Proteomics

Drothler Stephan, Rieser Caroline

October 2018

1 Introduction

Lung cancer cells (A549) are treated with TNF-α and compared to a untreated control group. The harvested cells are lysed in order to purify, denaturate, reduce and alkylate the proteome. After incubation the sample is concentrated so it can be measured with a HPLC-ESI-MS/MS setup.

2 Results

2.1 De-Novo Sequencing

De-novo sequencing was performed via the proteome discoverer (Figure 1), but was confirmed with manual calculation and amino acid assignment with Table 1 in the proteomics protocol of the institute.

$$AA = Y_x - Y_{x-1} \tag{1}$$

652.33009 Da - 489.26677 Da = 160.06332 Da (Y)
Table 1: 163.06333 Da = Y
489.26677 Da - 374.23982 Da = 115.02695 Da (D)
Table 1: 115.02695 Da = D
374.23982 Da - 303.20270 Da = 71.03712 Da (A)
Table 1: 71.03712 Da = A
303.20270 Da - 246.18123 Da = 57.02147 Da (G)
Table 1: 57.02147 Da = G
246.18123 Da - 147.11281 Da = 99.06842 Da (V)
Table 1: 99.06842 Da = V
147.11281 Da - 19.015 Da = 128.09781 Da (K)
Table 1: 128.09497 Da = K

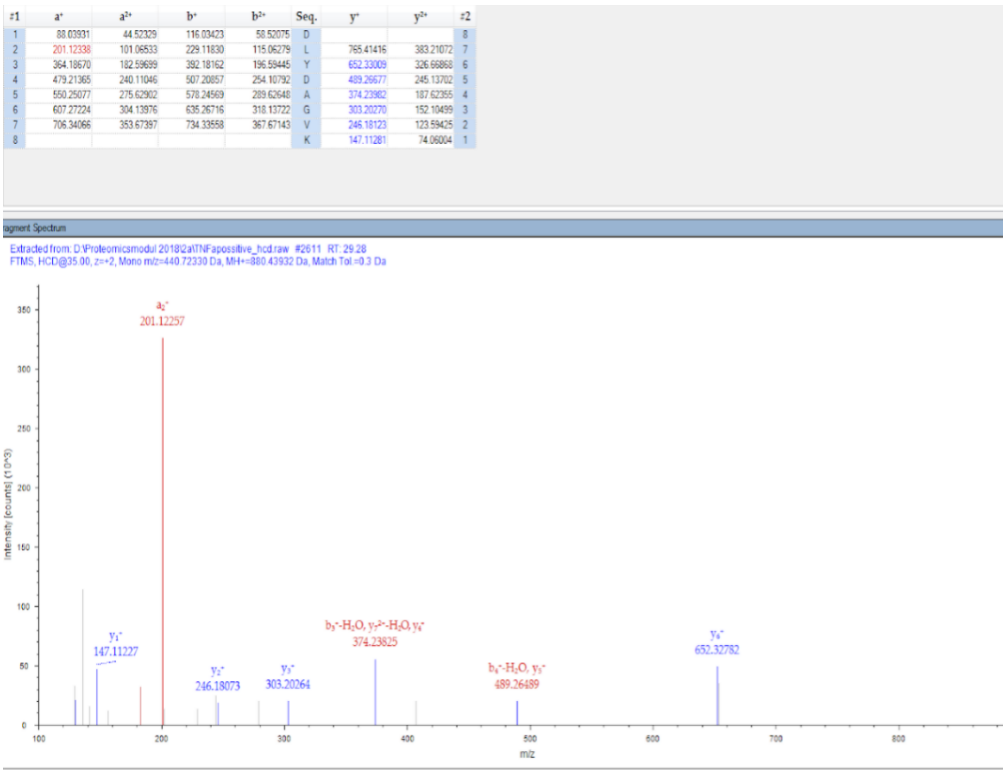


Figure 1: De-Novo Sequencing

2.2 Analysis

2.2.1 R Studio

After MS-Analysis of adenocarcinomic lung epithelial A-549 cells, the generated data sets were analyzed with in silico tools. TNFα (OnlyT) showed 61 exclusively expressed proteins whereas 55 solely expressed proteins were identified in the negative group. We also compared two differently measured positive groups with R Studio. The 2 μl positive specimen (compare lab journal) and the HCD positive sample were compared with the treated group. The results showed 100 unique proteins in the 2μl-group and 44 in the HCD-group.

