

# Combination of temsirolimus with cisplatin as anti-cancer agents through inducing apoptosis and altering cell cycle distribution in Hela cell line

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**Abstract.** Combination treatment is the most effective therapy strategy on cancer by dramatically reducing drug toxicity and enhancing efficacy. In this study, we assessed whether temsirolimus, an mTOR inhibitor enhanced anti-cancer effects of cisplatin on human cervical Hela cell line or not. The combination of temsirolimus and cisplatin, to a large extent, reduced proliferation rate of cervical Hela cells in comparison with single chemotherapeutics cisplatin in vitro and resulted in synergistic effects ( $CI < 1$ ). Flow cytometry results indicated that temsirolimus markedly increased cisplatin-induced apoptosis. Thus, the combination chemotherapy using cisplatin and temsirolimus could be a novel strategy for synergistic effects against human cervical cancer.

**Keywords:** Temsirolimus, Cisplatin, Cervical cancer, mTOR pathway, Apoptosis, Cell cycle distribution.

## 1. Introduction

Cervical cancer is the most common malignant tumor among women worldwide. Although some therapy methods are developed to treat advanced cervical cancer including surgery, radiotherapy, and the combination with cisplatin-based chemotherapy, the 5-year overall survival rate of patients remains low (50%)[1]. Therefore, the main attention is paid to discover a novel and effective combination strategy to prevent tumor invasion and improve mediate drug resistance.

Many novel combination treatments that target specific cellular pathways are being studied and one of the most hopeful combination methods is the mammalian target of mTOR inhibitors [2]. Among those best-studied mTOR inhibitors, such as rapamycin, everolimus (RAD001) and temsirolimus (CCI-779), temsirolimus is the first mTOR inhibitor approved to treat renal cell carcinoma [3]. Based on some vitro and vivo experiments, temsirolimus could promote cytotoxic efficacy of chemotherapy regimen [18, 34, 39].

Given its great potential in anti-cancer field, it is hypothesized that mTOR is a feasible therapy target in human cervical cancer and temsirolimus can act as an adjunctive agent in chemotherapy. Therefore, we investigated the cytotoxic efficacy of temsirolimus combined with cisplatin in the treatment of human cervical cancer cells in vitro.

