.

The gel pieces in the Eppendorf vials were covered with 100 µl 50% ACN/water, shaked for 15 minutes and then after the removal of the ACN/water they covered with 100 µl 50mM ABS and shaked again for 15 minutes. This step repeated twice for the destaining of the gel pieces and the removal of residual SDS. Next step was the reduction with 100 µl 10mM DTT dissolved in ABS for 45 minutes at 56^{o}C. Then, the samples were alkylated with 55mM IAA dissolved in ABS for 45min at room

temperature in the dark. The gel pieces washed again with ACN/water and ABS for 3 times and after the removal of the last volume of ABS, they covered with 30-40 µl diluted Trypsin solution and incubated at 37°C overnight. The solution of Trypsin made with 20µg of lyophilized Trypsin into 20µl of storage buffer solution (50mM acetic acid/water). The volume of the solution needed diluted 260x with ABS. Next day, the supernatants transferred to new eppendorf vials and the gel pieces washed with nanopure water, then with 50% ACN and finally acidified with 0,1% TFA in 50% ACN/water. Meanwhile, these washes and TFA were transferred into the new vials. At the end, the samples dried using Speedvac and stored at 4°C for the nanoLC-MS/MS analysis.

2.2.2 Gel-free Method: Filter Aided Sample Preparation (FASP)

12 samples were used in this part of the study, 4 for each cell line, 2 treated and 2 untreated with N3a. The concentration of the total protein in the samples measured with the Bradford protein assay. The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible color change.

Sample ID	Cell Line +/- N3a	µg of protein	Trypsin needed for FASP (enzyme to protein ratio 1:100)
PRO118 NG001_01	MDA-V control	78,694	0,8
PRO118 NG001_02	MDAV + N3a	52,234	0,5
PRO118 NG001_03	MDA-V control	91,434	0,9
PRO118 NG001_04	MDAV + N3a	58,114	0,6
PRO118 NG001_05	JMP-1 control	34,594	0,3
PRO118 NG001_06	JMP-1 + N3a	94,374	0,9
PRO118 NG001_07	JMP-1 control	114,954	1,1
PRO118 NG001_08	JMP-1 + N3a	80,654	0,8
PRO118 NG001_09	SUP-M2 control	40,474	0,4

PRO118 NG001_10	SUP-M2 + N3a	73,794	0,7
PRO118 NG001_11	SUP-M2 control	42,434	0,4
PRO118 NG001_12	SUP-M2 + N3a	66,934	0,7

Table 2: Protein concentration (μg) of the secretome samples calculated with the Bradford Protein Assay and Trypsin needed for FASP.

FASP is a method for the generation of tryptic peptides from crude lysates for LC-MS analysis. With this protocol, samples were mixed into a filter unit (Microcon YM-30, Millipore) in a collection tube with 200 μl UA solution (urea 8M dissolved in 0.1 M Tris/HCl pH 8.5) and centrifuged at 14,000 x g for 15 min at 20°C. This step repeated and then the flow through discarded. 100 μl IAA solution (0.05 M iodoacetamide in UA) added for alkylation and samples centrifuged at 14,000 x g for 10 min at 20°C. Another 100 μl UA added to the filter unit and centrifuged at 14,000 x g for 15 min at 20°C (x2). Then, 100 μl ABS added (50mM NH4HCO in water) and centrifuged at 14,000 x g for 10 min at 20°C (x2). 50 μl ABS with Trypsin (enzyme to protein ratio 1:100) added and samples incubated in a wet chamber at 37oC overnight. Next day, the filter units transferred to new collection tubes, centrifuged at 14,000 x g for 20 min at 20°C, 40 μl ABS added and centrifuged at 14,000 x g for 10 min at 20°C (x2). The samples acidified with CF_3COOH (TFA), dried with Speed Vac and Store samples at $4^{o}C$.

2.2.3 Desalting

To clear the samples from buffer salts, urea, SDS, DTT and other small molecules that might have left in them and can damage the LC column and make the MS analysis difficult, the desalting protocol was used.

First, samples diluted in V > 40μl 5% Formic Acid, sonicated in water bath for 10 minutes and quickly centrifuged for 20 seconds (vortex). For the column preparation, columns for each sample were initialized with 20μl 90% ACN/5% FA. and again 20 μl 5% FA for re-equilibration. 20 μl of the sample were loaded in each column and the flow-through collected in a new Eppendorf. This step was repeated twice to be sure that as much amount as possible of the sample passed. For the sample washing, the columns were loaded with 20μl 5% FA and the step was repeated twice. Finally, the elution of the samples was performed with 20μl 90% ACN/5% FA, 3 times and the samples collected in new Eppendorf vials. Last step was the evaporation of the resulted desalted peptide solutions with SpeedVac.

2.3 Nano LC – MS/MS Analysis

Liquid chromatography tandem mass spectrometry (LC-MS/MS) is an analytical method where proteins are first separated by reversed phase liquid chromatography and detected by tandem mass spectrometry. MS is the most popular high-throughput methodology in proteomics and can generate gigabytes of data in several hours. It is mainly used to unambiguously identify a protein of interest by measuring the mass-to-charge ratio (m/z) of a charged peptide, and to thus determine the protein composition of a complex protein mixture (e.g., cell lysate, secretome, or tissue extract). In tandem mass spectrometry (MS/MS), the peptides are fragmented at their peptide bones, using a collision gas to generate product ions with different masses from the residue masses of the amino acids.

Two fundamental strategies are currently employed for protein identification and characterization by mass spectrometry. In bottom-up approaches, purified proteins or complex protein mixtures are subjected to proteolytic cleavage, and the peptide products are analyzed by MS. In top-down proteomics, intact protein ions or large protein fragments are subjected to gas-phase fragmentation for MS analysis.

In this study, the samples were first diluted with Buffer A (0,5% FA/H_2O) and then analyzed with nano flow liquid chromatography tandem mass spectrometry performed on an EASY-nLC system (Thermo Scientific) connected to a LTQ-Orbitrap-XL-ETD Mass Spectrometer (Thermo Scientific) through an electrospray ion source (ESI). An Xcalibur 2.0.7 software was used for instrument settings and data acquisition.

2.4 Bioinformatics Analysis

Bioinformatics tools (software and databases) are indispensable for data analysis and the construction of methodologies for interpreting secretome/proteome results. Dependable data interpretation is necessary for the formulation and investigation of hypotheses relating to biological processes, and for the proposal of disease biomarkers and discovery of new drug targets.

For this study, several bioinformatic tools were used for the identification, visualization and analysis of the secretome samples, such as Scaffold, Panther 14.0 Classification System from the Gene Ontology Database, Kegg Pathway, SignalP-5.0

and String. All the bioinformatic analysis was performed using the Uniprot Human database.

An important software tool is Scaffold, which is designed to help scientists identify and analyze proteins in biological samples. Using output files from MS/MS search engines, Scaffold validates, organizes, and interprets mass spectrometry data, allowing the user to more easily manage large amounts of data, compare samples, and search for protein modifications.

It is possible to collect essential information about the proteins of a secretome using Gene Ontology (GO). The GO project provides a set of hierarchical controlled vocabulary split into 3 categories:

- Biological process
- Molecular function
- Cellular component

In particular, GO analyses can determine how identified components might be related to specialized functions or processes, and whether any class of proteins is more likely to be found in a secretome. Furthermore, within a statistical framework, the GO methodology can be used to determine whether any GO term is significantly enriched in secretomes under various conditions. In a GO database, the molecular function is defined as the biochemical activity of a gene product, and biological pathways represent the integration of protein biochemical properties. Pathway analysis is an essential step for the systematic understanding of secretome specificity and functionality.

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a compendium of databases covering both annotated genomes and protein interaction networks for all sequenced organisms. Its integral part, KEGG pathway, is a compilation of manually verified pathway maps displaying both the molecular interactions and the biochemical reactions.

STRING is a free database of known and predicted protein interactions, including direct (physical) and indirect (functional) association that are derived from different sources. It quantitatively integrates interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable. The database currently covers more than 5,000,000 proteins from more than 1100 completely sequenced organisms.

3. Results

The following results as well as the discussion and conclusion section refer to the samples prepared by the FASP method. The samples managed with the 1DGE and in-gel digestion did not proceed in further analysis with LC-MS/MS due to machine damage.

3.1 Chromatographs – MS/MS spectrums

The chromatogram of a sample from SUPM-2 cell line (PRO118_NG001_09) that was extracted with the Xcalibur software is presented below:

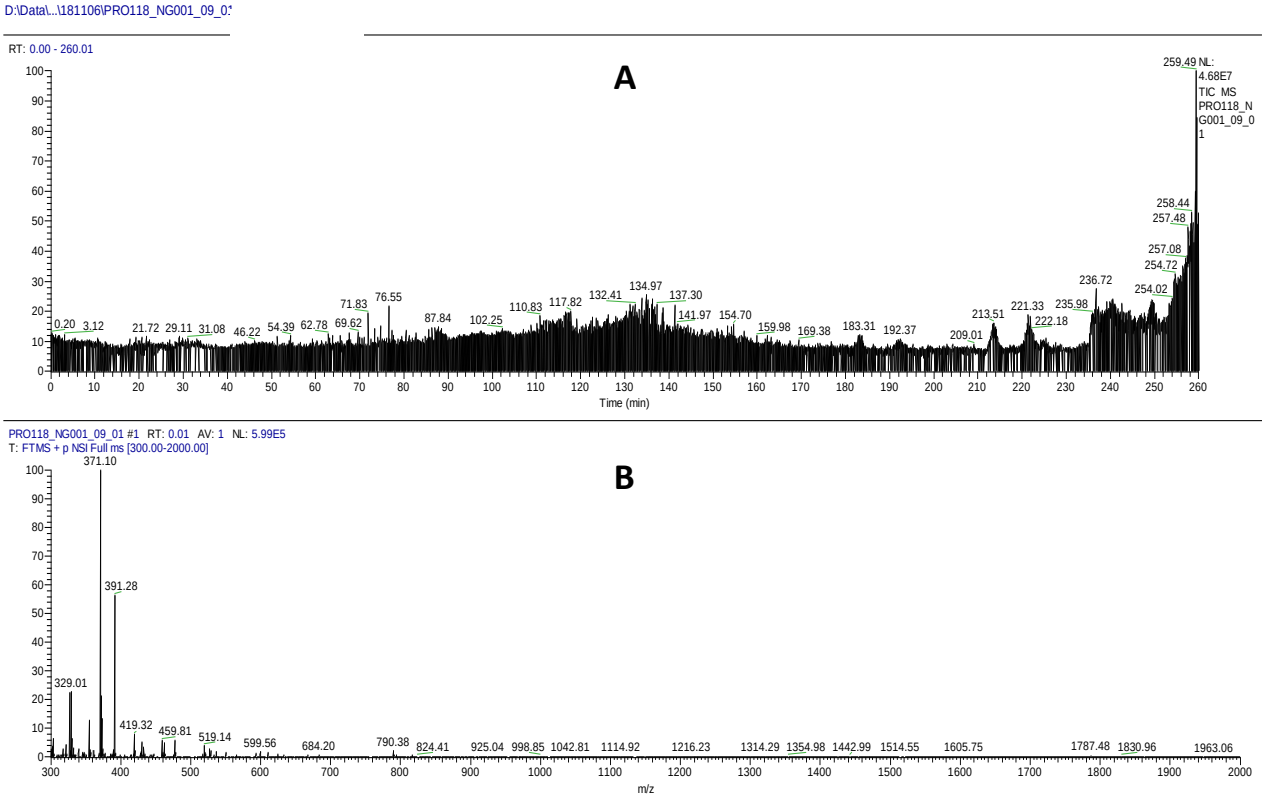


Figure 11: A. Chromatogram of sample PRO118_NG001_09 which is derived from the SUPM-2 cell line (control), B. The MS/MS spectrum of the same sample

The chromatograms of all the samples from the 3 cell lines (treated and untreated with N3a) can be found in the appendix section.

3.2 Label-free quantitative analysis

Using the Scaffold 4.0.7 software, 2326 proteins identified and 437 proteins found to have mapped IDs, using the Panther 14.0 Classification System from the

Gene Ontology Database. The secreted proteins were 89 (20%). Some of these proteins are presented in the table below:

Mapped IDs	Gene Name/ Gene Symbol	Species
P25774	Cathepsin S / **CTSS**	Homo sapiens
Q92583	C-C motif chemokine 17 / **CCL17**	Homo Sapiens
O00585	C-C motif chemokine 21 / **CCL21**	Homo sapiens

Table 3: Three of the secreted proteins that identified using the Panther Classification System from the Gene Ontology Database

The whole table of the proteins can be found in the appendix section.

3.3 Functional Analysis, Pathways and Protein Visualization

Using the Gene Ontology Database, these 437 proteins categorized according to their molecular function, the biological processes in which they participate and their position in the cell (cellular component). Also, they categorized according to the protein class. The results are presented in bar graphs which show the enrichment in each category.

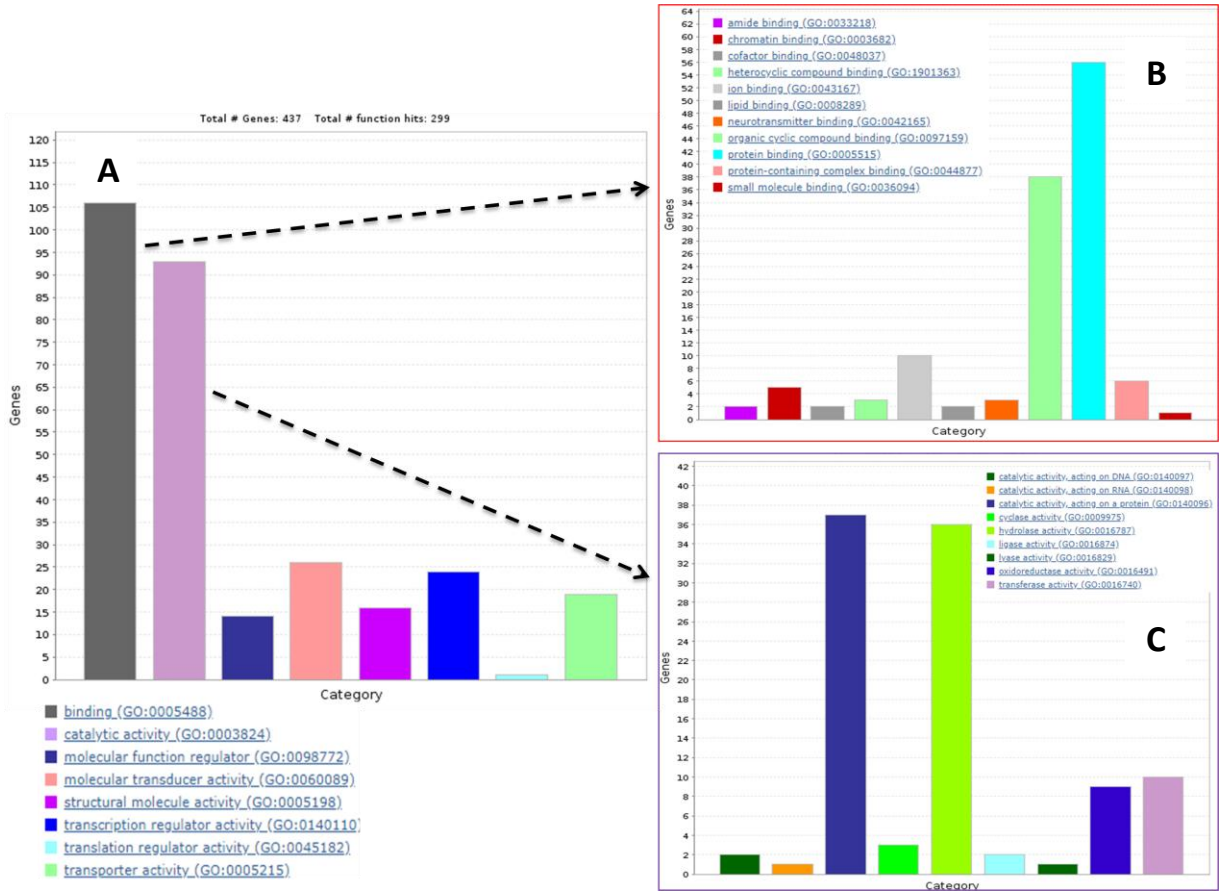


Figure 12: A) Bar Chart of the identified proteins according to their molecular function. The total number of genes in this ontology is 299. B) Binding, a category of molecular function with its subcategories. C) Catalytic Activity, a category of molecular function with its subcategories.

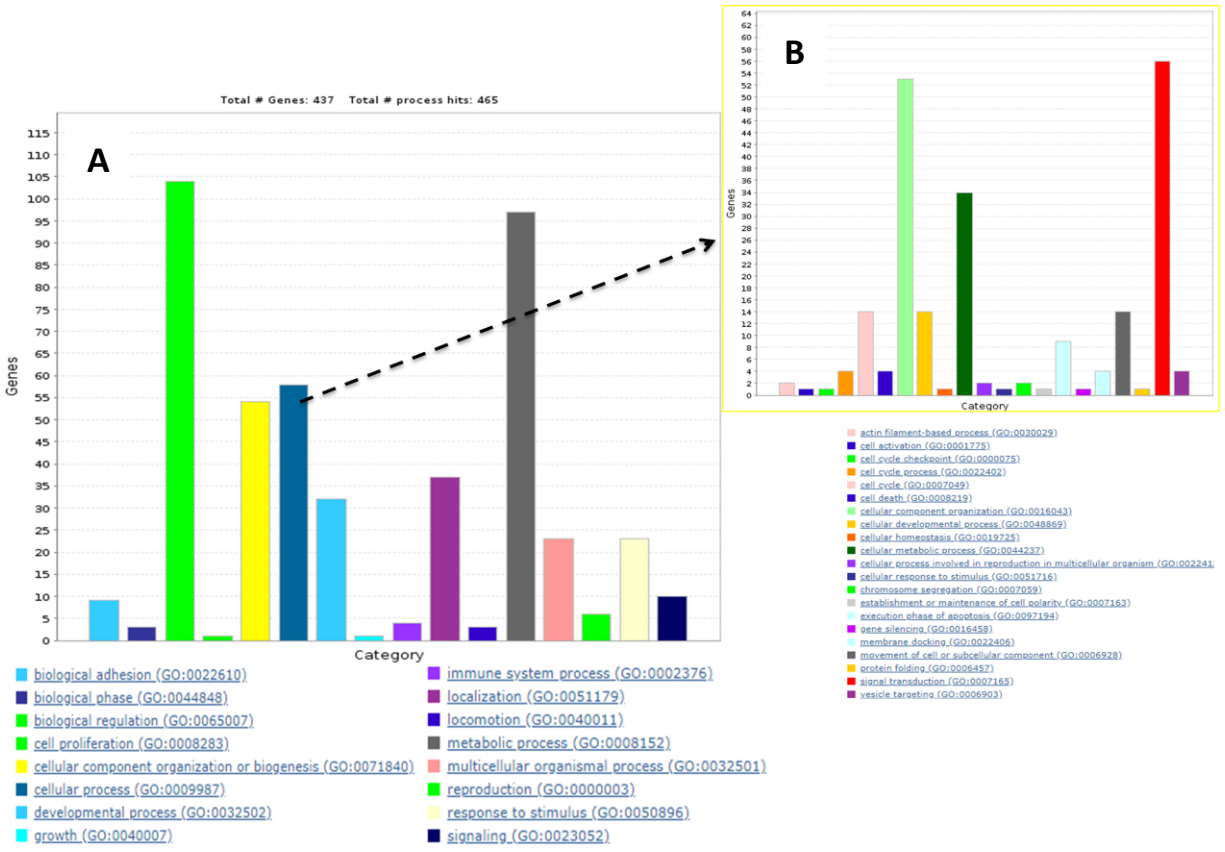


Figure 13: A) Bar Chart of the identified proteins according to the biological process that they participate. The total number of genes in this ontology is 465. B) Cellular Process, a category of biological process with its subcategories.

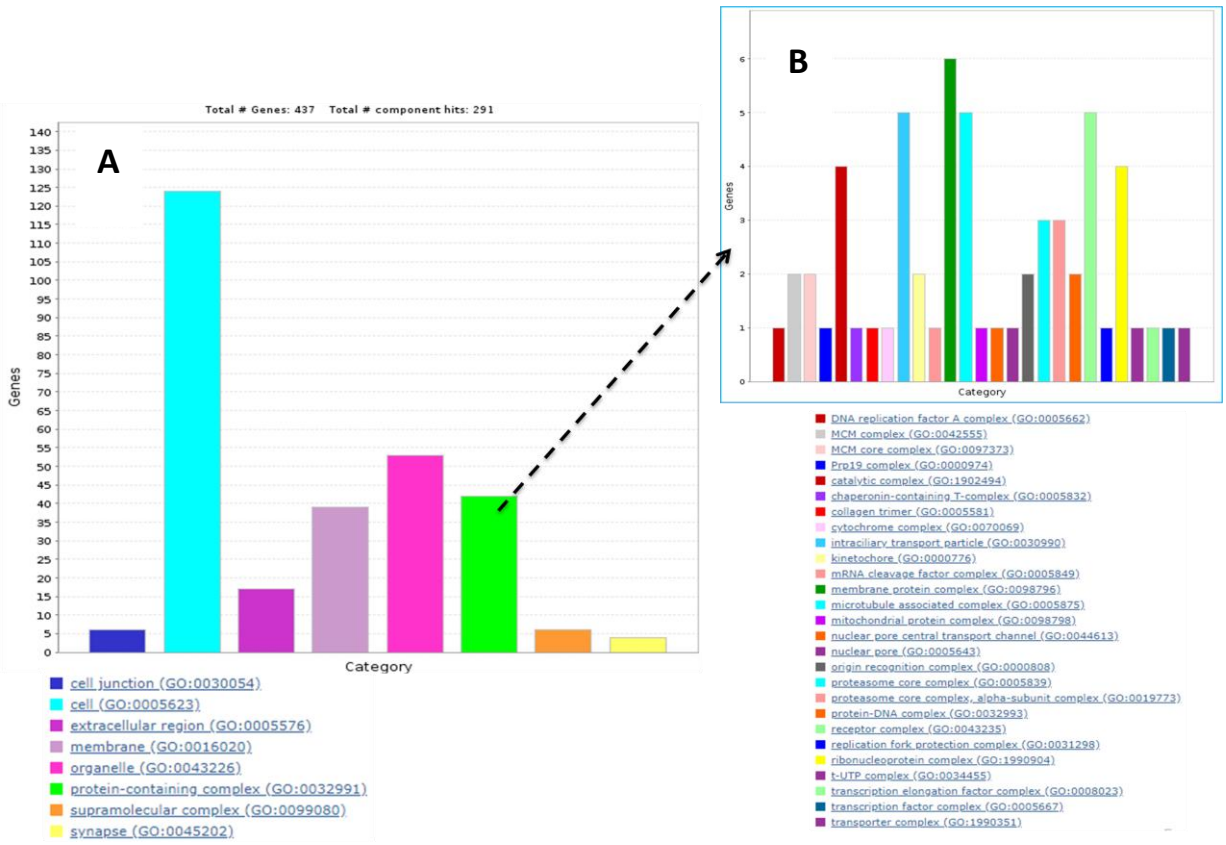


Figure 14: A) Bar Chart of the identified proteins according to their cell position (cellular component). The total number of genes in this ontology is 291. B)Protein-containing complex, a category of cellular component with its subcategories.

