The Anti-cancer Agent Weiteichun is a Novel and Selective AMPK Activator in Prostate Cancer Cells That Leads To a Decrease in Their Viability

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Introduction

Cancer is responsible for one in four deaths in the United States [1]. In men, prostate cancer is the most diagnosed form of cancer and the second leading cause of cancer-related deaths [2]. It is estimated in America that 241,740 men will be diagnosed with prostate cancer and 28,170 men will die of it in 2012 [1]. Our lab previously studied Alternol's effect on prostate cancer cells and found its actions were significantly mediated through AMP-activated protein kinase (AMPK) [3]. AMPK, a stress induced kinase, activates energy-producing pathways in times of metabolic need. Under low ATP conditions, AMPK phosphorylates the Raptor subunit of mTOR. This phosphorylation decreases the activity of P70S6K which inhibits mTOR's regulation of cell growth, cell proliferation, and metabolism. In cancer cells, mTOR is constitutively active; therefore the activation of AMPK can greatly affect cancer cells' viability [4]. Weiteichun (WTC), a compound structurally similar to Alternol, has been recently shown to successfully inhibit cancer cell growth. However, the mechanism of its action is unknown [unpublished work from Zhenhua Huang]. We hypothesize that because of its structural similarities to Alternol, WTC's anti-cancer properties are mediated through AMPK.

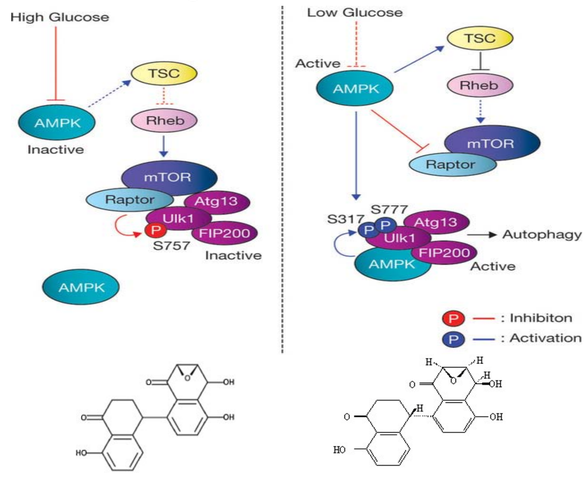

Fig. 1. (top) **AMPK Pathway in High Glucose vs. Low Glucose** [1]. (bottom left) **Structure of Alternol** [3]. (bottom right) **Structure of Weiteichun** [unpublished work from Zhenhua Huang].

Methods

Cell Culture

The C4-2 human prostate cancer cell line was purchased from Urocor, Oklahoma City, OK. The RWPE-1, PC-3, and DU-145 cell lines were purchased from ATCC. C4-2 cells were grown in RPMI medium containing 10% FBS, 100 units/mL penicillin and 100 mg/mL streptomycin. RWPE-1 cells were grown in complete KSFM supplemented with 100 units/mL penicillin and 100 mg/mL streptomycin (Gibco). PC-3 and DU-145 cells were grown in 10% FBS F-12 K medium and MEM. All cell lines were maintained at 37°C in a humidified incubator with a mixture of 95% air and 5% CO2 [3].

MTT Assay

Cell viability was measured by the MTT assay. This assay is based on the reduction of the yellow colored MTT by mitochondrial dehydrogenase of metabolically active cells to a purple–blue formazan. Exponentially growing cells were washed and resuspended in 1% FBS medium to a density of $1X10^5$ cells/L. One hundred microliters aliquots of cells with WTC was seeded in quadruplicate into 96-well flat bottom microculture plates (Costar, Corning) for 24 hr. The vehicle control group received the same amount of DMSO. At the end of the incubation period, 10mL of MTT (5 mg/mL) was added to each well and incubated for another 4 hr, and then 100 mL of SDS (10% w/v, in 0.01 M HCl) was added and mixed thoroughly to dissolve formazan crystals. After shaking the plates for 10 min and continually incubating at 37°C overnight, optical density (OD) was measured at 570 nm with a Microplate Reader (Spectra Max190, Molecular Devices, Sunnyvale, CA). Drug effect was quantified as the percentage of control absorbance of reduced dye at 570 nm. All four cell lines were pre-incubated with the AMPK inhibitor Compound C (1 mM) for 15 min before WTC treatment [3].

Western blots

Ice cold lysis buffer (containing protease inhibitors) was added and cells were scraped. Lysates were then centrifuged at 12,000rpm for 15 minutes at 4°C. The protein concentration was evaluated using the Bradford assay (Bio-Rad, Hercules CA). Equivalent protein (10ug/ul) and prestained molecular weight markers (GIBCO, Gaithersburg MD) were loaded onto each well of 10% SDS polyacrylamide gels in a mini-gel apparatus (Mini-PROTEAN II; Bio-Rad), electrophoresed at 100V for 90-120min, and then transferred to nitrocellulose membranes (0.2um pore size, Bio-Rad). Membranes were incubated for 1hr in a blocking solution (5% skim milk in Tris-buffered saline Tween-20 [TBST]) buffer before being rinsed and incubated with anti-pAMPK, anti-AMPK, anti-mTOR, and anti-P-P70S6K (Cell Signaling Technology, Beverly MA) antibodies overnight at 4°C. The intensities of the bands were measured with a scanning densitometer meter (Model GS-800; Bio-Rad) coupled to Bio-Rad scanning software.

