The Effects of ATM and AMPK Inhibitors on Bladder Cancer Cells Treated by Cisplatin

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Abstract

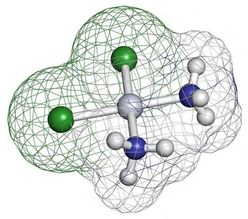
Bladder cancer is one of the most aggressive malignancies and chemotherapy is an effective strategy for its treatment. Cisplatin is widely used to treat bladder cancer, however the usage of cisplatin as an effective cancer treatment has been limited due to toxicity and developed drug resistance in cancer cells. To overcome the problem of resistance to Cisplatin, other drugs that induce chemosensitization in cancer cells are utilized. ATM which is a key factor in genome integrity by activating cell cycle checkpoints is beneficial to normal cells because of its role in the DNA damage response, so its inhibitor KU55933 can inhibit cancer cell proliferation and prevent them from surviving treatments. Due to its dual nature of being a cancer promoter and suppressor, AMPK activation can confer on cancer cells the plasticity to survive under metabolic stress such as hypoxia. However, AMPK inhibitor Compound C can work against the anti-apoptotic action of AMPK. The purpose of this study was to compare the efficacy of Cisplatin when used in combination of pharmacological compounds like ATM-inhibitor KU55933 and AMPK inhibitor Compound C that would act as chemosensitizers to stop the human bladder cancer cell proliferation in vitro. To analyze cellular responses to AMPK inhibitor compound C and ATM inhibitor KU55933 in a panel of 2 human urinary bladder carcinoma cell lines, T24 and UMUC3, a hemocytometer was used to measure the cell proliferation by counting the number of live cell after the treatments. The results show that Cisplatin used in combination with either one of the inhibitors was more potent in reducing the cell viability than just Cisplatin alone or just one of the inhibitors by themselves. Cisplatin + KU55933 or Cisplatin + Compound C has the potential as an anticancer agent for bladder cancer. Further studies must be conducted to analyze the added toxicity and effectiveness of the treatment in vivo.

Introduction

The global cancer burden is very concerning given cancer is the second leading cause of death worldwide. Bladder Cancer is one of the most common cancers, especially in men and has high incidence and fatality rates. In 2023, an estimated 82,290 adults in the United States will be diagnosed with bladder cancer. Effective ways to treat bladder cancer include using treatments in chemotherapy that kill rapidly multiplying cells. One such treatment is Cisplatin, also called cis-diamminedichloroplatinum, that has been proven to have anti-cancer and cytotoxic properties like its abilities in inhibiting cell division, damaging DNA, inhibiting DNA synthesis and mitosis, and inducing apoptotic cell death. Cisplatin acts by reacting with purine DNA bases and can cause DNA damage and prevent cell division. Cisplatin produces cytotoxicity by inducing reactive oxygen species that trigger apoptosis or induce cell death through autophagy and in extreme cases lead to necrosis, which is when cells die due to injury or disease.

AMPK, or AMP-activated protein kinase, is a main regulator of energy metabolism. When ATP is being depleted, AMPK is activated and inhibits biosynthetic pathways that consume a lot of ATP and enhances ATP-generating pathways. However, studies show that activation of this energy sensor can give cancer cells the ability to survive metabolic crisis and make them resistant to apoptosis. AMPK may work against Cisplatin by combating apoptosis in bladder cancer cells, making Cisplatin less effective for cancer treatment. Therefore, other chemosensitizing drugs will be needed to enhance the activity of Cisplatin selectively in the tumor cells. Compound C, a small molecule inhibitor of AMPK inhibits the proliferation of many types of cancers by restricting the main anabolic processes that sustain cancer cell proliferation and growth. It also sensitize cancer cells to other anti-cancer drugs in combined treatment. Studies also indicated that Compound C induced apoptotic or autophagic death in certain types of cancer cells.

Figure A: Molecular Structure of Cisplatin



ATM, or Ataxia-Telangiectasia Mutated Kinase, is responsible for the genome stability as it initiates and coordinates the DNA-damage response. It phosphorylates many protein kinases that play a part in DNA damage response (DDR), or those that arrest the cell cycle so that the cells don't divide with damaged DNA. ATM has ability to modulate oxidative stress responses and maintain mitochondrial homeostasis. However, studies indicated that ATM sustains cancer stem cells survival by promoting the autophagic flux and ATM kinase activity is enhanced in some tumors. ATM inhibitor KU-55933 inhibits cancer cell proliferation by inducing apoptosis by triggering G(1) cell cycle arrest. KU55933, also causes cellular radio and chemosensitization, making it a excellent combination to be used with other chemotherapy drugs.