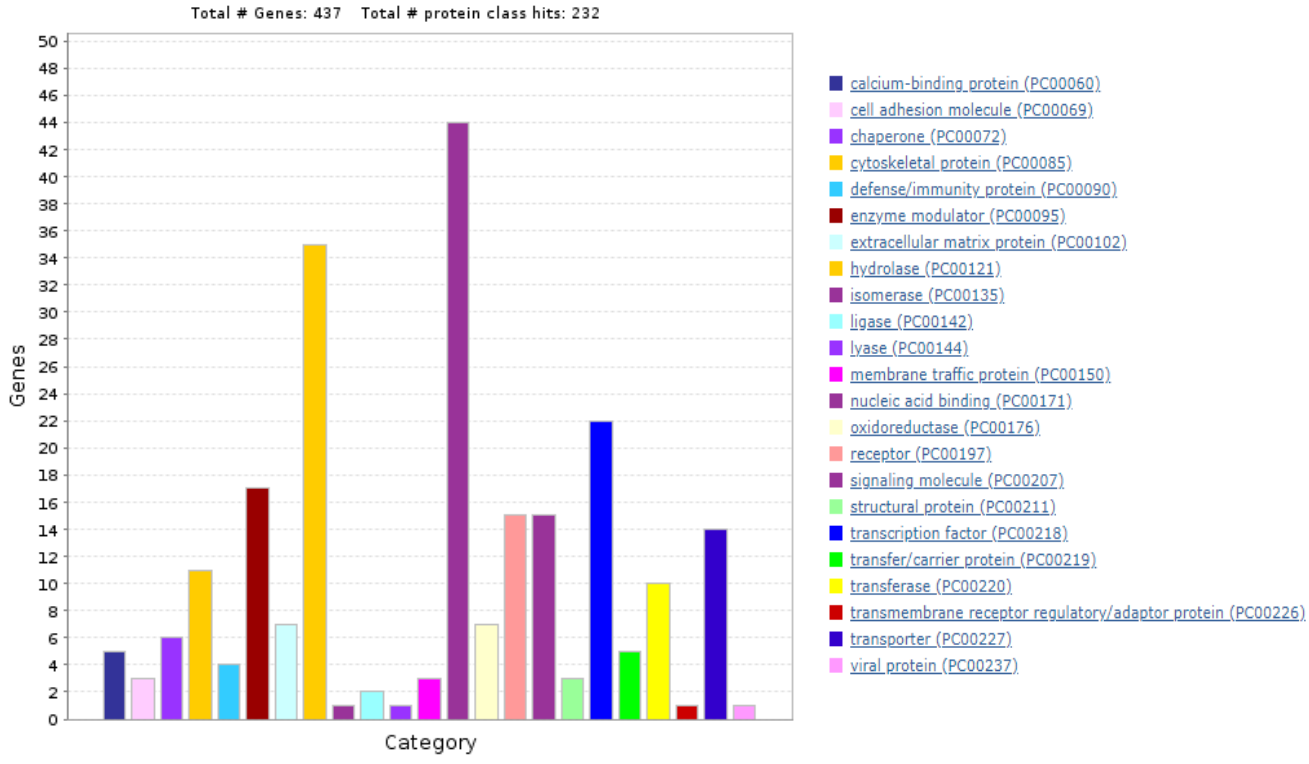


Figure 15: A) Bar Chart of the class of the identified proteins

In figure 16 is presented the number of the genes in each of the 3 categories of GO and in figure 17 the number of the common ones using Venny 2.1.

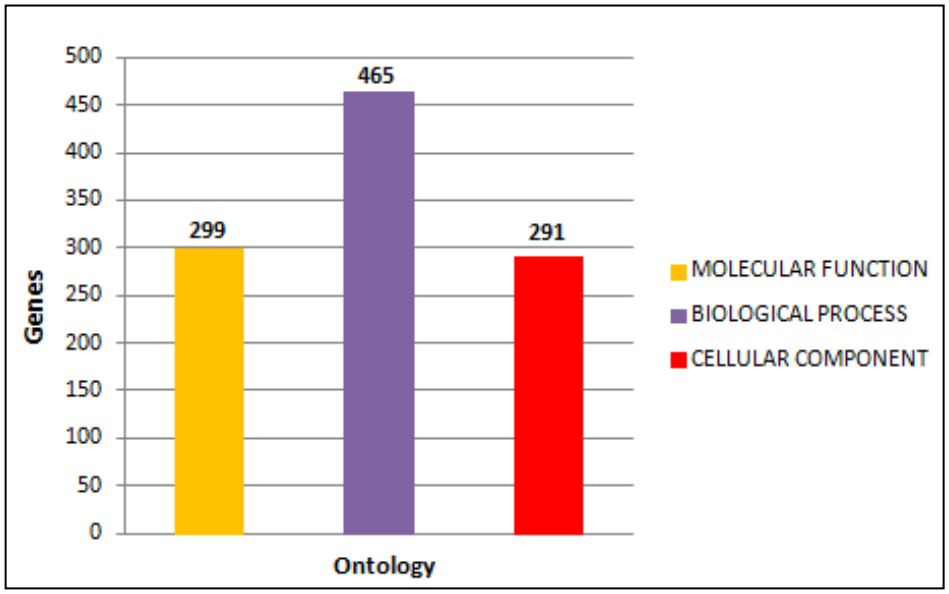


Figure 16: Bar Chart of the 3 ontologies described above and the number of the genes in each one.

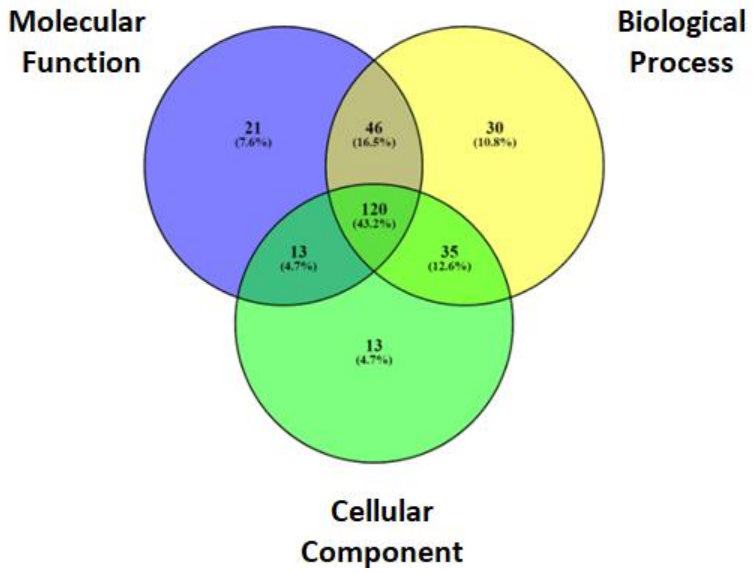


Figure 17: Venn Diagram of the 3 ontologies described above with the number of the unique and common genes between them. 120 common proteins found at the 3 ontologies, 46 between molecular function and biological process, 13 between molecular function and cellular component and 35 biological process and cellular component. This diagram was created with Venny 2.1.0.

89 out of the 437 identified proteins (20%), found to be in the extracellular region and could characterized as secreted. The proteins that have a signal peptide and

were secreted therefore with the classical secretory pathway were found through the SignalP-5.0 server. The rest of them that don't have a signal peptide were secreted by the non classical secretory pathway. (Figure 18).

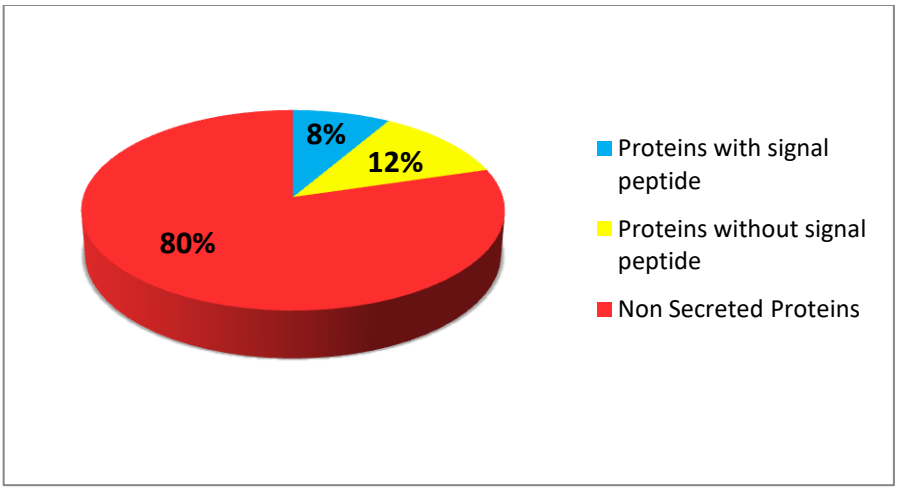


Figure 18: Pie chart with the presentage of the proteins with and without signal peptide (SP). 37 proteins (8%) out of the 437 that identified, were found to have a signal peptide and 52 (12%) were found not to have one. The rest 348 (80%) are non-secreted proteins.

In the following graph is presented the position of the signal peptide into the protein sequence of the CCL17 protein that identified (a C-C motif chemokine). The cleavage site position is found between the 23rd and the 24th amino acid.

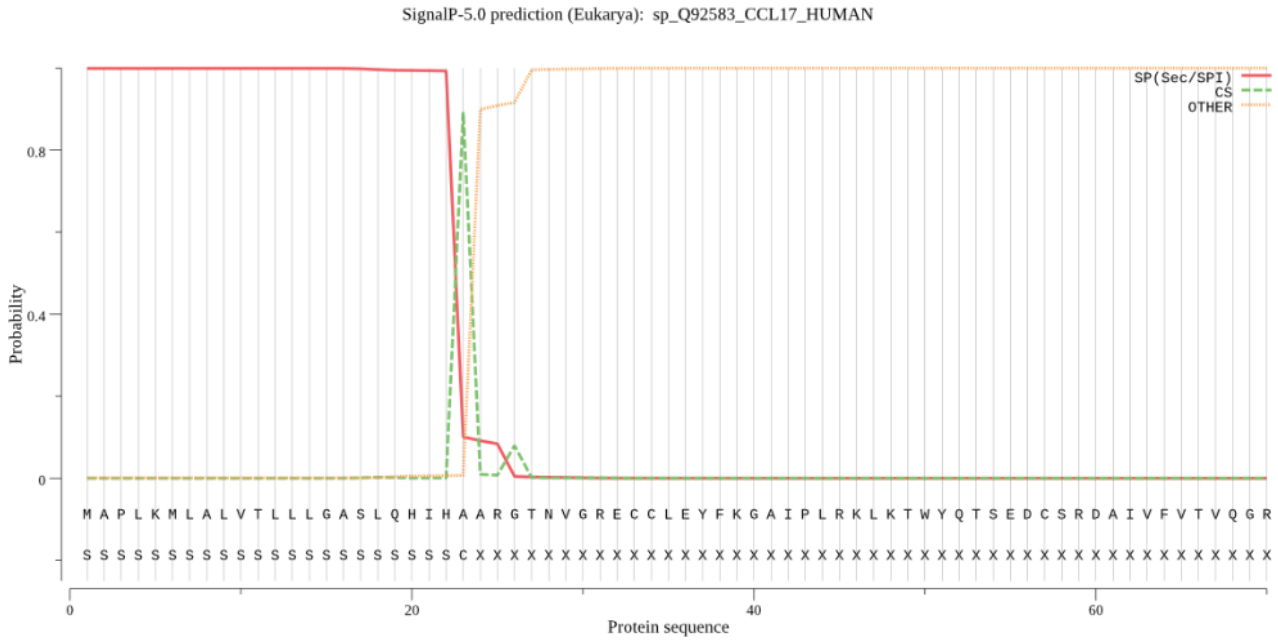


Figure 19: Plot of the signal peptide position in CCL17 protein. Three likelihood probabilities are reported on the plot, i.e. SP(Sec/SPI), CS (the cleavage site) and OTHER (the probability that the sequence does not have any kind of signal peptide).

From these secreted proteins, some belong to HL samples, some to MCL samples, some to ALCL samples and some are common at the 3 lymphoma subtypes. In figure 20 is presented the number of the unique and common secreted proteins between HL, MCL and ALCL.

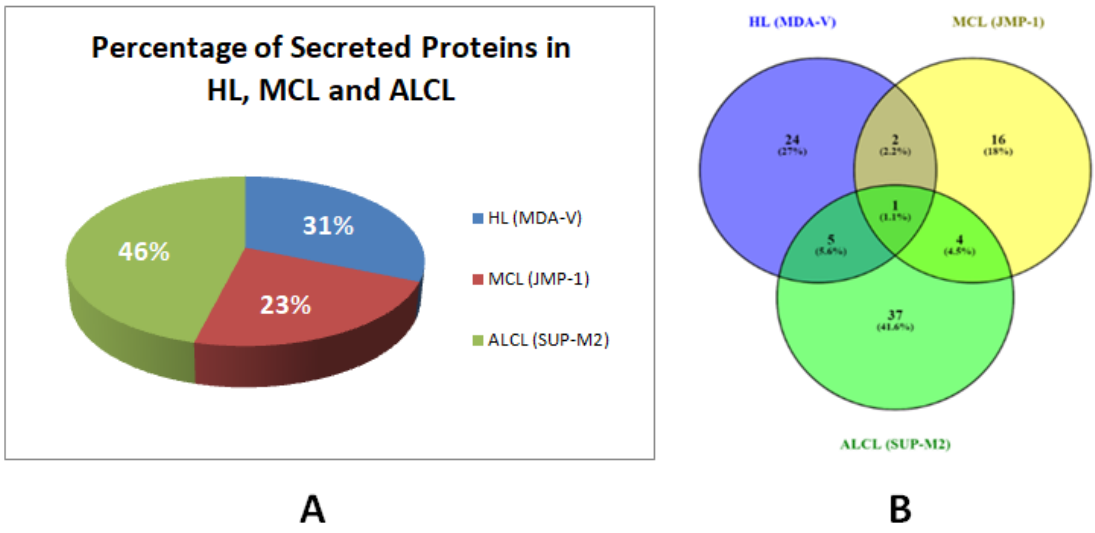


Figure 20: A) Pie chart with the percentage of the secreted proteins in the three lymphoma subtypes used (HL, MCL, ALCL) with their corresponding cell lines. B) Venn diagram that shows the number of the unique and common secreted proteins between HL, MCL and ALCL with their corresponding cell lines. Only 1 protein found common in all the cell lines. Created by Venny 2.1.0.

Also, protein number differs between samples treated with Nutlin-3a and control samples. In the bar graph below, it is presented the number of the secreted proteins in each lymphoma subtype and cell line before and after treatment with N3a.

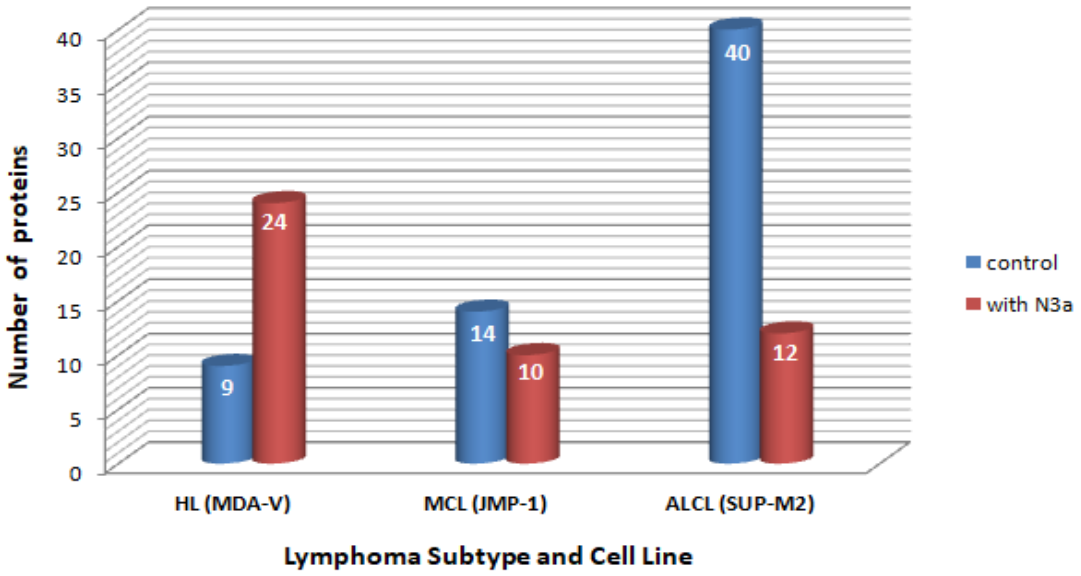
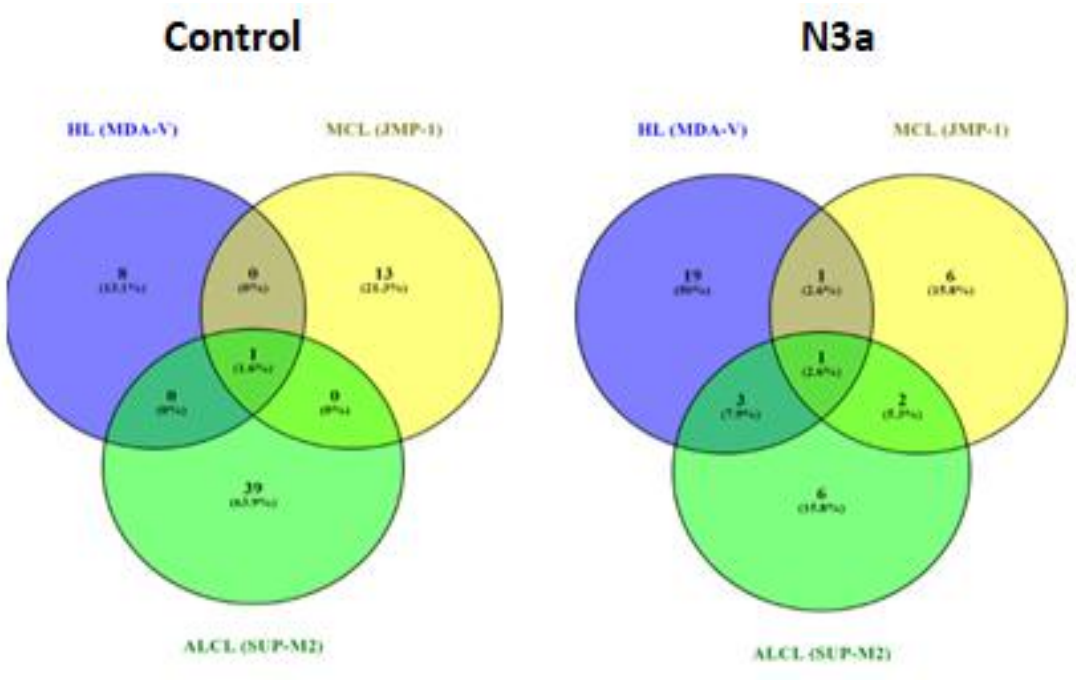
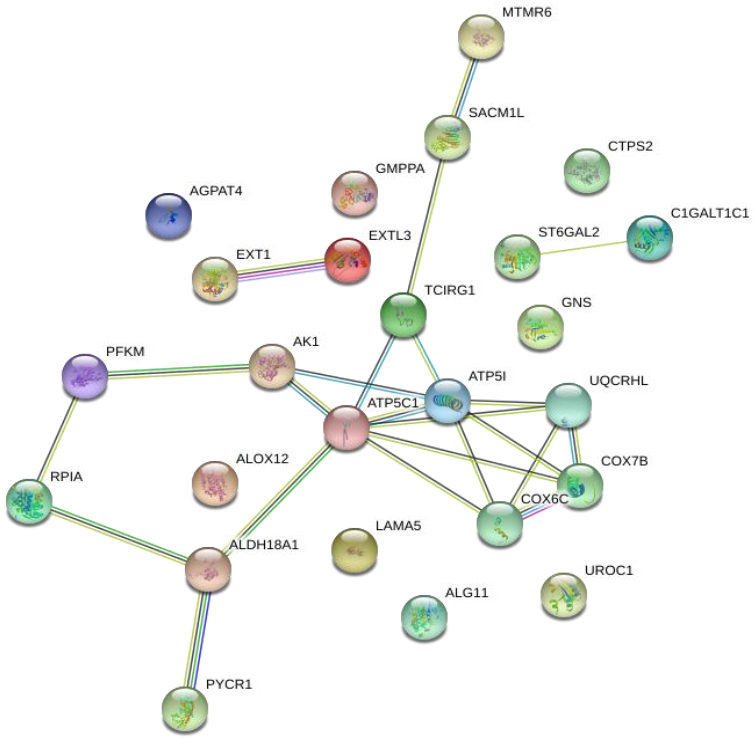


Figure 21: Bar chart describing the number of the secreted proteins that found in each cell line before (control) and after treatment with N3a.