TNFαnegative	55
TNFαpositive	61
TNFαpositive 2μ	100
TNFαpositive hcd	44

Table 1: Quantity unique proteins

2.2.2 DAVID Bioinformatic Database

To find out which pathways the expressed proteins contribute to, we use the DAVID database. It turned out that all four samples are mostly involved in three pathways. Most interactions are involved in acetylation, poly (A) RNA binding and the extracellular exosomes.

	acetylation	poly-(A) RNA binding	extracelluar exosomes
TNFαnegative	197 proteins / 78,8 %	132 proteins / 52,8%	172 proteins / 68,8%
TNFαpositive	206 proteins / 82,1 %	130 proteins / 51,8%	180 proteins / 71,7%
TNFαpositive 2μl	227 proteins / 79,4%	146 proteins / 51,0%	198 proteins / 69,2%
TNFαpositive hcd	130 proteins / 82,3%	89 proteins / 56,3%	123 proteins / 77,8%

Table 2: Pathway Analysis

Additionally a detailed examination on a acetylation-related gene, in terms of its biological function and its connection to TNF- α was performed. We chose randomly among the most integrated proteins and obtained AHNAK nucleoprotein.

1. function: May be required for neuronal cell differentiation
2. similarity: Contains one PDZ (DHR) domain
3. subunit: Interacts with DYSF; the interaction is direct and (Ca^{2+})-independent

"The protein encoded by this gene is a large (700 kDa) structural scaffold protein consisting of a central domain with 128 aa repeats. The encoded protein may play a role in such diverse processes as blood-brain barrier formation, cell structure and migration, cardiac calcium channel regulation, and tumor metastasis. A much shorter variant encoding a 17 kDa isoform exists for this gene, and the shorter isoform initiates a feedback loop that regulates alternative splicing of this gene." [1]

2.2.3 STRING

To identify the link between TNF- α and AHNAK in a human organism, we use a STRING network [2]

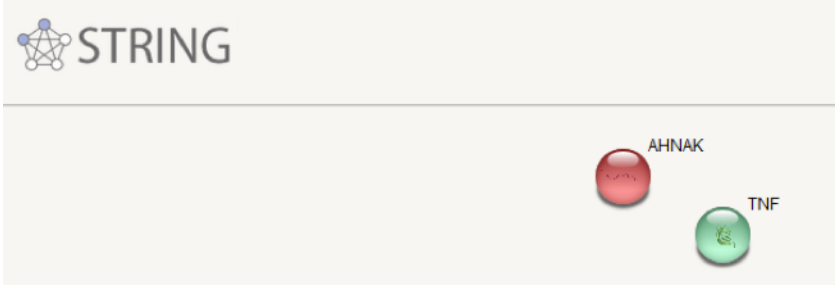


Figure 2: Link TNFα and AHNAK

At first glance, the String database initially has no interaction between the two proteins, so we continue:

After further analysis we have an interaction between TNF and AHNAK. AHNAK is connected to the network by RELA and NFKB1, which is a query protein and first shell of interactor.

RELA:

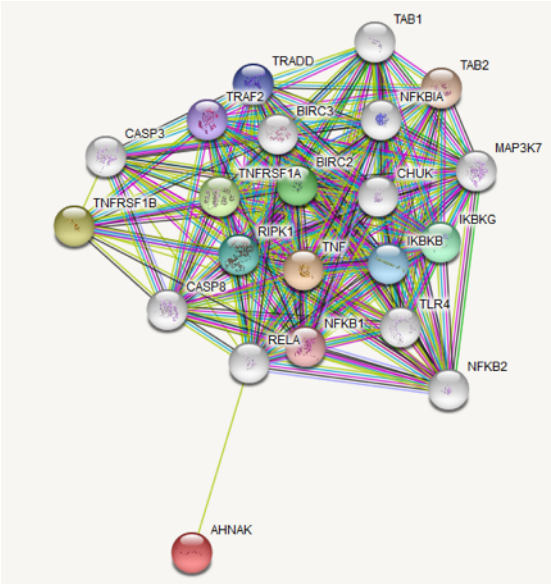
V-rel reticuloendotheliosis viral oncogene homolog A (avian) [2]

NFKB1:

Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1; NF-kappa-B is apleiotropic transcription factor present in almost all cell types and is the endpoint of a series of signal transduction events that are initiated by a vast array of stimuli related to many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. [2]

