Brief Report

Deciphering the role of autophagy and exosomes in human lymphoma

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Abstract

The orchestrated homeostatic action of autophagy and exosome biogenesis provides a potential causal relation with lymphomagenesis. This study aims to characterize the differently expressed proteins associated with both above-mentioned pathways, as well as to explore their potential implication in therapeutic response controlling lymphomagenesis via N3a-induced re-activated p53 in different lymphoma subtypes.

Keywords: lymphoma, Nutlin-3a, proteomics, autophagy, exosomes

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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

Lymphoma consists of a group of B- and T- cell haematologic malignancies with increasing incidence rates that, unlike most cancers, commonly harbor a functionally inactivated wild-type (wt) p53. This feature promotes the rationale strategy for a p53-restoration lymphoma treatment as a promising therapeutic strategy. Accumulating data demonstrate that the non-genotoxic re-activation of wt p53 using Nutlin-3a (N3a) in lymphomas has a promising potential compromising their progression. N3a is a small molecule, antagonist of p53's physiological negative regulator, MDM2 (murine double minute 2), that occupies the p53-binding site of MDM2, disrupting the p53-MDM2 interaction (Vassilev, 2004). More specifically, several *in vitro* and *in vivo* studies demonstrate that N3a promotes cell cycle arrest and apoptosis (Drakos *et al.*, 2007; Drakos, Atsaves, Li, *et al.*, 2009; Drakos, Atsaves, Schlette, *et al.*, 2009), while autophagy activity is shown to be cell type-dependent and context-dependent (Zhang, 2018). Autophagy is a highly conserved intracellular pathway that takes place at basal levels in normal cells, allowing defective protein and organelle degradation. In lymphomas, autophagy acts as a two-edged sword, working either in a cytoprotective manner or promoting growth arrest and/or cell death, depending on the circumstances (Zhang, 2018). Interestingly, autophagy regulators have been shown to modulate exosome release, while the cellular metabolic state has been suggested to fine tune the balance between exosome release and autophagy (Baixauli and Carlos Lσpez-Otvn and Maria Mittelbrunn, 2014). The cell-derived exosomes serve as a cell-to-cell communication system that delivers molecular and genetic signals to close or remote cells reprograming their functions (Boyiadzis and Whiteside, 2017). The release of these extracellular nanovesicles, recently implicated in the spread of harmful proteins and in cross-talks with cancer-related pathways, has been shown to modulate lymphoma cell susceptibility to antibody therapy (Aung *et al.*, 2011). The coordinated action of autophagy and exosome biogenesis, regulation and secretion play an essential role in preserving cellular fitness. The secretion of exosomes may operate in close cooperation with the autophagic pathway to maintain protein and RNA homeostasis, and to aid the propagation of neighboring-cell signaling process, in order to orchestrate systemic responses at the organismal level (Baixauli and Carlos Lσpez-Otvn and Maria Mittelbrunn, 2014), thus providing a potential causal relation with lymphomagenesis.

Recent advances in the field of mass spectrometry (MS)-based proteomics coupled with progress in proteomics experimental and bioinformatics pipelines, have allowed the hypothesis-free study of specific processes of interest in detail. To this end, MS-based quantitative proteomics holds incremental value in deciphering the heterogenous and complex deregulated molecular mechanisms and deregulated pathways in human lymphoma (Psatha *et al.*, 2017). Therefore, the investigation of the autophagy and exosomal proteomic profile in wt p53 lymphomas may facilitate the decryption of how the secretion of exosome-content is linked to the autophagy pathway, shedding light in the field of lymphoma biopathology.

In this perspective report, we discuss such a study that aims to detect the differentially expressed proteins associated with autophagy and exosomal pathways, to evaluate their functional relationship, and explore their potential implication in therapeutic response controlling