<u>Figure 22:</u> Venn diagram that shows the number of the unique and common secreted proteins between the three lymphoma subtypes (HL, MCL, ALCL), before (left) and after treatment with N3a (right). Only 1 protein found in all the cell lines. Created by Venny 2.1.0.

Diagrams of protein-protein interactions in different pathways were created, using the String 11.0 database.



<u>Figure 23:</u> Proteins that participate in Metabolic Pathways and the interactions between them, using the String 11.0 database. Proteins are shown as nodes and the edges represent the predicted functional associations.

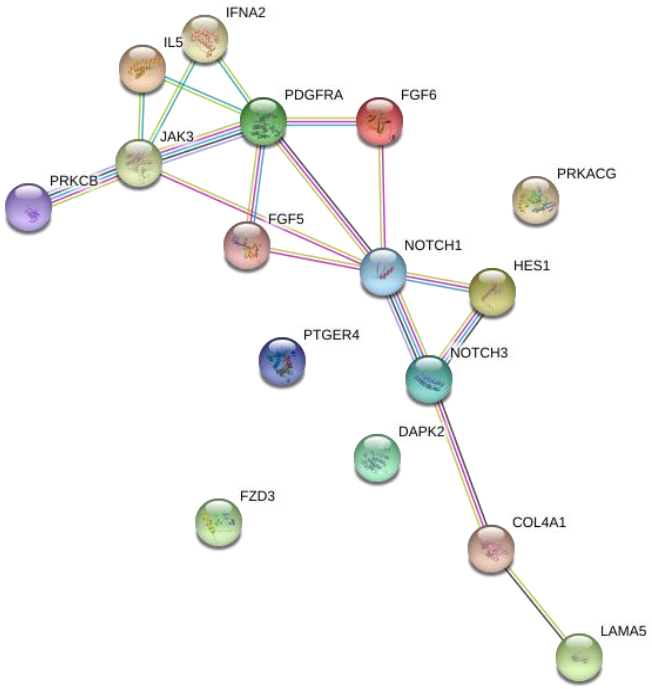


Figure 24: Proteins that participate in Pathways in Cancer and the interactions between them, using the String 11.0 database. Proteins are shown as nodes and the edges represent the predicted functional associations.

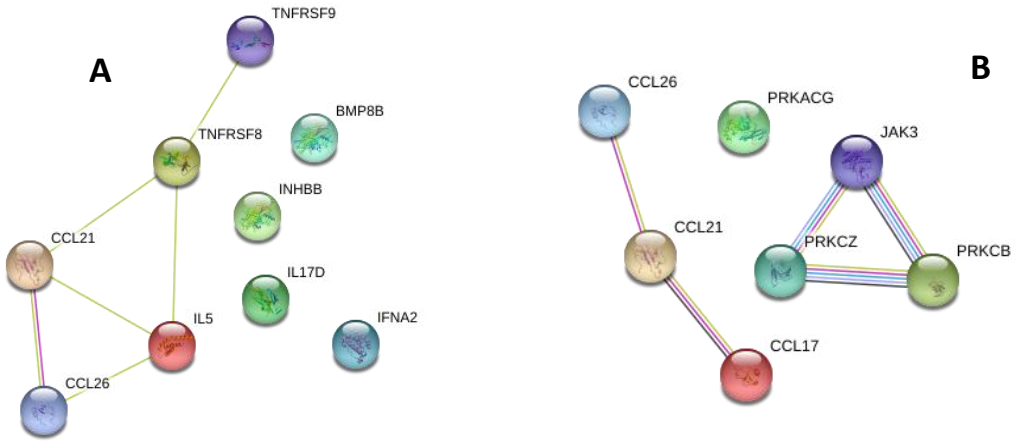
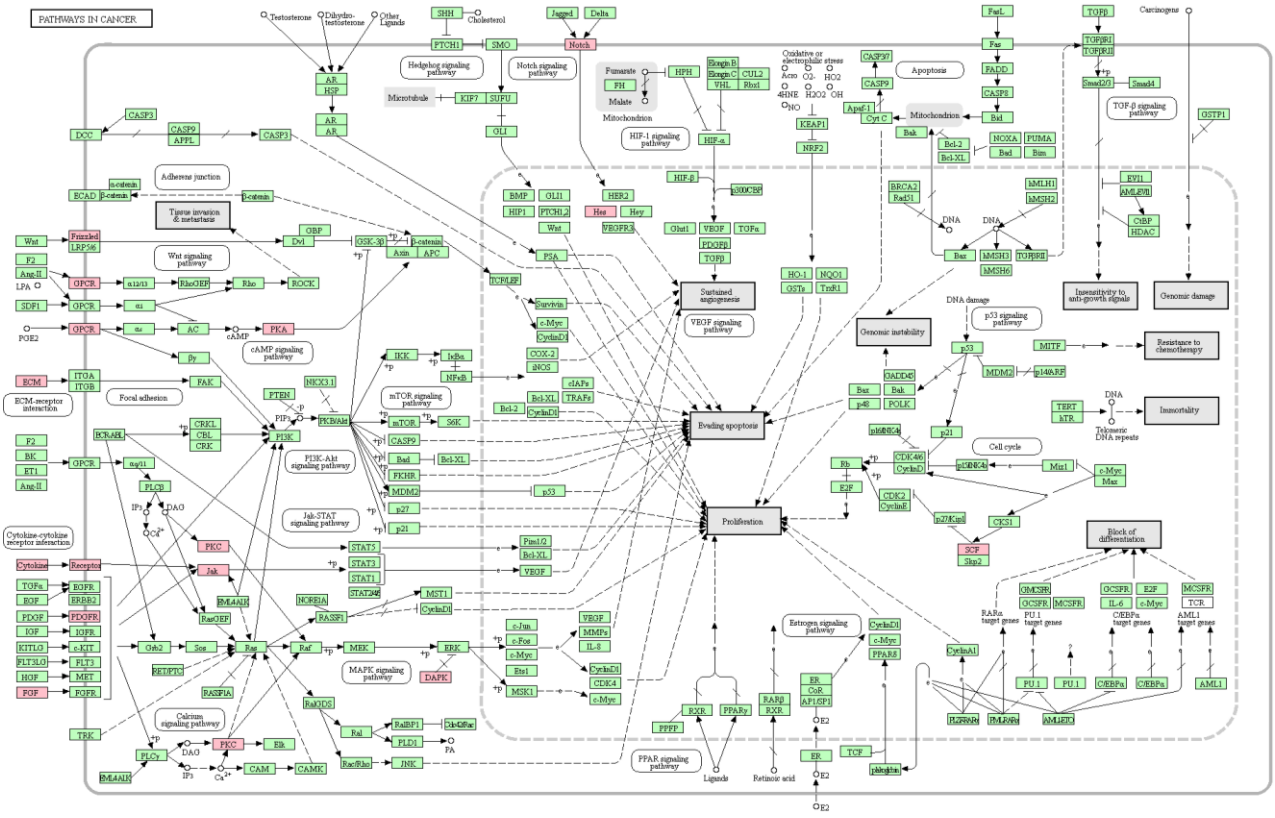


Figure 25: A) Proteins that participate in cytokine - cytokine receptor interaction pathway and the interactions between them, B) Proteins that participate in chemokine signaling pathway and the interactions between them. Diagrams were created using the String 11.0 database. Proteins are shown as nodes and the edges represent the predicted functional associations.

Moreover, with the help of another bioinformatic tool, KEGG, followed the creation of pathway maps for some of the important pathways in which the identified proteins participate.



*<u>Figure 26</u>: Kegg Map of Pathways in Cancer**.** Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them**.***

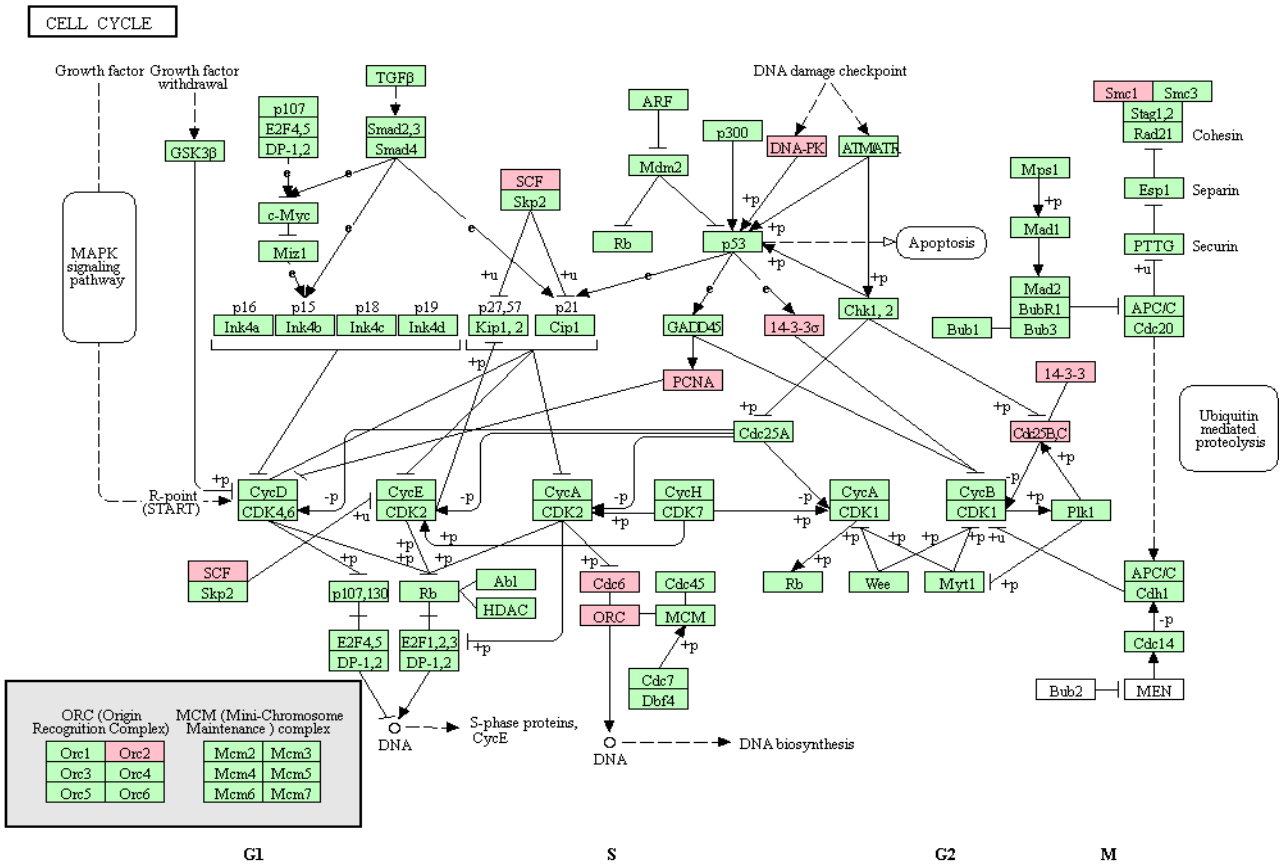


Figure 27: Kegg Map of the Cell Cycle pathway. Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them.

More pathway maps can be found in the appendix section.

4. Discussion

It is commonly known that proteins secreted by cancer cells play important roles in cell interaction, adhesion, and invasion. Secretome analysis of 3 different human cell lines of Hodgkin and Non-Hodgkin Lymphoma treated or not with N3a, was performed to discover the total proteins released from the cells and which ones are probably involved in lymphomagenesis. In addition, this study aims to evaluate the differences of the secretome profile, before and after treatment with N3a and between Hodgkin and Non Hodgkin lymphoma and their subtypes. As a result, 2326 differentially expressed proteins were identified in the 12 analyzed samples, from which 437 identified by the Gene Ontology Database and 89 of them found by the GO terms to be secreted.

As it can be seen from the chromatograms, the separation of the peaks is quite good, as well as the MS/MS fragmentation, which indicates the favorable identification of many peptides. The background in the chromatograms probably derives from keratins or plastic, which are difficult to be omitted, due to the MS instrument's sensitivity or human's factors. However, a lot of background can be excluded during the bioinformatic analysis.

Using the Panther 14.0 Classification System from the Gene Ontology Database, the identified proteins categorized according to their molecular function, the biological processes in which they participate, their position in the cell (cellular component) and the protein class. In the molecular function term (figure 12), proteins seem to involve in many categories, such as binding, catalytic activity, structural molecule activity, transcription and translation regulation activity. However, binding is the category with the majority of the genes and especially protein binding, such as signaling receptor binding, cytoskeletal protein binding and enzyme binding. According to the biological process term (figure 13), proteins participate in cellular component organization or biogenesis, immune system process, localization, signaling, biological regulation, cellular process, metabolic process etc. The last 3 categories have the higher numbers of genes. From the cellular component bar chart (figure 14), it can be seen that most of the proteins are in the category "cell", in the intracellular part and the plasma membrane. Last but not least, the classification according to protein class (figure 15) showed that many proteins are hydrolases, nucleic acid binding proteins and transcription factors.

Comparing the three GO terms (figure 16), it seems that biological process is the category with the most genes out of the total 437. However, they have enough proteins in common according to the Venn diagram and especially in particular 120. (Figure 17)

Furthermore, 20% of the proteins found to be known secreted proteins. The 8% of the proteins found to include a signal peptide, that indicates that they were probably secreted out of the cell by the classical secretory pathway, from the ER to Golgi apparatus and finally to the extracellular space. The rest 12% that do not have a signal peptide, were probably secreted by the non-classical secretory pathway, with the help of exosomes, microvesicles, apoptotic bodies, autophagosome-like vesicles, lysosomes etc. or with direct translocation across the plasma membrane. 80% of the identified proteins found to be known non-secreted proteins. Comparing the three lymphoma subtypes (HL, MCL, ALCL), 32 proteins (31%) found to be known secreted proteins in HL, 23 proteins (23%) in MCL and 47 proteins (46%) in ALCL (Figure 20). ALCL (SUP-M2 cell line) was the subtype with the most proteins,

secreted or not and MCL the subtype with the less proteins. From these secreted proteins only 1 was common in the 3 subtypes (Figure 20). This protein is Hemicentin-1, a protein that in humans is encoded by the HMCN1 gene.

Between control samples and samples treated with N3a, it seems that in HL the number of secreted proteins increased with the addition of N3a. In particular, 9 proteins found to be known secreted proteins in HL and 24 in HL treated with N3a. Instead, in MCL and ALCL, subtypes of Non-Hodgkin Lymphoma, the number of proteins decreased after N3a. 14 proteins found to be known secreted proteins in MCL and 10 proteins after treatment with N3a. In ALCL we identified 40 known secreted proteins and 12 known secreted proteins after adding N3a (Figure 21). Hemicentin-1 was common in the samples without N3a and also in the samples with N3a (Figure 22).

According to the article “Proteomics analysis of Hodgkin lymphoma: identification of new players involved in the cross-talk between HRS cells and infiltrating lymphocytes” (Blood, 2008) by Ma Y. et. al., two of the known secreted proteins found in this study, Cathepsin S and CCL17 (TARC), may serve as potential biomarkers in Hodgkin Lymphoma to evaluate prognosis or disease activity. Feasibility of one of these proteins as potential biomarker has already been demonstrated by Weihrauch et al, who found that significantly increased serum CCL17 levels in majority of HL patients were related to disease activity. Cathepsin S secretion by the HRS cells found that might be closely related to angiogenic activity and basic fibroblast growth factor expression observed in HL.

Cathepsin S is a lysosomal enzyme that belongs to the papain family of cysteine proteases and acts as an important protagonist of cancers, according to multiple studies. It has been shown to participate in dissolution and remodelling of connective tissue and basement membranes, resulting in the process of tumor growth, cell invasion, metastasis and angiogenesis. Therefore, cathepsin S has been suggested as a potential therapeutic target in cancer therapy. Although cathepsin S is primarily localized in lysosomes, it can be translocated to the cell surface and subsequently secreted into the extracellular milieu, leading to the degradation of various extracellular matrix proteins such as laminin, fibronectin, elastin, osteocalcinand and some collagens, resulting in the promotion of tumor cell invasion and metastasis. As a member of lysosomal cathepsins, cathepsin S also plays an important role in the apoptosis of cancer cells. The apoptosis induced by targeting cathepsin S was reportedly attributed to different apoptotic pathways.

The chemokine CCL17, also known as thymus and activation-related chemokine (TARC), emerges to have important biological functions, as it is expressed in high amounts by HRS cells and highly elevated in the serum of HL patients. CCL17 recruits Th2 cells and regulatory T cells that account for a beneficial microenvironment for HRS cells. Chemokines produced by HRS cells play a major role in leukocyte trafficking.

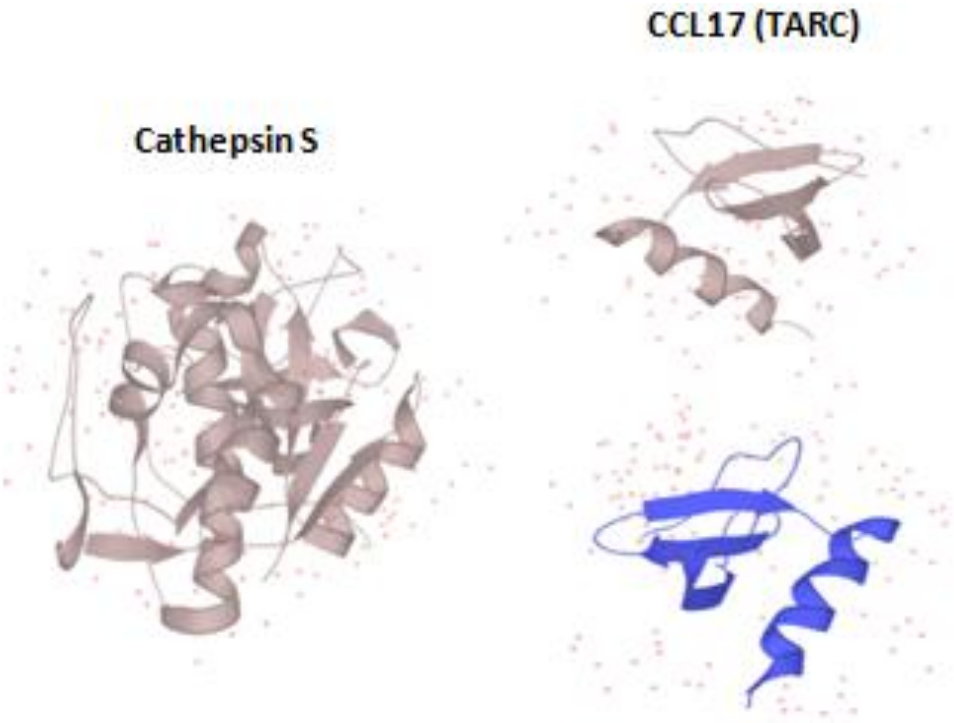


Figure 28: 3D structure of Cathepsin S (left) and CCL17 (right)

Looking at the NSAF values by Scaffold 4.0.7 analysis, the presence of these 2 proteins confirmed only in the MDA-V samples that correspond to Hodgkin Lymphoma, as expected. Normalized spectral abundance factors (NSAFs) provide an improved measure of relative abundance and the ability to compare the abundance of proteins within a sample.

From the KEGG pathway maps, it seems that many of the analyzed proteins participate in important pathways such as apoptosis, cell cycle, pathways in cancer, chemokine signaling pathway, cytokine-cytokine receptor interaction, Jak-Stat Signaling Pathway etc. In particular, Cathepsin S is participating in the apoptosis and lysosome pathway and CCL17 in cytokine-cytokine receptor interaction, chemokine signaling pathway, C-type lectin receptor signaling pathway and IL-17 signaling pathway.

5. Conclusions

In conclusion, 437 proteins identified in the Hodgkin and Non Hodgkin lymphoma samples after filter aided sample preparation and nanoLC-MS/MS analysis. Although the samples used derived from the cell secretome, only the 20% of these proteins were known secreted proteins and the rest 80% were known non-secreted. Most of the proteins were found in the ALCL, subtype of Non-Hodgkin lymphoma that corresponds to SUP-M2 cell line. Some important proteins between them were Cathepsin S and CCL17, which may act as potential biomarkers in lymphoma for evaluating prognosis or disease activity. These proteins appeared only in Hodgkin lymphoma cells (MDA-V cell line) as expected and found to participate in important pathways such as apoptosis, chemokine signaling pathway, cytokine-cytokine receptor interaction.