Biological Process (GO)		
pathway description	*count in gene set*	*false discovery rate*
TRIF-dependent toll-like receptor signaling pathway	15	1.78e-28
toll-like receptor 3 signaling pathway	15	1.79e-28
toll-like receptor 4 signaling pathway	15	2.14e-27
positive regulation of NF-kappaB transcription factor activity	14	2.08e-23
pattern recognition receptor signaling pathway	14	8.03e-23
		(more ...)
Molecular Function (GO)		
pathway description	*count in gene set*	*false discovery rate*
ubiquitin protein ligase binding	7	3.35e-06
scaffold protein binding	4	8.87e-05
identical protein binding	9	0.000188
IkappaB kinase activity	2	0.000908
protein binding	15	0.00148
		(more ...)
Cellular Component (GO)		
pathway description	*count in gene set*	*false discovery rate*
membrane raft	9	2.22e-09
cytosol	18	3.06e-09
I-kappaB/NF-kappaB complex	4	1.91e-08
death-inducing signaling complex	4	2.86e-08
receptor complex	8	8.56e-08

(a) Functional Enrichment



(b) Interaction between TNFα und AHNAK

Figure 3: String data

2.2.4 PANTHER:

For more information about the molecular function from our samples but also about the relationship between TNFα and AHNAK in terms of gene ontology we use the PANTHER tools: [3]

