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Antitumor activity of paclitaxel is significantly enhanced by a novel proapoptotic agent in non–small cell lung cancer

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ABSTRACT

Background: Newer targeted agents are increasingly used in combination chemotherapy regimens with enhanced survival and improved toxicity profile. Taxols, such as paclitaxel, independently potentiate tumor destruction via apoptosis and are used as first line therapy in patients with advanced non–small cell lung cancer (NSCLC). Procaspase-3-activating compound-1 (PAC-1) is a novel proapoptotic agent that directly activates procaspase-3 (PC-3) to caspase-3, leading to apoptosis in human lung adenocarcinoma cells. Hence, we sought to evaluate the antitumor effects of paclitaxel in combination with PAC-1.

Methods: Human NSCLC cell lines (A-549 and H-322m) were incubated in the presence of PAC-1 and paclitaxel. Tumor cell viability was determined by a tetrazolium-based colorimetric assay (MTT assay). Western blot and flow cytometric analysis were performed to evaluate expression of PC-3 and the proportion of apoptotic cells, respectively. A xenograft murine model of NSCLC was used to study the *in vivo* antitumor effects of PAC-1.

Results: PAC-1 significantly reduced the inhibitory concentration 50% of paclitaxel from 35.3 to 0.33 nM in A-549 and 8.2 to 1.16 nM in H-322m cell lines. Similarly, the apoptotic activity significantly increased to 85.38% and 70.36% in A-549 and H322m, respectively. Significantly enhanced conversion of PC-3 to caspase-3 was observed with PAC-1 paclitaxel combination ($P < 0.05$). Mice treated with a drug combination demonstrated 60% reduced tumor growth rate compared with those of controls ($P < 0.05$).

Conclusions: PAC-1 significantly enhances the antitumor activity of paclitaxel against NSCLC. The activation of PC-3 and thus the apoptotic pathway is a potential strategy in the treatment of human lung cancer.

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1. Background

Lung cancer remains the leading cause of cancer-related death in both men and women and is responsible for 1.3 million deaths worldwide annually [1]. Although surgical resection of early stage lung cancer provides the highest survival rates in patients with non–small lung cancer (NSCLC), many patients eventually develop progressive disease and require additional treatment [2]. During the last two decades, the introduction of molecular targeting agents has tremendously evolved the treatment paradigm of NSCLC. These molecular targeting agents are now being increasingly used as a personalized anticancer strategy [3–5]. Combination chemotherapy regimens, including cytotoxic chemotherapy and/or molecular targeting agents, may delay disease progression and prolong survival in advanced NSCLC, however with varying results [6,7].

Traditional cytotoxic agents, such as taxols, exert antiproliferative effects by inducing cell death in all rapidly dividing cell types, and are commonly used as the first line chemotherapy agent against NSCLC [8]. However, emerging evidence based on the underlying molecular mechanisms regulating cell-cycle suggest that rather than being intrinsically toxic, most anticancer drugs including paclitaxel merely stimulate tumor cells to self-destruct via apoptosis [9,10]. Essentially, these apoptotic pathways converge on the activation of executioner caspase(s) 3 and 7 [11]. Hence, procaspase-3-activating compounds (PACs) provide an attractive antitumor strategy by essentially bypassing the often redundant upstream pathways in lung cancer cells leading directly to apoptotic cell death. PAC-1 and the related analogs are small molecules that directly activate procaspase-3 (PC-3) through the chelation of inhibitory zinc ions [12,13]. As cancer cells have much higher levels of PC-3 compared with those of normal counterparts, PAC-1's mechanism of action imparts a greater likelihood of success in inducing selective tumor cell death.

PAC-1 has shown significant efficacy in inducing apoptotic cell death in various cultured cancer cell lines as well as *in vivo* murine and canine tumor models [12,14,15]. Hence, we sought to further characterize the efficacy of PAC-1 in combination with a known cytotoxic agent, paclitaxel. To our knowledge, this is the first study to evaluate the potential of targeted proapoptotic agents such as PAC-1 in a combination chemotherapy regimen.

2. Methods

2.1. Cell culture and reagents

We evaluated the effects of PAC-1 in two different human non–small cell lung adenocarcinoma cell lines. A-549 was acquired from American Type Culture Collection (Manassas, VA), and H-322m was acquired from National Cancer Institute (Frederick, MD). Both cell lines possess high levels of PC-3 and have been extensively studied in cancer chemotherapy against NSCLC [16,17]. Cell lines were cultured according to standard guidelines. Cells were grown in Dulbecco Modified Eagle's Medium (Invitrogen, Carlsbad, CA), supplemented with 10% heat inactivated fetal bovine serum, glutamine, penicillin (100 U/mL), and streptomycin (100 U/mL) in a humidified incubator containing 5% CO_2 at 37°C.

2.2. Viability assays

Cell viability was determined using the MTT assay (Invitrogen). A-549 and H-322m cells were plated at a concentration of 5×10^3 cells/200 μL and 1×10^4 cells/200 μL per well separately in 96-well plates (Fisher Scientific, Pittsburgh, PA). Three replicated wells were used for each assay. Six wells were used with only the culture medium to blank the spectrophotometer, and it was determined that the drug(s) alone or in combination did not influence the background color of the medium. The following day, cells were treated with fresh growth medium containing PAC-1 (Cayman Chemical, Ann Arbor, MI) at increasing concentrations of 0–20 μM in combination with paclitaxel (Bristol-Myers Squibb, New York, NY) or dimethyl sulfoxide (DMSO) (0.1%) for 72 h to achieve antiproliferative effects. After the treatment, 20 μM of MTT (freshly prepared 5 mg/mL in phosphate-buffered saline [PBS]) was added to each well and was kept in culture incubator for 4 h. After the careful removal of media, 150 μM of MTT solvent (4 mM HCl, 0.1% nondet P-40 [NP40] all in isopropranolol) was added. Optical density, which directly correlates to the viable cell proportion, was read at 590 nm with a reference filter of 620 nm.

2.3. Flow cytometry

The cells were stained with annexin V-FITC and propidium iodide (PI) before flow cytometry as described in the apoptosis detection kit protocol (ApoScreen Annexin V Apoptosis Kit; Southern Biotech Company, Birmingham, AL). A-549 and H-322m cell lines were plated at a concentration of 2×10^5 cells per well and 4×10^5 cells per well in six-well plates (Corning Cell Culture Plates; Sigma–Aldrich, St. Louis, MO). Three replicated wells were used for each assay. The following day, cells were treated with fresh growth medium containing PAC-1 and paclitaxel at varying dose concentrations for 24–36 h to achieve antiproliferative effects. All cells (adherent and nonadherent) were harvested by centrifugation and washed twice in PBS, stained with annexin V-FITC and PI, and flow cytometry performed to analyze apoptosis according to manufacturer's instructions. Approximately 10,000 events (cells) were evaluated for each sample.

2.4. Western blot

Cultured cell protein extracts were prepared by cell pellets in cell lysis buffer (150-mM sodium chloride, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 50 mM Tris, pH 7.5) with protein inhibitor mix (Roche Diagnostics, Indianapolis, IN). Extracts were centrifuged at 12,000 rpm for 15 min at 4°C. Supernatant fractions were assayed for protein concentration by bicinchoninic acid assay with bovine serum albumin as reference standard (Thermo Scientific, Waltham, MA). Proteins were separated in a 4%–20% Tris-Glycine gel (Invitrogen Corporation) and