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7. Appendix

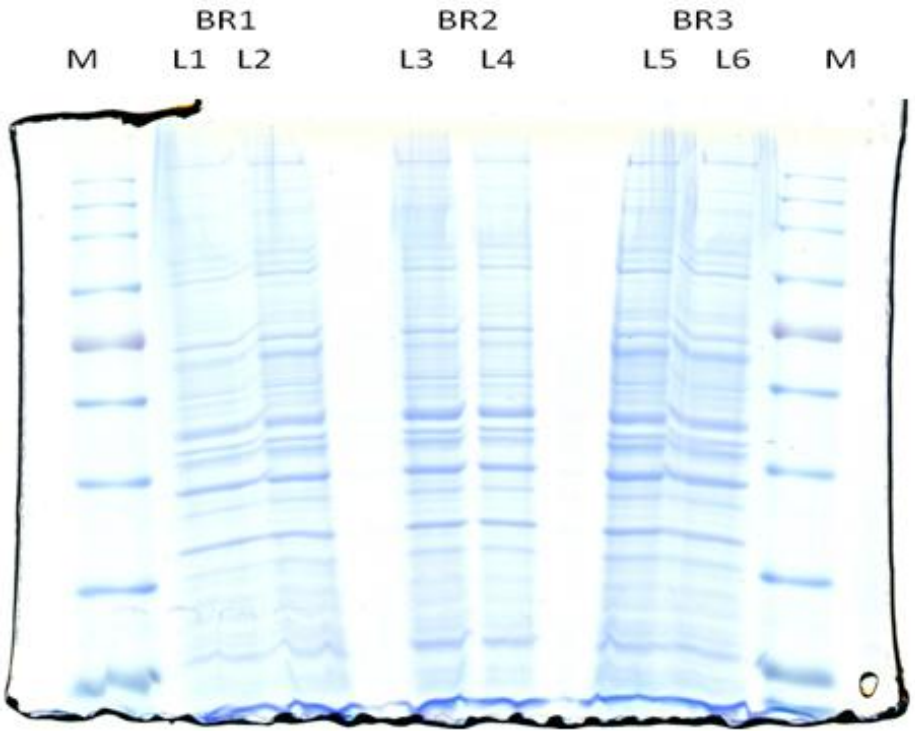


Figure 29: SDS-PAGE gel of MDA-V cell line (PRO118_1DG001) after 24 h incubation in media free of FBS. BR: Biological Replicate, M: Marker

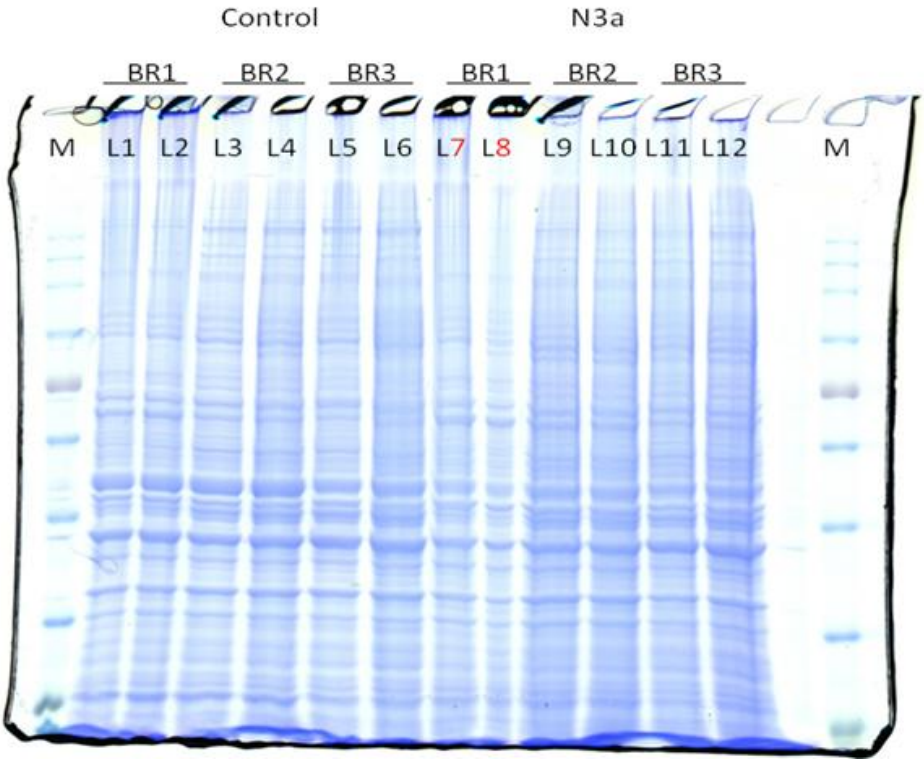
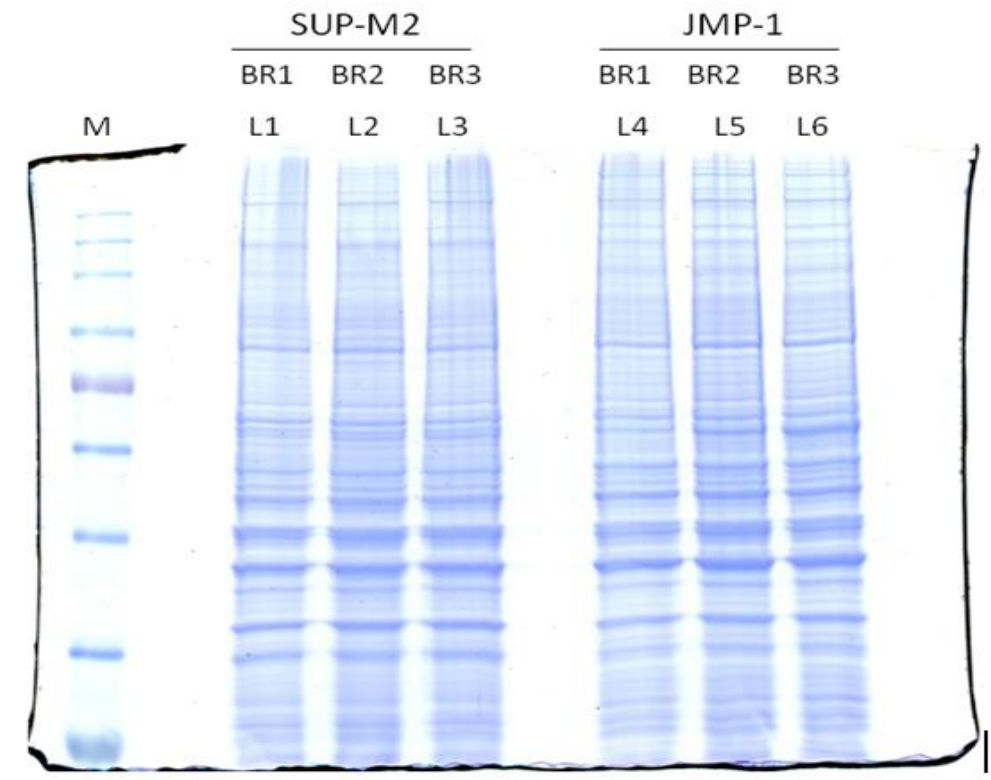
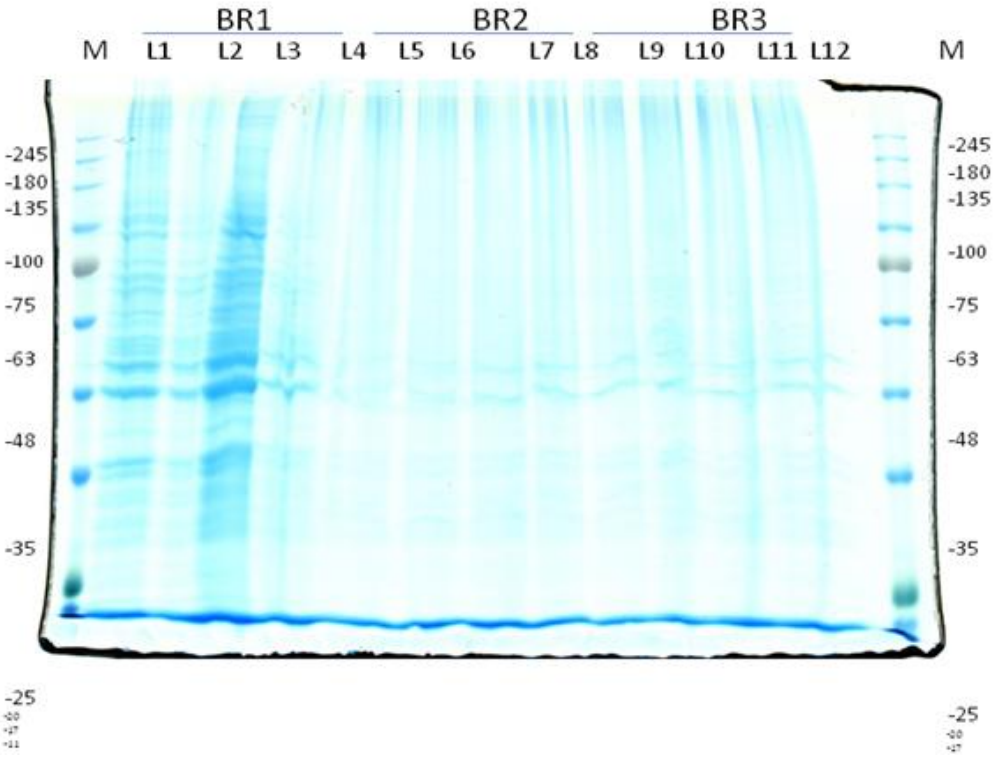


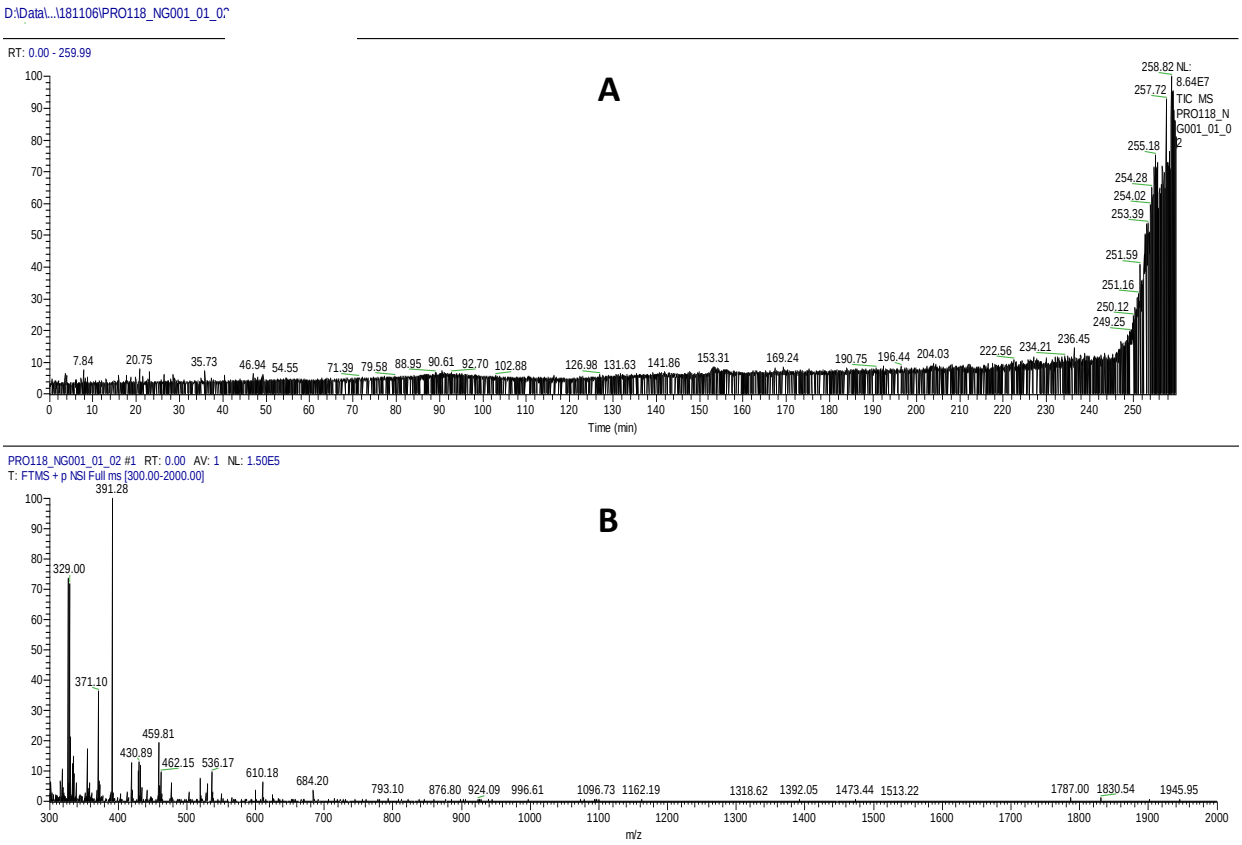
Figure 30: SDS-PAGE gel of MDA-V cell line (PRO118_1DG002) after 24 h incubation in media free of FBS. L7-L12 were treated with N3a. BR: Biological Replicate, M: Marker



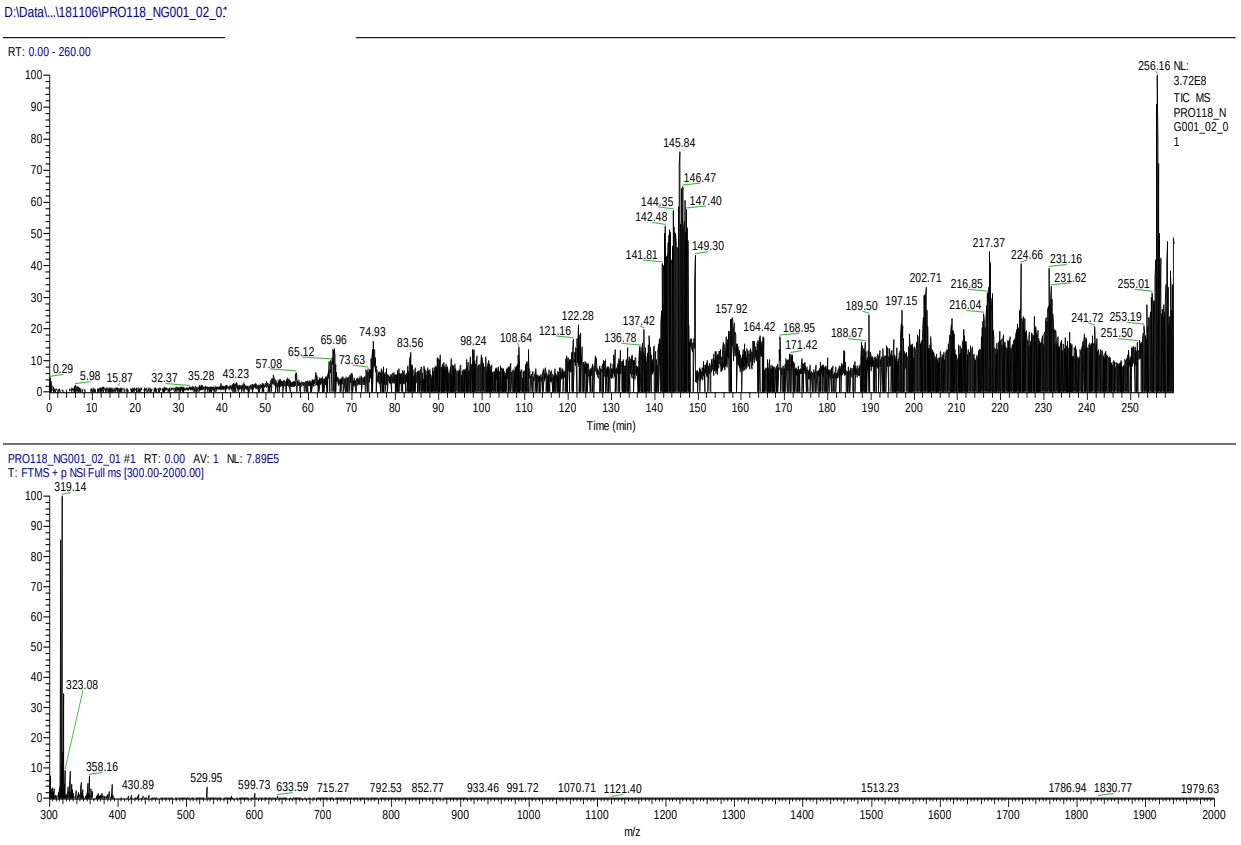
<u>Figure 31:</u> SDS-PAGE gel of SUP-M2 and JMP-1 cell lines (PRO118_1DG003) after 24 h incubation in media free of FBS. L1-L3 refer to SUP-M2 and L4-L6 refer to JMP-1. BR: Biological Replicate, M: Marker



<u>Figure 32:</u> SDS-PAGE gel of SUP-M2 cell line (PRO118_1DG005) after 24 h incubation in media free of FBS. L3, L4, L7, L8, L11, L12 were treated with N3a. BR: Biological Replicate, M: Marker



<u>Figure 33:</u> A. Chromatogram of sample PRO118_NG001_01 which is derived from the MDA-V cell line (control), B. The MS/MS spectrum of the same sample



<u>Figure 34:</u> A. Chromatogram of sample PRO118_NG001_02 which is derived from the MDA-V cell line treated with N3a, B. The MS/MS spectrum of the same sample

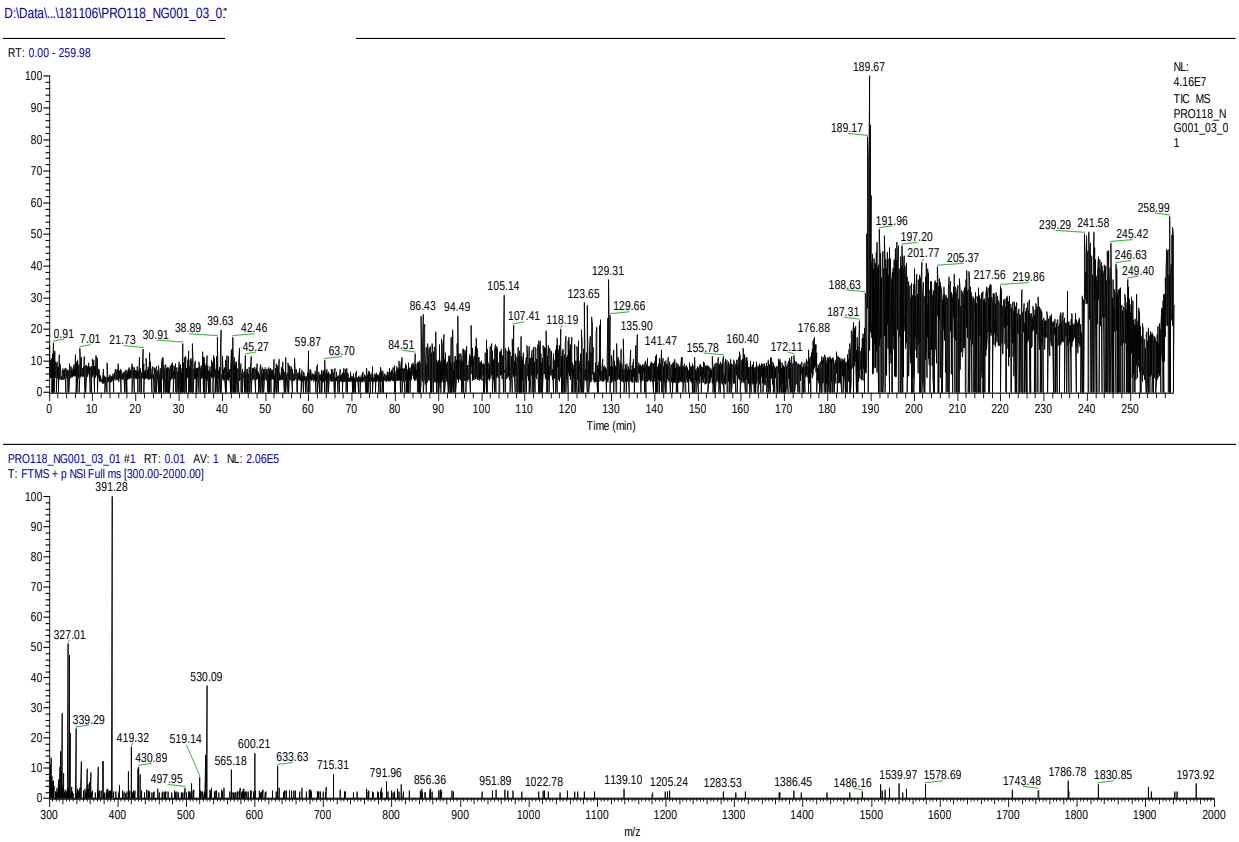


Figure 35: A. Chromatogram of sample PRO118_NG001_03 which is derived from the MDA-V cell (control), B. The MS/MS spectrum of the same sample