Finally, kinase inhibitors like KU55933 and Compound C promote chemosensitization, a process in which a drug is used to enhance the activity of another selectively in the tumor cells, while limiting any undesired toxicity or side effects in normal cells. This research aims to test the ability of each of these pharmacological compounds to stop the proliferation of T24 and UMUC3 urinary bladder cancer cells by themselves, and then test the effectiveness of Cisplatin when used in combination with ATM-inhibitor KU55933 and AMPK-inhibitor Compound C.

Methodology

Materials:

1. T24 cell line from human urinary bladder carcinoma
   UMUC3 cell line from human transitional cell carcinoma of the bladder
2. AMPK inhibitor Compound C
3. ATM inhibitor KU55933
4. Cisplatin.
5. Hemocytometer
6. Trypan blue

Methods:

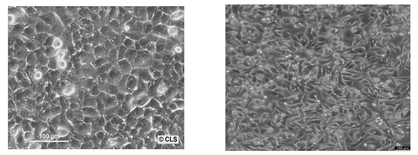
T24 cell line from human urinary bladder carcinoma and UM-UC-3 cell line from human transitional cell carcinoma of the bladder are acquired from tissue biopsies of patients with bladder cancer. These cells are cultured in vitro. Four 12-well plates were used to house all of the T24 and UMUC3 cells, 2 plates for T24 and 2 for UMUC3. Each well is seeded at 0.15 million cells.

T24 Cell line Experiment: In the T24 experiment, two 12-well plates were used with 0.15 million cells per microliter - 3 wells were untreated and this will be used as the control group. 3 wells were treated with 10uM of Cisplatin, 3 wells were treated with 5uM of KU55933, 3 wells had 5uM of Compound C, 3 wells had a combination of 5uM of KU55933 and 10uM of Cisplatin, and the final 3 wells are treated with the combination of 5uM of Compound C and 10uM of Cisplatin.

UMUC3 Cell line Experiment: Similar to the T24 Cell line experiment, for the UMUC3 experiment, two 12-well plates were used with 0.15 million cells per microliter - 3 wells were untreated and this will be used as the control group. 3 wells were treated with 10uM of Cisplatin, 3 wells were treated with 5uM of KU55933, 3 wells had 5uM of Compound C, 3 wells had a combination of 5uM of KU55933 and 10uM of Cisplatin, and the final 3 wells are treated with the combination of 5uM of Compound C and 10uM of Cisplatin.

After the cells were treated with Cisplatin and/or an inhibitor, they were cultured overnight for 24 hours. The cells were then given Trypan blue, a cell stain to assess cell viability which only penetrates the cell membrane if the cell is dead. After waiting 15 minutes, the remaining live cells in each well were counted using a hemocytometer. The cell counts in each well were collected and analyzed.

Figure B1: Bladder Cancer cells lines T24 and UMUC3 used in the experiment



Images from: https://cls.shop

Figure B2: Materials used in the experiment

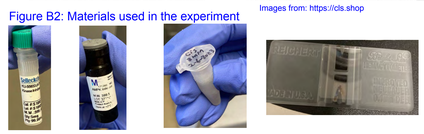
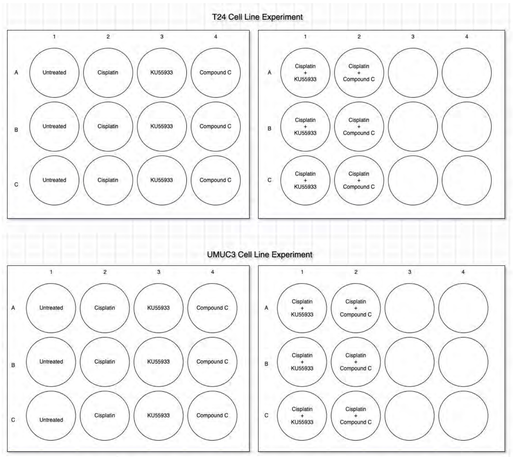


