Liproxstatin-1 induces cell cycle arrest, apoptosis, and caspase-3/GSDME-dependent secondary pyroptosis in K562 cells

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Abstract. Leukemia is a fatal hematopoietic disorder with a poor prognosis. Drug resistance is inevitable after the long-term use of chemotherapeutic agents. Liproxstatin-1, commonly known as a ferroptosis inhibitor, has never been reported to have anticancer effects. In the present study, the antileukemic role of liproxstatin-1 in K562 leukemia cells was investigated. Liproxstatin-1 inhibited K562 cell proliferation in a dose- and time-dependent manner. RNA sequencing revealed several pathways that were affected by liproxstatin-1, such as the G1/S transition of the mitotic cell cycle and extrinsic or intrinsic apoptotic signaling pathways. The results of flow cytometry indicated that liproxstatin-1 arrests the cell cycle at the G1 phase, and even at the G2/M phase. $p21^{WAF1/CIP1}$, a cyclin-dependent kinase inhibitor, was upregulated. It was also determined that liproxstatin-1 induced BAX and TNF-α expression, which was accompanied by cleavage of caspase-3 and PARP. The caspase-3-specific inhibitor z-DEVD-FMK rescued some of the apoptotic cells. Interestingly, K562 cells were characterized by swelling and plasma membrane rupture when treated with a high concentration of liproxstatin-1, which was inconsistent with the typical apoptotic appearance. Thus, it was hypothesized that apoptosis-mediated pyroptosis occurs during liproxstatin-1-induced cell death. The expression of the hallmark of pyroptosis, the cleaved N-terminal GSDME, increased. Additionally, it was observed that endoplasmic reticulum stress and autophagy were involved in liproxstatin-1-induced cell death. Collectively, liproxstatin-1 induced cell cycle arrest, apoptosis, and caspase-3/GSDME-dependent secondary pyroptosis in K562 leukemia cells, which provides new hope for the treatment of leukemia.

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Introduction

Leukemia is a life-threatening hematopoietic malignancy characterized by the malignant proliferation of hematopoietic stem cells and dysregulation of regulated cell death (RCD) (1-3). The age-standardized 5-year relative survival rate of Chinese patients with leukemia increased from 19.6% in 2003-2005 to 25.4% in 2012-2015 owing to progression in pathogenesis and therapeutic strategies for leukemia (4). However, side effects and drug resistance are inevitable after the long-term use of chemotherapeutic drugs (5). Thus, new antileukemic drugs need to be discovered.

A delicate balance between RCD and cell proliferation is essential to maintain a healthy cell population; however, this balance is out of control in leukemia cell populations. Conventional drugs, such as cytarabine and daunorubicin, are used to treat leukemia via the induction of DNA damage and cell cycle arrest (6). The novel oral drug venetoclax, which targets the apoptotic pathway, has been approved by the Food and Drug Administration for acute myeloid leukemia (7,8).

Pyroptosis is a novel form of RCD characterized by cell swelling, plasma membrane rupture, and DNA condensation and fragmentation (9,10). Gasdermins play a pivotal role in pyroptosis. Apart from APJK (DFNB59), all gasdermins (GSDMA-E) share the gasdermin N-terminal effector domain (11-13). The N-terminal effector domain is activated by the caspase-dependent cleavage of GSDMD or GSDME and exhibits pore-forming, intrinsic cytotoxic, and antibacterial properties (11,12). Activated caspase-1 and caspase-4/5/11 are responsible for canonical and non-canonical GSDMD-related pyroptosis, respectively (14,15). Activated caspase-3 that is induced by tumor necrosis factor (TNF)-α or DNA-damaging chemotherapeutic drugs are responsible for GSDME-related pyroptosis (10,13,16). Preclinical studies involving GSDMD-related pyroptosis induced by DPP8/9 inhibitors and GSDME-related pyroptosis induced by pyridoxine, aimed at the treatment of acute myeloid leukemia, have indicated that pyroptosis is a new target for the management of leukemia (17-19). Apoptosis, characterized by cytoplasmic shrinkage and small apoptotic bodies, also commonly known as type I cell death, is a classical form of RCD mediated by