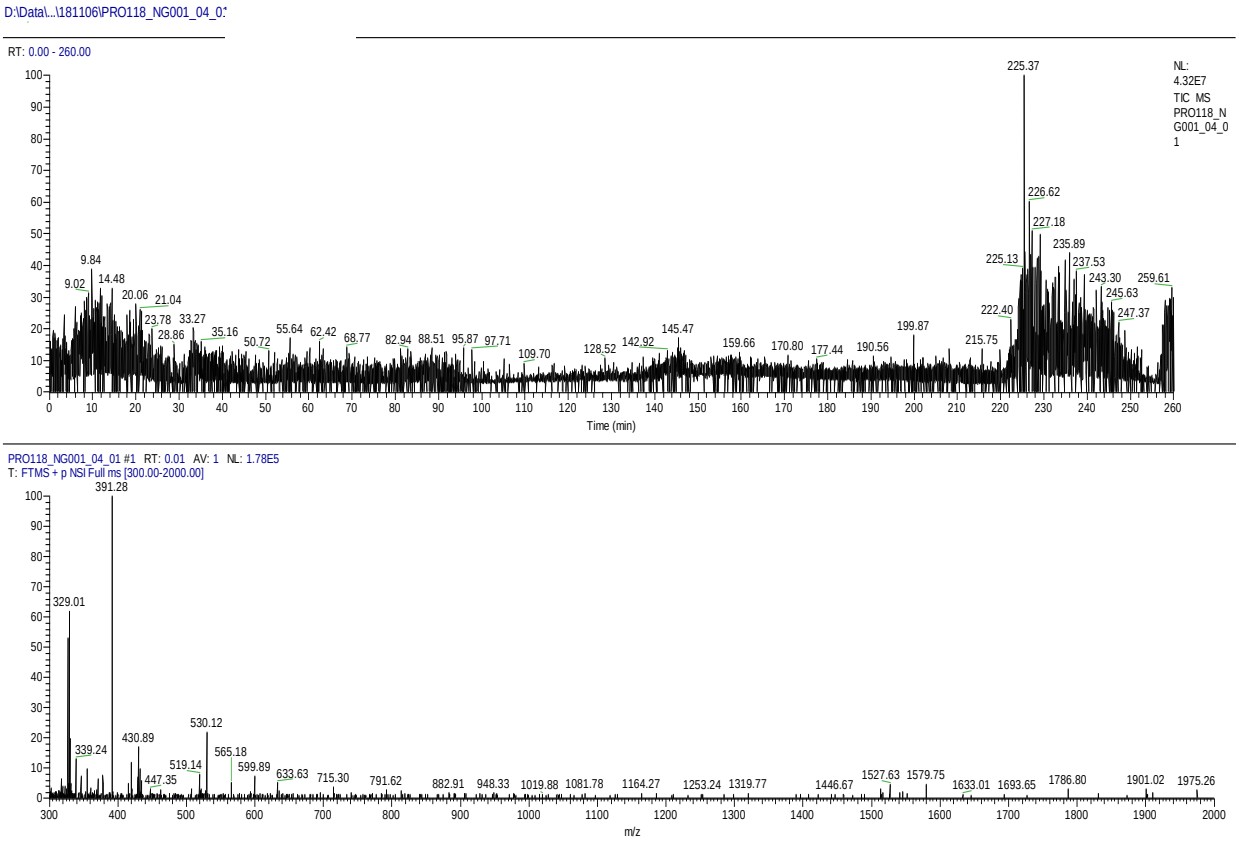
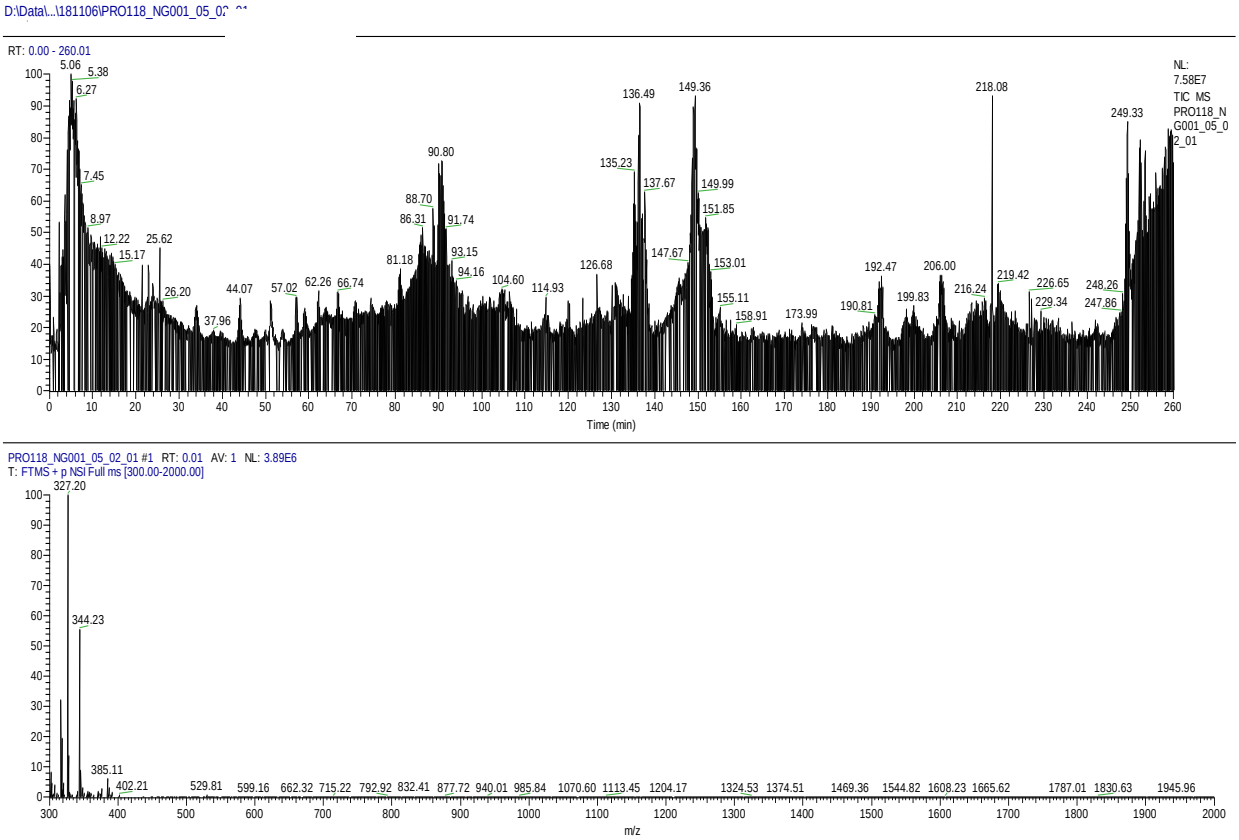
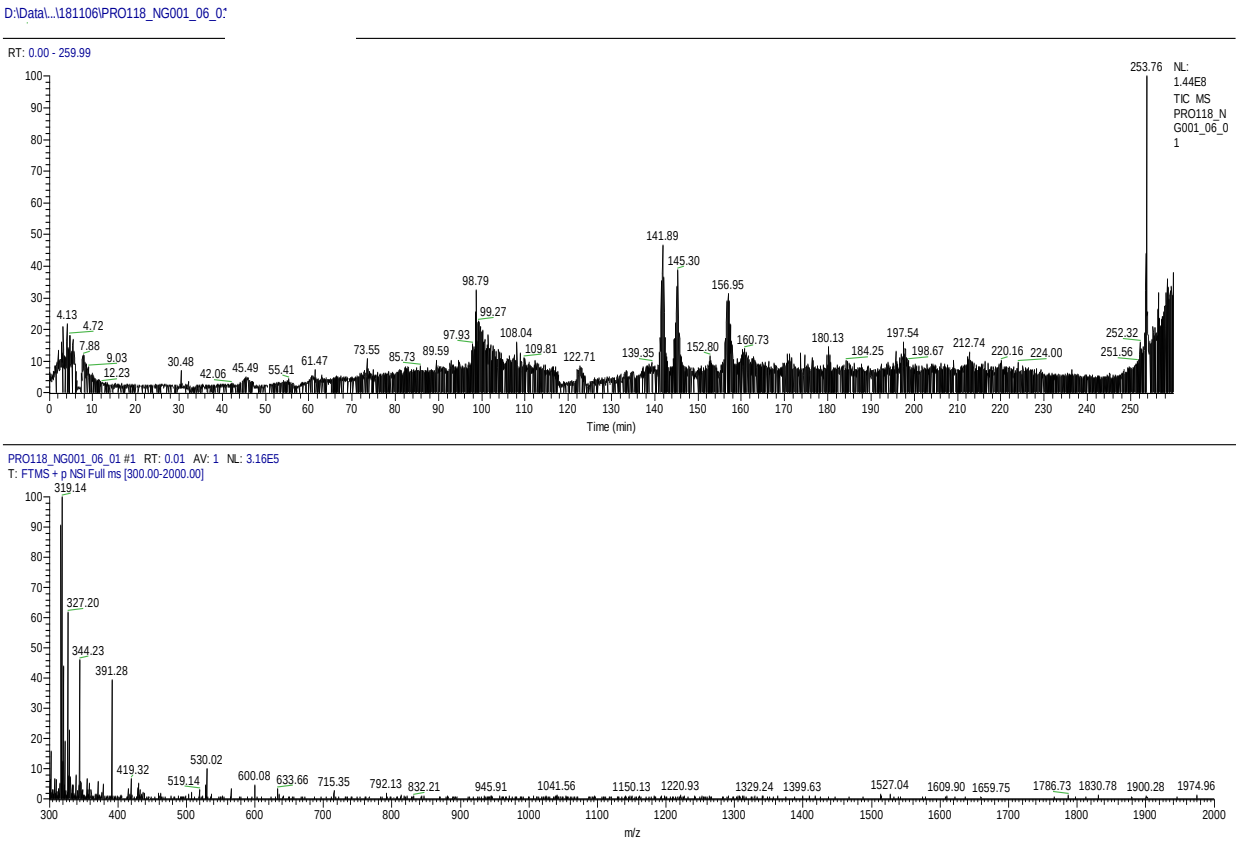


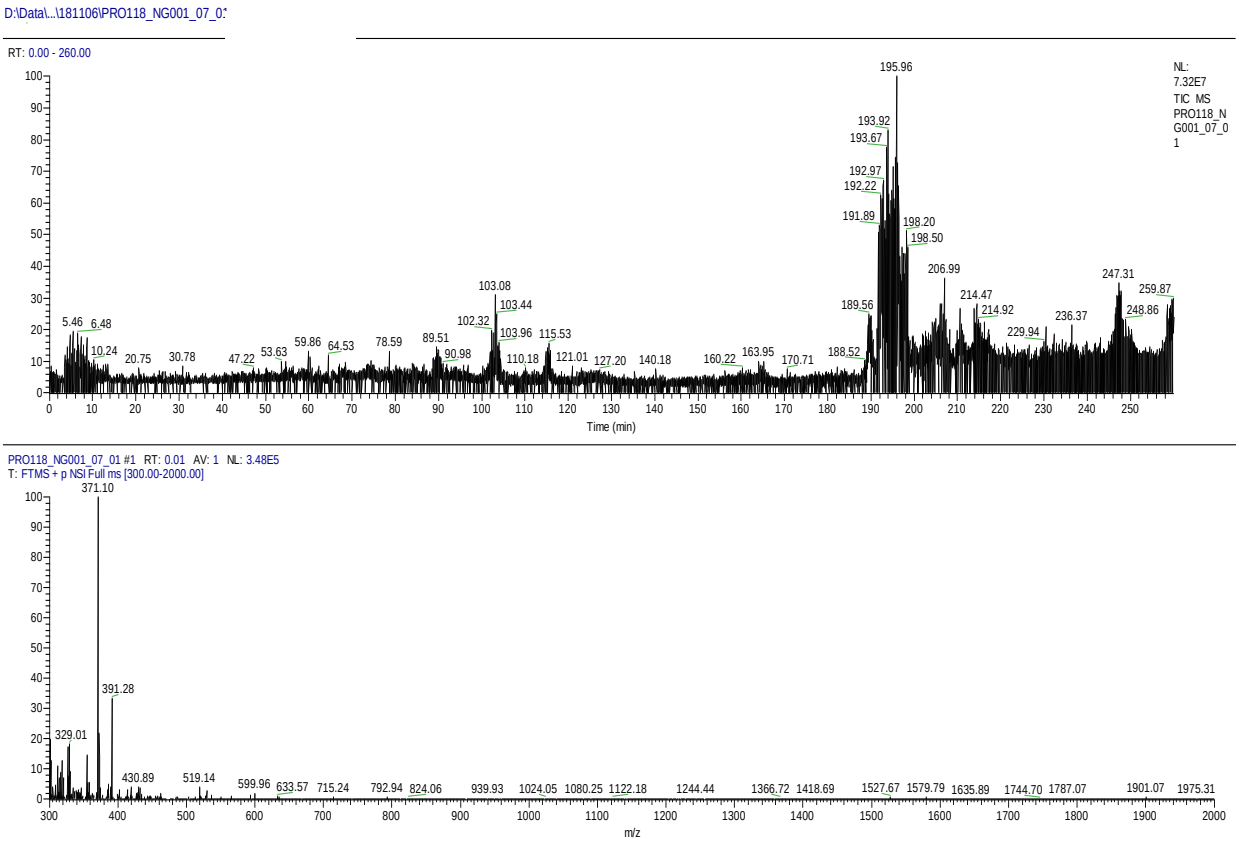
Figure 36: A. Chromatogram of sample PRO118_NG001_04 which is derived from the MDA-V cell line treated with N3a, B. The MS/MS spectrum of the same sample



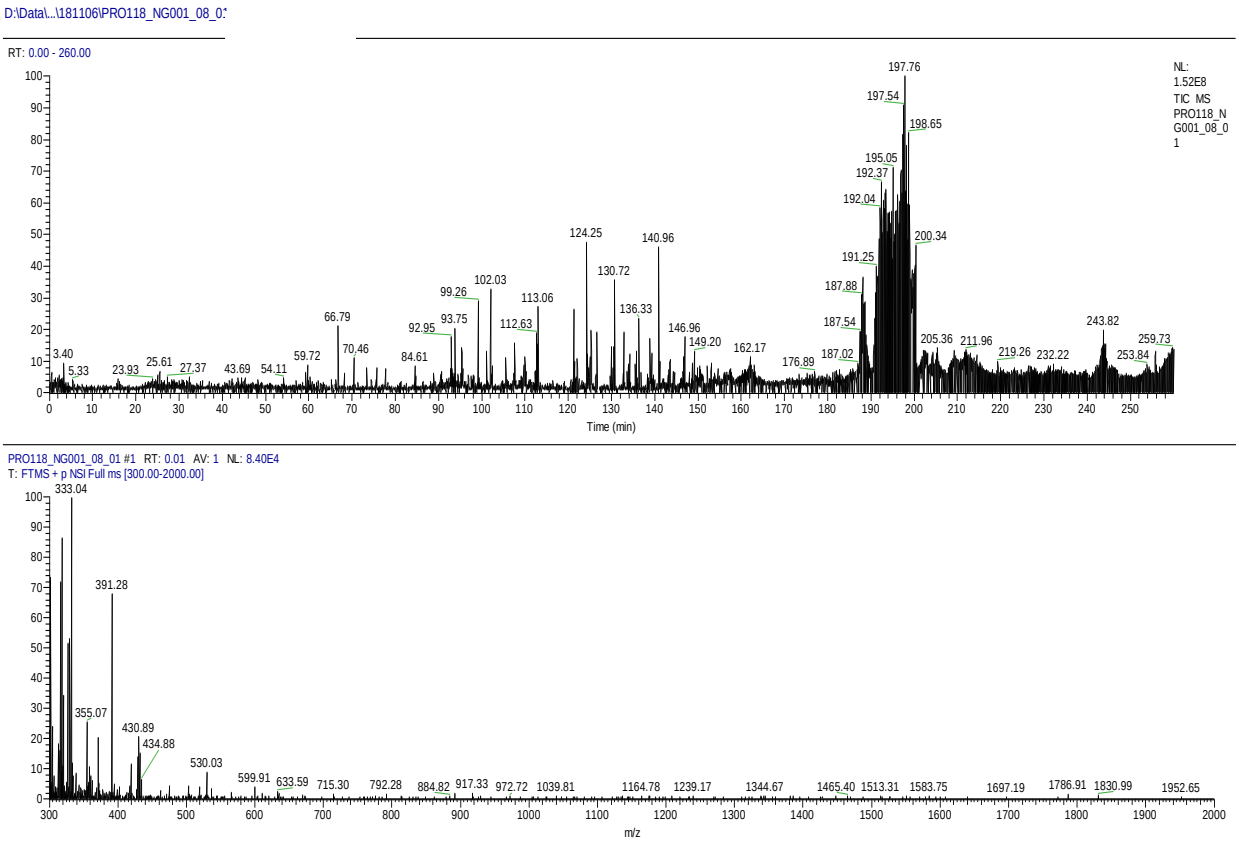
<u>Figure 37:</u> A. Chromatogram of sample PRO118_NG001_05 which is derived from the JMP-1 cell line (control), B. The MS/MS spectrum of the same sample



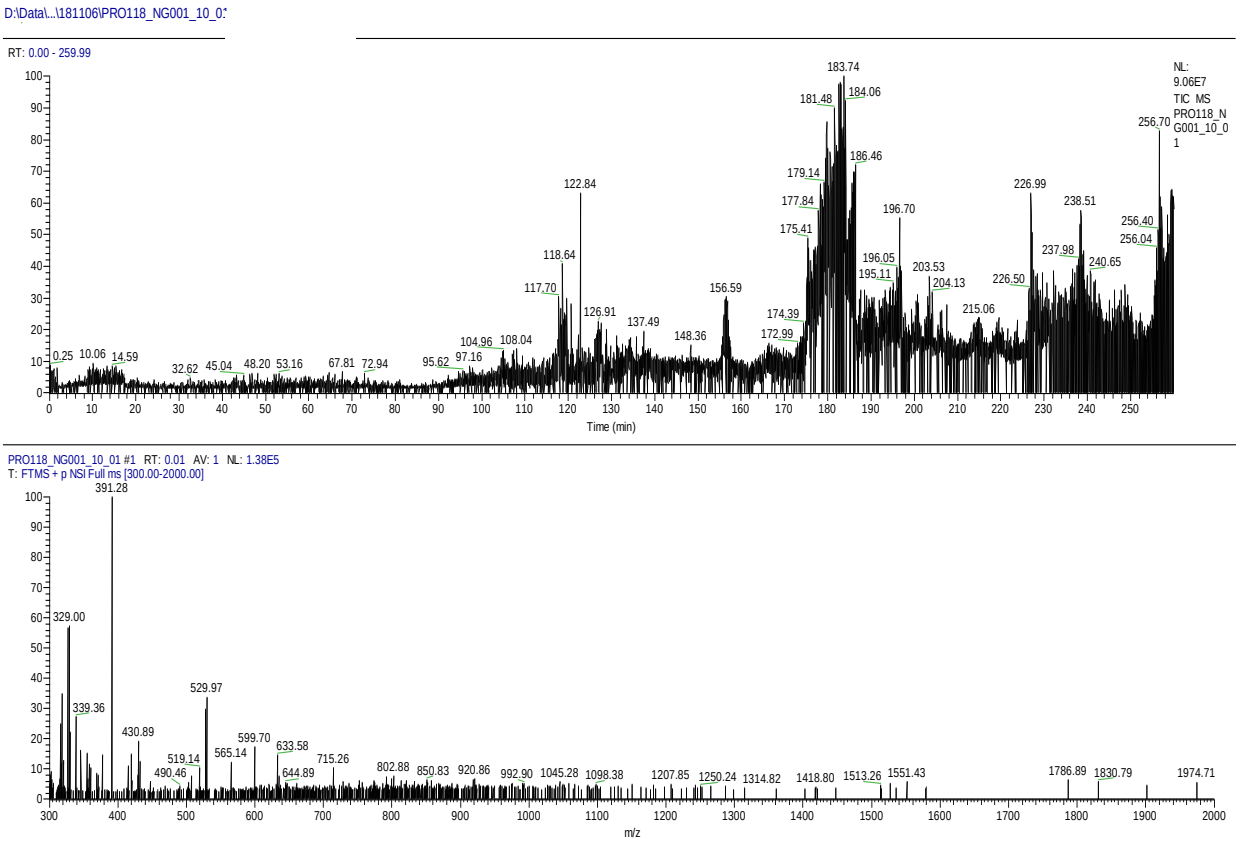
<u>Figure 38:</u> A. Chromatogram of sample PRO118_NG001_06 which is derived from the JMP-1 cell line treated with N3a, B. The MS/MS spectrum of the same sample



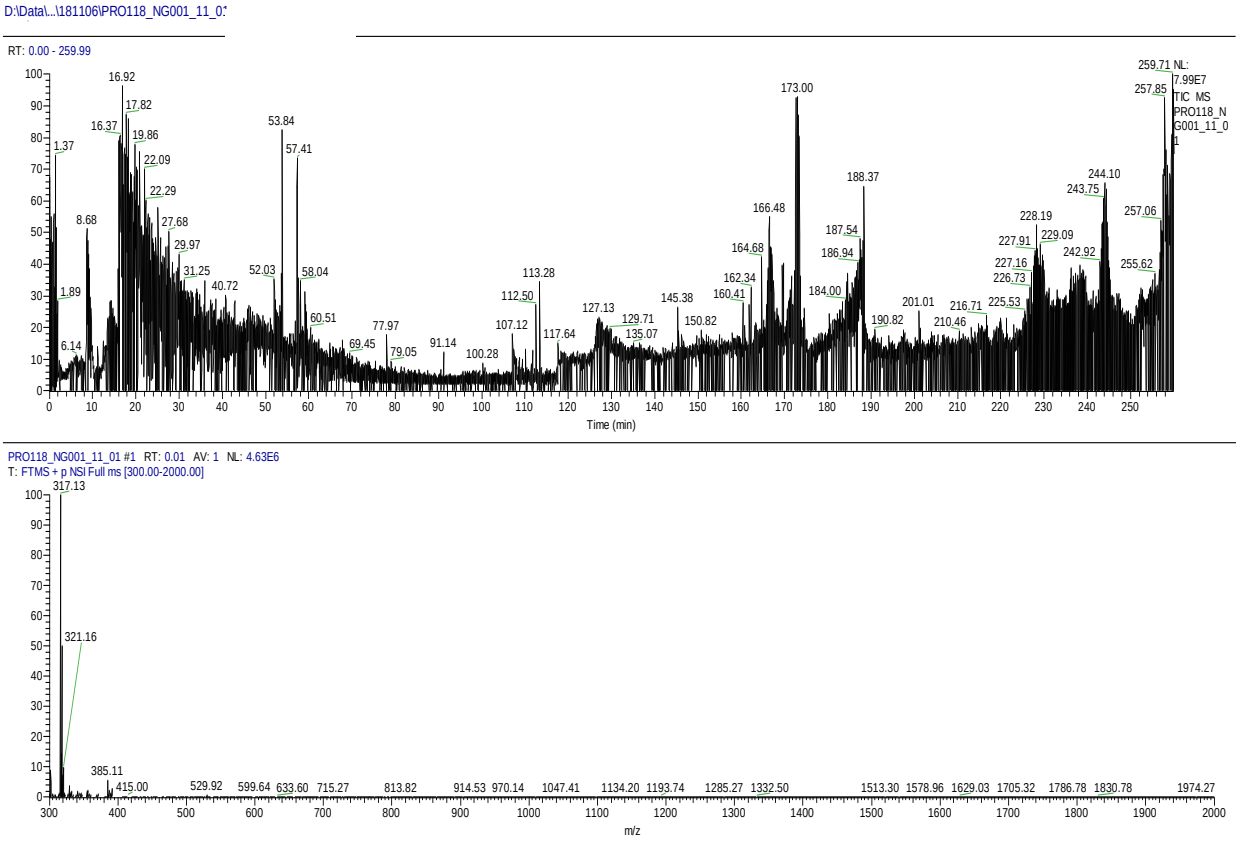
<u>Figure 39:</u> A. Chromatogram of sample PRO118_NG001_07 which is derived from the JMP-1 cell line (control), B. The MS/MS spectrum of the same sample



