Effect of TUBB2A and TUBB3 Knockdown on Paclitaxel Sensitivity of Ovarian Cancer Cells

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Abstract

The high mortality rate of ovarian cancer and the evolution of resistance to traditional chemotherapeutic drugs like paclitaxel make it a key concern in oncology. The purpose of this work was to determine whether downregulating TUBB2A or TUBB3, two important β-tubulin isotypes, will further amplify the cell death that paclitaxel induces in ovarian cancer cells. After transfecting A2780 ovarian cancer cells with siRNA directed against TUBB2A, TUBB3, or a control gene, paclitaxel treatment was administered. Crystal Violet staining was used to evaluate the viability of the cells. The hypothesis that TUBB2A downregulation would cause more paclitaxel-induced cell death than TUBB3 downregulation was not supported by the results, which was unexpected. The control group showed an unusually high rate of cell mortality in the absence of paclitaxel treatment, perhaps as a result of variable cell plating. Despite this discrepancy, siTUBB3-transfected cells treated with paclitaxel had the lowest viability, followed by control siRNA and siTUBB2A. These results imply that paclitaxel sensitivity in ovarian cancer cells may be strongly influenced by factors other than TUBB2A and TUBB3 expression levels. The observed discrepancies challenge existing hypotheses regarding the relationship between TUBB2A, TUBB3, and paclitaxel resistance. This highlights the need for more research to understand the molecular mechanisms underlying paclitaxel resistance and to find viable therapeutic targets to overcome drug resistance in ovarian cancer.

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Introduction

Ovarian cancer is the most lethal female reproductive cancer. According to estimates made by the American Cancer Society, there will be about 19,710 new diagnoses of ovarian cancer in 2023, with about 67 percent of them concluding in death, which is 3 percent higher than last year's estimates. Dubbed a "silent killer", its symptoms, such as bloating, abdominal pain, and an upset stomach, are often recognizable at the more severe stages of cancer where tumor metastasis is already widespread. Thus, almost 80% of women are diagnosed at either stage III or IV out of four stages.

In addition, standard chemotherapy has been rendered nearly useless due to drug resistance. Paclitaxel is a drug with a history dating back to 1962 when it was isolated from the bark of the Pacific yew tree, and is FDA-approved in the treatment of various common solid tumors including ovarian cancer. When paclitaxel binds to β-tubulin, it causes the tubulin subunits within the microtubules to become rigid, meaning they become unable to be attached to or removed from the microtubules. As a result, these structures become dysfunctional, leading to cell cycle arrest. If this situation is not corrected, the cell undergoes apoptosis (Yang et al., 2018). However, approximately 60-75% of ovarian cancer patients experience a relapse of symptoms within two years after receiving paclitaxel treatment because they develop resistance to paclitaxel. Paclitaxel is not as effective as it is preferred to be because cancer cells often overexpress tubulin genes to develop resistance to paclitaxel.

The human genes TUBB2A and TUBB3 code for class IIa β-tubulin and class III β-tubulin respectively – key structural components of microtubules. Microtubules are long, hollow tube-like structures within cells, composed of tubulin subunits. They are necessary for proper mitotic spindle formation during both metaphase and anaphase of mitosis and help to ensure the even distribution of chromosomes to the daughter cells. There are six types of tubulins — alpha, beta, gamma, delta, epsilon, and zeta — and there are eight isotypes of beta-tubulin, with TUBB2A and TUBB3 being two commonly researched examples of these.

TUBB3 is often overexpressed in ovarian cancer (Gao et al., 2012). When TUBB3 is overexpressed, it triggers the production of tubulin, increasing the chance that microtubules will function correctly in a cell. This is believed to be the main reason for paclitaxel resistance (Gao et al., 2012). TUBB2A is a lesser-researched isotype of beta-tubulin, but literature suggests that there was an inverse correlation between TUBB2A expression and paclitaxel-induced apoptosis

in lymphoblastoid cell lines (Leandro-Garcia et al., 2012). However, it is not known which tubulin downregulation will lead to a higher degree of ovarian cancer cell death upon paclitaxel resistance. Interestingly, according to the Kaplan-Meier plotter (https://kmplot.com) high levels of TUBB2A are significantly associated with poor survival outcome of ovarian cancer patients while high levels of TUBB3 are not. This implies that TUBB2A might serve as a better therapeutic target than TUBB3 to overcome paclitaxel resistance in ovarian cancer.