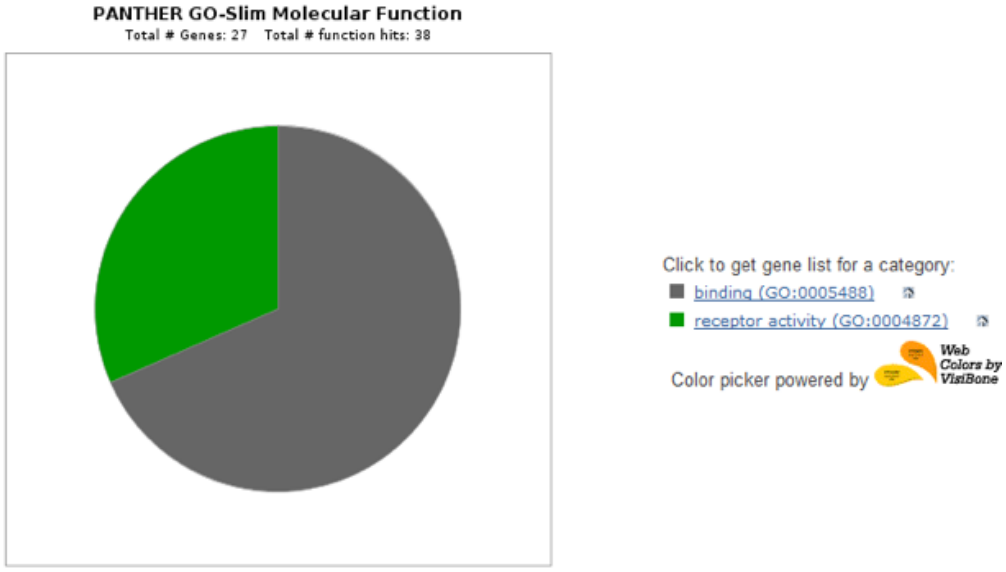


Figure 4: TNFα/AHNAK Molecuar Fuction

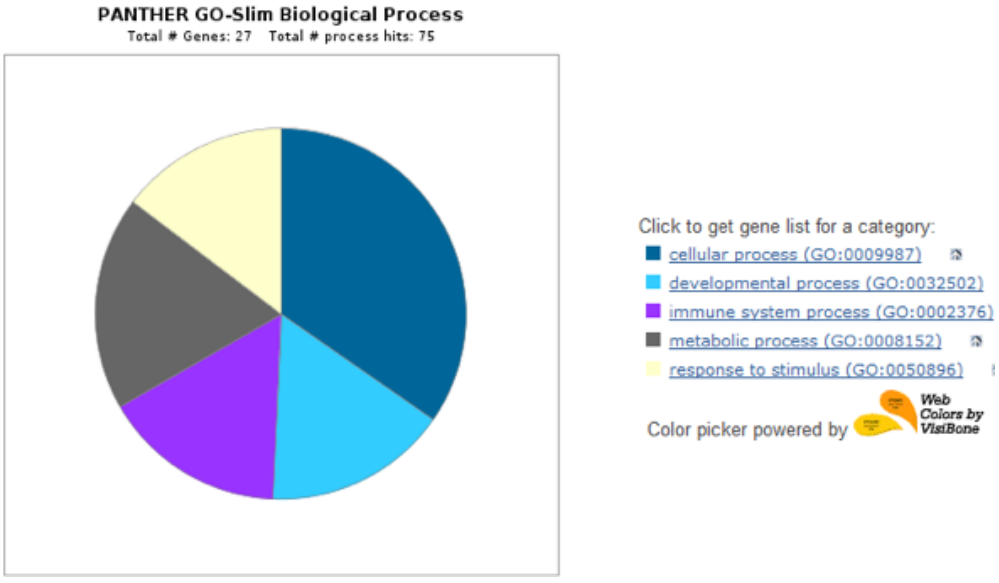


Figure 5: TNFα/AHNAK Biological Process

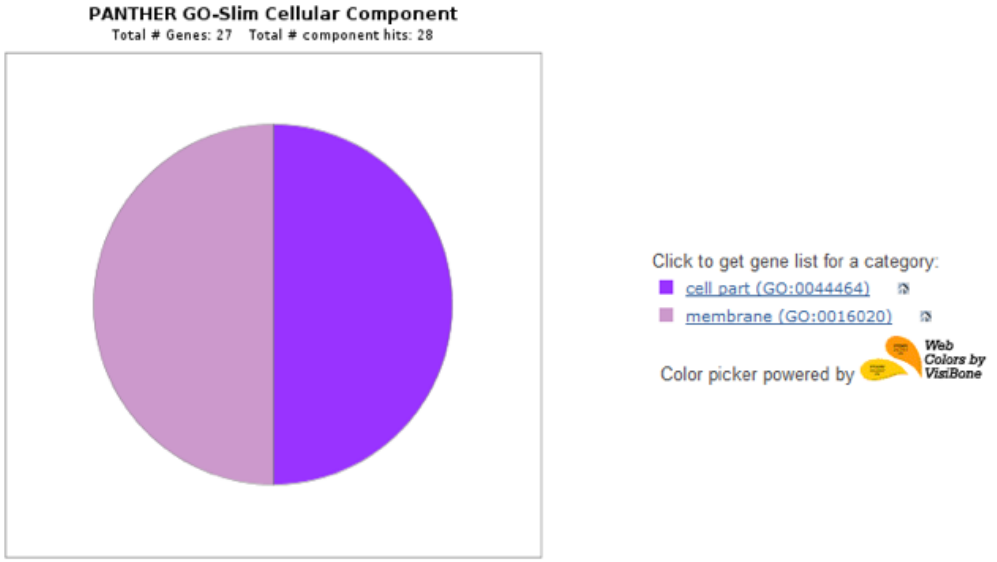


Figure 6: TFNa/AHNAK Celluar Component

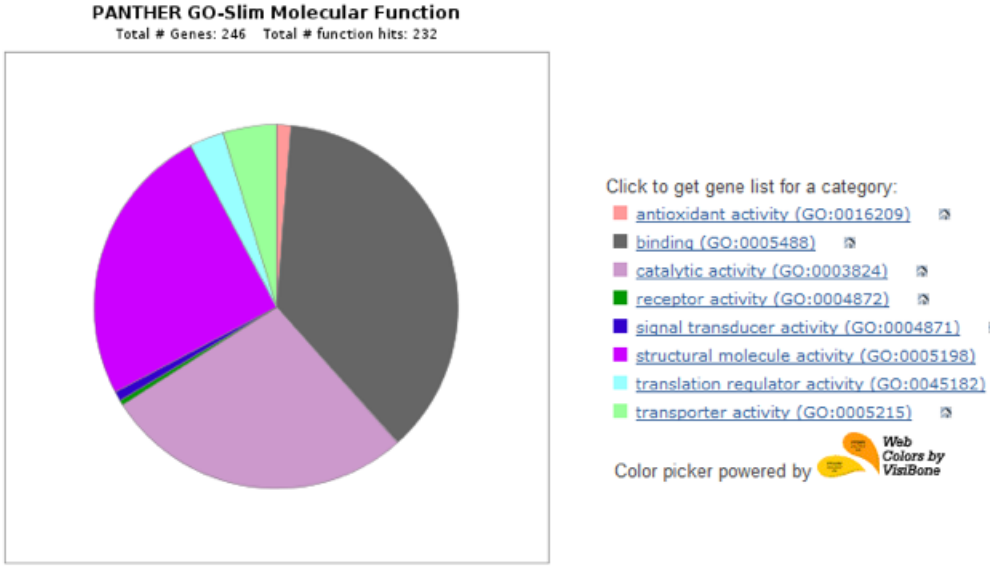


Figure 7: TNFα negative Molecular Function

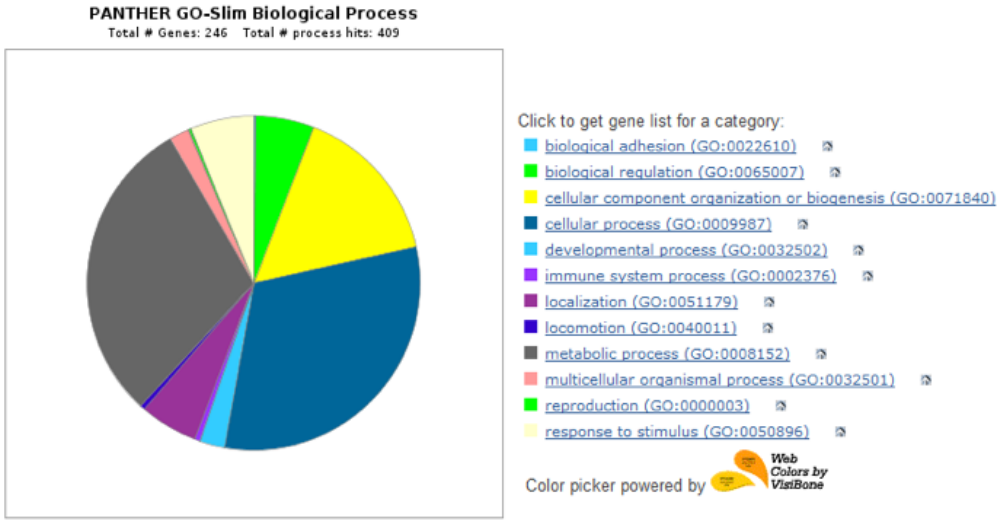