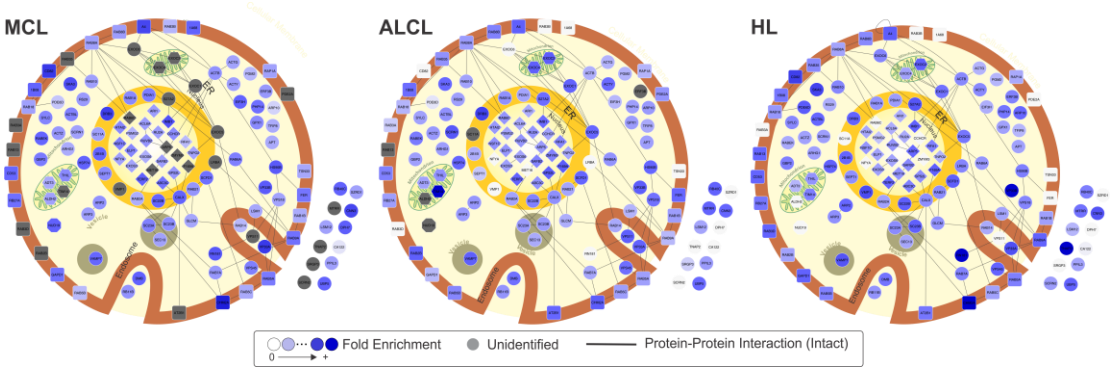

Figure 1: Topological protein interaction network of autophagy, mitophagy and exosomes proteins in (Hodgkin lymphoma (HL), mantle cell lymphoma (MCL) and anaplastic large cell lymphoma (ALCL). All the autophagy, mitophagy and exosomes proteins identified in HL, MCL and ALCL were mapped on a topological protein interaction map, indicating the known or predicted interaction, as well as their cellular topology. The colors indicate the protein identification and their relative abundance in the different lymphoma cell-types after N3a-treatment. As it is shown in the inset box, white is the lowest abundance where dark blue is the highest. Grey indicates no identification of the specific protein in the given lymphoma type.

lymphomagenesis using N3a to re-activate p53 in different lymphoma subtypes.

Initially, *in vitro* human lymphoma cell lines that were used as model system of three different lymphoma subtypes (Hodgkin lymphoma (HL), mantle cell lymphoma (MCL) and anaplastic large cell lymphoma (ALCL)), were screened for the antiproliferative effect of N3a related to p53's restoration, exhibiting enhanced apoptotic death. Next, total proteome corresponding to the three lymphoma subtypes before and after N3a-treatment was subjected to comparative proteomic analyses, and selected proteins were confirmed by immunoblotting.

Global proteomic analysis detected more than 4000 proteins, while functional pathway analysis revealed more than 32 proteins to be related to autophagy (including mitophagy) and 77 to be related to exosomal pathways. N3a induced autophagic stimulation in HL, MCL, ALCL, in a different, cell-type-dependent manner, involving increased levels of well-known p53 targets, such as DRAM and PRKAB1. DRAM, a p53-mediated modulator of autophagy, holds a critical role for programmed cell death, linking autophagy to p53 signaling pathway and apoptosis (Crighton *et al.*, 2006). The identified PRKAB1 is the beta subunit of AMP-activated protein kinase (AMPKb), a central energy sensor. AMPK activates serine/threonine kinase ULK1, playing a key role in autophagy promotion (Lee *et al.*, 2010). Moreover, treatment with N3a was associated, among other proteins, with the deregulation of several Rab GTPases, known to play a direct and significant role in the endocytic and exosome secretion pathways, such as Rab3, Rab13 and Rab27 (Blanc and Vidal, 2018). Generally, the resulting data indicate that endosome-lysosome fusion is possibly increased in all lymphoma cell lines after treatment, as well as exosome secretion. Vesicle fusion is suggested to be higher in ALCL and HL cell line, whose trafficking may be regulated by Rab13. In addition, protein interaction analysis revealed the degree of functional correlation and interconnections among exosomes- and autophagy-related proteins and their topology, suggesting potential targeting markers against lymphoma

development (Figure 1).

Our hypothesis-free approach seeks to investigate the influence autophagy and exosomes biogenesis and secretion have in the context of human lymphomas, and lymphomas that are more tolerant to therapeutic approaches, using the nongenotoxic restoration of p53 as a therapeutic strategy. Overall, the resulting data indicate that N3a is a potent agent that induces significant lymphoma cell death and promotes autophagy. Autophagy induction may increase the secretion and the intercellular spreading of selected proteins in exosomes that contribute to the advance of the lymphoma neoplastic transformation. This is another example of the many different escape mechanisms lymphomas evolve to avoid host anti-lymphoma surveillance, leaving several questions for further investigation.

Acknowledgements

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

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