Brief Report

Dissecting human lymphoma using an integrated network analysis

Charikleia Seitanidou[#1], Georgia Orfanoudaki[#2], Dimitra Mavridou[4], Aikaterini Pitsikali[4], Elias Drakos[3], Konstantina Psatha[2], Michalis Aivaliotis[4,5]

[1] *Department of Chemistry, University of Crete, Heraklion, Greece*
[2] *Institute of Molecular Biology and Biotechnology Foundation for Research and Technology-Hellas, Heraklion, Greece*
[3] *School of Medicine, University of Crete, Heraklion, Greece*
[4] *Laboratory of Biochemistry Department of Biological Sciences and Preventive Medicine, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[5] *Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Balkan Center, Thessaloniki, Greece*
[#]*Equal Contribution*

Abstract

Lymphomas are cancers that originate in the lymphatic system. Despite the heterogeneity, lymphomas are characterized by the deregulation of the p53-signaling pathway. This study describes a systems-wide network analysis of the key mal-functioning p53-centered network of interactions involved in lymphoma pathogenesis through integration of transcriptomic and proteomic data.

Keywords: lymphoma, p53, integration, transciptomics, proteomics

Corresponding authors:
Prof. Dr. Michalis Aivaliotis
Laboratory of Biological Chemistry, Faculty of Health Sciences, School of Medicine, Aristotle University of Thessaloniki, Building 16a, 3rd Floor, GR-54124, Thessaloniki, Greece; Functional Proteomics and Systems Biology (FunPATh), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Balkan Center, Buildings A & B, Thessaloniki, 10th km Thessaloniki-Thermi Rd, P.O. Box 8318, GR 57001; E-mail: aivaliot@auth.gr; Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology, Vassilika Vouton, 70013 Heraklion, Crete, GREECE; Tel: +30-2810-391063; E-mail: aivaliotis@imbb.forth.gr
Dr Konstantina Psatha
Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology, Vassilika Vouton, 70013 Heraklion, Crete, GREECE; Tel: +30-2810-391063; E-mail: konstantina_psatha@imbb.forth.gr

Abbreviations:

N3a: Nutlin-3a

wt p53: wild-type p53

MDM2: Murine double minute 2

HL: Hodgkin lymphoma

MCL: Mantle cell lymphoma

ALCL: Anaplastic large cell lymphoma

Lymphomas constitute a heterogeneous type of haematological cancer derived from lymphocytes in the lymphatic system, with high diversity in both clinical and biological terms (Jaffe et al., 2008). One of the major characteristics of this blood cancer is the deregulation of wild type (wt) p53-dependent signaling pathways. P53 is a transcription factor involved in various cellular functions, such as cell cycle, apoptosis and DNA repair pathways that maintain cellular integrity and defense against cancer.

P53 deregulation is frequently attributed to the cessation of wt p53-functionality by overexpressed modifiers, such as MDM2 (p53's main negative regulator) (Fahraeus and Olivares-Illana, 2014). MDM2 interacts with the wt p53 and promotes its ubuquitin-mediated degradation (Fahraeus and Olivares-Illana, 2014) (Figure 1A). Nutlin-3a (N3a), a small molecule and a MDM2-antagonist with considerable promise in clinical trials, has proven to efficiently re-activate non-functional wt p53 in lymphomas, demonstrating anti-lymphoma effect exhibited by cell cycle arrest and apoptosis (Drakos et al., 2009, Drakos et al., 2011) (Figure 1A).

Our aim is to understand lymphoma's pathobiology and progression under the prism of a systemic approach and by focusing on the identification of the key mal-functioning p53-centered network of protein-protein interactions. The current brief report describes a network-based analysis and integration of data generated at two different levels: proteome and transcriptome of three lymphoma cell lines (Hodgkin lymphoma (HL), anaplastic large cell lymphoma (ALCL) and mantle cell lymphoma (MCL)), before and after the effect of N3a treatment.

Geared towards our goal, microarray transcriptomic and mass spectrometry-based proteomic datasets were generated that enabled the development of network-based integrated analysis of the effect of N3a-treatment on three model lymphoma cell lines (HL, ALCL and MCL) (Figure 1B). The integrative analysis of lymphoma transcriptomics and proteomics data was based on unpublished work of Dr Konstantina Psatha. As a first step in our analysis, the identified and relatively quantified transcripts and proteins in the N3a-treated cells were statistically tested against the untreated ones and the differentially expressed transcripts and proteins were defined. The statistical inference was applied both on the proteomic and transcriptomic quantitation values, and the resulted data were merged into a final list, creating a unified reference dataset.

