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発表者（※学会発表の場合のみ記載、共同発表者の氏名も記載すること）	○Yue Cai[1], Ryu Yamada[1], Yuki Marugame[1], Satoshi Yano[1], Haruhisa Kikuchi[2], Yuzuru Kubohara[3], Taichi Hara[1]
発表題目（※学会発表の場合のみ記載）	Identification and Mechanistic Analysis of DIF Derivatives that Induce Autophagy and Ferroptosis

発表の概要と成果（抄録を公開している URL がある場合、「概要・成果」を記載した上で、URL を末尾に記してください。また、抄録 PDF は別途ご提出ください。なお、抄録 PDF は Web 上には公開されません。）

【Introduction】

Cancer drug resistance remains a major barrier to durable therapy, creating a need for strategies that can eliminate cancer cells even when classical resistance mechanisms are engaged. Autophagy and ferroptosis are stress-responsive pathways that may be leveraged to overcome resistance, and small molecules that modulate autophagy–ferroptosis crosstalk are of particular interest. Differentiation-inducing factors (DIFs) and their derivatives have reported anti-tumor activities, but how they regulate autophagy and ferroptosis in cancer cells is not fully understood. Therefore, this study aimed to (i) screen synthetic DIF derivatives to identify candidates that robustly enhance autophagic flux, and (ii) perform mechanistic analyses of the lead compound, focusing on autophagic flux regulation and its association with mitochondrial/iron stress and ferroptosis-like cancer cell death.

【Methods and Results】

We first performed an autophagic flux screen using HeLa cells stably expressing the GFP-LC3–RFP-LC3ΔG (GLR) reporter. Cells were treated for 24 h with vehicle (0.1% DMSO), Torin1 (1 μM), Bafilomycin A1 (200 nM), native DIF-1 (5 μM), and 15 synthetic DIF derivatives; autophagic flux was quantified by the GFP/RFP ratio. Bu-DIF-3 produced the strongest reduction in the GFP/RFP ratio and was selected as the lead compound, and its autophagy-inducing effect was also confirmed in Hap1-GLR cells. To test whether Bu-DIF-3–induced autophagy is linked to canonical mTORC1 inhibition, HeLa WT cells were treated for 24 h with Torin1 (1 μM) or Bu-DIF-3, followed by Western blotting of mTORC1 substrates p-p70S6K and p-4E-BP1. Torin1 strongly reduced these phosphorylation signals, whereas Bu-DIF-3 did not significantly decrease them compared with control, supporting an mTORC1-independent mechanism. RNA-seq analysis in Hap1 cells (24 h Bu-DIF-3 treatment) revealed up-regulation of stress-responsive gene sets, including autophagy regulation and ER stress, as well as enrichment of the L-serine biosynthetic process (linked to GSH synthesis). Upstream regulator analysis predicted enhanced pro-oxidant/ROS-generating signaling. Finally, we assessed oxidative stress, mitochondrial function, and labile iron. In HeLa WT cells, Bu-DIF-3 increased CellROX Green fluorescence (ROS), which was strongly suppressed by N-acetylcysteine (NAC), indicating NAC-sensitive oxidative stress. Bu-DIF-3 also reduced mitochondrial membrane potential (MitoMP) and increased FerroOrange signal, indicating accumulation of labile Fe^{2+}—features consistent with ferroptosis-like cell death.

【Conclusions, summary and Highlights】

Overall, Bu-DIF-3 was identified as a lead DIF derivative that robustly enhances autophagic flux without clear inhibition of canonical mTORC1 signaling. Mechanistically, Bu-DIF-3 activates a transcriptional stress program (autophagy/ER stress/L-serine–GSH axis) and induces ROS-dependent mitochondrial dysfunction and Fe^{2+} accumulation, collectively pointing to a ferroptosis-like death program and suggesting potential utility in overcoming drug resistance. Next steps will focus on (i) tracing the cellular sources of ROS and labile Fe^{2+} (e.g., mitochondria, lysosomal/ferritinophagy-related pools, ER-derived stress) using time-course imaging, selective probes, and pathway-specific inhibitors, and (ii) dissecting the functional contribution of autophagy/mitophagy to Bu-DIF-3–induced cell death by manipulating core autophagy genes and monitoring ferroptosis markers.