<u>Figure 40:</u> A. Chromatogram of sample PRO118_NG001_08 which is derived from the JMP-1 cell line treated with N3a, B. The MS/MS spectrum of the same sample



<u>Figure 41:</u> A. Chromatogram of sample PRO118_NG001_10 which is derived from the SUP-M2 cell line treated with N3a, B. The MS/MS spectrum of the same sample



<u>Figure 42:</u> A. Chromatogram of sample PRO118_NG001_11 which is derived from the SUP-M2 cell line (control), B. The MS/MS spectrum of the same sample

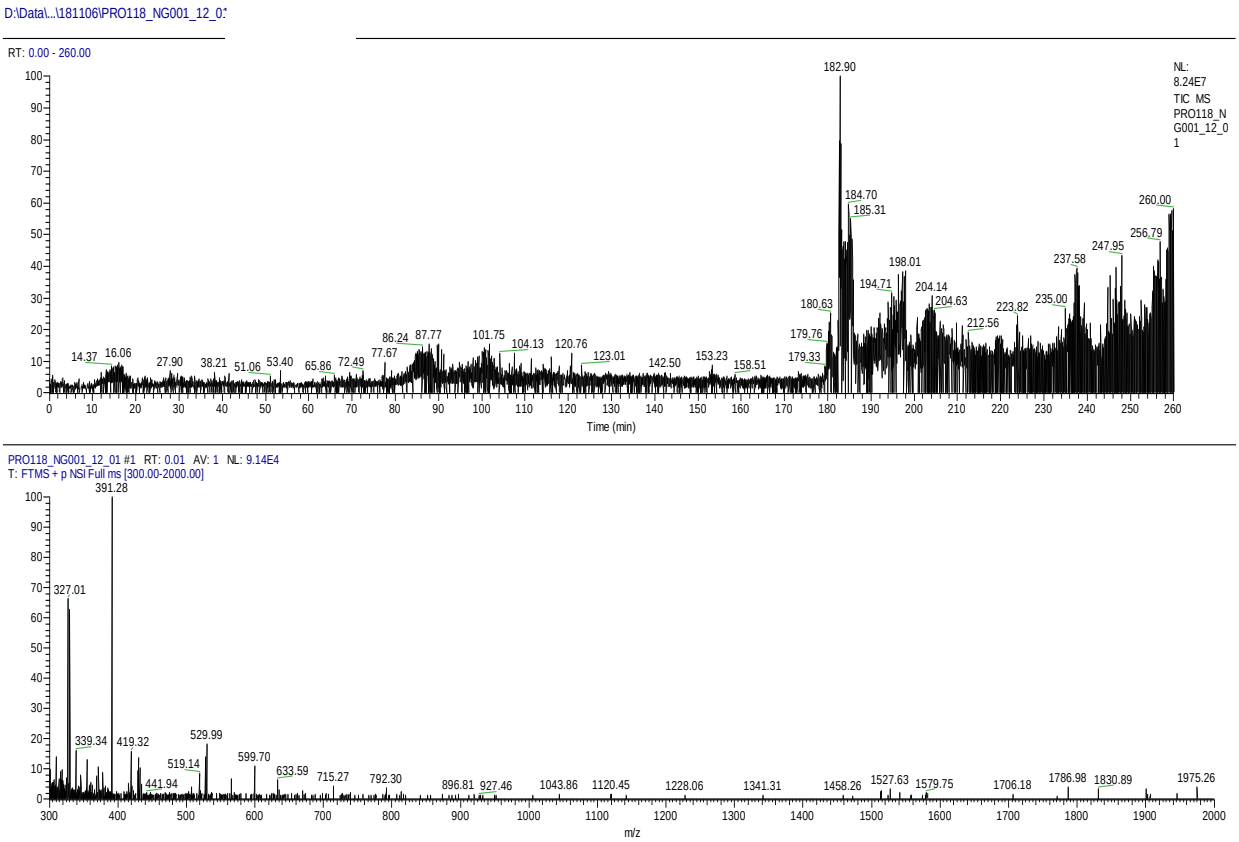


Figure 43: A. Chromatogram of sample PRO118_NG001_12 which is derived from the SUP-M2 cell line treated with N3a, B. The MS/MS spectrum of the same sample.

#	Mapped IDs	Gene Name; Gene Symbol; Ortholog	Species
1	**D6RIA3**	Uncharacterized protein C4orf54;C4orf54;ortholog	Homo sapiens
2	**Q96JF0**	Beta-galactoside alpha-2,6-sialyltransferase 2;ST6GAL2;ortholog	Homo sapiens
3	**P46531**	Neurogenic locus notch homolog protein 1;NOTCH1;ortholog	Homo sapiens
4	**Q9NU22**	Midasin;MDN1;ortholog	Homo sapiens
5	**P56385**	ATP synthase subunit e, mitochondrial;ATP5I;ortholog	Homo sapiens
6	**Q5SZB4**	Uncharacterized protein C9orf50;C9orf50;ortholog	Homo sapiens
7	**Q9NRZ5**	1-acyl-sn-glycerol-3-phosphate acyltransferase delta;AGPAT4;ortholog	Homo sapiens
8	**Q9Y6D0**	Selenoprotein K;SELENOK;ortholog	Homo sapiens
9	**A2RUB1**	Meiosis-specific coiled-coil domain-containing protein MEIOC;MEIOC;ortholog	Homo sapiens
10	**Q9C0B2**	Cilia- and flagella-associated protein 74;CFAP74;ortholog	Homo sapiens
11	**P00568**	Adenylate kinase isoenzyme 1;AK1;ortholog	Homo sapiens
12	**Q8IWA6**	Coiled-coil domain-containing protein 60;CCDC60;ortholog	Homo sapiens
13	**P23396**	40S ribosomal protein S3;RPS3;ortholog	Homo sapiens
14	**Q5VWT5**	FYN-binding protein 2;FYB2;ortholog	Homo sapiens
15	**Q15306**	Interferon regulatory factor 4;IRF4;ortholog	Homo sapiens
16	**O76039**	Cyclin-dependent kinase-like 5;CDKL5;ortholog	Homo sapiens
17	**Q2LD37**	Uncharacterized protein KIAA1109;KIAA1109;ortholog	Homo sapiens

18	**Q86YT6**	E3 ubiquitin-protein ligase MIB1;MIB1;ortholog	Homo sapiens
19	**P31512**	Dimethylaniline monooxygenase [N-oxide-forming] 4;FMO4;ortholog	Homo sapiens
20	**P52333**	Tyrosine-protein kinase JAK3;JAK3;ortholog	Homo sapiens
21	**P09529**	Inhibin beta B chain;INHBB;ortholog	Homo sapiens
22	**P10767**	Fibroblast growth factor 6;FGF6;ortholog	Homo sapiens
23	**Q8N2E2**	von Willebrand factor D and EGF domain-containing protein;VWDE;ortholog	Homo sapiens
24	**Q8TB73**	Protein NDNF;NDNF;ortholog	Homo sapiens
25	**Q8TE73**	Dynein heavy chain 5, axonemal;DNAH5;ortholog	Homo sapiens
26	**A1X283**	SH3 and PX domain-containing protein 2B;SH3PXD2B;ortholog	Homo sapiens
27	**A6NI15**	Mesogenin-1;MSGN1;ortholog	Homo sapiens
28	**Q9NRK6**	ATP-binding cassette sub-family B member 10, mitochondrial;ABCB10;ortholog	Homo sapiens
29	**Q9H2Z4**	Homeobox protein Nkx-2.4;NKX2-4;ortholog	Homo sapiens
30	**O95613**	Pericentrin;PCNT;ortholog	Homo sapiens
31	**A6NMT0**	Homeobox protein DBX1;DBX1;ortholog	Homo sapiens
32	**Q9ULW8**	Protein-arginine deiminase type-3;PADI3;ortholog	Homo sapiens
33	**P48634**	Protein PRRC2A;PRRC2A;ortholog	Homo sapiens
34	**Q6P2D8**	X-ray radiation resistance-associated protein 1;XRRA1;ortholog	Homo sapiens
35	**Q96FG2**	ELMO domain-containing protein 3;ELMOD3;ortholog	Homo sapiens
36	**A8MZG2**	Uncharacterized protein C16orf90;C16orf90;ortholog	Homo sapiens
37	**P62805**	Histone H4;HIST1H4A;ortholog	Homo sapiens
38	**Q5T848**	Probable G-protein coupled receptor 158;GPR158;ortholog	Homo sapiens
39	**Q5VWG9**	Transcription initiation factor TFIID subunit 3;TAF3;ortholog	Homo sapiens
40	**P25774**	Cathepsin S;CTSS;ortholog	Homo sapiens
41	**B2RTY4**	Unconventional myosin-IXa;MYO9A;ortholog	Homo sapiens
42	**Q5VZ72**	Izumo sperm-egg fusion protein 3;IZUMO3;ortholog	Homo sapiens
43	**Q9GZW5**	Putative SCAN domain-containing protein SCAND2P;SCAND2P;ortholog	Homo sapiens
44	**Q6UX72**	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 9;B3GNT9;ortholog	Homo sapiens
45	**Q5VTR2**	E3 ubiquitin-protein ligase BRE1A;RNF20;ortholog	Homo sapiens
46	**P0C7P3**	Protein SLFN14;SLFN14;ortholog	Homo sapiens
47	**P24347**	Stromelysin-3;MMP11;ortholog	Homo sapiens
48	**Q5SZK8**	FRAS1-related extracellular matrix protein 2;FREM2;ortholog	Homo sapiens
49	**O15020**	Spectrin beta chain, non-erythrocytic 2;SPTBN2;ortholog	Homo sapiens
50	**A8MXV6**	CMT1A duplicated region transcript 15 protein-like protein;CDRT15L2;ortholog	Homo sapiens
51	**H3BQB6**	Stathmin domain-containing protein 1;STMND1;ortholog	Homo sapiens
52	**Q14BN4**	Sarcolemmal membrane-associated protein;SLMAP;ortholog	Homo sapiens
53	**Q3I5F7**	Putative acyl-coenzyme A thioesterase 6;ACOT6;ortholog	Homo sapiens
54	**P56180**	Putative tyrosine-protein phosphatase TPTE;TPTE;ortholog	Homo sapiens
55	**Q9UGU0**	Transcription factor 20;TCF20;ortholog	Homo sapiens
56	**A0A0B4J1R7**	BORCS7-ASMT readthrough (NMD candidate);BORCS7-ASMT;ortholog	Homo sapiens

57	**Q8IWB6**	Inactive serine/threonine-protein kinase TEX14;TEX14;ortholog	Homo sapiens
58	**A6NL88**	Protein shisa-7;SHISA7;ortholog	Homo sapiens
59	**Q6P4F7**	Rho GTPase-activating protein 11A;ARHGAP11A;ortholog	Homo sapiens
60	**Q13185**	Chromobox protein homolog 3;CBX3;ortholog	Homo sapiens
61	**Q16478**	Glutamate receptor ionotropic, kainate 5;GRIK5;ortholog	Homo sapiens
62	**Q9BY21**	G-protein coupled receptor 87;GPR87;ortholog	Homo sapiens
63	**O14980**	Exportin-1;XPO1;ortholog	Homo sapiens
64	**Q07011**	Tumor necrosis factor receptor superfamily member 9;TNFRSF9;ortholog	Homo sapiens
65	**A6NCK2**	Tripartite motif-containing protein 43B;TRIM43B;ortholog	Homo sapiens
66	**Q96NL6**	Sodium channel and clathrin linker 1;SCLT1;ortholog	Homo sapiens
67	**P62913**	60S ribosomal protein L11;RPL11;ortholog	Homo sapiens
68	**P98164**	Low-density lipoprotein receptor-related protein 2;LRP2;ortholog	Homo sapiens
69	**O00585**	C-C motif chemokine 21;CCL21;ortholog	Homo sapiens
70	**Q2M1Z3**	Rho GTPase-activating protein 31;ARHGAP31;ortholog	Homo sapiens
71	**Q96JB3**	Hypermethylated in cancer 2 protein;HIC2;ortholog	Homo sapiens
72	**Q5T3J3**	Ligand-dependent nuclear receptor-interacting factor 1;LRIF1;ortholog	Homo sapiens
73	**Q3SY17**	Solute carrier family 25 member 52;SLC25A52;ortholog	Homo sapiens
74	**O60469**	Down syndrome cell adhesion molecule;DSCAM;ortholog	Homo sapiens
75	**Q7RTU4**	Class A basic helix-loop-helix protein 9;BHLHA9;ortholog	Homo sapiens
76	**P30414**	NK-tumor recognition protein;NKTR;ortholog	Homo sapiens
77	**Q7KZ85**	Transcription elongation factor SPT6;SUPT6H;ortholog	Homo sapiens
78	**O14983**	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1;ATP2A1;ortholog	Homo sapiens
79	**P24311**	Cytochrome c oxidase subunit 7B, mitochondrial;COX7B;ortholog	Homo sapiens
80	**Q9Y272**	Dexamethasone-induced Ras-related protein 1;RASD1;ortholog	Homo sapiens
81	**P37802**	Transgelin-2;TAGLN2;ortholog	Homo sapiens
82	**K7EM74**	Uncharacterized protein (Fragment);unassigned;ortholog	Homo sapiens
83	**Q14191**	Werner syndrome ATP-dependent helicase;WRN;ortholog	Homo sapiens
84	**Q99784**	Noelin;OLFM1;ortholog	Homo sapiens
85	**Q99250**	Sodium channel protein type 2 subunit alpha;SCN2A;ortholog	Homo sapiens
86	**Q96C11**	FGGY carbohydrate kinase domain-containing protein;FGGY;ortholog	Homo sapiens
87	**P28074**	Proteasome subunit beta type-5;PSMB5;ortholog	Homo sapiens
88	**Q8NH64**	Olfactory receptor 51A7;OR51A7;ortholog	Homo sapiens
89	**Q8ND23**	Capping protein, Arp2/3 and myosin-I linker protein 3;CARMIL3;ortholog	Homo sapiens
90	**A0A087WXM9**	Meiosis-specific kinetochore protein;MEIKIN;ortholog	Homo sapiens
91	**Q6PF05**	Tetratricopeptide repeat protein 23-like;TTC23L;ortholog	Homo sapiens
92	**Q2QGD7**	Zinc finger protein ZXDC;ZXDC;ortholog	Homo sapiens
93	**P78527**	DNA-dependent protein kinase catalytic subunit;PRKDC;ortholog	Homo sapiens
94	**Q14997**	Proteasome activator complex subunit 4;PSME4;ortholog	Homo sapiens
95	**Q9C0G6**	Dynein heavy chain 6, axonemal;DNAH6;ortholog	Homo sapiens
96	**Q8IZC6**	Collagen alpha-1(XXVII) chain;COL27A1;ortholog	Homo sapiens

97	**P30048**	Thioredoxin-dependent peroxide reductase, mitochondrial;PRDX3;ortholog	Homo sapiens
98	**Q8HWS3**	DNA-binding protein RFX6;RFX6;ortholog	Homo sapiens
99	**O14818**	Proteasome subunit alpha type-7;PSMA7;ortholog	Homo sapiens
100	**Q7LFL8**	CXXC-type zinc finger protein 5;CXXC5;ortholog	Homo sapiens
101	**Q05823**	2-5A-dependent ribonuclease;RNASEL;ortholog	Homo sapiens
102	**Q96J65**	Multidrug resistance-associated protein 9;ABCC12;ortholog	Homo sapiens
103	**P46779**	60S ribosomal protein L28;RPL28;ortholog	Homo sapiens
104	**Q5T0Z8**	Uncharacterized protein C6orf132;C6orf132;ortholog	Homo sapiens
105	**Q96DN2**	von Willebrand factor C and EGF domain-containing protein;VWCE;ortholog	Homo sapiens
106	**A0PJX8**	Transmembrane protein 82;TMEM82;ortholog	Homo sapiens
107	**A0A096LP55**	Cytochrome b-c1 complex subunit 6-like, mitochondrial;UQCRHL;ortholog	Homo sapiens
108	**P26012**	Integrin beta-8;ITGB8;ortholog	Homo sapiens
109	**O95425**	Supervillin;SVIL;ortholog	Homo sapiens
110	**P34820**	Bone morphogenetic protein 8B;BMP8B;ortholog	Homo sapiens
111	**Q96KM6**	Zinc finger protein 512B;ZNF512B;ortholog	Homo sapiens
112	**P08922**	Proto-oncogene tyrosine-protein kinase ROS;ROS1;ortholog	Homo sapiens
113	**Q9BXM7**	Serine/threonine-protein kinase PINK1, mitochondrial;PINK1;ortholog	Homo sapiens
114	**Q9NYZ2**	Mitoferrin-1;SLC25A37;ortholog	Homo sapiens
115	**A7E2V4**	Zinc finger SWIM domain-containing protein 8;ZSWIM8;ortholog	Homo sapiens
116	**Q9Y2J4**	Angiomotin-like protein 2;AMOTL2;ortholog	Homo sapiens
117	**M0QYG6**	Uncharacterized protein (Fragment);unassigned;ortholog	Homo sapiens
118	**Q9H1B4**	Nuclear RNA export factor 5;NXF5;ortholog	Homo sapiens
119	**Q96EU7**	C1GALT1-specific chaperone 1;C1GALT1C1;ortholog	Homo sapiens
120	**O15230**	Laminin subunit alpha-5;LAMA5;ortholog	Homo sapiens
121	**Q9C0J8**	pre-mRNA 3' end processing protein WDR33;WDR33;ortholog	Homo sapiens
122	**Q96AG4**	Leucine-rich repeat-containing protein 59;LRRC59;ortholog	Homo sapiens
123	**P00390**	Glutathione reductase, mitochondrial;GSR;ortholog	Homo sapiens
124	**Q96LM5**	Uncharacterized protein C4orf45;C4orf45;ortholog	Homo sapiens
125	**Q8TEA1**	Putative methyltransferase NSUN6;NSUN6;ortholog	Homo sapiens
126	**Q15149**	Plectin;PLEC;ortholog	Homo sapiens
127	**O60493**	Sorting nexin-3;SNX3;ortholog	Homo sapiens
128	**P0C7P4**	Putative cytochrome b-c1 complex subunit Rieske-like protein 1;UQCRFS1P1;ortholog	Homo sapiens
129	**A6NFN9**	Protein ANKUB1;ANKUB1;ortholog	Homo sapiens
130	**A5D8V7**	Coiled-coil domain-containing protein 151;CCDC151;ortholog	Homo sapiens
131	**Q86YV9**	Hermansky-Pudlak syndrome 6 protein;HPS6;ortholog	Homo sapiens
132	**Q9NZI8**	Insulin-like growth factor 2 mRNA-binding protein 1;IGF2BP1;ortholog	Homo sapiens
133	**Q9H7S9**	Zinc finger protein 703;ZNF703;ortholog	Homo sapiens
134	**Q6NY19**	KN motif and ankyrin repeat domain-containing protein 3;KANK3;ortholog	Homo sapiens
135	**O60840**	Voltage-dependent L-type calcium channel subunit alpha-	Homo sapiens

		1F;CACNA1F;ortholog	
136	**Q96LR2**	Leucine rich adaptor protein 1;LURAP1;ortholog	Homo sapiens
137	**P63244**	Receptor of activated protein C kinase 1;RACK1;ortholog	Homo sapiens
138	**Q8IU99**	Calcium homeostasis modulator protein 1;CALHM1;ortholog	Homo sapiens
139	**Q8NE79**	Blood vessel epicardial substance;BVES;ortholog	Homo sapiens
140	**P35408**	Prostaglandin E2 receptor EP4 subtype;PTGER4;ortholog	Homo sapiens
141	**A6NGR9**	Maestro heat-like repeat-containing protein family member 6;MROH6;ortholog	Homo sapiens
142	**P09327**	Villin-1;VIL1;ortholog	Homo sapiens
143	**P12004**	Proliferating cell nuclear antigen;PCNA;ortholog	Homo sapiens
144	**Q9HCK8**	Chromodomain-helicase-DNA-binding protein 8;CHD8;ortholog	Homo sapiens
145	**O14686**	Histone-lysine N-methyltransferase 2D;KMT2D;ortholog	Homo sapiens
146	**O15061**	Synemin;SYNM;ortholog	Homo sapiens
147	**Q8NCF5**	NFATC2-interacting protein;NFATC2IP;ortholog	Homo sapiens
148	**A1L190**	Synaptonemal complex central element protein 3;SYCE3;ortholog	Homo sapiens
149	**P25788**	Proteasome subunit alpha type-3;PSMA3;ortholog	Homo sapiens
150	**O60812**	Heterogeneous nuclear ribonucleoprotein C-like 1;HNRNPCL1;ortholog	Homo sapiens
151	**Q8NGL9**	Olfactory receptor 4C16;OR4C16;ortholog	Homo sapiens
152	**P54762**	Ephrin type-B receptor 1;EPHB1;ortholog	Homo sapiens
153	**Q92583**	C-C motif chemokine 17;CCL17;ortholog	Homo sapiens
154	**Q96MC4**	CEP295 N-terminal-like protein;CEP295NL;ortholog	Homo sapiens
155	**Q7L590**	Protein MCM10 homolog;MCM10;ortholog	Homo sapiens
156	**O43283**	Mitogen-activated protein kinase kinase kinase 13;MAP3K13;ortholog	Homo sapiens
157	**Q9Y5Z0**	Beta-secretase 2;BACE2;ortholog	Homo sapiens
158	**P53384**	Cytosolic Fe-S cluster assembly factor NUBP1;NUBP1;ortholog	Homo sapiens
159	**A8TX70**	Collagen alpha-5(VI) chain;COL6A5;ortholog	Homo sapiens
160	**Q13410**	Butyrophilin subfamily 1 member A1;BTN1A1;ortholog	Homo sapiens
161	**Q6NSI1**	Putative ankyrin repeat domain-containing protein 26-like protein;ANKRD26P1;ortholog	Homo sapiens
162	**Q6NUP7**	Serine/threonine-protein phosphatase 4 regulatory subunit 4;PPP4R4;ortholog	Homo sapiens
163	**P62241**	40S ribosomal protein S8;RPS8;ortholog	Homo sapiens
164	**Q8N4B4**	F-box only protein 39;FBXO39;ortholog	Homo sapiens
165	**Q96L46**	Calpain small subunit 2;CAPNS2;ortholog	Homo sapiens
166	**Q86W10**	Cytochrome P450 4Z1;CYP4Z1;ortholog	Homo sapiens
167	**Q01101**	Insulinoma-associated protein 1;INSM1;ortholog	Homo sapiens
168	**Q96LA8**	Protein arginine N-methyltransferase 6;PRMT6;ortholog	Homo sapiens
169	**P16401**	Histone H1.5;HIST1H1B;ortholog	Homo sapiens
170	**Q8NEA5**	Uncharacterized protein C19orf18;C19orf18;ortholog	Homo sapiens
171	**Q6ZUS5**	Coiled-coil domain-containing protein 121;CCDC121;ortholog	Homo sapiens
172	**Q9NQH7**	Probable Xaa-Pro aminopeptidase 3;XPNPEP3;ortholog	Homo sapiens
173	**Q8WWQ8**	Stabilin-2;STAB2;ortholog	Homo sapiens
174	**P21796**	Voltage-dependent anion-selective channel protein	Homo sapiens

