

Synergistic Effect of YK-4-279 and Paclitaxel on A549 Cell Line [†]

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Abstract: Lung cancer, among all cancer types around the world, is listed as the major cause of death with high mortality rate. ERG transcription factor has an important role in the Epithelial Mesenchymal Transition (EMT), which is one of the most important mechanisms in lung cancer progression. It is known that a small molecule inhibitor YK-4-279 is a potential inhibitor of ERG expression in prostate cancer. In this study, the possible synergistic effect of YK-4-279 and another anticancer drug, Paclitaxel is analysed in non-small cell lung cancer (NSCLC) cell line, A549.

Keywords: lung cancer; ERG; EMT; YK-4-279; paclitaxel; synergistic effect

1. Introduction

Cancer is the most common cause of death worldwide. Cancer arises when cells acquire a series of mutations that lead to uncontrolled cell growth and division [1]. Lung carcinoma is accepted as the most common cancer in the world and is divided into two major groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [2].

Epithelial Mesenchymal Transition (EMT) is a process that is observed in many cellular activities such as embryonic development, wound healing and drug resistance. During the EMT process, epithelial cells lose their intercellular contacts and become motile [3]. However, in cancer progression, EMT leads to changes in phenotype of cancer cells in terms of their proliferation, motility, invasion and morphology [4].

ERG transcription factor is a member of ETS family, and has an important role in cell proliferation, apoptosis, invasion and angiogenesis [5]. In prostate cancer, it has been demonstrated that elevated expression level of ERG is correlated with invasion and metastasis. ERG transcriptional activity is inhibited by YK-4-279, which is a small molecule inhibitor that targets cellular protein-protein interactions and leads to reduced cell motility and invasion [6].

Paclitaxel is a mitotic inhibitor which leads to inhibition of cell division by binding to tubulin and stabilizing microtubules in the polymerized form [7]. Paclitaxel is widely used in treating patients with non-small lung cancer cells [8]. Furthermore, Paclitaxel has been used in combination with other anticancer agents for the treatment of advanced NSCLC.

In this study, the possible synergistic effect of YK-4-279 and Paclitaxel is analysed in A549 NSCLC cell line.

2. Materials and Methods

A549 cell line was obtained from the American Tissue and Cell Collection (ATCC, Manassas, VA, USA). The cells were cultured in F12-K media supplemented with 10% fetal bovine serum (Wisent, Saint-Jean-Baptiste, QC, Canada) at 37 °C in a humidified incubator with 5% CO₂. YK-4-279 (purity > 99%) and Paclitaxel (purity ≥ 95%) were purchased from Tocris (Bristol, UK) and Biovision (Milpitas, CA, USA), respectively, and dissolved in DMSO. The final concentration of DMSO in all experiment set-ups was 0.1%.

For Cell Proliferation Assay, cells were seeded at a density of 5×10^3 – 1×10^4 cells/well in a 96-well plate and incubated for 24 h. The next day, cells were treated in combination with different concentrations of YK-4-279 and Paclitaxel for 48 h. After treatment, WST-1 reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to each well. Absorbance at (420–480 nm) was measured using MultiScan GO Microplate Spectrophotometer. And the half maximal inhibitory concentration (IC₅₀) values of each YK-4-279 and Paclitaxel were calculated using GraphPad Prism software program. The potential synergistic effects of YK-4-279 and Paclitaxel were analysed again by using GraphPad Prism and assessed using combination index (CI) equation. When CI is <1, the combination of the drugs is defined as synergistic. If CI is ≥1, the combination of the drugs is considered as antagonist or additive.

3. Results

The effect of YK-4-279 and Paclitaxel alone or in combination on A549 cell line was studied using WST-1 Cell Proliferation Assay. The IC₅₀ value of YK-4-279 on A549 cell line was 6.5 μM, whereas the IC₅₀ value of Paclitaxel was 13 μM. Results showed that treatment of YK-4-279 in combination with Paclitaxel had a less effect on A549 cells growth than YK-4-279 alone. Whereby, the CI value was 2.8. Figure 1 shows IC₅₀ graphs for each of YK-4-279 and Paclitaxel. And Figure 2 shows the graph of drugs in combination.

