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Traditional herbal medicine-derived sulforaphene promotes mitophagic cell death in lymphoma cells through CRM1-mediated p62/SQSTM1 accumulation and AMPK activation

Haina Wang[a], Fuqiang Wang[b], Sijin Wu[a], Zhiheng Liu[c], Tingting Li[c], Lei Mao[d], Jie Zhang[b], Cheng Li[c,**], Caigang Liu[e,***], Yongliang Yang[a,*]

[a] Center for Molecular Medicine (CMM), School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116023, China
[b] College of Medicine, Xiamen University, Xiamen, Fujian 361102, China
[c] School of Life Sciences, Peking University, Beijing 100871, China
[d] DrivingForce Therapeutics, Venture Harbor, Dalian 116085, China
[e] Department of Breast Surgery, Shengjing Hospital of China Medical University, Shenyang 110004, China

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ABSTRACT

Sulforaphene (LFS-01) is the major chemical constituent of *Raphanus sativus*, a medicinal herb used for over a thousand years in traditional Chinese medicine. Here we identified that LFS-01 can selectively eradicate lymphoma cells while sparing normal lymphocytes by triggering concomitant mitophagy and apoptosis. We demonstrated that LFS-01 can retain Nrf2 in the nucleus by covalently modulating CRM1 and consequently upregulate p62/SQSTM1, an essential structural component of the autophagosomes during mitophagic process. We found that LFS-01 treatment also stimulated AMPK and thereby inhibited the mTOR pathway. On the contrary, we revealed that AMPK inhibition can severely impair the LFS-01-mediated mitophagy. Transcriptomic studies confirmed that 15 autophagy-associated genes such as *p62/SQSTM1*, *VCP* and *BCL2* were differentially expressed after LFS-01 treatment. Furthermore, protein interactome network analysis revealed that the events of apoptosis and the assembly of autophagy vacuole were significant upon LFS-01 exposure. Lastly, we found that LFS-01 exhibited strong efficacy in xenograft mouse model yet with the lack of apparent toxicity to animals. We concluded that LFS-01 triggered mitophagic cell death via CRM1-mediated p62 overexpression and AMPK activation. Our findings provide new insights into the mechanism of action for LFS-01 and highlight its potential applications in treating major human diseases.

1. Introduction

The medicinal effects of *Lai Fu Zi* (*Raphanus sativus*) have been documented since the Song dynasty for over one thousand years in traditional Chinese medicine to treat symptoms such as coughing and food stagnation [1]. The major active component of *Lai Fu Zi* is a naturally-derived isothiocyanate compound named as sulforaphene (LFS-01, Fig. 1a), which has recently raised considerable research interests worldwide for its excellent therapeutic activities yet with minimal side effects. However, the exact mechanism of action for LFS-01 in malignant cells is still poorly understood and remains to be clearly defined. Interestingly, we found that LFS-01 can induce apoptosis in numerous lymphoma cell lines while sparing normal lymphocytes isolated from healthy donors. More interestingly, the effects of LFS-01 in lymphoma cells are only partially suppressed by caspase inhibitors, strongly implicating that LFS-01 may also exert its potent antitumor effects through caspase-independent cellular death.

Mitophagy refers to the selective degradation of defective mitochondria by autophagy in response to stress conditions [2]. This cargo-specific autophagy involves a few distinct steps to selectively engulf damaged mitochondria into double-membraned vesicles called autophagosomes, in which LC3-II and p62 act as essential structural components [3]. Noteworthy, a very recent study particularly suggests that p62 is crucial for mitophagic clearance and depletion of p62 in cells directly disrupts mitophagy [4]. Furthermore, mammalian target of rapamycin (mTOR) kinase was recently identified as a master

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: cheng_li@pku.edu.cn (C. Li), angel-s205@163.com (C. Liu), everbright99@foxmail.com (Y. Yang).

