American Journal of Clinical Cancer Research

American Journals of
Clinical Cancer Research
http://ivyunion.org/index.php/ajcre
Vol. 1, Article ID 201300065, 6 pages

Research Article

Retroviral Interferon- α Gene Transfer Potentiates Paclitaxel against Ovarian Cancer Cells

Linus T. Chuang[1*], Sylvia Shenouda[2], David A Fishman[1], Nimesh Nagarsheth[1], Konstantin Zakashansky[1], Xiaohua Wu[3], and Nader G. Abraham[2]

[1] Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Science, Mt. Sinai School of Medicine, New York, United States
[2] Department of Pharmacology, New York Medical College, Valhalla, United States
[3] Department of Gynecologic Oncology, Shanghai Fudan Cancer Center, Shanghai, China

Abstract

Objective: To analyze the cytotoxic effects of paclitaxel following introduction of the retroviral interferon- α (IFN- α) gene into epithelial ovarian cancer cells.

Design: Experimental molecular study.

Setting: University hospital research center. Sample: Epithelial ovarian cancer cell lines OV-2774 and SKOV3. Empty vector was used as control.

Methods: The cytotoxic effects of paclitaxel on ovarian cancer cells were studied prior to and after transfection with the retrovirus-mediated inteferon- α gene. RT/PCR of the interferon gene, cell survival and cell death were analyzed to assess retroviral interferon- α gene expression after transfection.

Results: Paclitaxel inhibited cell growth in a dose dependent manner with half maximal inhibitory concentration (IC50) of 7.5 ng/ml. Retroviral inteferon- α gene transfer-transduced cells potentiated paclitaxel response against both ovarian cancer cell lines by 68%.

Conclusion: Retrovirus-mediated IFN- α gene transfer enhanced paclitaxel cytotoxicity on ovarian cancer cells. Retroviral IFN- α gene transfer in combination with paclitaxel may have significant clinical utility for the treatment of epithelial ovarian cancers.

Keywords: Epithelial ovarian cancers; retroviral Interferon- α Gene Transfer; paclitaxel; ovarian cancer cell lines; anti-proliferation; cytotoxicity

Abbreviations: IFN- α, Retroviral interferon- α; OV-2774, Ovarian cancer cell line OV-2774; SKOV3, Ovarian cancer cell line SKOV3

Peer Reviewers: Riyaz M. Basha, PhD, Experimental Therapeutics Laboratory, The University of Texas MD Anderson Cancer Center Orlando, United States; Imtiaz Ahmed Wani, MD, District Hospital, Directorate Health services, Kashmir, India

Received: December 15, 2012; **Accepted**: March 2, 2013; **Published:** March 20, 2013

Competing Interests: The authors have declared that no competing interests exist.

Funding: This study was funded in part by NIH grant HL-54138.

***Correspondence to**: Linus T. Chuang, Division of Gynecologic Oncology, Department of Obstetrics, Gynecology, and Reproductive Science, Mt. Sinai School of Medicine, New York NY, United States, Email: linus.chuang@mssm.edu

Introduction

Ovarian cancer is estimated to have been responsible for slightly above half (52%) of gynecologic cancer-related deaths, according to the American Cancer Society for the year 2013 [1]. Epithelial ovarian cancer (EOC) is a specific type of ovarian cancer that develops in the epithelium and comprises 90% of all ovarian cancers [2]. The vast majority of patients diagnosed and treated for EOC develop recurrences [3]. While various therapies have been established, the current standard of care for epithelial ovarian cancer following cytoreductive surgery is combination platinum and paclitaxel therapies, although rigorous research yielding more favorable long-lasting outcomes continues to develop, with plenty of room for improvements [4-6].

Interferon-α (IFN-α) via retroviral gene transfer in vitro is a novel therapeutic approach receiving attention for its effects in a wide variety of cancers [7-10]. As epithelial ovarian cancer is confined within the peritoneal cavity, gene therapy using the delivery of IFN-α through viral vectors into the peritoneal cavity would appear to offer an alternative treatment option in treating ovarian cancers. In this study the anti-tumor activity of interferon-α over-expression using the retrovirus-mediated gene transfer was first assessed. Subsequently we evaluated the effects of interferon-α gene expression in combination with paclitaxel treatments on ovarian cancer cell lines OV2774 and SKOV3.

