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ScienceDirect
Cancer Letters 254 (2007) 119–127
CANCER Letters
www.elsevier.com/locate/canlet

Parthenolide induces significant apoptosis and production of reactive oxygen species in high-risk pre-B leukemia cells

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Received 17 January 2007; accepted 2 March 2007

Abstract

We investigated whether parthenolide, the principal bioactive component of the herb feverfew (*Tanacetum parthenium*) induced apoptosis in pre-B acute lymphoblastic leukemia (ALL) lines, including cells carrying the t(4;11)(q21;q23) chromosomal translocation. Parthenolide induced rapid apoptotic cell death distinguished by loss of nuclear DNA, externalization of cell membrane phosphatidylserine, and depolarization of mitochondrial membranes at concentrations ranging from 5 to 100 μM. Using reactive oxygen species (ROS)-specific dyes, an increase in nitric oxide and superoxide anion was detected in the cells by 4 h after exposure to parthenolide. Parthenolide-induced elevation of hypochlorite anion was observed only in the two t(4;11) lines. These data suggest parthenolide may have potential as a potent and novel therapeutic agent against pre-B ALLs.

Keywords: Apoptosis; Mitochondria; Leukemia; Parthenolide; Reactive oxygen species, ROS

1. Introduction

Chromosomal abnormalities in the ALL-1 gene (also known as MLL, HRX, and HTRX1) on chromosome 11 are frequently involved in childhood ALL. The chromosomal translocation t(4;11)-(q21;q23) is found in greater than 60% of infants, 2% of children, and 3–6% of adults diagnosed with ALL and the presence of this chromosomal abnormality is strongly associated with an exceedingly poor prognosis [1]. More recently, the frequency of the t(4;11) translocation in infants with ALL was placed at 85% [2]. The t(4;11) ALLs are often classified as pre-B cells, but display a mixed-lineage phenotype with both B and myeloid cell surface markers. Dual-lineage markers suggest that these leukemias may represent pluripotent hematopoietic progenitors that can potentially differentiate into lymphoid, myeloid, or other hematopoietic cells. This high-risk subgroup of ALL is highly resistant to conventional chemotherapeutics and has an exceedingly poor prognosis.

Parthenolide is a sesquiterpene lactone and the principal bioactive component of the medicinal herb

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doi:10.1016/j.canlet.2007.03.002

feverfew (*Tanacetum parthenium*). Feverfew has been used for centuries as a folk medicine to treat migraines and rheumatoid arthritis [3]. Parthenolide has shown anti-inflammatory and anti-cancer activities [4,5]. Potent anti-cancer activity of this substance is due in part to its ability to inhibit the transcription factor NF-κB, thereby reducing survival potential in a number of cancer cells [6,7]. Parthenolide-induced generation of reactive oxygen species (ROS) in cancer cells has also been shown to play a role in promoting apoptotic cell death [8].

We have previously shown that a number of phytochemicals can effectively induce apoptotic cell death in cell lines that were established from patients with high-risk, B-lineage acute lymphoblastic leukemia (ALL) carrying the t(4;11)(q21;q23) chromosomal translocation, as well as other ALL lines without the translocation [9–11]. In the current study, the ability of parthenolide from the herb feverfew to induce apoptosis was examined in two t(4;11) pre-B ALL-derived cell lines and a pre-B ALL line without the translocation. We hypothesized that parthenolide would be effective in killing these leukemia cells. Parthenolide produced significant apoptotic death accompanied by mitochondrial dysfunction in the ALL lines and the t(4;11) lines were more sensitive to this agent than cells without the translocation. Parthenolide increased the production of nitric oxide and superoxide anion in the ALL lines. The production of the potent oxidant hypochlorite anion was also increased in the mixed-lineage t(4;11) lines, but not the cells without the translocation, in response to parthenolide treatment. These data suggest that parthenolide may be useful as a novel therapeutic agent against high-risk t(4;11) as well as other ALLs.

2. Materials and methods

2.1. Reagents

Parthenolide was purchased from EMD Biosciences (San Diego, CA) and stock solutions were dissolved dimethylsulfoxide (DMSO, Sigma Chemical Co., St. Louis, Mo.) before use. JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide) was purchased from Molecular Probes (Eugene, OR) and stock solutions were prepared in DMSO. Alexa Fluor 488-conjugated Annexin V and propidium iodide (PI) were obtained from Molecular Probes and Sigma, respectively. The cell permeable dye 4-amino-5-methylamino-20, 70-difluorofluorescein diacetate (DAF-FM DA), dihydroethidium (DHE), 3′-(*p*-aminophenyl) fluorescein (APF), 3′-(*p*-hydroxyphenyl) fluorescein (HPF), BODIPY 581/591 C11, and dihydrorhodamine 123 (DHR123) were purchased from Molecular Probes.

2.2. Cell culture

SEM and RS4;11 are established cell lines from patients diagnosed with pre-B cell acute lymphoblastic leukemia (ALL) containing the chromosomal translocation t(4;11)(q21;q23) [12,13]. The REH cell line (pre-B cell ALL without the translocation) was obtained from American Type Culture Collection (Manassas, VA). All cell lines were maintained at 37 °C, 5% CO_2 in RPMI 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma), 50 IU/ml penicillin, 50 μg/ml streptomycin, 0.25 μg/ml amphotericin B, 1 mM sodium pyruvate, and 2 mM L-glutamine (Invitrogen). For each experiment, unless otherwise noted, the cells were split to a density of 0.5×10^6/ml before treatment. As a control for the dissolving medium used for each chemical, an equivalent amount of the specific diluent was always added to a control cell population in every experiment (designated untreated).

2.3. Flow cytometric analysis of cell death

Immunofluorescence analysis was performed on a FACSCanto fluorescence activated cell sorter (FACS) using FACSDiva software (Becton Dickinson, Mountain View, CA). Cells were incubated with vehicle (0.1% DMSO) or concentrations of parthenolide ranging from 5 to 100 μM and aliquots from each treatment were removed at 24, 48, and 72 h. Cell death was measured by lysing the cells in a hypotonic solution containing 1 mg/ml sodium citrate, 0.1% Triton X-100, and 50 μg/ml propidium iodide (PI, Sigma) and analyzing the resulting nuclei by FACS. The extent of cell death (percentage) was determined by measuring the fraction of nuclei that contained sub-diploid DNA content. Ten thousand events were collected for each sample stained with propidium iodide.

To determine whether cell death was due to apoptosis, cells were plated in 96-well microtiter plates and treated with concentrations of parthenolide ranging from 5 to 100 μM. Control cells were treated with 0.1% DMSO. After 24 h, whole cells were stained with Alexa Fluor 488-conjugated Annexin V (Invitrogen) and PI according to the manufacturer's protocol. All analyses of whole cells were performed using appropriate scatter gates to exclude cellular debris and aggregated cells. Ten thousand events were collected for each sample stained with Annexin V and PI.

2.4. Analysis of mitochondrial membrane potential

JC-1 dye was used to measure changes in mitochondrial membrane potential as previously described [9]. Cells were plated in 96-well microtiter plates and treated with