Next, we sought to investigate the functional roles of the differentially expressed transcripts and proteins by analyzing the protein-protein interaction networks in each lymphoma subtype, based on previous knowledge available in curated protein-protein interaction databases. This led us to the underlying protein-protein interactions, delineating specific and overlapping molecular signatures among the different lymphoma subtypes. We employed two distinct network clustering approaches, the "Hypothesis Free" and the "Targeted". In the "Hypothesis free" approach, the Markov Cluster Algorithm in Cytoscape was applied in the initial total network, using the default parameters. Markov Cluster Algorithm organized the network into different number of distinct protein clusters, and from this, the cluster containing p53 protein was chosen for further functional analysis. The "Targeted" approach used sequentially two algorithms: first the MCODE in direct mode, and then the Markov Cluster Algorithm on the resulted

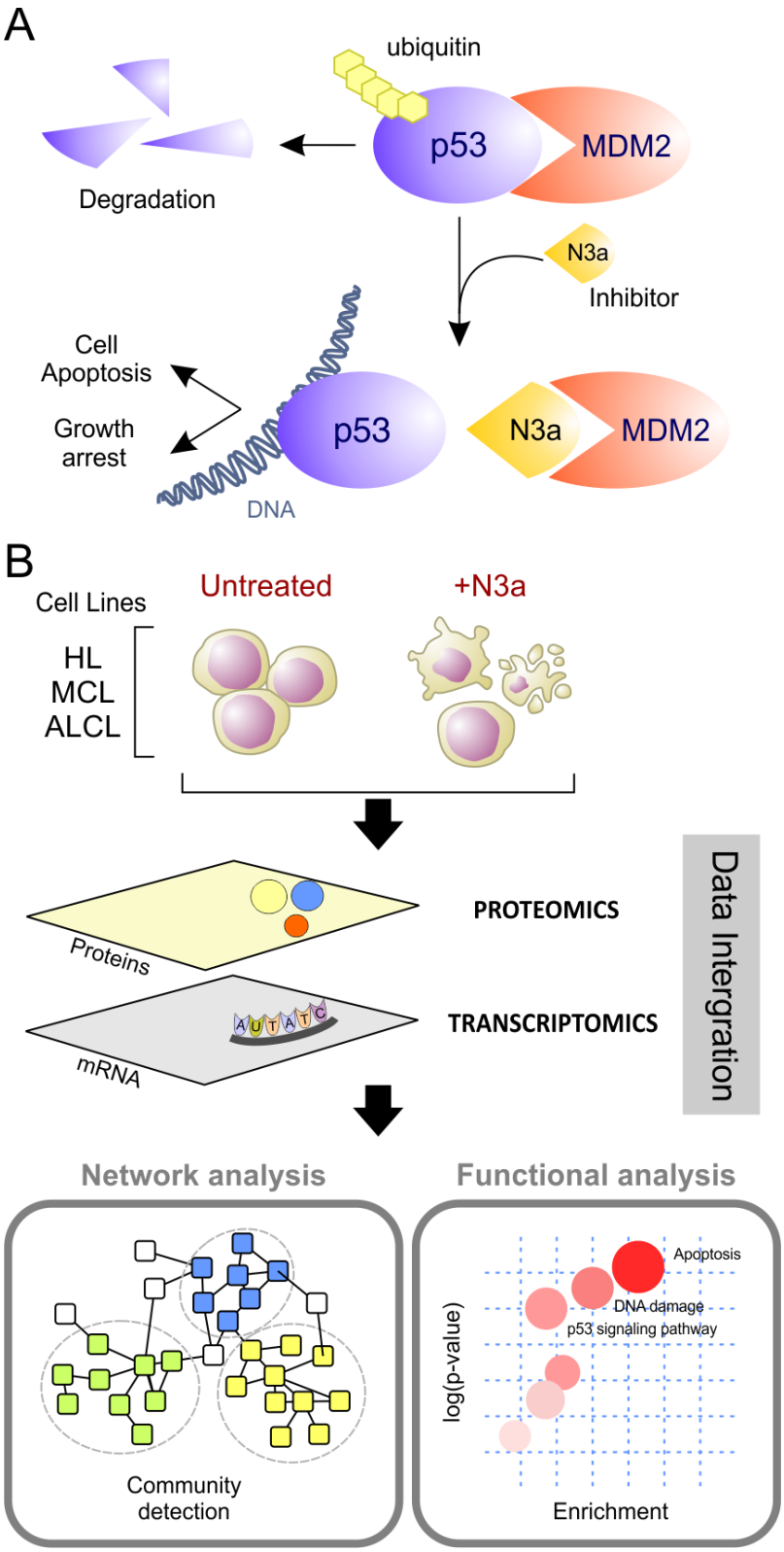

Figure 1: Integrated *in silico* analysis of –omic data generated from Nutlin-3a (N3a) treated lymphoma cell lines.

A. The p53–MDM2 autoregulatory feedback loop governs p53 amounts. Overexpression of MDM2 in human cancer, e.g., gene amplification of MDM2, targets p53 for ubiquitin-dependent protelytic degradation to disable the p53 network. Nutlin-3a complexes with MDM2 and inhibits its interaction with p53

B. Bioinformatics workflow of intergration of transcriptomic and proteomic data that were generated from three lymphoma subtypes (ALCL, HL, MCL) which were treated with Nutlin-

3a (N3a). Differentially expressed genes were statistically inferred after comparing the untreated with the N3a-treated cells. Related protein-protein interaction (PPIs) sub-networks were retrieved from STRING database via Cytoscape platform. Network clustering analysis and functional/pathway enrichment analysis was performed on the specific PPI networks per lymphoma subtype.

network, in which protein interactions around key proteins were examined. Analysis and visualization of differentially expressed transcript and protein networks was achieved using specific network clustering plugins and algorithms for Cytoscape (Shannon et al., 2003). A comparison of pathway and Gene Ontology enrichment information deriving from different data sources and analyses methods, identified and implicated many key protein and network modules in the three lymphoma subtypes. The detected abnormalities suggest potential directional changes and molecular signatures for each cell line, underlying the differential corresponding clinical outcome.