Statement of Purpose

It was hypothesized that TUBB2A downregulation would induce more paclitaxel-induced ovarian cancer cell death compared to TUBB3 downregulation. This research will provide novel information about how to better combat paclitaxel resistance among ovarian cancer patients, in hopes of improving the survival outcome of ovarian cancer patients.

Methods and Materials

Two 24-well plates were utilized:

Plate 1: No paclitaxel treatment

siTUBB3	siTUBB3	siTUBB3	siTUBB3	siTUBB3	siTUBB3
siTUBB2A	siTUBB2A	siTUBB2A	siTUBB2A	siTUBB2A	siTUBB2A
Control siRNA	Control siRNA	Control siRNA	Control siRNA	Control siRNA	Control siRNA
Unoccupied	Unoccupied	Unoccupied	Unoccupied	Unoccupied	Unoccupied

Plate 2: Paclitaxel treatment

siTUBB3 Paclitaxel	siTUBB3 Paclitaxel	siTUBB3 Paclitaxel	siTUBB3 Paclitaxel	siTUBB3 Paclitaxel	siTUBB3 Paclitaxel
siTUBB2A Paclitaxel	siTUBB2A Paclitaxel	siTUBB2A Paclitaxel	siTUBB2A Paclitaxel	siTUBB2A Paclitaxel	siTUBB2A Paclitaxel
Control siRNA Paclitaxel	Control siRNA Paclitaxel	Control siRNA Paclitaxel	Control siRNA Paclitaxel	Control siRNA Paclitaxel	Control siRNA Paclitaxel
Unoccupied	Unoccupied	Unoccupied	Unoccupied	Unoccupied	Unoccupied

Initially, approximately 50,000 A2780 ovarian cancer cells were cultured in RPMI-1640 growth medium supplemented with fetal bovine serum (FBS) and antibiotics.

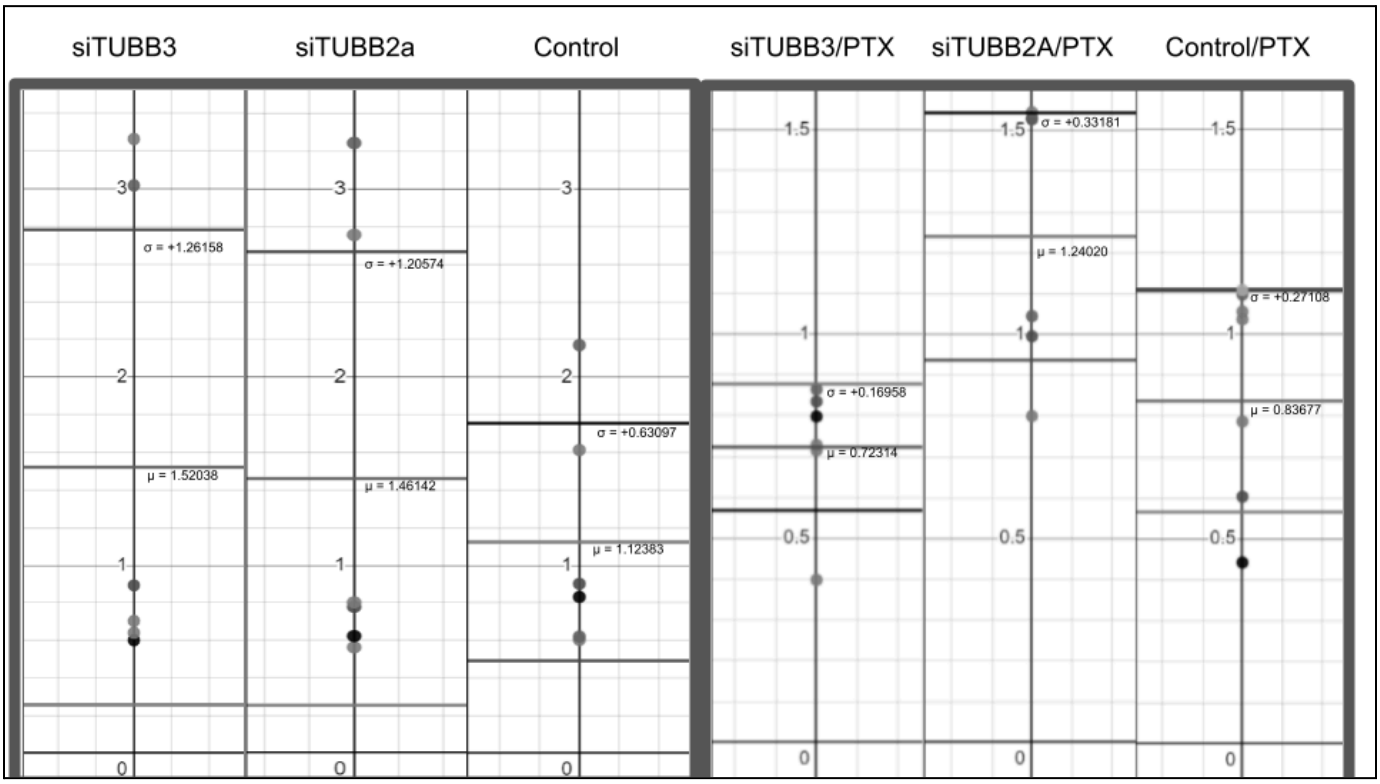
24 hours later, siRNA transfections targeting TUBB3, TUBB2A, and an unimportant control gene were performed on the designated wells in both plates.