Figure 8: TNFα negative Biological Process

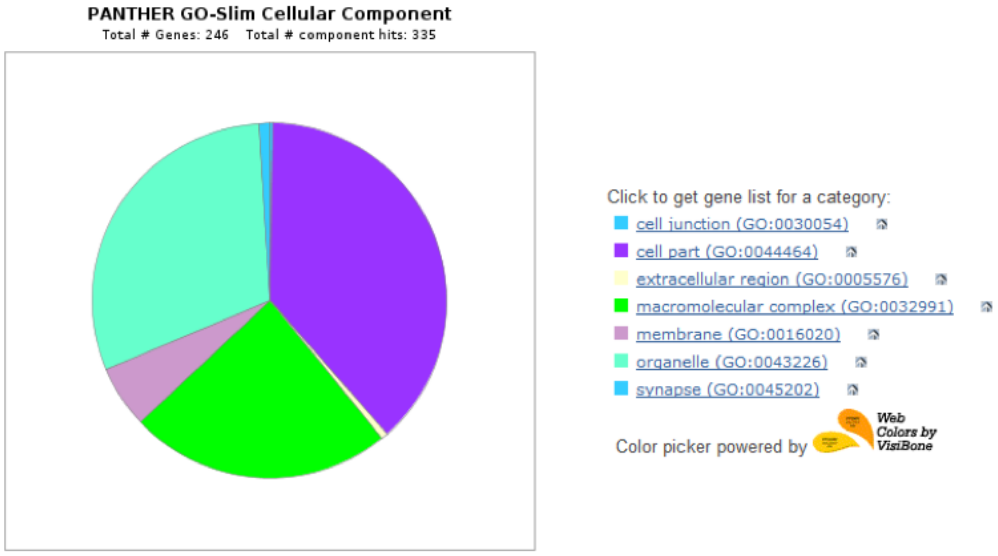


Figure 9: TNFα negative Celluar Component

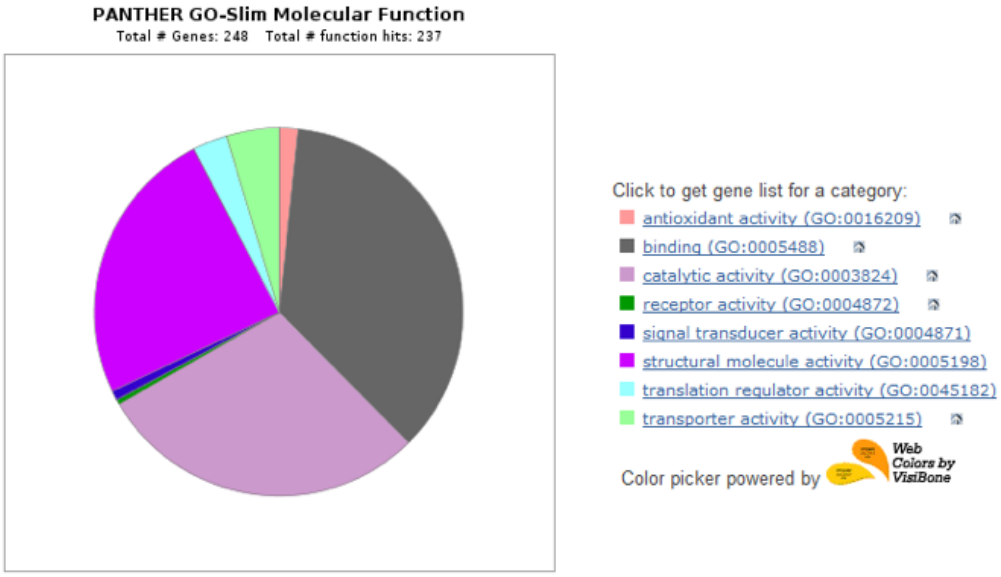


Figure 10: TNFα positive Molecular Function

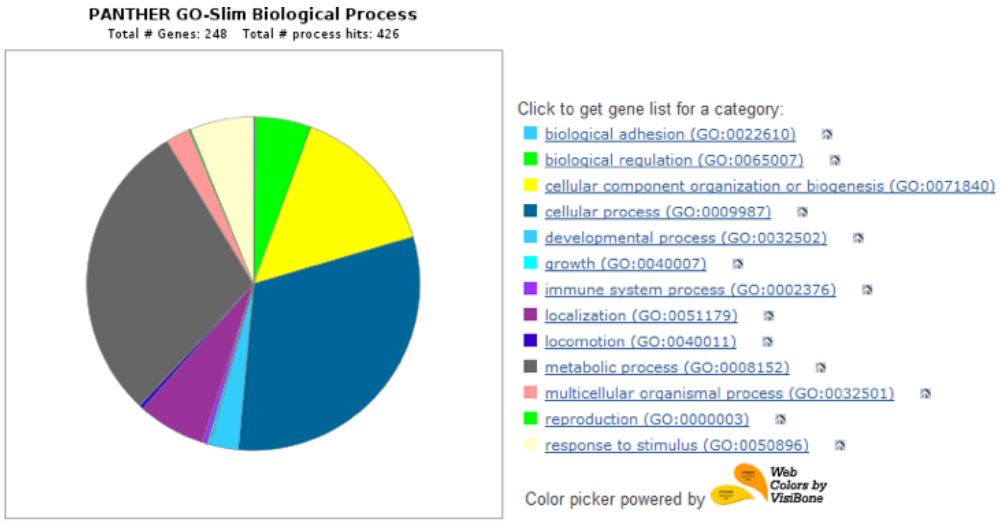


Figure 11: TNFα positive Biological Process

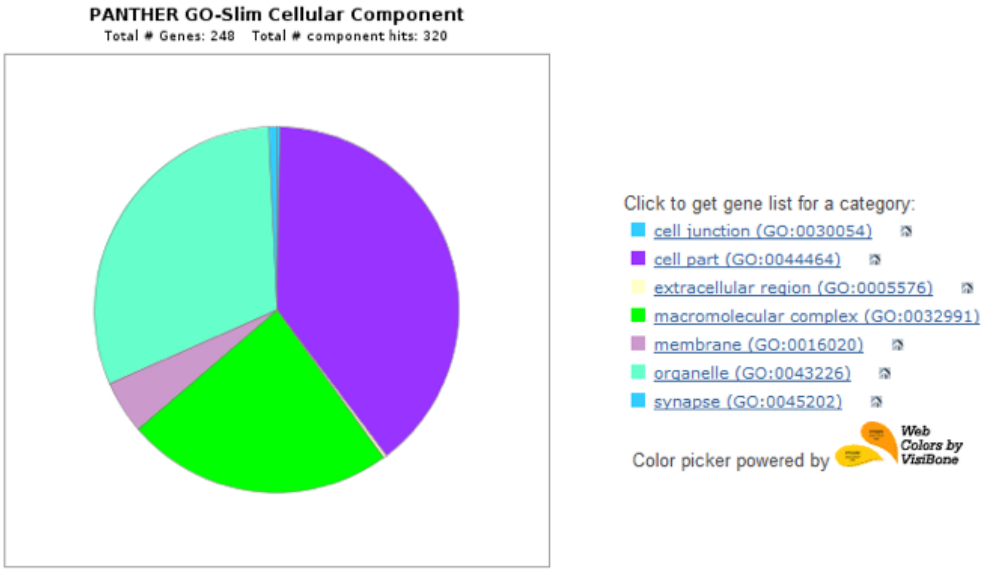


