

Label-free Global Proteomics Comparison of Interferon Alpha and Beta Responses in Human Liver Carcinoma Huh7 Cells: Relevant to Antiviral Therapy

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Overview

Label-free proteomics applied to characterize IFN-induced protein changes affords insights into human innate immune responses and potential new antiviral therapeutics.

Introduction

- Interferons (IFNs) are key components of the innate immune response and the first line of the host's defense against viral infection. Currently, IFNs are being used therapeutically as antiviral remedies against viruses such as hepatitis C.
- We utilized a label-free, comparative proteomics approach (spectral counting) involving extensive sample fractionation and LC-MS/MS to identify alterations in host protein expression following IFN type I (IFN- α and IFN- β) treatment.
- Results revealed increased protein abundances directly associated with the IFN-mediated antiviral response, as well as decreased cellular functions associated with all viral replication steps.
- In addition to advancing knowledge on host immunity, this study demonstrated the utility of a label-free approach for identifying potential protein targets of therapeutic relevance.

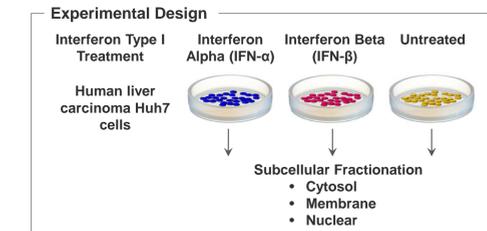
Methods

- Human liver carcinoma Huh7 cells ($2\text{-}2.5 \times 10^7$ cells) were treated for 20 h (at 37 °C) with either IFN- α (INTRONA; 100U/mL) or IFN- β (100U/mL), or were untreated.
- The treatment was repeated to collect additional cells; 3×10^8 cells were collected for each of the three treatment groups.
- Cells were gently lysed prior to differential centrifugation to obtain nuclear, membrane, and cytoplasmic fractions. Protein extracts were typically digested, fractionated by strong cation-exchange chromatography, and analyzed using LC-MS/MS.
- Spectra were searched against the UniProtKB/Swiss-Prot database, and significant protein differences between treated and control groups were identified by applying stringent filtering criteria and a ≥ 2.5 -fold change cutoff value.
- Detailed spectral count data filters used to identify significant changes between IFN treatment and untreated controls are provided in the table below.

Fold change of Treated* vs. Untreated	Treated* - Untreated
2.5 fold < x > 5 fold	≥ 9
5 fold or greater	≥ 5

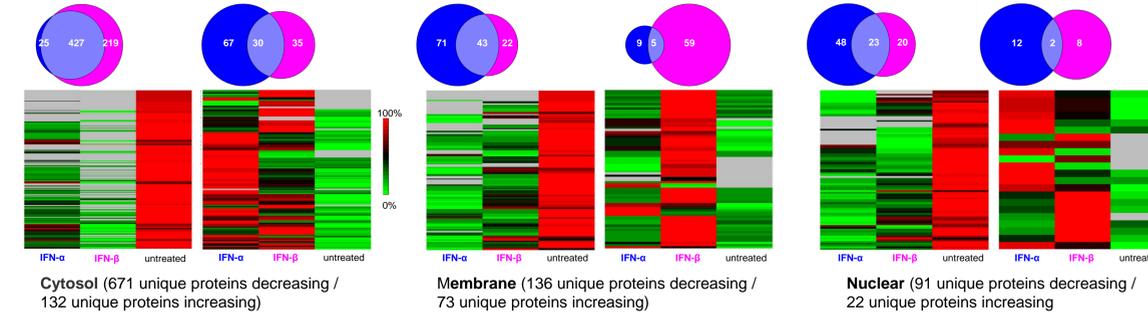
*Treatment IFN- α or IFN- β

Methodology overview



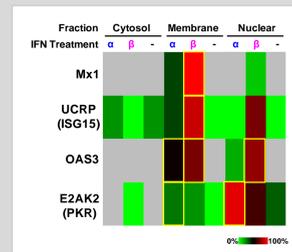
Spectral counting results

- This study identified 6,860 proteins from 41,210 unambiguous peptides (i.e., peptides that matched to only one protein in the database)
- Following IFN treatment, significant differences in protein abundances were observed, with 223 proteins increasing and 851 proteins decreasing in abundance