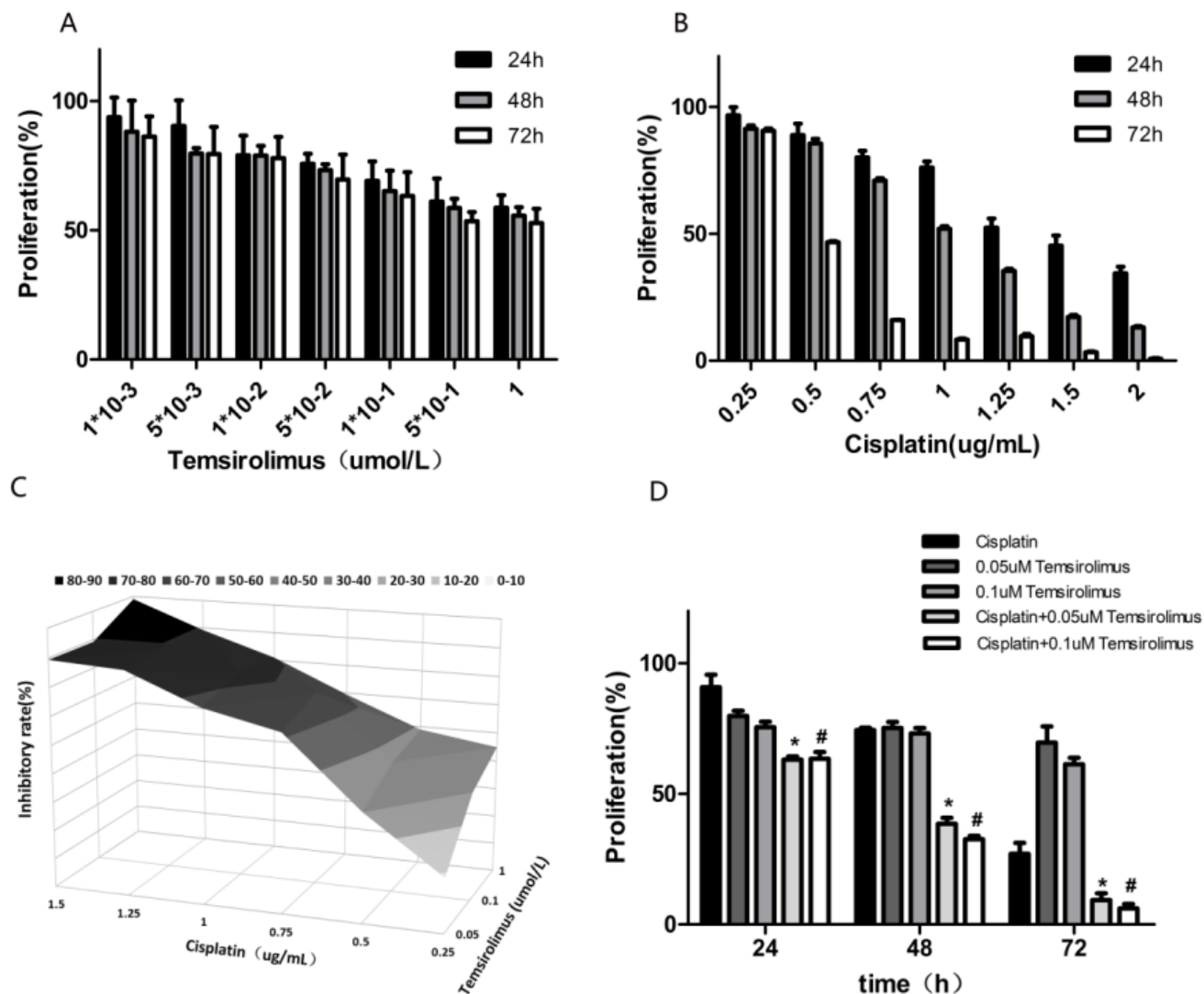
## 2. Results

### 2.1. Combination of temsirolimus with cisplatin resulted in synergistic effects on Human cervical Hela cell line.

We did 48h MTT survival assays to investigate whether the combination of temsirolimus and cisplatin might exert synergistic effects in terms of inhibition in short term. In Hela cells, mainly synergistic anticancer effects ( $CI < 1$ ) were observed independence of the responsiveness of the Hela cells to both single agents. Antagonistic effects ( $CI > 1$ ) were only observed at high temsirolimus dosages, which might be explained by that high concentrations of temsirolimus changed the cell cycle induced by cisplatin.

Temsirolimus at 1.45 $\mu$ M and cisplatin at 1.31 $\mu$ g/ml when used alone induced nearly 67% growth inhibition. The same amount of inhibition was observed with the combination of temsirolimus and

cisplatin at 0.1  $\mu$ M and 0.75  $\mu$ g/ml. Therefore, Temeirolimus reduced the dose of cisplatin by nearly half. The CI for this combination group was 0.64 and Fa was 0.67.



**Fig. 1** The anti-proliferative effect of cisplatin and temsirolimus

## 2.2. The increased inhibition rate of cisplatin combination with temsirolimus was achieved through enhanced apoptosis in Human cervical Hela cell line.

To consider the induction of apoptosis is a significant feature during cancer chemotherapy, we studied whether the addition of temsirolimus could enhance the apoptosis by cisplatin in Hela cells for 48h. Our flow cytometer analysis after Annexin V-FITC/PI double staining showed that the population of Annexin V-positive and PI-negative cells (in early and late apoptotic stage) in two combination treatment groups (20.11 $\pm$ 4.95%, 34.62 $\pm$ 6.20%) were significantly increased compared with those in untreated control (13.7 $\pm$ 1.23%), two different dosage temsirolimus (15.64 $\pm$ 3.22%, 16.25 $\pm$ 4.06%) and cisplatin (18.18 $\pm$ 2.48) single treatment groups ( $p$ <0.05, Fig.2).

