



COMMENTARY

ISGylation orchestrates the degradation flux of cellular cargo toward lysosome

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Post-translational modification (PTM) is a critical step for nascent protein processing, which accompanies with proteins lifetime to modulate their functions dynamically. Currently, there are over 300 species of PTMs been identified, including chemical group modification (e.g., phosphate, acetyl, methyl, or succinyl groups) and ubiquitin-like (UBL) group modification (e.g., ubiquitination, SUMOylation, and ISGylation).¹ The UBL modification covalently linked to the side chain of amino acid residue, like lysine, that signal for multiple biological progresses. Ubiquitin is firstly identified as a modification on protein, that involved in a variety of metabolic processes, such as mitophagy. ISGylation, like is a unique PTM among UBL conjugation that known to be induced by specific inflammatory stimuli, such as interferon signaling pathway,² its novel function on mediating mitophagy and exosome secretion was recently uncovered, in particular on tumorigenesis.

Interferon-stimulated gene 15 (ISG15) exists in two distinct statuses: conjugated to client proteins and as a free molecular (intracellular and extracellular). As a kind of UBL modifier, pro-ISG15 is specifically cleaved at Gly157 and Gly158 to form a mature ISG15 carboxyl terminal, which allows its binding to the client proteins at the C-terminal to form an isopeptide bond. The binding of ISG15 to the target protein is mediated by the continuous interaction from E1 activase (Ube1L), E2 binding enzyme (UbCH8) to E3 ligase (mHERC6/hHERC5).³ The balance of protein ISGylation and de-ISGylation is maintained with specific isopeptidases USP18 and UBP43.³ Interestingly, the level of free and bound ISG15, as well as their subcellular distribution, is highly relied on cell types. In fact, ISGylation-mediated biological function and its role in cancer remains poorly understand.

ISG15 as a driver of lysosomal degradation

Against this backdrop, two studies in *Nature Communications*,^{4,5} sought to define a critical role of UBL modifier ISG15 as a signal for cargo fate determinant in cell - ISGylated cargo is assigned to lysosomal degradation. In the first study, Alcala and colleagues demonstrated that ISG15 is key for sustaining pancreatic cancer stem cells (PaCSCs) stemness, tumorigenesis and metabolic plasticity, which elevates the metabolic function of mitochondria, and removes the damaged mitochondria via

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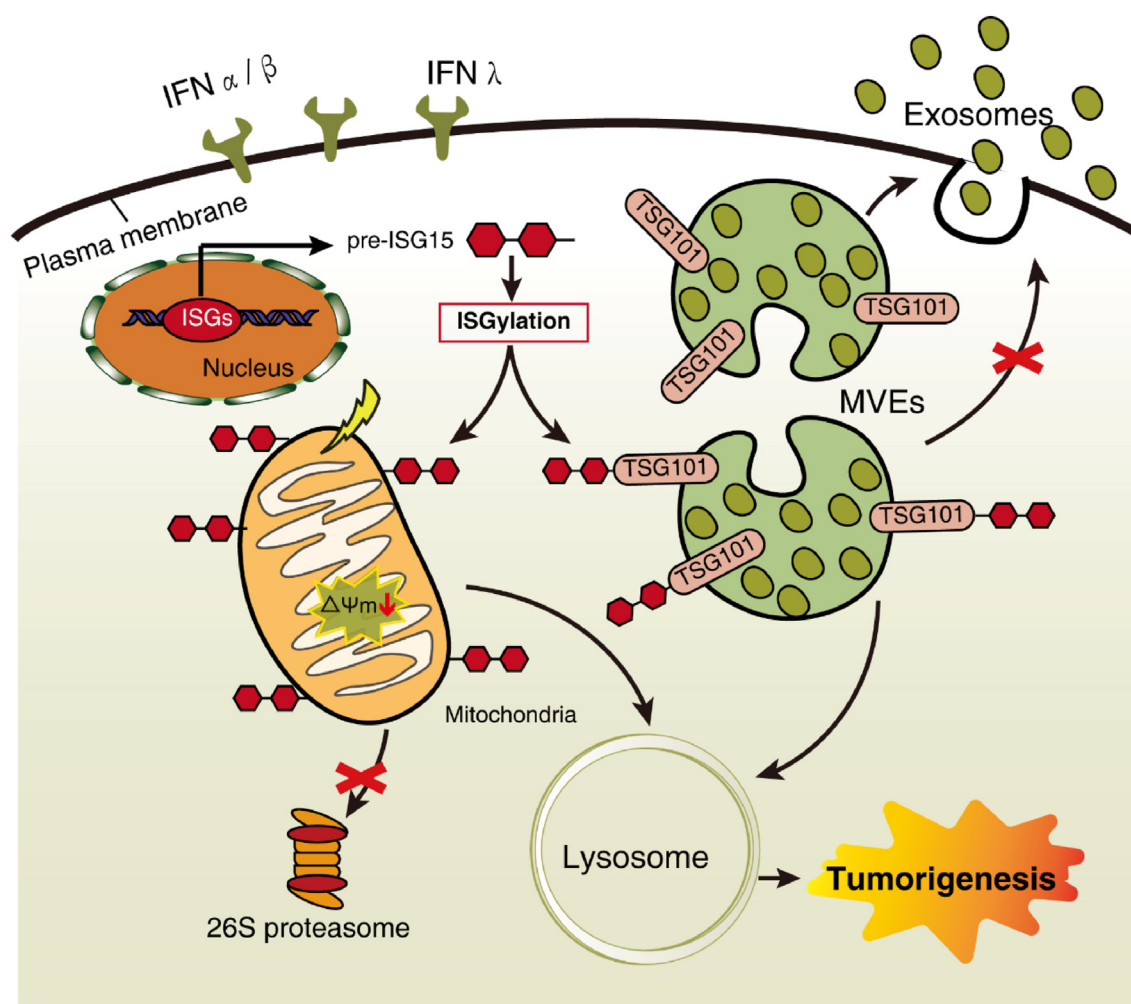