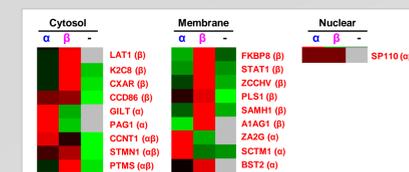
Biological Implications

Known antiviral effectors



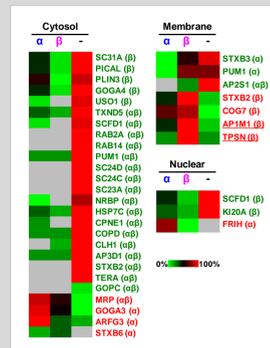
- Proteins directly associated with IFN-mediated antiviral response displayed increased abundances
- The proteins GTPase Mx1 (myxovirus resistance1), ISG15 (IFN-stimulated protein of 15kDa), OAS3 (2'-5'-oligoadenylate synthase 3) and protein kinase R (PKR, also known as EIF2 α 2K) all increased with IFN treatment
- These proteins have well-known roles in blocking viral transcription, modifying protein function, degrading viral RNA, and inhibiting translation, respectively
- On the basis of these findings, we anticipate that the 223 proteins identified in our study that exhibited increased abundances could provide a more complete repertoire of antiviral effectors

Immune response, response to virus, and interspecies interaction



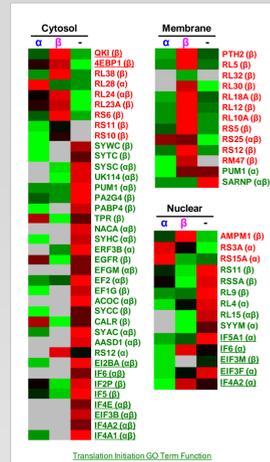
Gene Ontology (GO) annotations [1] of each protein illuminated how interferon treatment decreased host cellular functions associated with all steps of viral replication, including vesicle trafficking, ubiquitin-proteasome proteolytic activity, and translation initiation. Given their simplicity, viruses have well-developed strategies for hijacking the biosynthetic machinery of the host cell.

Vesicle trafficking



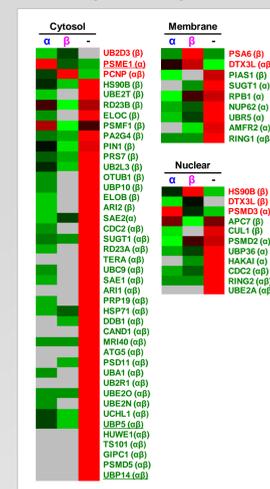
- IFN decreases vesicle trafficking among the plasma membrane, endosomes, Golgi, and endoplasmic reticulum
- For proteins with GO processes related to vesicle trafficking, 27 proteins decreased and 9 proteins increased following IFN-treatment
- Two proteins which increased in the membrane fraction, Tapasin (TPSN) and adaptor-related protein complex 1, mu 1 subunit (AP1M1), are associated with the major histocompatibility complex I (MHC-I) [2, 3]

Translation



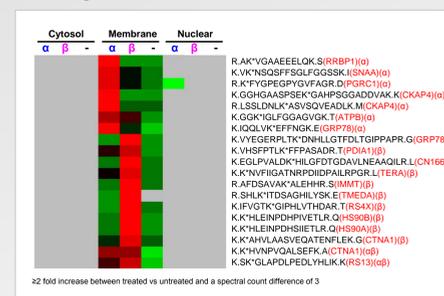
- IFN decreases translation initiation
- All 11 proteins with translational initiation factor activity GO term function decreased in abundance
- Two of the proteins increasing with IFN treatment, Eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) and Protein quaking (QKI), function as translation regulators
- QKI acts as a translational repressor [4] 4EBP1 regulates eIF4E activity by preventing its assembly into the eIF4F complex [5]

Ubiquitin-proteasome proteolytic activity



- For proteins with GO processes related to ubiquitin-proteasome proteolytic activity, 54 proteins decreased and 8 proteins increased in abundance
- One protein which increased in the cytosol, proteasome (prosome, macropain) activator subunit 1 (PA28 alpha) (PSME1), is implicated as a member of the immunoproteasome which is responsible for processing class I MHC peptides [6]
- Two decreasing proteins, Ubiquitin carboxyl-terminal hydrolase 14 (protein UBP14, gene USP14) and Ubiquitin carboxyl-terminal hydrolase 5 (protein UBP5, gene USP5) are able to hydrolyze ubiquitin-like protein ISG15 conjugates based on previous experiments where they bound to the suicidal probe ISG15-VS [6]

ISGylation



- Unlike ubiquitinylation, ISGylations does not promote degradation of target proteins, but instead has activating antiviral effects
- In this study we searched for potential protein targets of ISG15 by identifying the 114 Da diglycine tag remaining on Lysine (K) residues following trypsin digestion
- Nineteen modified membrane peptides (16 proteins) showed an increase with IFN treatment
- Three of these proteins, Heat shock protein HSP 90-alpha and beta (HS90A and HS90B) and Transitional endoplasmic reticulum ATPase (TERA), have been previously reported to be ISGylated [7]

Conclusions

- Host proteins with functional roles in viral replication decrease with IFN (α and β) treatment
- Many known antiviral proteins increase with IFN (α and β) treatment
- Many of the identified 223 proteins with increasing abundances are currently believed to have unknown antiviral roles, which will be investigated by future research

Acknowledgements

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