Biochemical Pharmacology 140 (2017) 41–52

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Lipoxygenase inhibitors protect acute lymphoblastic leukemia cells from ferroptotic cell death

Lukas Probst [a,1], Jasmin Dächert [a,1], Barbara Schenk [a], Simone Fulda [a,b,c,*]

[a] Institute for Experimental Cancer Research in Pediatrics, Goethe-University, Komturstr. 3a, 60528 Frankfurt, Germany
[b] German Cancer Consortium (DKTK), Partner Site Frankfurt, Germany
[c] German Cancer Research Center (DKFZ), Heidelberg, Germany

ARTICLE INFO

Article history:
Received 28 March 2017
Accepted 2 June 2017
Available online 6 June 2017

Keywords:
Ferroptosis
Cell death
Acute leukemia
ROS
Redox

ABSTRACT

Ferroptosis has recently been identified as a mode of programmed cell death. However, little is yet known about the signaling mechanism. Here, we report that lipoxygenases (LOX) contribute to the regulation of RSL3-induced ferroptosis in acute lymphoblastic leukemia (ALL) cells. We show that the glutathione (GSH) peroxidase 4 (GPX4) inhibitor RSL3 triggers lipid peroxidation, production of reactive oxygen species (ROS) and cell death in ALL cells. All these events are impeded in the presence of Ferrostatin-1 (Fer-1), a small-molecule inhibitor of lipid peroxidation. Also, lipid peroxidation and ROS production precede the induction of cell death, underscoring their contribution to cell death upon exposure to RSL3. Importantly, LOX inhibitors, including the selective 12/15-LOX inhibitor Baicalein and the pan-LOX inhibitor nordihydroguaiaretic acid (NDGA), protect ALL cells from RSL3-stimulated lipid peroxidation, ROS generation and cell death, indicating that LOX contribute to ferroptosis. RSL3 triggers lipid peroxidation and cell death also in FAS-associated Death Domain (FADD)-deficient cells which are resistant to death receptor-induced apoptosis indicating that the induction of ferroptosis may bypass apoptosis resistance. By providing new insights into the molecular regulation of ferroptosis, our study contributes to the development of novel treatment strategies to reactivate programmed cell death in ALL.

1. Introduction

ALL is the most frequent type of malignant neoplasm in childhood [1,2]. The prognosis of children with very high-risk or relapsed disease is still dismal, thus calling for innovative therapeutic approaches. This includes new concepts to trigger programmed cell death, since evasion of leukemic cells to undergo cell death represents a frequent cause of treatment failure [3].

Several forms of programmed cell death have been described [4]. Ferroptosis is a recently defined mode of regulated cell death that depends on iron and is characterized by the generation of lipid-based ROS and lipid peroxidation [5]. Several ROS-generating enzymes contain iron or iron derivatives as essential co-factors for their proper function, for example LOX, nicotinamide adenine dinucleotide phosphate hydride (NADPH) oxidases (NOX), xanthine oxidase, and cytochrome P450 enzymes [6]. In addition, redox-active labile iron pools can directly catalyze free radical formation via Fenton chemistry [7].

LOX are key enzymes that catalyze the oxygenation of polyunsaturated fatty acyl groups to lipid hydroperoxides [8], while GPX family members are responsible for the reduction of hydrogen and lipid peroxides [9]. Among them, only GPX4 can directly reduce lipid hydroperoxides within biological membranes and therefore plays a crucial role in regulating the redox state of the cell membrane [9]. In addition, non-enzymatic lipophilic antioxidants such as α-Tocopherol (α-Toc) can scavenge membrane peroxyl radicals [10]. The membrane redox state is governed on the one side by the generation of lipid peroxides and on the other side by membrane-associated enzymatic and non-enzymatic peroxide scavengers. Disturbance of this homeostasis can lead to permeabilization of membranes such as the cell membrane and subsequently to cell death.

Ferroptosis has been implicated as a cell death process in a number of human diseases [5]. In cancer, the induction of ferroptosis may represent a therapeutic option, especially in those types of cancer that are refractory to other forms of programmed cell death, as ferroptosis proceeds independently of caspases. However, little is yet known about the signaling pathways that control ferroptosis

* Corresponding author at: Institute for Experimental Cancer Research in Pediatrics, Goethe-University, Komturstr. 3a, 60528 Frankfurt, Germany.
E-mail address: simone.fulda@kgu.de (S. Fulda).
[1] Shared first authorship.

