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Methyl jasmonate induces cell death with mixed characteristics of apoptosis and necrosis in cervical cancer cells

Tatiana Kniazhanski, Anna Jackman, Alina Heyfets, Pinhas Gonen, Eliezer Flescher, Levana Sherman *

Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel

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Abstract

In the present study the effectiveness of methyl jasmonate (MJ) against cervical cancer cell lines was investigated. We show that MJ is cytotoxic to a range of cervical cancer lines including SiHa, CaSki and HeLa that carry human papillomavirus (HPV) DNA and wild type p53, and C33A that is negative for HPV and contains mutant p53. Primary human foreskin keratinocytes were almost resistant to the drug. Cytotoxicity of MJ was dose and time dependent, and associated mainly with the induction of cell death and to a less extent with inhibition of cell growth. Cell death induced by MJ displayed features characteristic to both apoptosis and necrosis, and was associated with different changes in the levels of p53, p21, bcl-2 and bax in the various cervical cancer lines. In conclusion, MJ a novel anticancer agent, acts via multiple pathways to induce death of cervical cancer cells, thus making it a promising candidate for treatment of cervical cancer.

Keywords: Jasmonates; Cervical cancer; Necrosis; Apoptosis; HPV E6 and E7; p53; p21; Bcl-2; Bax

1. Introduction

Cervical cancer has a major impact on women's live worldwide, particularly in developing countries where it is the leading cause of cancer deaths among women [1,2]. Development of anogenital cancer is strongly associated with infection by certain types of human papillomavirus (HPV) referred to as high-risk or oncogenic. More than 90% of the cervical cancers contain HPV DNA. The major viral oncogenes contributing to the carcinogenic process are HPV E6 and E7. These viral genes are always present and continuously expressed in HPV positive cancers and have been shown to contribute to the initiation and maintenance of the transformed cancer cell phenotype [3,4]. The ability of the E6 and E7 proteins of the oncogenic HPVs to interact with and facilitate the degradation of cellular proteins that regulate the cell cycle and apoptosis such as p53 and pRb, respectively, is a potential mechanism by which these viral proteins induce tumors [3,4].

Treatment of locally advanced cervical cancer consists of radiotherapy plus cisplatin-based chemotherapy. Although results are better with this combination than with radiotherapy alone, 5-year overall survival is still around 52% [5] and the treat-

* Corresponding author. Tel.: +972 3 6405194; fax: +972 3 6409160.
E-mail address: lsherman@post.tau.ac.il (L. Sherman).

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ment still results in substantial morbidity [6]. Further improvement in survival through intensification of the standard treatment is limited by intrinsic and acquired tumor resistance and by short-term and long-term side effects [6–8]. Therefore, alternatives are needed that can lengthen the antitumor effect with few toxic effects. A series of phase II studies has been performed in locally advanced or recurrent/ metastatic cervical cancers in order to evaluate the effectiveness of a number of compounds of recent development as single and combination agents [8,9]. So far however, cisplatin was more effective as a single agent or in combination with other compounds. Several novel molecular approaches using inhibitors of survival pathways and activators of apoptotic pathways are being studied at present as potential options for the treatment of cervical cancer [8].

Many anticancer drugs act through the induction of apoptosis [10,11]. Cervical cancer cells pose a particular problem in the induction of apoptosis. The key apoptotic regulators, p53 and pRb are inactivated by over expressed viral E6 and E7 proteins, respectively. Other pro-apoptotic regulators such as Bak, Myc, Bax, the tumor necrosis factor receptor (TNFR1), are also downregulated by the HPV E6 protein [12].Therefore, genetic approaches or drugs which downregulate HPV expression could potentially restore these tumor suppressor pathways and increase sensitivity to apoptosis [13]. Novel drugs which activate other pathways of programmed cell death, including necrosis [14,15] may be more effective. Recently it was discovered that plant stress hormones called jasmonates, that regulate cell death in stressed plants, possess anticancer activities in vitro and in vivo [16–20]. Jasmonates induced suppression of cell proliferation and death in a variety of cancer cell lines [16–20], as well as in leukemia cells from chronic lymphocytic leukemia patients [18]. The cytotoxic effects of jasmonates were selective for transformed cells, and shown to be independent of transcription, translation and p53 expression [21]. The mechanism of action of jasmonates is not fully understood and different cell death signaling pathways were described in different cancer cells [18,20,22,23].

In this study we investigated the effect of methyl jasmonate (MJ) on a panel of cell lines derived form cervical carcinoma including cells which contain (SiHa, CaSki, HeLa) or lack (C33A) HPV DNA. We show that MJ displays significant, though differential, cytotoxicity to cervical cancer cells with almost no effect on normal primary human keratinocytes. MJ treatment induced cell death in all tested cell lines and only slightly inhibited cell-growth of SiHa and CaSki. Cell death induced by MJ displayed characteristics of apoptosis as well as necrosis and was accompanied with different changes in the expression levels of apoptosis control proteins, p53, p21, bax, and bcl-2. This study suggests that MJ may be a useful option to current treatment of cervical carcinoma irrespective of the HPV or p53 status.

2. Materials and methods

2.1. Reagents

Methyl jasmonate (MJ) and cisplatin (CDDP) were obtained from Sigma Chemicals (Sigma-Aldrich, St Louis, MO). 4′6-diamidine-2-phenylindole-dihydrochloride (DAPI) was purchased from Boehringer-Mannheim (Mannheim, Germany). Primary antibodies to proteins were purchased from the following companies: Bax rabbit polyclonal antibody (sc-493), p53 DO-1 monoclonal antibody (mAb) (sc-126), bcl-2 mouse monoclonal antibody (C-2)(sc-7382), p21 rabbit polyclonal antibody (C-19) (sc-397), caspase-3 rabbit polyclonal antibody (H-277) (sc-7148) and caspase-9 p10 (H-83) (sc-7885), all from Santa Cruz Biotechnology (Santa Cruz, CA). Actin mouse monoclonal antibody (A 5316) was obtained from Sigma (St. Louis, MO) and PARP rabbit polyclonal antibody (Cat No 9542) from Cell Signaling Technology Inc (Denver, MA).

2.2. Cells

CaSki, SiHa, HeLa, C33A cervical carcinoma cell lines, Molt-4, a human T lymphoblastic leukemia cell line, MCF 7, a human breast carcinoma cell line, and 3LL, Lewis lung carcinoma cell line (mouse) were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Foreskin primary human keratinocytes (PHKs) were prepared freshly as described previously [24]. Molt-4 were maintained in RPMI 1640 supplemented with 10% (v/v) foetal bovine serum, PHKs were maintained in keratinocyte serum free medium (KSFM) supplemented with epidermal growth factor (EGF)(5 ng ml^{-1}) and bovine pituitary extract (BPE) (50 μg ml^{-1}) (all from Invitrogen, Paisely, Scotland). All other cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated foetal bovine serum and penicillin/streptomycin.

2.3. Viability assays

The non-radioactive 96-well cell proliferation assay with XTT reagent kit (Biological Industries, Israel, Beit