Materials and methods

Cell Lines, Paclitaxel Treatment

Two human epithelial ovarian cancer cell lines, OV2774 and SKOV3, were obtained from MD Anderson Cancer Center, Houston, Texas (courtesy of Judith Wolf, M.D.). Cell cultures were grown at 37 ℃ in 5% CO2 environment. OV2774 and SKOV3 were adjusted to 2x 106 cells/ml and were cultured in RPMI 1640 medium supplemented by 10% fetal calf serum. The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented by 10% heat-inactivated fetal bovine serum (FBS; Life Technologies, Rockville, Md.), 100 U/ml penicillin and streptomycin 100 μg/ml. The drugs was kept at ambient temperature and made fresh in 0.9% NaCl at the time of use [11]. For cell culture experiments, dissolved agents were mixed with RPMI medium containing 10% FBS just before use. At the beginning of the treatment, the medium overlying the monolayers was removed, and the cells were covered with 2ml RPMI medium containing 10% FBS at pH 7.4 + 0.1 and then treated with the drug for 4 h at 37 ℃. Based on what we previously published, paclitaxel were used at the concentrations of 2, 5, 7.5, 10, and 12 ng/ml [11]. At the end of the treatments, the cells were washed and assayed for viability.

Retroviral vector and recombinant virus production

The retrovirus vector encoding IFN-α was constructed in our laboratory as previously described [9]. Briefly, based on the backbones of retroviral vectors pLXSN and pLNCX (clontech, Palo Alto, CA) the replication-deficient retroviral vector (LSN-IFN-α) containing the human gene was constructed by inserting a 677-bp Xhol-Xhol human 5cDNA fragment from BMG neo IFN-α vector in the Xhol site of retroviral LXSN. PA 317 retroviral packaging cells (3 x 105) were seeded into 60-mm dishes and incubated for 24h, then washed twice with serum reduced Opti-MEM (GIBCO-BRL). In the current study, 5 g of each of the retroviral vectors PA 317/LSN-IFN-α -H3, which produced the highest viral titer of 11x105 cfu/ml, was incubated in 20 μl plasmid Lipofectamine reagent (Boehringer Mannheim, Mannheim Germany) for 30 min at ambient temperature, added to the culture dish cells, and incubated at 37 ℃ for 5 h. After incubation, 3 ml DMEM containing 20% FBS was added to the culture dish, which was reincubated for 18 h. The media were replaced by complete fresh media and reincubated for another 24 h. The cells were then passed 1 to 10 times into G418-containing medium (500μg/ml). After 14 days' culture at 37°C, 5% CO2, the transduced cells were subjected to a single cell cloning. For each isolated cells, the viral

titer was determined by real-time quantitative PCR as described [12]. Viral supernatants were collected, passed through 0.22m filters, and stored in aliquots at -80 ℃.

Cytotoxicity and Cell Death Assay

The cytotoxic effect of paclitaxel was studied both in cell cultures transfected with the interferon-α gene and those transfected with the empty vector. RT/PCR of the interferon gene, cell survival and cell death were analyzed to assess for interferon-α gene expression after infection [10, 11]. Cytotoxicity assays were performed with a combination of drug and the interferon-α gene. The combinations were analyzed according to the multiplication concept of drug interaction in both cultures [11, 13].

Statistical Analysis

All values were from at least three independent experiments in triplicate and are expressed as the mean + standard error of the mean. The probabilities of significant differences upon comparison of the groups were determined by two-tailed Student's t-tests. Significance for the comparisons was inferred at $p<0.05$.

Results

We first examined the antiproliferative effects of paclitaxel on OV2774 and SKOV3 cell lines. The respective antiproliferative effect of paclitaxel is seen in Figure 1. Paclitaxel exerts a dose-dependent cytotoxic effect on ovarian cancer cell lines with 50% inhibition at a concentration of 7.5 ng/ml for 24-hour cultures (Figure 1).