Fig C. Schematic representation of in vitro experiment design



Results

Results indicate that untreated cancer cells continue to proliferate and more than doubled in count from the initial cell count of .15 million/mL. Cisplatin, when used alone, almost halved the amount of cells compared to the untreated cancer cells, however the cancer cells continue to proliferate. This could be an indication that cells likely developed resistance to Cisplatin. Similarly, the inhibitors, KU55933 and Compound C, when used alone showed minimal impact on stopping the cell proliferation. On the other hand, Cisplatin used in combination with either of the inhibitors significantly reduced the viability of T24 and UMUC3 cells. No significant differences were observed when using the ATM inhibitor, KU55933, or the AMPK inhibitor, Compound C, along with Cisplatin. In addition, both cell lines, T24 and UMUC3, respond similarly to Cisplatin, KU55933, and Compound C treatments.

Table A: Cell counts from T24 cell line Experiment

	Cell count (Million/ml)			Average	STD
T24 UN	0.33	0.31	0.34	0.33	0.015
T24 Cis 24Hr 10uM	0.19	0.22	0.18	0.20	0.021
T24 KU55933	0.28	0.27	0.30	0.28	0.015
T24 KU55933 + Cis	0.06	0.13	0.11	0.10	0.036
T24 Comp C	0.27	0.25	0.24	0.25	0.015
T24 Comp C + Cis	0.11	0.10	0.08	0.10	0.015

Table B: Cell counts from UMUC3 cell line Experiment

	Cell count (Million/ml)			Average	STD
UMUC3 UN	0.38	0.36	0.35	0.36	0.015
UMUC3 Cis 24Hr 10uM	0.21	0.18	0.18	0.19	0.017
UMUC3 KU55933	0.25	0.31	0.28	0.28	0.030
UMUC3 KU55933 + Cis	0.08	0.09	0.06	0.08	0.015
UMUC3 Comp C	0.26	0.28	0.24	0.26	0.020
UMUC3 Comp C + Cis	0.10	0.07	0.08	0.08	0.015

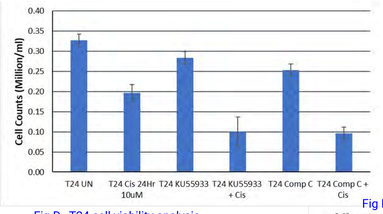
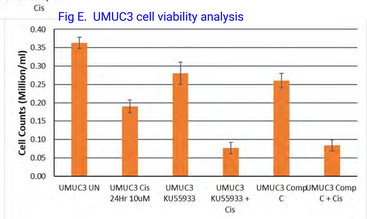


Fig D. T24 cell viability analysis

Fig E. UMUC3 cell viability analysis



Conclusion

In conclusion, both KU55933 and Compound C are radio and chemosensitizing agents, allowing Cisplatin to be more effective at diminishing cancer cell viability. This study proved that the chemotherapy agent Cisplatin is more effective in preventing bladder cancer cell proliferation when it is used in conjunction with an ATM inhibitor KU55933 or an AMPK inhibitor Compound C. Moreover, there was no significant difference between how the two cell lines T24 and UMUC3 reacted to Cisplatin used in combination with an inhibitor KU55933 or Compound C, therefore making this treatment effective for multiple types of bladder cancers.

Further Studies

1. Experiments must be performed with varying experimental conditions used in this study to check if inhibition rate was significantly higher or lower
   - Different concentrations of Compound C, KU55933 other than 5 uM
   - Varying the concentrations of T24 and UMUC3 other than 0.15 million cells/mL
   - Increasing or decreasing cell culture times from 24 hours

2. Since current bladder cancer treatments include gemcitabine in combination with Cisplatin, effectiveness of Compound C and KU55933 can be tested on the combination of Cisplatin and gemcitabine vs Cisplatin alone

3. Other platinum based treatments like Carboplatin can be used in place of Cisplatin to analyze the chemosensitivity induced by Compound C and KU55933

4. Different cell viability counts such as measuring the ATP before and after treatment with inhibitors can be used in place of hemocytometer cell counts

5. Further studies must be conducted to see if the combined therapy adds additional toxicity when compared to Cisplatin toxicity alone

6. Additional experiments must be performed In vivo to analyze if the in vitro behaviour is observed

Acknowledgements

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