		1;VDAC1;ortholog	
175	**J3QT63**	Uncharacterized protein (Fragment);unassigned;ortholog	Homo sapiens
176	**Q8TCI5**	Protein pitchfork;PIFO;ortholog	Homo sapiens
177	**Q9BZ81**	Melanoma-associated antigen B5;MAGEB5;ortholog	Homo sapiens
178	**P16234**	Platelet-derived growth factor receptor alpha;PDGFRA;ortholog	Homo sapiens
179	**P49755**	Transmembrane emp24 domain-containing protein 10;TMED10;ortholog	Homo sapiens
180	**O75326**	Semaphorin-7A;SEMA7A;ortholog	Homo sapiens
181	**Q8TBB6**	Probable cationic amino acid transporter;SLC7A14;ortholog	Homo sapiens
182	**Q9NZJ4**	Sacsin;SACS;ortholog	Homo sapiens
183	**Q9UPU9**	Protein Smaug homolog 1;SAMD4A;ortholog	Homo sapiens
184	**Q9NT22**	EMILIN-3;EMILIN3;ortholog	Homo sapiens
185	**Q8IY92**	Structure-specific endonuclease subunit SLX4;SLX4;ortholog	Homo sapiens
186	**Q9H8S5**	Cyclin N-terminal domain-containing protein 2;CNTD2;ortholog	Homo sapiens
187	**Q14469**	Transcription factor HES-1;HES1;ortholog	Homo sapiens
188	**P36542**	ATP synthase subunit gamma, mitochondrial;ATP5F1C;ortholog	Homo sapiens
189	**P28908**	Tumor necrosis factor receptor superfamily member 8;TNFRSF8;ortholog	Homo sapiens
190	**Q8IZ20**	Tissue-resident T-cell transcription regulator protein ZNF683;ZNF683;ortholog	Homo sapiens
191	**Q6ZS11**	Ras and Rab interactor-like protein;RINL;ortholog	Homo sapiens
192	**A0A0B4J277**	T cell receptor alpha variable 22;TRAV22;ortholog	Homo sapiens
193	**Q9NP90**	Ras-related protein Rab-9B;RAB9B;ortholog	Homo sapiens
194	**P43652**	Afamin;AFM;ortholog	Homo sapiens
195	**Q9BVG8**	Kinesin-like protein KIFC3;KIFC3;ortholog	Homo sapiens
196	**Q07954**	Prolow-density lipoprotein receptor-related protein 1;LRP1;ortholog	Homo sapiens
197	**Q15784**	Neurogenic differentiation factor 2;NEUROD2;ortholog	Homo sapiens
198	**Q9Y4C4**	Malignant fibrous histiocytoma-amplified sequence 1;MFHAS1;ortholog	Homo sapiens
199	**Q9Y4R8**	Telomere length regulation protein TEL2 homolog;TELO2;ortholog	Homo sapiens
200	**Q9UKJ8**	Disintegrin and metalloproteinase domain-containing protein 21;ADAM21;ortholog	Homo sapiens
201	**P12883**	Myosin-7;MYH7;ortholog	Homo sapiens
202	**Q6K0P9**	Pyrin and HIN domain-containing protein 1;PYHIN1;ortholog	Homo sapiens
203	**Q04760**	Lactoylglutathione lyase;GLO1;ortholog	Homo sapiens
204	**P38570**	Integrin alpha-E;ITGAE;ortholog	Homo sapiens
205	**P36405**	ADP-ribosylation factor-like protein 3;ARL3;ortholog	Homo sapiens
206	**O95661**	GTP-binding protein Di-Ras3;DIRAS3;ortholog	Homo sapiens
207	**Q8IXW0**	Lamin tail domain-containing protein 2;LMNTD2;ortholog	Homo sapiens
208	**O00244**	Copper transport protein ATOX1;ATOX1;ortholog	Homo sapiens
209	**P50583**	Bis(5'-nucleosyl)-tetraphosphatase [asymmetrical];NUDT2;ortholog	Homo sapiens
210	**Q8TD26**	Chromodomain-helicase-DNA-binding protein 6;CHD6;ortholog	Homo sapiens
211	**Q92887**	Canalicular multispecific organic anion transporter	Homo sapiens

		1;ABCC2;ortholog	
212	**P15085**	Carboxypeptidase A1;CPA1;ortholog	Homo sapiens
213	**Q15717**	ELAV-like protein 1;ELAVL1;ortholog	Homo sapiens
214	**Q8IVP5**	FUN14 domain-containing protein 1;FUNDC1;ortholog	Homo sapiens
215	**P40429**	60S ribosomal protein L13a;RPL13A;ortholog	Homo sapiens
216	**Q99584**	Protein S100-A13;S100A13;ortholog	Homo sapiens
217	**Q9NPF4**	Probable tRNA N6-adenosine threonylcarbamoyltransferase;OSGEP;ortholog	Homo sapiens
218	**Q9ULM3**	YEATS domain-containing protein 2;YEATS2;ortholog	Homo sapiens
219	**P49792**	E3 SUMO-protein ligase RanBP2;RANBP2;ortholog	Homo sapiens
220	**Q14140**	SERTA domain-containing protein 2;SERTAD2;ortholog	Homo sapiens
221	**Q9Y5L4**	Mitochondrial import inner membrane translocase subunit Tim13;TIMM13;ortholog	Homo sapiens
222	**A6NEL2**	Ankyrin repeat domain-containing protein SOWAHB;SOWAHB;ortholog	Homo sapiens
223	**Q9P242**	Neuronal tyrosine-phosphorylated phosphoinositide-3-kinase adapter 2;NYAP2;ortholog	Homo sapiens
224	**Q8TD57**	Dynein heavy chain 3, axonemal;DNAH3;ortholog	Homo sapiens
225	**Q96N23**	Cilia- and flagella-associated protein 54;CFAP54;ortholog	Homo sapiens
226	**H7C0C1**	Uncharacterized protein (Fragment);unassigned;ortholog	Homo sapiens
227	**Q8ND82**	Zinc finger protein 280C;ZNF280C;ortholog	Homo sapiens
228	**P51160**	Cone cGMP-specific 3',5'-cyclic phosphodiesterase subunit alpha';PDE6C;ortholog	Homo sapiens
229	**Q8N7R1**	POM121-like protein 12;POM121L12;ortholog	Homo sapiens
230	**Q9NX94**	WW domain binding protein 1-like;WBP1L;ortholog	Homo sapiens
231	**Q8WVD5**	RING finger protein 141;RNF141;ortholog	Homo sapiens
232	**Q9P225**	Dynein heavy chain 2, axonemal;DNAH2;ortholog	Homo sapiens
233	**Q7Z3I7**	Zinc finger protein 572;ZNF572;ortholog	Homo sapiens
234	**Q9NP55**	BPI fold-containing family A member 1;BPIFA1;ortholog	Homo sapiens
235	**P0C2Y1**	Putative neuroblastoma breakpoint family member 7;NBPF7;ortholog	Homo sapiens
236	**Q32MK0**	Myosin light chain kinase 3;MYLK3;ortholog	Homo sapiens
237	**P20749**	B-cell lymphoma 3 protein;BCL3;ortholog	Homo sapiens
238	**Q7Z333**	Probable helicase senataxin;SETX;ortholog	Homo sapiens
239	**Q9H211**	DNA replication factor Cdt1;CDT1;ortholog	Homo sapiens
240	**Q9BVQ7**	Spermatogenesis-associated protein 5-like protein 1;SPATA5L1;ortholog	Homo sapiens
241	**Q9BXM0**	Periaxin;PRX;ortholog	Homo sapiens
242	**Q674R7**	Autophagy-related protein 9B;ATG9B;ortholog	Homo sapiens
243	**P35410**	Mas-related G-protein coupled receptor MRG;MAS1L;ortholog	Homo sapiens
244	**Q76B58**	BMP/retinoic acid-inducible neural-specific protein 3;BRINP3;ortholog	Homo sapiens
245	**P61278**	Somatostatin;SST;ortholog	Homo sapiens
246	**Q9BYV6**	Tripartite motif-containing protein 55;TRIM55;ortholog	Homo sapiens
247	**P17787**	Neuronal acetylcholine receptor subunit beta-2;CHRNB2;ortholog	Homo sapiens

248	**Q8WUT4**	Leucine-rich repeat neuronal protein 4;LRRN4;ortholog	Homo sapiens
249	**O60240**	Perilipin-1;PLIN1;ortholog	Homo sapiens
250	**O15244**	Solute carrier family 22 member 2;SLC22A2;ortholog	Homo sapiens
251	**P36021**	Monocarboxylate transporter 8;SLC16A2;ortholog	Homo sapiens
252	**Q15825**	Neuronal acetylcholine receptor subunit alpha-6;CHRNA6;ortholog	Homo sapiens
253	**O60548**	Forkhead box protein D2;FOXD2;ortholog	Homo sapiens
254	**P63104**	14-3-3 protein zeta/delta;YWHAZ;ortholog	Homo sapiens
255	**B4DU55**	Zinc finger protein 879;ZNF879;ortholog	Homo sapiens
256	**Q5JVS0**	Intracellular hyaluronan-binding protein 4;HABP4;ortholog	Homo sapiens
257	**P62701**	40S ribosomal protein S4, X isoform;RPS4X;ortholog	Homo sapiens
258	**Q5SXM2**	snRNA-activating protein complex subunit 4;SNAPC4;ortholog	Homo sapiens
259	**Q7L8S5**	OTU domain-containing protein 6A;OTUD6A;ortholog	Homo sapiens
260	**Q7L9B9**	Endonuclease/exonuclease/phosphatase family domain-containing protein 1;EEPD1;ortholog	Homo sapiens
261	**Q12802**	A-kinase anchor protein 13;AKAP13;ortholog	Homo sapiens
262	**Q92604**	Acyl-CoA:lysophosphatidylglycerol acyltransferase 1;LPGAT1;ortholog	Homo sapiens
263	**Q86Y01**	E3 ubiquitin-protein ligase DTX1;DTX1;ortholog	Homo sapiens
264	**P01563**	Interferon alpha-2;IFNA2;ortholog	Homo sapiens
265	**Q6GPH6**	Inositol 1,4,5-trisphosphate receptor-interacting protein-like 1;ITPRIPL1;ortholog	Homo sapiens
266	**Q96I51**	RCC1-like G exchanging factor-like protein;RCC1L;ortholog	Homo sapiens
267	**Q14643**	Inositol 1,4,5-trisphosphate receptor type 1;ITPR1;ortholog	Homo sapiens
268	**Q5H9E4**	Solute carrier family 25 member 53;SLC25A53;ortholog	Homo sapiens
269	**Q3SXP7**	Protein shisa-like-1;SHISAL1;ortholog	Homo sapiens
270	**Q969Y0**	NXPE family member 3;NXPE3;ortholog	Homo sapiens
271	**P57082**	T-box transcription factor TBX4;TBX4;ortholog	Homo sapiens
272	**Q68DN1**	Uncharacterized protein C2orf16;C2orf16;ortholog	Homo sapiens
273	**P28066**	Proteasome subunit alpha type-5;PSMA5;ortholog	Homo sapiens
274	**Q9NQZ2**	Something about silencing protein 10;UTP3;ortholog	Homo sapiens
275	**O75592**	E3 ubiquitin-protein ligase MYCBP2;MYCBP2;ortholog	Homo sapiens
276	**P05771**	Protein kinase C beta type;PRKCB;ortholog	Homo sapiens
277	**A2VCL2**	Coiled-coil domain-containing protein 162;CCDC162P;ortholog	Homo sapiens
278	**P62258**	14-3-3 protein epsilon;YWHAE;ortholog	Homo sapiens
279	**P12034**	Fibroblast growth factor 5;FGF5;ortholog	Homo sapiens
280	**Q9BQM9**	Uncharacterized protein C20orf144;C20orf144;ortholog	Homo sapiens
281	**Q9NPG1**	Frizzled-3;FZD3;ortholog	Homo sapiens
282	**Q5SQS8**	Uncharacterized protein C10orf120;C10orf120;ortholog	Homo sapiens
283	**A0A0B4J268**	T cell receptor alpha variable 4;TRAV4;ortholog	Homo sapiens
284	**Q9Y232**	Chromodomain Y-like protein;CDYL;ortholog	Homo sapiens
285	**Q9Y3P9**	Rab GTPase-activating protein 1;RABGAP1;ortholog	Homo sapiens
286	**Q9P1P5**	Trace amine-associated receptor 2;TAAR2;ortholog	Homo sapiens
287	**P62424**	60S ribosomal protein L7a;RPL7A;ortholog	Homo sapiens
288	**B2RXH4**	BTB/POZ domain-containing protein 18;BTBD18;ortholog	Homo sapiens

289	**A0PK11**	Clarin-2;CLRN2;ortholog	Homo sapiens
290	**Q96CP7**	Calfacilitin;TLCD1;ortholog	Homo sapiens
291	**Q9C0G0**	Zinc finger protein 407;ZNF407;ortholog	Homo sapiens
292	**P18054**	Arachidonate 12-lipoxygenase, 12S-type;ALOX12;ortholog	Homo sapiens
293	**Q96AQ8**	Mitochondrial calcium uniporter regulator 1;MCUR1;ortholog	Homo sapiens
294	**Q9Y573**	Actin-binding protein IPP;IPP;ortholog	Homo sapiens
295	**Q587I9**	Vesicle transport protein SFT2C;SFT2D3;ortholog	Homo sapiens
296	**Q99929**	Achaete-scute homolog 2;ASCL2;ortholog	Homo sapiens
297	**A6NMB1**	Sialic acid-binding Ig-like lectin 16;SIGLEC16;ortholog	Homo sapiens
298	**Q8WUY1**	Protein THEM6;THEM6;ortholog	Homo sapiens
299	**Q9NTJ5**	Phosphatidylinositide phosphatase SAC1;SACM1L;ortholog	Homo sapiens
300	**Q5T6X5**	G-protein coupled receptor family C group 6 member A;GPRC6A;ortholog	Homo sapiens
301	**Q9H7R5**	Zinc finger protein 665;ZNF665;ortholog	Homo sapiens
302	**Q9UJK0**	Ribosome biogenesis protein TSR3 homolog;TSR3;ortholog	Homo sapiens
303	**O43164**	E3 ubiquitin-protein ligase Praja-2;PJA2;ortholog	Homo sapiens
304	**Q8IX06**	Putative exonuclease GOR;REXO1L1P;ortholog	Homo sapiens
305	**Q8WXG9**	G-protein coupled receptor 98;GPR98;ortholog	Homo sapiens
306	**Q16394**	Exostosin-1;EXT1;ortholog	Homo sapiens
307	**Q9UPQ0**	LIM and calponin homology domains-containing protein 1;LIMCH1;ortholog	Homo sapiens
308	**Q9P2V4**	Leucine-rich repeat, immunoglobulin-like domain and transmembrane domain-containing protein 1;LRIT1;ortholog	Homo sapiens
309	**A0A0A6YYL3**	POTE ankyrin domain family member B;POTEB;ortholog	Homo sapiens
310	**O75052**	Carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase protein;NOS1AP;ortholog	Homo sapiens
311	**Q8N5S1**	Solute carrier family 25 member 41;SLC25A41;ortholog	Homo sapiens
312	**Q5M9N0**	Coiled-coil domain-containing protein 158;CCDC158;ortholog	Homo sapiens
313	**A6NNS2**	Dehydrogenase/reductase SDR family member 7C;DHRS7C;ortholog	Homo sapiens
314	**P31947**	14-3-3 protein sigma;SFN;ortholog	Homo sapiens
315	**P02462**	Collagen alpha-1(IV) chain;COL4A1;ortholog	Homo sapiens
316	**O75533**	Splicing factor 3B subunit 1;SF3B1;ortholog	Homo sapiens
317	**B4DGG1**	cDNA FLJ60496;unassigned;ortholog	Homo sapiens
318	**Q9Y258**	C-C motif chemokine 26;CCL26;ortholog	Homo sapiens
319	**Q8NBH2**	Kyphoscoliosis peptidase;KY;ortholog	Homo sapiens
320	**P0DM35**	Metallothionein 1H-like protein 1;MT1HL1;ortholog	Homo sapiens
321	**P49247**	Ribose-5-phosphate isomerase;RPIA;ortholog	Homo sapiens
322	**Q68DV7**	E3 ubiquitin-protein ligase RNF43;RNF43;ortholog	Homo sapiens
323	**P61106**	Ras-related protein Rab-14;RAB14;ortholog	Homo sapiens
324	**P51149**	Ras-related protein Rab-7a;RAB7A;ortholog	Homo sapiens
325	**Q9UKX3**	Myosin-13;MYH13;ortholog	Homo sapiens
326	**Q96N76**	Urocanate hydratase;UROC1;ortholog	Homo sapiens
327	**A9Z1Z3**	Fer-1-like protein 4;FER1L4;ortholog	Homo sapiens
328	**P62917**	60S ribosomal protein L8;RPL8;ortholog	Homo sapiens

329	**Q5JU85**	IQ motif and SEC7 domain-containing protein 2;IQSEC2;ortholog	Homo sapiens
330	**Q2TAA5**	GDP-Man:Man(3)GlcNAc(2)-PP-Dol alpha-1,2-mannosyltransferase;ALG11;ortholog	Homo sapiens
331	**Q92526**	T-complex protein 1 subunit zeta-2;CCT6B;ortholog	Homo sapiens
332	**Q5SQQ9**	Ventral anterior homeobox 1;VAX1;ortholog	Homo sapiens
333	**Q02505**	Mucin-3A;MUC3A;ortholog	Homo sapiens
334	**A0A0B4J235**	T cell receptor alpha variable 13-2;TRAV13-2;ortholog	Homo sapiens
335	**Q8NCU4**	Coiled-coil domain-containing protein 191;CCDC191;ortholog	Homo sapiens
336	**P0CW27**	Coiled-coil domain-containing protein 166;CCDC166;ortholog	Homo sapiens
337	**Q9UM47**	Neurogenic locus notch homolog protein 3;NOTCH3;ortholog	Homo sapiens
338	**P20340**	Ras-related protein Rab-6A;RAB6A;ortholog	Homo sapiens
339	**O15399**	Glutamate receptor ionotropic, NMDA 2D;GRIN2D;ortholog	Homo sapiens
340	**Q5HY64**	Putative protein FAM47C;FAM47C;ortholog	Homo sapiens
341	**P35268**	60S ribosomal protein L22;RPL22;ortholog	Homo sapiens
342	**O75897**	Sulfotransferase 1C4;SULT1C4;ortholog	Homo sapiens
343	**Q3KNS6**	Zinc finger protein 829;ZNF829;ortholog	Homo sapiens
344	**P08519**	Apolipoprotein(a);LPA;ortholog	Homo sapiens
345	**P56730**	Neurotrypsin;PRSS12;ortholog	Homo sapiens
346	**Q08211**	ATP-dependent RNA helicase A;DHX9;ortholog	Homo sapiens
347	**Q86YC3**	Negative regulator of reactive oxygen species;NRROS;ortholog	Homo sapiens
348	**P62906**	60S ribosomal protein L10a;RPL10A;ortholog	Homo sapiens
349	**Q9C0D2**	Centrosomal protein of 295 kDa;CEP295;ortholog	Homo sapiens
350	**Q14324**	Myosin-binding protein C, fast-type;MYBPC2;ortholog	Homo sapiens
351	**P0C7X3**	Putative cyclin-Y-like protein 3;CCNYL3;ortholog	Homo sapiens
352	**O60890**	Oligophrenin-1;OPHN1;ortholog	Homo sapiens
353	**Q9Y217**	Myotubularin-related protein 6;MTMR6;ortholog	Homo sapiens
354	**Q9P2P6**	StAR-related lipid transfer protein 9;STARD9;ortholog	Homo sapiens
355	**O43692**	Peptidase inhibitor 15;PI15;ortholog	Homo sapiens
356	**P27348**	14-3-3 protein theta;YWHAQ;ortholog	Homo sapiens
357	**Q8N7U6**	EF-hand domain-containing family member B;EFHB;ortholog	Homo sapiens
358	**Q9BRZ2**	E3 ubiquitin-protein ligase TRIM56;TRIM56;ortholog	Homo sapiens
359	**A6NCW0**	Ubiquitin carboxyl-terminal hydrolase 17-like protein 3;USP17L3;ortholog	Homo sapiens
360	**Q53TQ3**	INO80 complex subunit D;INO80D;ortholog	Homo sapiens
361	**Q9NPE3**	H/ACA ribonucleoprotein complex subunit 3;NOP10;ortholog	Homo sapiens
362	**Q14197**	Peptidyl-tRNA hydrolase ICT1, mitochondrial;MRPL58;ortholog	Homo sapiens
363	**P0CG22**	Putative dehydrogenase/reductase SDR family member 4-like 1;DHRS4L1;ortholog	Homo sapiens
364	**Q5UIP0**	Telomere-associated protein RIF1;RIF1;ortholog	Homo sapiens
365	**A8MSY1**	TMEM110-MUSTN1 readthrough;TMEM110-MUSTN1;ortholog	Homo sapiens
366	**Q8N961**	Ankyrin repeat and BTB/POZ domain-containing protein 2;ABTB2;ortholog	Homo sapiens
367	**Q6ZR37**	Pleckstrin homology domain-containing family G member 7;PLEKHG7;ortholog	Homo sapiens
368	**Q12849**	G-rich sequence factor 1;GRSF1;ortholog	Homo sapiens