In the following set of experiments, we assessed the effect of retrovirus-mediated interferon gene construct on survival of OV2774 and SKOV3 cell lines. Supernatant of packing cell line PA317 cells at 105-106 pfu/ml failed to achieve a high infection on the SKOV3 and OV2774 cell lines. Retroviral particles were centrifuged to acquire a higher concentration of 109-1010 pfu/ml (17). Infection of OV2774 and SKOV3 cells with the higher concentration achieved a higher infectivity and cell death. Using the ELISA kit to measure the secretion of Interferon-α, the maximal secretion was achieved after 3-4 days of infection. As seen in Figure 2, the interferon protein produced by retroviral construct resulted in time-dependent OV2774 and SKOV3 cell death. Maximum cell death was achieved after 96 hours of incubations.

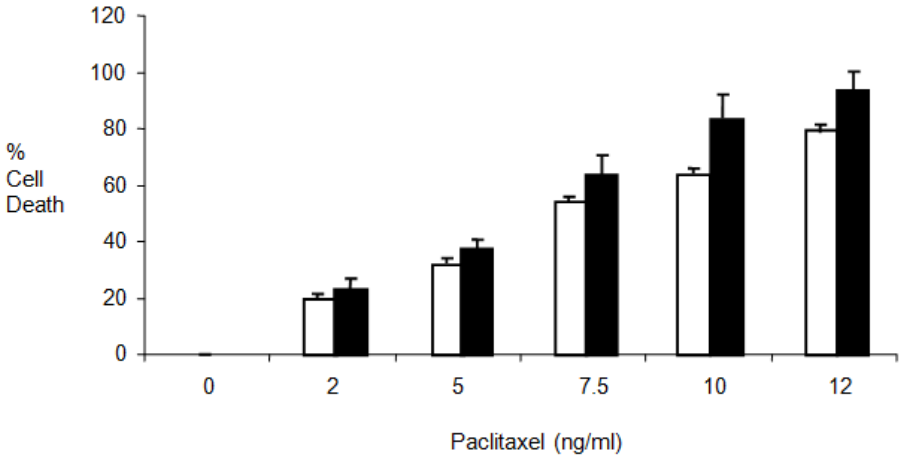

Figure 1 Effect of different Paclitaxel concentrations on ovarian cancer cells OV2774 (opened column) and SKOV3 (solid column) survival. Ovarian cancer cell lines were cultured and treated with Paclitaxel and analyzed for cell death after 24 hours of drug exposure as described in the materials and methods. There is a dose dependent cytotoxicity of Paclitaxel on both OV2774 and SKOV3 ($P<0.001$).

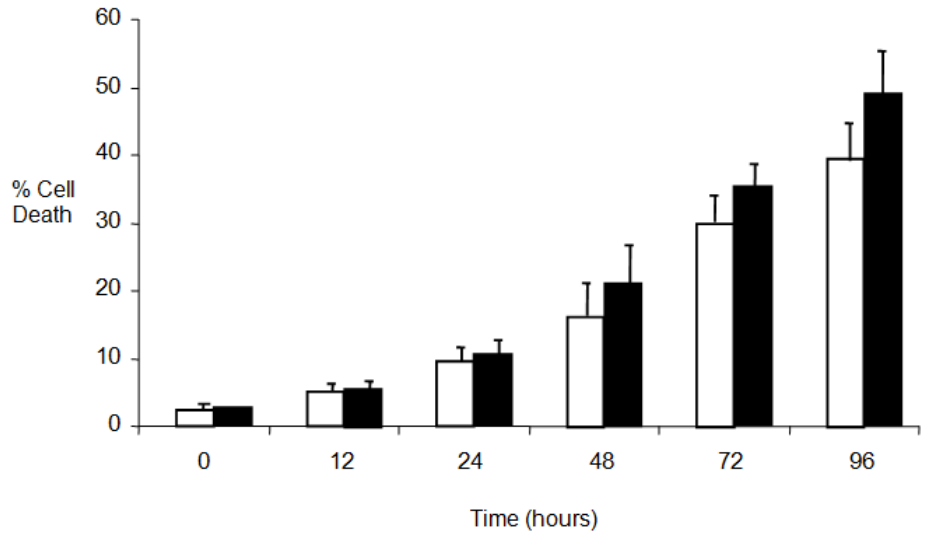

Figure 2 Effect of durations of IFN-α incubations on ovarian cancer cell OV2774 (opened column) and SKOV3 (solid column) (P<0.001)