Our systems biology point of view explored the regulatory pathways and protein interaction networks affecting multiple cellular processes in the lymphoma pathophysiology. Although the two analyses showed a very low degree of overlap, regarding the number of common proteins, functional analysis revealed high overlap in biological processes dominating the p53 interactome every time, confirming the validity of global transcriptomic and proteomic profiling in revealing already reported proteins and pathways, along with novel ones. Cell cycle, apoptosis, p53 signaling pathway were among the pathways validated by our pipeline as having altered activity upon N3a treatment, in all three lymphoma subtypes. Protein transport and folding, as well as carbon metabolism appeared to be also affected in the presence of N3a. Taking into consideration the resulting data, both developed workflows hold great potential for unraveling new biological insights, thus fostering an in depth understanding of a system under study. The potential impact of such holistic approaches encourages the notion that molecular identification of unique and common characteristics between different lymphoma entities and other diseases, may lead future research to the development of new diagnostic, prognostic and/or therapeutic schemes, promoting new drug discovery and/or drug repurposing.

Acknowledgements

Konstantina Psatha was funded by a "Stavros Niarchos Foundation – FORTH Fellowships" for Postdoctoral Researchers ARCHERS-SNF-IMBB".

References

Drakos, E., Atsaves, V., Schlette, E., Li, J., Papanastasi, I., Rassidakis, G. Z. , Medeiros, L. J. (2009). The therapeutic potential of p53 reactivation by nutlin-3a in ALK+ anaplastic large cell lymphoma with wild-type or mutated p53. Leukemia, 23**,** 2290-9

Drakos, E., SinghI, R. R., Rassidakis, G. Z., Schlette, E., Li, J., Claret, F. X., Ford, R. J., JR., Vega, F., Medeiros, L. J. (2011). Activation of the p53 pathway by the MDM2 inhibitor nutlin-3a overcomes BCL2 overexpression in a preclinical model of diffuse large B-cell lymphoma associated with t(14;18)(q32;q21). Leukemia, 25**,** 856-67

Fahraeus, R. and Olivares-Illiana, V. (2014). MDM2's social network. Oncogene, 33**,** 4365-76

Jaffe, E. S., Harris, N. L., Stein, H., Isaacson, P. G. (2008). Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. Blood, 112**,** 4384-99

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ragage, D., Amin, N., Schwikowski, B. & Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 13**,** 2498-504.