369	**Q8N0Z8**	tRNA pseudouridine synthase-like 1;PUSL1;ortholog	Homo sapiens
370	**P54886**	Delta-1-pyrroline-5-carboxylate synthase;ALDH18A1;ortholog	Homo sapiens
371	**P62820**	Ras-related protein Rab-1A;RAB1A;ortholog	Homo sapiens
372	**P26374**	Rab proteins geranylgeranyltransferase component A 2;CHML;ortholog	Homo sapiens
373	**Q8NDA2**	Hemicentin-2;HMCN2;ortholog	Homo sapiens
374	**O75628**	GTP-binding protein REM 1;REM1;ortholog	Homo sapiens
375	**Q02086**	Transcription factor Sp2;SP2;ortholog	Homo sapiens
376	**Q53QZ3**	Rho GTPase-activating protein 15;ARHGAP15;ortholog	Homo sapiens
377	**Q684P5**	Rap1 GTPase-activating protein 2;RAP1GAP2;ortholog	Homo sapiens
378	**Q8N3Z0**	Inactive serine protease 35;PRSS35;ortholog	Homo sapiens
379	**P05141**	ADP/ATP translocase 2;SLC25A5;ortholog	Homo sapiens
380	**P23471**	Receptor-type tyrosine-protein phosphatase zeta;PTPRZ1;ortholog	Homo sapiens
381	**O60882**	Matrix metalloproteinase-20;MMP20;ortholog	Homo sapiens
382	**A0A087X0R7**	SENP3-EIF4A1 readthrough (NMD candidate) (Fragment);SENP3-EIF4A1;ortholog	Homo sapiens
383	**H7BZ55**	Putative ciliary rootlet coiled-coil protein 2;CROCC2;ortholog	Homo sapiens
384	**Q96BM1**	Ankyrin repeat domain-containing protein 9;ANKRD9;ortholog	Homo sapiens
385	**Q02779**	Mitogen-activated protein kinase kinase kinase 10;MAP3K10;ortholog	Homo sapiens
386	**Q8NDL9**	Cytosolic carboxypeptidase-like protein 5;AGBL5;ortholog	Homo sapiens
387	**P24588**	A-kinase anchor protein 5;AKAP5;ortholog	Homo sapiens
388	**Q9BYE9**	Cadherin-related family member 2;CDHR2;ortholog	Homo sapiens
389	**A6NNY8**	Ubiquitin carboxyl-terminal hydrolase 27;USP27X;ortholog	Homo sapiens
390	**Q6ZMY3**	SPOC domain-containing protein 1;SPOCD1;ortholog	Homo sapiens
391	**P28072**	Proteasome subunit beta type-6;PSMB6;ortholog	Homo sapiens
392	**Q49A33**	Putative zinc finger protein 876;ZNF876P;ortholog	Homo sapiens
393	**P23284**	Peptidyl-prolyl cis-trans isomerase B;PPIB;ortholog	Homo sapiens
394	**Q9NQM4**	Protein PIH1D3;PIH1D3;ortholog	Homo sapiens
395	**P57078**	Receptor-interacting serine/threonine-protein kinase 4;RIPK4;ortholog	Homo sapiens
396	**Q9Y2F5**	Little elongation complex subunit 1;ICE1;ortholog	Homo sapiens
397	**Q9GZQ6**	Neuropeptide FF receptor 1;NPFFR1;ortholog	Homo sapiens
398	**P55081**	Microfibrillar-associated protein 1;MFAP1;ortholog	Homo sapiens
399	**Q6Q759**	Sperm-associated antigen 17;SPAG17;ortholog	Homo sapiens
400	**A0PK00**	Transmembrane protein 120B;TMEM120B;ortholog	Homo sapiens
401	**Q8IY22**	C-Maf-inducing protein;CMIP;ortholog	Homo sapiens
402	**Q9H1X3**	DnaJ homolog subfamily C member 25;DNAJC25;ortholog	Homo sapiens
403	**Q7Z2Z1**	Treslin;TICRR;ortholog	Homo sapiens
404	**Q9C0C6**	CLOCK-interacting pacemaker;CIPC;ortholog	Homo sapiens
405	**Q05513**	Protein kinase C zeta type;PRKCZ;ortholog	Homo sapiens
406	**P26373**	60S ribosomal protein L13;RPL13;ortholog	Homo sapiens
407	**P10244**	Myb-related protein B;MYBL2;ortholog	Homo sapiens
408	**Q8IZT6**	Abnormal spindle-like microcephaly-associated protein;ASPM;ortholog	Homo sapiens

409	**Q8WWL2**	Protein spire homolog 2;SPIRE2;ortholog	Homo sapiens
410	**Q4G0X9**	Coiled-coil domain-containing protein 40;CCDC40;ortholog	Homo sapiens
411	**Q96RW7**	Hemicentin-1;HMCN1;ortholog	Homo sapiens
412	**O75396**	Vesicle-trafficking protein SEC22b;SEC22B;ortholog	Homo sapiens
413	**Q8IYD1**	Eukaryotic peptide chain release factor GTP-binding subunit ERF3B;GSPT2;ortholog	Homo sapiens
414	**Q8N5N4**	Uncharacterized protein C3orf22;C3orf22;ortholog	Homo sapiens
415	**P0C7P0**	CDGSH iron-sulfur domain-containing protein 3, mitochondrial;CISD3;ortholog	Homo sapiens
416	**Q8NEV4**	Myosin-IIIa;MYO3A;ortholog	Homo sapiens
417	**P58397**	A disintegrin and metalloproteinase with thrombospondin motifs 12;ADAMTS12;ortholog	Homo sapiens
418	**Q96NZ8**	WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 1;WFIKKN1;ortholog	Homo sapiens
419	**Q8NCN4**	E3 ubiquitin-protein ligase RNF169;RNF169;ortholog	Homo sapiens
420	**Q8WTR8**	Netrin-5;NTN5;ortholog	Homo sapiens
421	**Q8WXD0**	Relaxin receptor 2;RXFP2;ortholog	Homo sapiens
422	**A1A5C7**	Solute carrier family 22 member 23;SLC22A23;ortholog	Homo sapiens
423	**Q9H9B1**	Histone-lysine N-methyltransferase EHMT1;EHMT1;ortholog	Homo sapiens
424	**Q86YV5**	Tyrosine-protein kinase PRAG1;PRAG1;ortholog	Homo sapiens
425	**Q8IZQ8**	Myocardin;MYOCD;ortholog	Homo sapiens
426	**P22612**	cAMP-dependent protein kinase catalytic subunit gamma;PRKACG;ortholog	Homo sapiens
427	**O43909**	Exostosin-like 3;EXTL3;ortholog	Homo sapiens
428	**Q7Z7G8**	Vacuolar protein sorting-associated protein 13B;VPS13B;ortholog	Homo sapiens
429	**Q9Y5G5**	Protocadherin gamma-A8;PCDHGA8;ortholog	Homo sapiens
430	**Q5QGS0**	Neurite extension and migration factor;NEXMIF;ortholog	Homo sapiens
431	**Q8NDV3**	Structural maintenance of chromosomes protein 1B;SMC1B;ortholog	Homo sapiens
432	**Q9C0D4**	Zinc finger protein 518B;ZNF518B;ortholog	Homo sapiens
433	**Q9BX26**	Synaptonemal complex protein 2;SYCP2;ortholog	Homo sapiens
434	**Q9NRG7**	Epimerase family protein SDR39U1;SDR39U1;ortholog	Homo sapiens
435	**Q15075**	Early endosome antigen 1;EEA1;ortholog	Homo sapiens
436	**P0CB47**	Upstream-binding factor 1-like protein 1;UBTFL1;ortholog	Homo sapiens
437	**P62753**	40S ribosomal protein S6;RPS6;ortholog	Homo sapiens

Table 4: Total identified proteins by Panther 14.0 Classification System (GO). The table includes the protein accession (Uniprot), the gene name and symbol and the species.

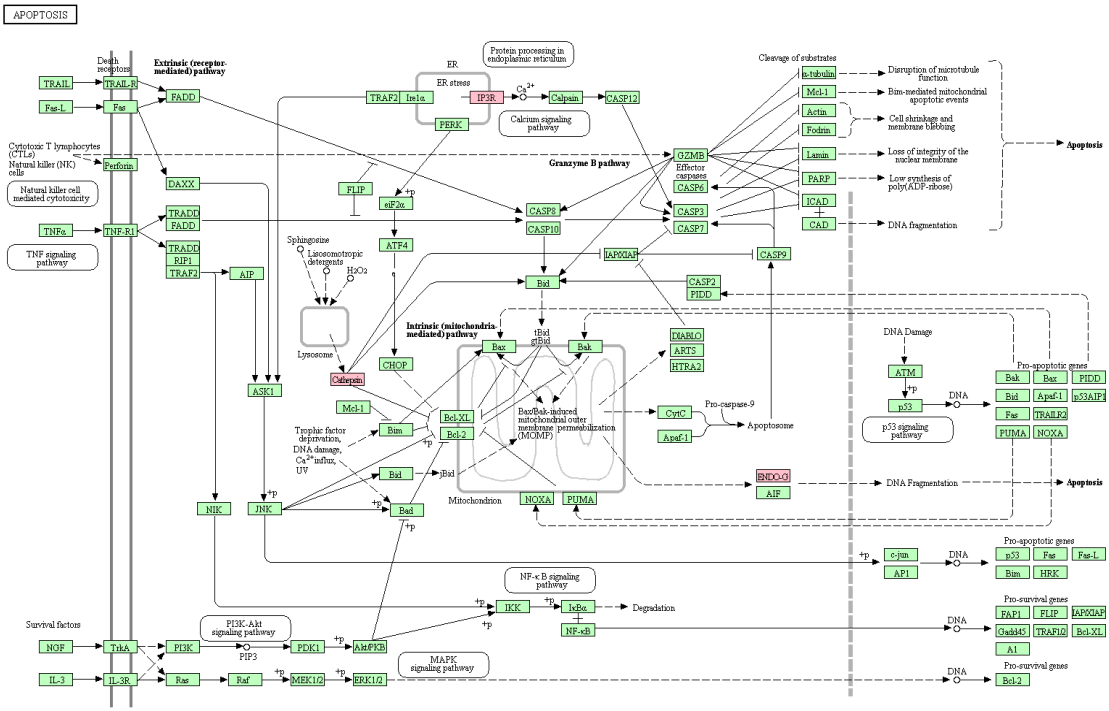


Figure 44: Kegg Map of the Apoptosis Pathway. Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them.

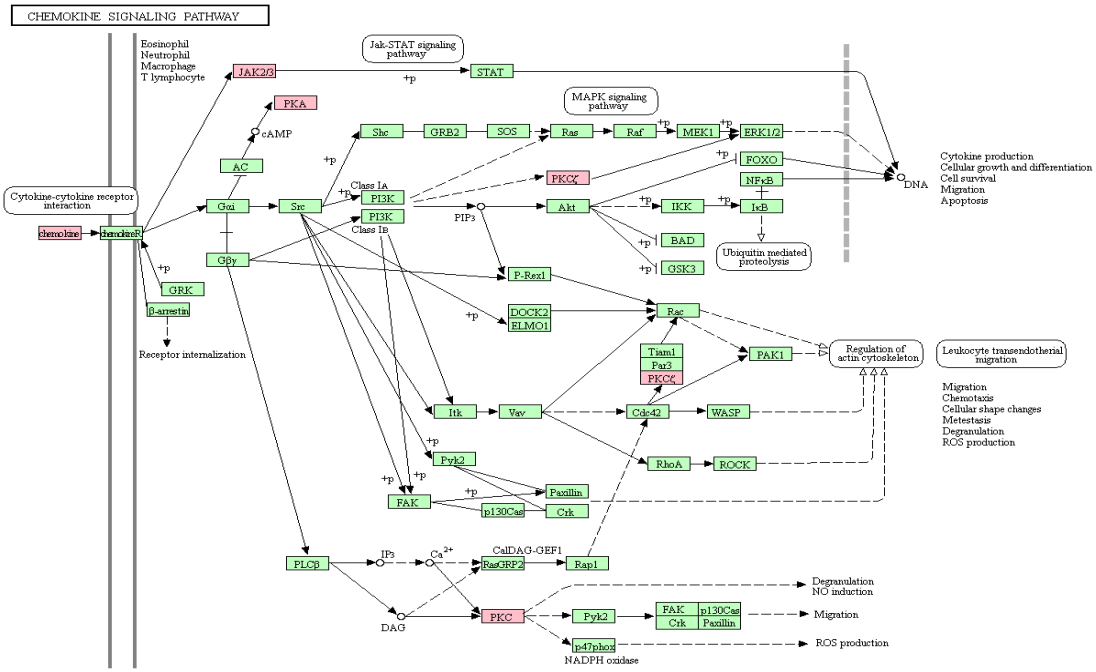


Figure 45: Kegg Map of the Chemokine Signaling Pathway. Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them.

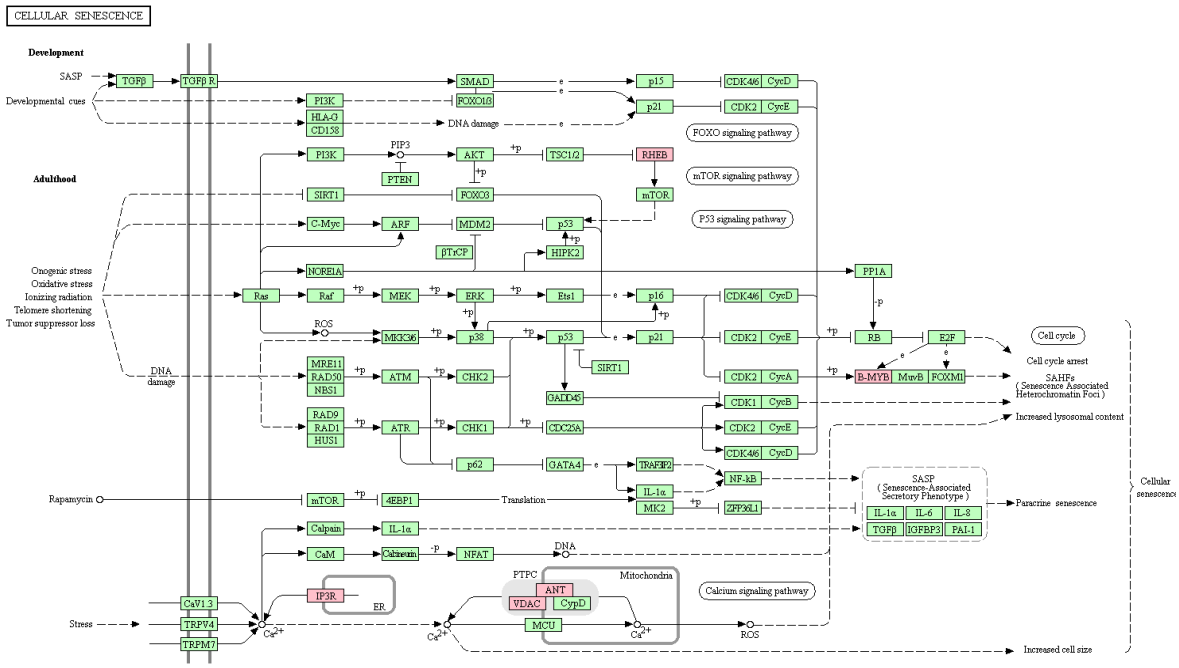


Figure 46: Kegg Map of the Cellular Senescence Pathway.. Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them.

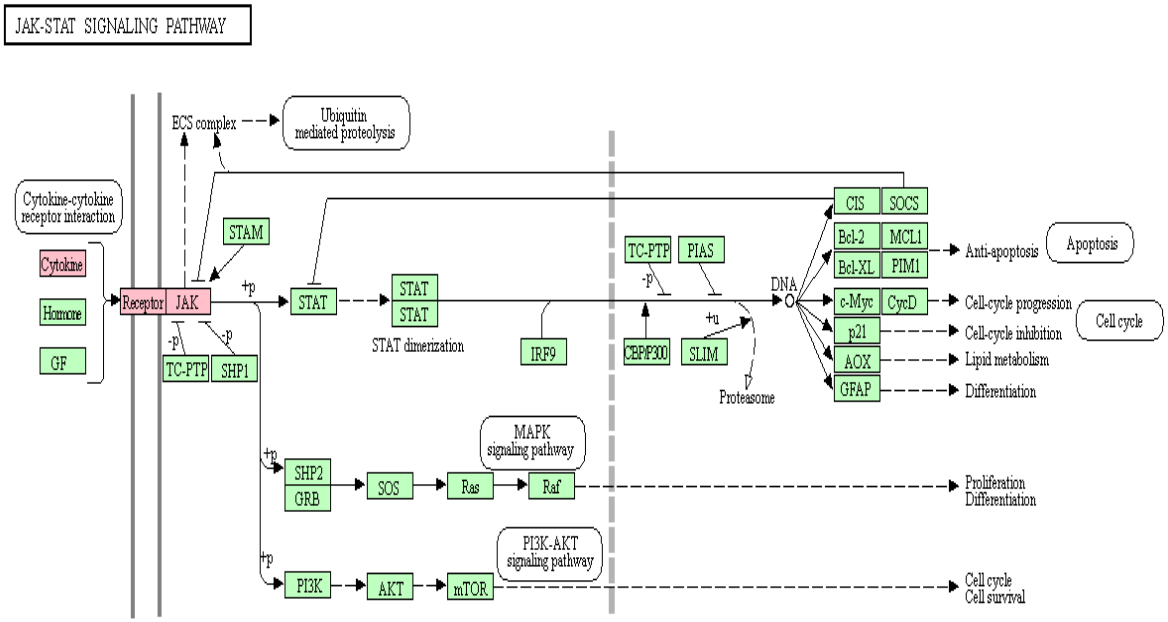
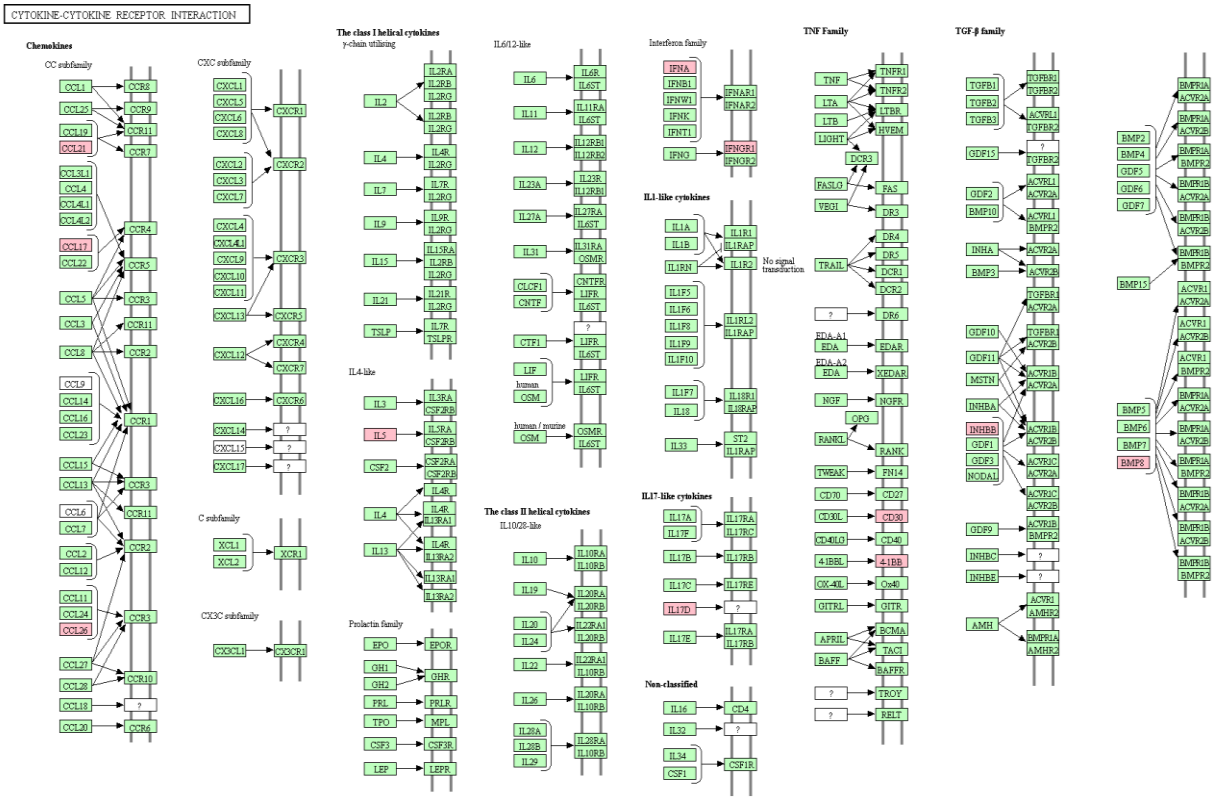


Figure 47: Kegg Map of the Jak – Stat Singlaning Pathway.. Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them.



<u>Figure 48:</u> Kegg Map of the Cytokine - Cytokine receptor interaction pathway. Green boxes are hyperlinked to genes entries by converting K numbers (KO identifiers) to gene identifiers in the reference pathway, indicating the presence of genes in the genome and also the completeness of the pathway. Pink boxes present the genes found in this study. The arrows show the relations and molecular interactions between them.