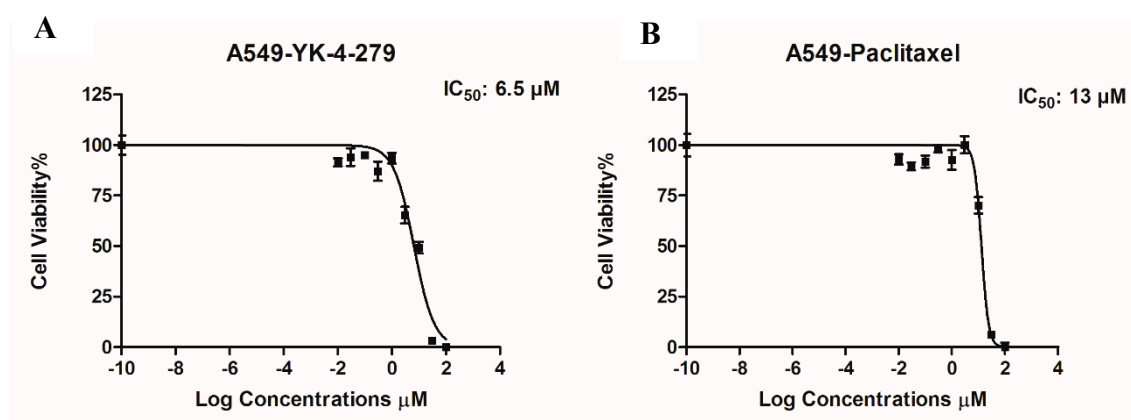


Figure 1. (A) IC₅₀ graph of A549 cell line treated with YK-4-279. (B) IC₅₀ graph of A549 cell line treated with Paclitaxel.

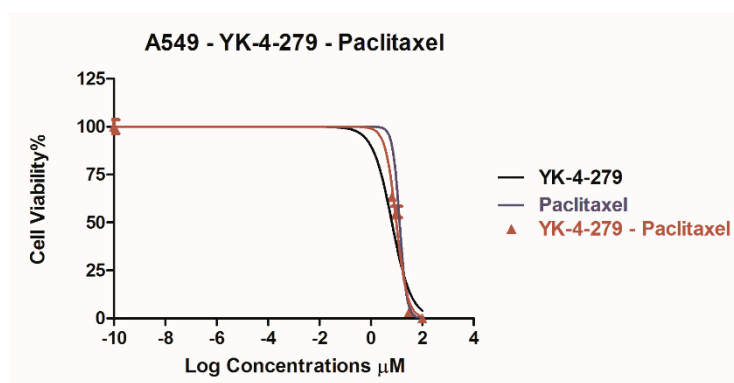


Figure 2. Combination graph of YK-4-279 and Paclitaxel (CI = 2.8).

4. Discussion

Previous studies demonstrated that the small molecule inhibitor, YK-4-279, has a powerful role in inducing mitotic arrest in prometaphase by inhibiting the formation of kinetochore microtubules and resulting in cell death in neuroblastoma [7]. Paclitaxel, which is an anticancer agent that binds to tubulin and leads to cell death, has been used alone and in combination with other chemotherapeutic agents in different types of cancer.

Although we hypothesised that YK-4-279 can synergize with Paclitaxel and this drug combination can be more effective than treatment with Paclitaxel alone, our data demonstrated that the combination of YK-4-279 and Paclitaxel had a less potent inhibitory effect on the growth of A549 cells than either drug used alone. Moreover, results showed an antagonist effect of the drugs in combination according to the “Combination Index” value. Hence, in A549 cell line, using YK-4-279 alone has a stronger effect on inducing apoptosis and causing cell death than in combination with Paclitaxel.

5. Conclusions

In this study, the potential of YK-4-279 to synergize with another anticancer agent, Paclitaxel, in A549 (NSCLC) cell line was tested. The results showed an antagonist effect between these two drugs. Thus, YK-4-279 can potentially be used alone or be tested for a possible synergistic effect with another chemotherapy drug in different NSCLC cell lines.

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Conflicts of Interest: The authors declare no conflict of interest.

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