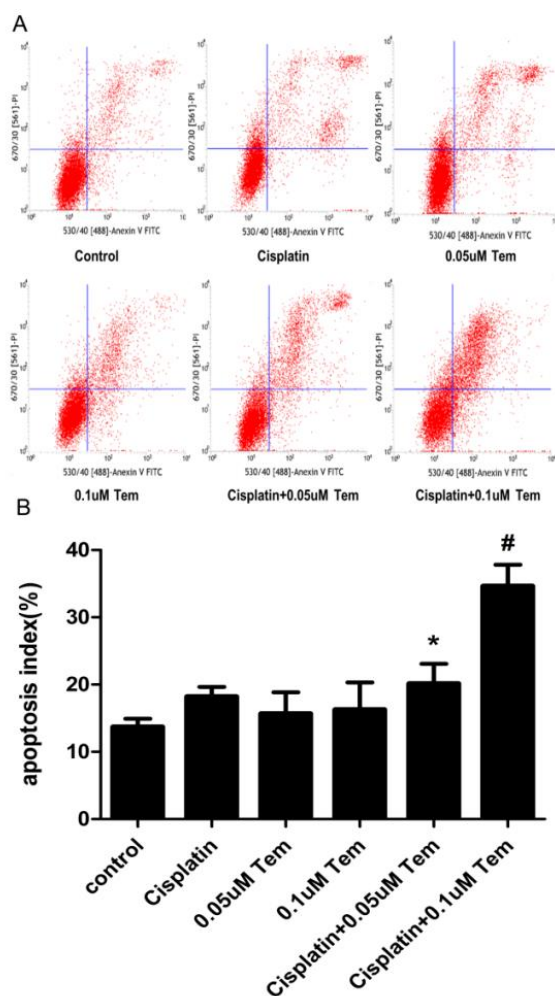


Fig. 2 The apoptosis effect of combination groups

### 3. Discussion

Cisplatin, as a DNA-damaging agent, displays clinical activity against a wide variety of tumor types [4]. However, the application of cisplatin is limited by cytotoxic side effects [5]. Therefore, the mainly concern is its narrow therapeutic window: too low dose has no effect, whereas too high a dose is cytotoxic. Combination treatment is the most effective therapy strategy in anti-cancer.

Early studies suggested that mTOR inhibitor rapamycin and its analogs everolimus, temsirolimus may be potential agents for the combination treatment with cisplatin. Rapamycin combined with cisplatin had significantly effects on a series of tumor types [2, 6-10]. Similarly, recent studies have proved temsirolimus and cisplatin exerted synergistic inhibition of the mTOR downstream signals and increased cell anti-proliferation and apoptosis in mesothelioma cell lines [11].

Thus, we speculated that temsirolimus had the pharmacological advantages as an adjuvant to cisplatin in cervical cancer. Moreover, mTOR pathway may contribute to the chemo-sensitizing potential of cisplatin in cervical cancer. However, as its mechanisms is still not clear, we cannot speculate that temsirolimus could affect the metastasis in cervical cancer, and further researches in vitro animal models are needed.

In conclusion, the combinations of low doses of temsirolimus and cisplatin had dramatically effects on the inhibition of cancer cell proliferation and apoptosis. Moreover, our data showed that the inhibition of the mTOR signaling pathway enhance the sensitivity of human cervical Hela cells to chemotherapy cisplatin. Therefore, this combination therapy may hold great promise for developing as an effective and novel chemotherapeutic approach in cervical cancer, and clinical researches with low doses are warranted to test and verify this hypothesis.

## Acknowledgments

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