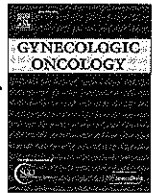




ELSEVIER

Contents lists available at ScienceDirect

Gynecologic Oncology

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

Suberoylanilide hydroxamic acid (SAHA) potentiates paclitaxel-induced apoptosis in ovarian cancer cell lines^{☆,☆☆}

Charles S. Dietrich III^{a,*}, Victoria L. Greenberg^b, Christopher P. DeSimone^b, Susan C. Modesitt^c, John R. van Nagell^b, Rolf Craven^b, Stephen G. Zimmer^b

^a Gynecologic Oncology Service, Department of Obstetrics and Gynecology, Tripler Army Medical Center, 1 Jarrett White Road, Honolulu, HI 96859-5000, USA

^b Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Kentucky Markey Cancer Center, 800 Rose Street, 333 Whitney-Hendrickson Building, Lexington, KY 40536-0298, USA

^c Division of Gynecologic Oncology, University of Virginia Health System, P.O. Box 800712, Charlottesville, VA 22908, USA

ARTICLE INFO

Article history:

Received 17 July 2009

Available online 28 October 2009

Keywords:

Ovarian cancer

Histone deacetylase inhibitor

SAHA

Paclitaxel

Survivin

Bad

ABSTRACT

Objectives. To determine if SAHA, a histone deacetylase inhibitor, decreases ovarian cancer cell viability when combined with paclitaxel *in vitro*, and to explore molecular alterations of combined paclitaxel + SAHA treatment.

Methods. SKOV3 and Hey ovarian cancer cell lines were treated for 24 h with paclitaxel, then re-treated with SAHA or paclitaxel for an additional 48 h. Protein extracts were prepared at 48 h for western blot analysis. Cell viability was assessed at 72 h using the ApoAlert Annexin V Apoptosis Kit.

Results. SAHA causes G1 and G2 cell cycle arrest in ovarian cancer cell lines. Cell viability was significantly reduced by combined paclitaxel + SAHA treatment. In Hey cells, viability was reduced to 67% with paclitaxel, and to 48% with paclitaxel + SAHA ($p < 0.001$). In the SKOV3 cell line, viability was reduced to 70% with continuous paclitaxel treatment, and was further reduced to 57% in the combined treatment group ($p < 0.05$). Increased PARP cleavage was noted in the paclitaxel + SAHA groups. SAHA increased expression of p21^{cip1}/waf1 and p27^{Kip1}, down regulated cyclins A and B, and suppressed CDK1. Paclitaxel induced expression of survivin, an inhibitor of apoptosis protein, was reduced to baseline control levels with the addition of SAHA. The pro-apoptotic protein, Bad, was also increased with SAHA.

Conclusions. Paclitaxel + SAHA reduces cell viability in excess of either agent alone in ovarian cancer cell lines. Cell death is mediated via several mechanisms including G1/G2 arrest from CDK1 downregulation, inhibition of paclitaxel-induced survivin accumulation, and from increased Bad expression.

Published by Elsevier Inc.

Introduction

Ovarian cancer is the second most common gynecologic malignancy in the United States with 21,650 new cases estimated in 2008 [1]. Due to the lack of an effective screening algorithm and a paucity of definitive presenting symptoms, most patients are diagnosed in advanced stages. Standard therapy involves extensive surgical debulking followed by combination chemotherapy, usually with paclitaxel and a platinum-based agent [2,3]. While initial response to therapy approaches 80%, recurrences are common. Unfortunately, the 5-year survival rate for advanced stage disease approaches 30% [1]. Recurrent disease quickly develops chemo-resistance and

responds poorly to salvage regimens. Novel modalities are needed to improve treatment responses.

Histones, the core proteins of the nucleosome packaging DNA, play a key role in the regulation of gene transcription and expression [4]. Competing acetyltransferases and deacetylases tightly control modification of histones. Deacetylation maintains chromatin in a compacted state, limiting accessibility of DNA targets to transcription factors critical for cell regulation [5]. Histone deacetylase inhibitors (HDACI) are gaining attention as anti-neoplastic agents as they induce cell differentiation, cell cycle arrest, and apoptosis [6–12]. Suberoylanilide hydroxamic acid (SAHA, vorinostat) is a potent inhibitor of histone deacetylase (HDAC) 1, 2, 3, and 6 [13]. In phase I/II trials, SAHA has shown promising responses with limited toxicities and has recently been FDA approved for treatment of recurrent cutaneous T-cell lymphoma [14–16].

Pilot studies with ovarian cancer cell lines demonstrate an encouraging decrease in cell viability with the combination of paclitaxel and an HDACI (sodium butyrate and trichostatin-A) [17–19]. The purpose of this study was to determine if SAHA decreases cell viability and increases apoptosis when combined with

[☆] Presented as a poster at the Society of Gynecologic Oncologists Annual Meeting, March 2006, Palm Springs, CA.

^{☆☆} The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the United States Government.

* Corresponding author. Fax: +1 808 433 1552.

E-mail address: chuck.dietrich@us.army.mil (C.S. Dietrich).