Results

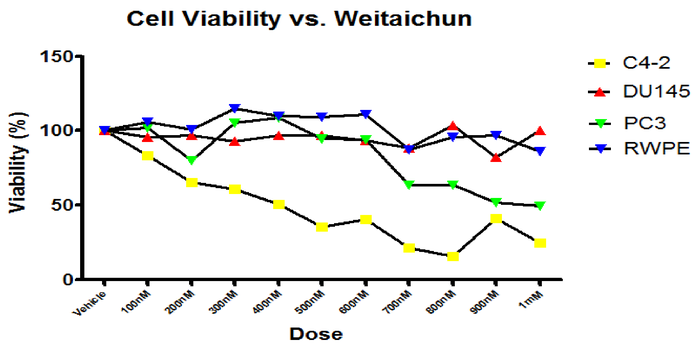

Fig. 2. Effects of WTC on multiple cell lines. C4-2, DU145, PC3 (prostate cancer cell lines), and RWPE (endothelial cell line) were treated with various doses of WTC for 24 hours and cell viability was assessed using the MTT assay. Progressive decreases in C4-2 and PC3 viability were seen with increasing dosage. No change was seen in DU145 and RWPE.

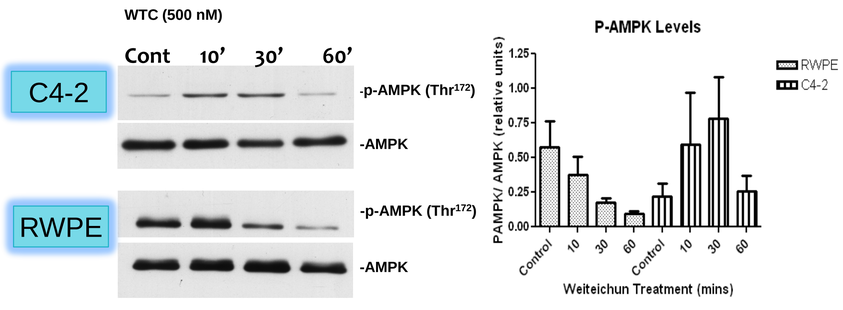

Fig. 3. WTC selectively activated AMPK in C4-2 cells. WTC was given to C4-2 and RWPE cells for 10, 30, and 60 minutes and AMPK activity was measured via Western blot. P-AMPK levels increased in C4-2 cells after 10 minutes, but decreased in RWPE cells after 30 minutes compared to basal.

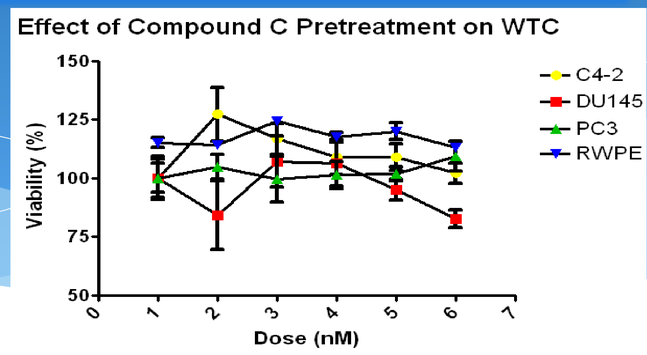

Fig. 4. Compound C pretreatment attenuated the effect of WTC on prostate cancer cells. C4-2, DU145, PC3, and RWPE were pretreated with Compound C , an AMPK inhibitor, 15 minutes before WTC treatment. C4-2 and PC3 prostate viability no longer decreased after WTC.

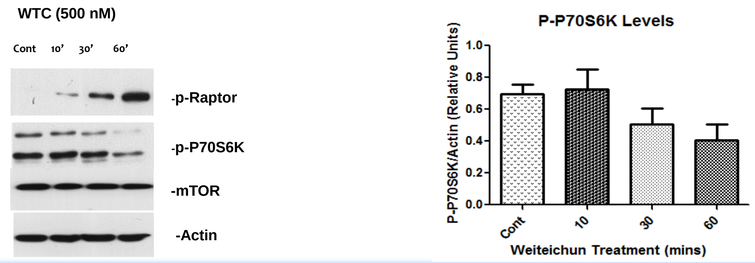

Fig. 5. WTC inhibits mTOR activity in C4-2 cells. WTC treatment increases the phosphorylation of Raptor, which is an inhibitory site on mTOR. Inhibition of mTOR results in the decreased phosphorylation of P70S6K. The phosphorylation of P70S6K is responsible for mTOR's stimulation of cell growth. Our data suggests that WTC is inhibiting cell growth in C4-2 cells through the inhibition of mTOR.

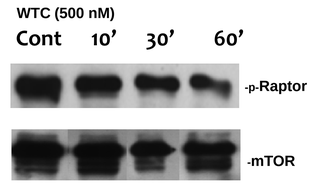

Fig. 6. WTC has an inverse effect on mTOR in RWPE cells. P-Raptor levels were examined in RWPE cells to look at the role of mTOR in WTC's action. P-Raptor levels decreased in the RWPE cells, suggesting mTOR activity was increased. Because the RWPE's viability was unaffected based on our MTT data, this data suggests mTOR inhibition as a main target of WTC's action.

Conclusions

- **WTC decreases the viability of C4-2 cells, a prostate cancer cell line**
- **WTC activates AMPK and inhibits mTOR activity in C4-2 cells, both of which are suggested pathways for cancer treatment**
- **Compound C attenuates WTC effects on C4-2 cells, suggesting a major role of AMPK**

References

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