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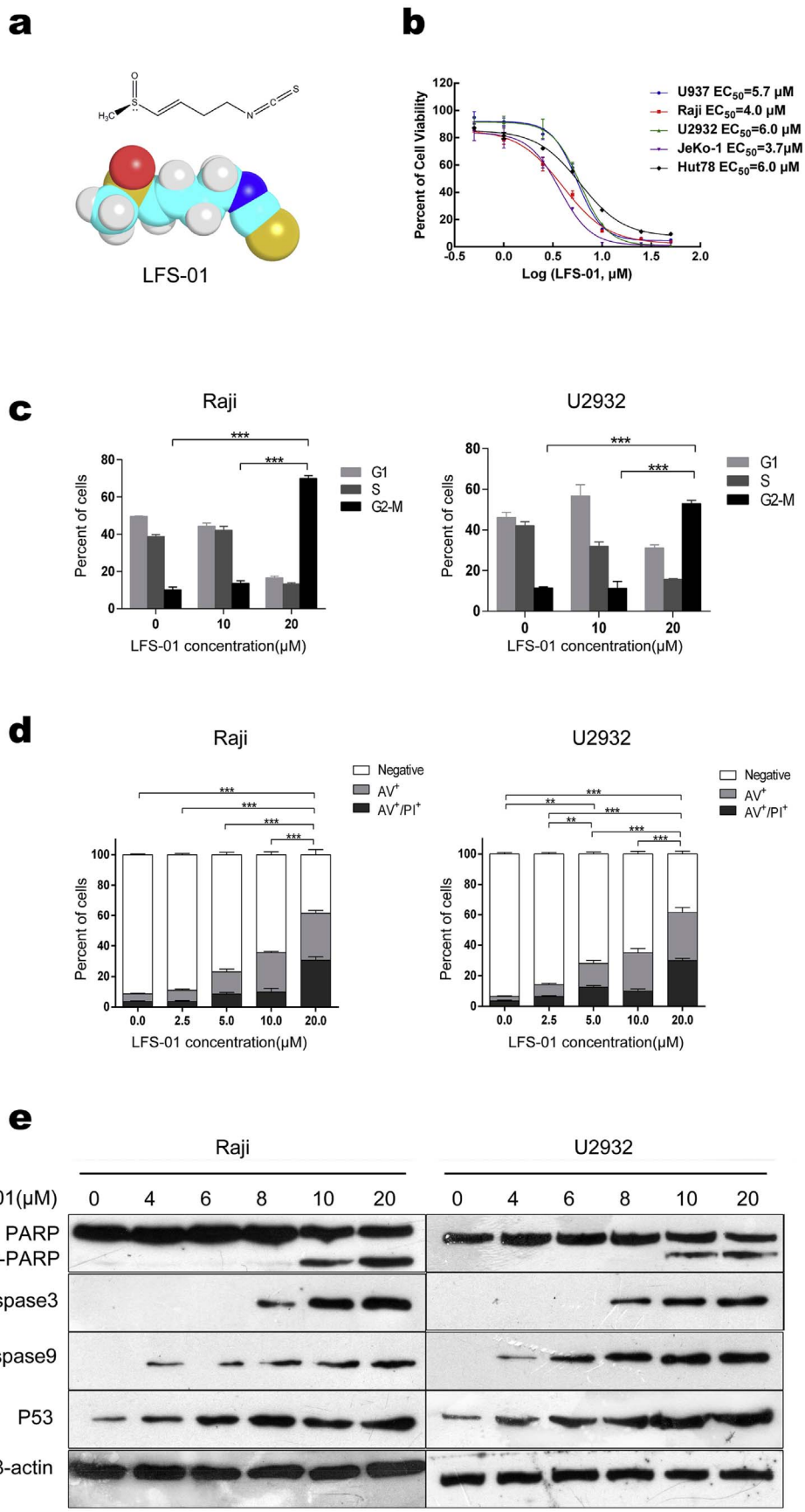

Fig. 1. LFS-01 inhibits lymphoma cell growth by inducing cell cycle arrest and apoptosis. **(a)** Chemical structure and CPK model of LFS-01. **(b)** Suppression of lymphoma cells growth by LFS-01. Different types of lymphoma cells were treated with the indicated concentrations of LFS-01 for 72 h. Cell viability was measured by CCK8 kit in triplicate wells. **(c)** LFS-01 induces cell cycle arrest in G2-M phase. Raji and U2932 cells were treated with the indicated concentrations of LFS-01 for 24 h. Cells were then harvested, stained with propidium iodide (PI) and analyzed by flow cytometry. *P < .05, **P < .01, ***P < .001 by one-way ANOVA, followed by Dunnett's test or Tukey's test. N = 3. Error bars = SEM. **(d)** LFS-01 induces apoptosis. Raji and U2932 cells were treated with the indicated concentrations of LFS-01 for 24 h. Cells were then harvested, stained with Annexin V and PI and analyzed by flow cytometry. *P < .05, **P < .01, ***P < .001 by one-way ANOVA, followed by Dunnett's test or Tukey's test. N = 3. Error bars = SEM. AV, Annexin V. PI, Propidium iodide. **(e)** Representative Western blot showing levels of P53 and apoptosis proteins. Cells were treated with LFS-01 at the indicated concentrations for 24 h. Whole cell proteins were extracted and subjected to immunoblotting analysis. Actin serves as loading control.