Figure 12: TNFα positive Cellular Component

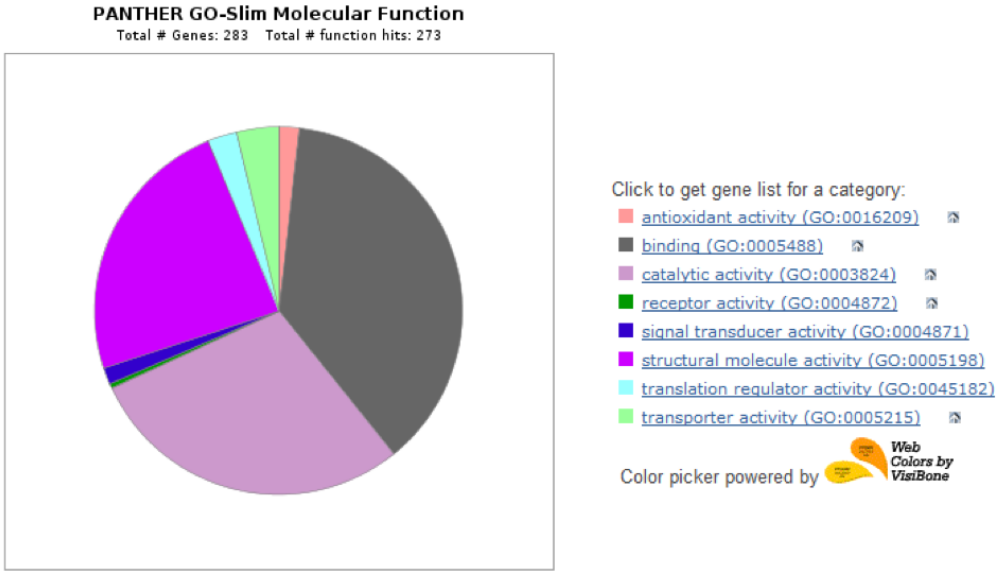


Figure 13: TNFα positive 2ul Molecular Function

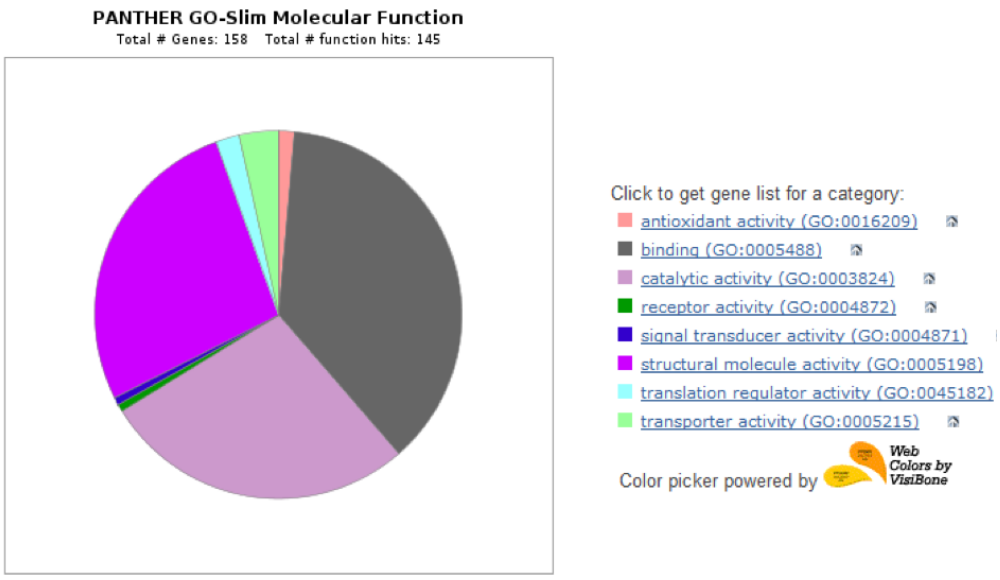


Figure 14: TNFα positive HCD Molecular Function

Pathway name
Interleukin-10 signaling
Interleukin-4 and Interleukin-13 signaling
TNFR1-mediated ceramide production
TNFR1-induced proapoptotic signaling
Signaling by Interleukins
TNFR1-induced NFkappaB signaling pathway
Regulation of TNFR1 signaling
TNF signaling
Cytokine Signaling in Immune system
TNFR2 non-canonical NF-kB pathway
Transcriptional regulation of white adipocyte differentiation
Death Receptor Signalling
Immune System
Developmental Biology
Signal Transduction