24 hours after initiating the transfection process, 16 nM paclitaxel was added to the designated wells of the paclitaxel treatment plate.

48 hours after treatment, cells were stained with Crystal Violet, and absorbance at 600 nm was utilized to quantify live cell count. The mean and standard deviation of absorbance was calculated across all six treatment groups, and the cell viability percentage for each type of siRNA knockdown was determined.

Results

One absorbance reading for each of six wells in each of six treatment groups was recorded, and the mean and standard deviation of the absorbance values were recorded for each treatment group:

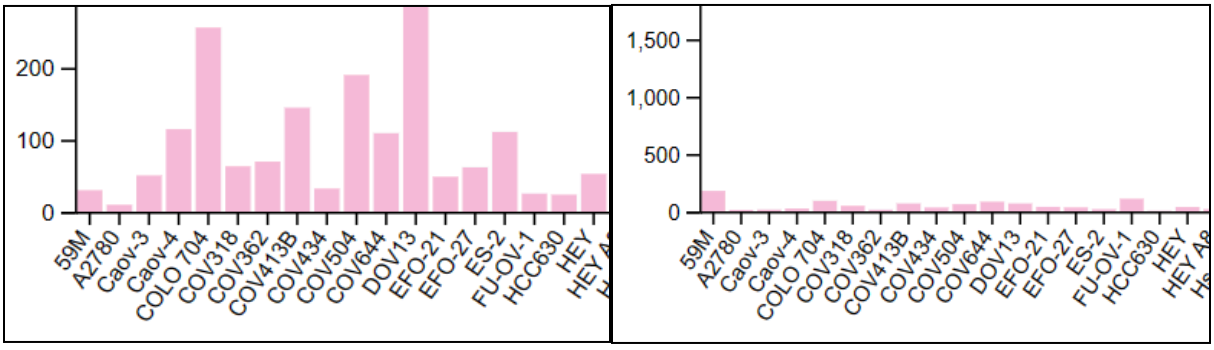


In addition, the absorbance value for each of the non-paclitaxel treatment groups was used as a benchmark for comparison with that of each of the paclitaxel treatment groups to measure cell viability for each type of siRNA knockdown:

Transfected siRNA	Mean Absorbance (no PTX)	Mean Absorbance (PTX)	% Cell Viability
siTUBB3	1.52038	0.72314	47.563%
siTUBB2A	1.46142	1.24020	84.8627%
Control siRNA	1.12383	0.83677	74.4570%

Discussion

In the absence of paclitaxel treatment, unexpectedly, the control group exhibited higher cell death. This inconsistency may have resulted from inadequate resuspension of the cell solution during addition to each well, resulting in variable cell numbers being plated. To mitigate these effects, cell viability percentages were calculated across all three types of transfected siRNA. Among the paclitaxel/no-paclitaxel treatments, cells transfected with siTUBB3 showed the least viability, followed by the control siRNA and then siTUBB2A. Generally, these results did not support the hypothesis that the degree of paclitaxel-induced cell death of ovarian cancer cells is larger upon TUBB2A knockdown compared to TUBB3 knockdown. However, a plausible reason is that the A2780 cells that were utilized exhibit a severely low expression of both TUBB3 (9.7 nTPM) and TUBB2A (12.8 nTPM) such that the difference in nTPM (normalized transcripts per million) can be considered negligible.



TUBB3 TUBB2A

Conclusion

There is no sufficient evidence to support, from my experiment, that the degree of paclitaxel-induced cell death of ovarian cancer cells is larger upon TUBB2A knockdown compared to TUBB3 knockdown.

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