Figure 1 A schematic illustration of how ISG15/ISGylation drives degradation flux of intracellular cargo to lysosome. ISG15 is transcriptionally induced by IFN signaling, the generated pre-ISG15 with two ubiquitin-like domains (red) is cleaved at Gly157-Gly158 peptide bond to exposes its carboxy-terminal LRLRG motif, which is essential for conjugation to lysine residues of client proteins/organelles. The damaged mitochondria or proteins underwent ISGylation are degraded by lysosome, in addition, ISG15 directs the degradation route of macromolecules in MVEs (e.g., TSG101) toward lysosome, and blocks exosome secretion and 26S proteasome activity simultaneously in cell. The ISG15/ISGylation-mediated high-level of lysosomal degradation is essential for sustaining overall metabolic plasticity of cancer cell.

mitophagy. Firstly, they proved that ISG15 is increased in CD133⁺ cells through RT-qPCR. Moreover, both mRNA and protein level of ISG15, as well as ISGylation, are increased in PaCSCs. Clinical study of ISG15 indicated that ISG15/ISGylation is highly expressed in pancreatic ductal adenocarcinoma (PDAC) and negatively predicts patient survival. They further asked whether ISG15/ISGylation involved in mitochondria-related pathways in PaCSCs. They performed GSEA, FACS and Western blotting assays and observed that ISG15/ISGylation is indeed associated with mitochondria-related pathways. The colony formation ability and self-renewal ability of cells lacking ISG15 are significantly reduced, RNA-seq analysis of ISG15 knockout cells demonstrated that ISG15 involved in the EMT, invasion or migration of PDAC.

To further investigate how ISG15/ISGylation regulates metabolic pathways in PaCSCs, CRISPR-Cas9 technology was performed to establish PDAC cell lines with ISG15 deletion

in panc185 and panc354 cell lines. It was found that the absence of ISG15/ISGylation dysregulates mitochondrial metabolism and reduces mitophagy, thereby inhibiting the metabolic plasticity of PaCSCs. Transmission electron microscope images showed that knockout of ISG15 increases number of dysfunctional mitochondria, indicating that loss of ISG15/ISGylation contributes to the accumulation of dysfunctional mitochondria and affects the mitochondria and metabolic status of PaCSCs. Next, they measured the mitochondrial autophagy flux in ISG15-KO cells, and found that mitophagy is impaired in the deletion of ISG15. Treatment of mitochondrial inhibitor metformin in the cell with or without ISG15 knockout, and found that ISG15-KO cells are more sensitive to exposure of metformin. This study provides strong evidences to demonstrate the role of ISG15 in sustaining metabolic plasticity in PaCSCs, which directs the lysosomal degradation of damaged mitochondria by mitophagy.

A related study by Villarroya-Beltri and colleagues revealed that ISG15/ISGylation plays a critical role in exosome release, which ISGylation induced by IFN-I treatment decreases multivesicular bodies (MVB) numbers and impairs exosome secretion.⁴ They mutated the carboxyterminal "LRLRGG" motif of ISG15, a fragment that required for ISGylation, and found that ISG15 conjugation to proteins, but not free ISG15, suppresses exosomal secretion. The role of ISG15 in suppressing exosome secretion was also confirmed by ISG15-knockout mice. Confocal microscopy analysis demonstrated that ISG15 conjugation not only decreases MVB number, but also promotes protein aggregation that degraded by lysosomes. Mechanically, they found that ISGylation of TSG101 is sufficient to impair exosome secretion, but accelerates cargo aggregation and lysosomal degradation. This study uncovers the role of ISG15 in switching cargo fate from exosomal secretion to lysosomal degradation, a progression that resemble to blocking the exit of viral particles. This finding provides new understanding of how ISGylation controls cellular vesicular trafficking route.

Perspectives

Both distinct studies depict the essential role of ISG15/ISGylation in mediating injured mitochondria and aggregated protein toward lysosomal degradation. Interestingly, accumulating evidences reveal an antagonistic role of ISGylation in ubiquitylation, which suppresses proteasome-mediated protein degradation.³ ISG15 seems to be a signal to direct degradation route of damaged macromolecules/organelles to lysosome, by means of turning off exosomal secretion and 26S proteasome pathway simultaneously (Fig. 1). It's worth noting that lysosomes are highly activated in majority of cancer cell, which exhibit high level of turnover-ratio by removal of metabolic waste. These studies highlight the potential value of new approaches to target ISG15 in those tumor cells that highly rely on ISGylation-mediated degradation. Last but not least, there are sever critical issues remained to be solved: The specific

ISG15-binding motifs have yet to be identified, and globally identification of cellular ISGylated sites is critical need for better understanding of ISGylation biology. It is also interesting to ask whether any PTMs (phosphorylation, acetylation, SUMOylation, etc.) that occurred on ISG15, its achievement will provide a rationale for PTMs cross-talk during tumorigenesis.

Author contributions

Y.W., J.Z. M.Y. H and L.OY. conceived and wrote the paper.

Conflict of interests

The authors have no conflict of interest.

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