http://dx.doi.org/10.1016/j.bcp.2017.06.112
0006-2952/

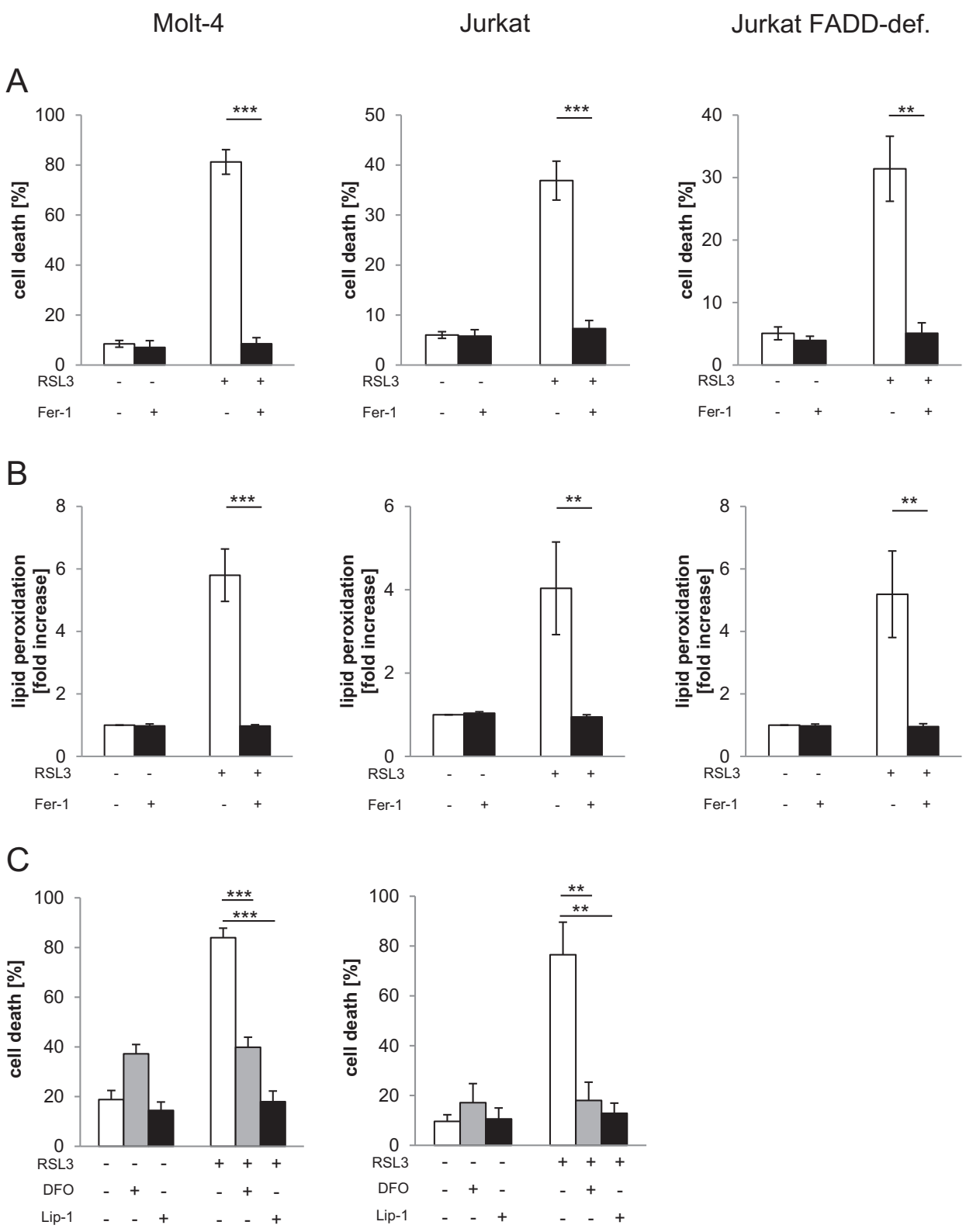

Fig. 1. RSL3 induces lipid peroxidation and ferroptotic cell death independently of FADD in ALL cells. ALL cells were treated for 24 h with RSL3 (A-F; Molt-4: 0.2 μM, Jurkat: 0.3 μM, Jurkat FADD-def: 0.3 μM), Etoposide (100 μM) and ABT737 (25 μM) (G), TNFα (Jurkat: 1 ng/ml, Molt-4: 50 ng/ml) and BV6 (4 μM) (H) or with FasL (500 ng/ml) and BV6 (4 μM) (I) in the presence or absence of 5 μM Fer-1 (A, B), 25 μM DFO (C), 25 nM Lip-1 (C, D), 20 μM zVAD.fmk (E, G) or 30 μM Nec-1s (F, H), which were added two hours before treatment. Cell death was determined by FSC/SSC analysis (A, C, E-I) or PI staining (D) using flow cytometry. Lipid peroxidation was assessed by flow cytometry in PI-negative cells using the fluorescent dye BODIPY-C11 and is shown as fold increase compared to untreated cells (B). Mean and SD of three experiments performed in duplicate are shown; *, $P < 0.05$; **, $P < 0.01$; ***; $P < 0.001$.