Figure 15: Pathways TNFa/AHNAK

Pathway name
Peptide chain elongation
Eukaryotic Translation Elongation
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)
Viral mRNA Translation
Formation of a pool of free 40S subunits
SRP-dependent cotranslational protein targeting to membrane
GTP hydrolysis and joining of the 60S ribosomal subunit
Translation initiation complex formation
Ribosomal scanning and start codon recognition
Major pathway of rRNA processing in the nucleolus and cytosol
Nonsense-Mediated Decay (NMD)
Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)
L13a-mediated translational silencing of Ceruloplasmin expression
Eukaryotic Translation Termination
Eukaryotic Translation Initiation

Figure 16: Pathways Control

Pathway name
Peptide chain elongation
Eukaryotic Translation Elongation
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)
Viral mRNA Translation
Formation of a pool of free 40S subunits
SRP-dependent cotranslational protein targeting to membrane
GTP hydrolysis and joining of the 60S ribosomal subunit
Translation initiation complex formation
Ribosomal scanning and start codon recognition
Major pathway of rRNA processing in the nucleolus and cytosol
Nonsense-Mediated Decay (NMD)
Activation of the mRNA upon binding of the cap-binding complex and eIFs, and subsequent binding to 43S
Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)
Cap-dependent Translation Initiation
Eukaryotic Translation Initiation

Figure 17: Pathways Treated

2.2.5 Reactome Database:

We use the reactome database to investigate a molecule interaction between TNF and AH-NAK as well as the proteome of the treated and control sample. (Figure 15,16,17)

3 Discussion

Interestingly there were 100 different proteins in the 2μl specimen, compared to the regular MS run.(Table 1) The DAVID analysis did not bring up any significant differences in major pathway cascades within the different samples.(Table 2) Using the string tool, a connection from AHNAK to TNF α via RELA and NFKB1 has been identified. Since AHNAK may play a role in cell differentiation and NFKB1 is known to be potentially tumopromoting, a possible biological connection might be present. In the PANTHER analysis no major differences in the gene ontology has been identified. As expected the reactome investigation of the treated and control sample yieled very similar results. The examination of AHNAK with those tools validated expected the biological and molecular attributes from the previous steps. Further investigation and validation could potentially identify AHNAK as an interesting candidate in cancer cell treatments.

List of Figures

1 De-Novo Sequencing . . . 2
2 Link TNFα and AHNAK . . . 3
3 String data . . . 4
4 TNFα/AHNAK Molecuar Fuction . . . 5
5 TNFα/AHNAK Biological Process . . . 5
6 TFNa/AHNAK Celluar Component . . . 6
7 TNFα negative Molecular Function . . . 6
8 TNFα negative Biological Process . . . 7
9 TNFα negative Celluar Component . . . 7
10 TNFα positive Molecular Function . . . 8
11 TNFα positive Biological Process . . . 8
12 TNFα positive Cellular Component . . . 9
13 TNFα positive 2ul Molecular Function . . . 9
14 TNFα positive HCD Molecular Function . . . 10
15 Pathways TNFa/AHNAK . . . 10
16 Pathways Control . . . 11
17 Pathways Treated . . . 11

List of Tables

1 Quantity unique proteins . . . 2
2 Pathway Analysis . . . 3

References

[1] Da Wei Huang, Brad T Sherman, and Richard A Lempicki. Systematic and integrative analysis of large gene lists using david bioinformatics resources. *Nature protocols*, 4(1):44, 2008.

[2] Damian Szklarczyk, Annika L Gable, David Lyon, Alexander Junge, Stefan Wyder, Jaime Huerta-Cepas, Milan Simonovic, Nadezhda T Doncheva, John H Morris, Peer Bork, Lars J Jensen, and Christian vonMering. String v11: proteinprotein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research*, page gky1131, 2018.

[3] Paul D THOMAS, Michael J CAMPBELL, Anish KEJARIWAL, MI HUAIYU, Brian KARLAK, Robin DAVERMAN, Karen DIEMER, Anushya MURUGANUJAN, and Apurva NARECHANIA. Panther: A library of protein families and subfamilies indexed by function. *Genome research*, 13(9):2129–2141, 2003.