Next, we examined whether Interferon-α gene transfer may potentiate the cytotoxic effects of paclitaxel on OV2774 and SKOV3 cell lines. Cells were first infected with either retroviral empty vector or retrovirus mediated Interferon-α. After 12 hours of infection, paclitaxel was added for an additional 48 hours and cell death was measured. We did not observe any cell death in control cells or cells infected with the empty viral vector. In our experiments, the retrovirus-mediated interferon gene potentiates the cytotoxicity of Paclitaxel on pretreated OV2774 and SKOV3 cells (Figure 3). In Figue 3, the cytotoxicity of Paclitaxel at 5ng/ml was enhanced by 68% when the ovarian cancer cells were pretreated with the retrovirus-mediated interferon gene. Our results show that expression of a functional interferon gene may enhance cell cytotoxicity when it is administered in combination with paclitaxel.

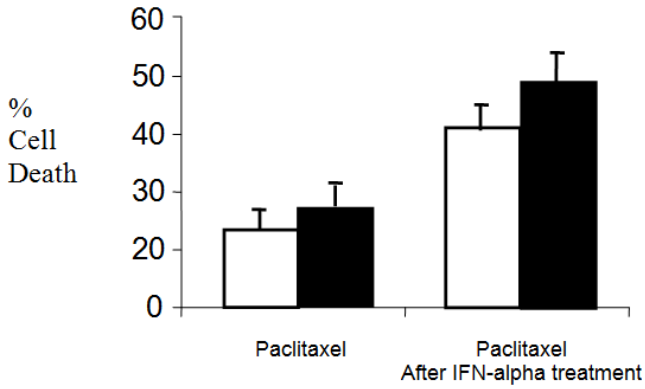

Figure 3 Combinatory effect of Paclitaxel, and Paclitaxel after IFN-α gene transduction on Ovarian Cancer Cell OV2774 (open column) and SKOV3 (solid column) (P<0.001)

Discussion

Here we presented a unique approach using paclitaxel in combination with gene transfer as we demonstrate how the construction of a functional retroviral mediated interferon gene delivery resulted in enhancement of interferon secretion.

Local expression of interferon in OV2774 and SKOV3 cell lines sensitizes the cells to paclitaxel. The combination of interferon transfer using retroviral vectors shows synergy in cell death, which may be of clinical significance. This may allow us to avoid the cytotoxicity in normal cells that is usually associated with using higher doses of paclitaxel alone. In previous retroviral studies IFN-α has been shown to have a short half-life of 3 hours, low detection rate near the site of tumors and toxic side effects, so both dosage and timing needed to be fine-tuned in order to yield higher response rates [14-16], which currently stands at a mere 30% over 5 years [5, 17].

The exact molecular mechanisms of the inhibitory effects of interferon are not fully understood; studies had supported host-mediated antitumor mechanisms induced by the local production of IFN-α. Other studies have shown immunomodulatory effects of IFN-α result in IFN-induced antitumor immunity [18]. Some data have demonstrated that IFN-α binds to the surface receptors and activates the interferon signaling cascade. It is suggested that part of this cascade involves proteins that act as tumor suppressors. Interferon is thought to activate receptors associated with tyrosine kinases. The best evidence of this model is the observation that interferon signaling is lost in cells deficient in a particular kinase, JAK 2. One substrate for the interferon activated tyrosine kinase is a multi-subunit transcription factor called ISGF-3. Upon tyrosine phosphorylation of multi-subunits, this factor assembles and moves from the cytoplasm to the nucleus, where it binds to specific sequences called interferon sequence response elements (ISRE). This results in the promotion of various interferon-α inducible genes initiating new gene transcription that may include tumor suppressor genes. The tumor suppression genes may well be activated in presence of paclitaxel. The revolutionary use of interferon gene transfer to assure cellular gene expression seems promising.

The results of our study show that the retrovirus-mediated IFN-α gene transfer enhanced paclitaxel cytotoxicity on ovarian cancer cells. Retroviral interferon-α gene transfer in combination with paclitaxel may have significant clinical utility for the treatment of epithelial ovarian cancers. It is possible that present study demonstrating the potential benefits of gene transfer in cell cultures will lead to further development of molecular therapies and new treatments using existing chemotherapeutic drugs in the management of cancer. Future studies including gene expression and microarray expression of these genes will facilitate investigation into mechanism of dual treatment of chemotherapy and gene therapy of ovarian cancer.

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