Long non-coding RNA regulation of therapeutic response in metastatic cells

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Introduction

- ***Gastric cancer*** is a multifactorial disease, where genetic and environmental factors influence its occurrence and development, presenting a poor overall survival (1, 2).
- ***LncRNA CCAT2*** involves in tumor development and progression.
- ***AZD6738*** is a selective inhibitor of ATR which inhibits the growth of tumor cells by limiting their ability to repair damaged DNA (3).
- ***DNA repair and damage*** -bypass mechanisms protect the DNA by either removing or tolerating the damage to ensure an overall survival - disruption or deregulation caused cancer (4).
- ***Oxaliplatin***, a third-generation platinum, exerts its cytotoxic effects mostly through DNA damage (5).

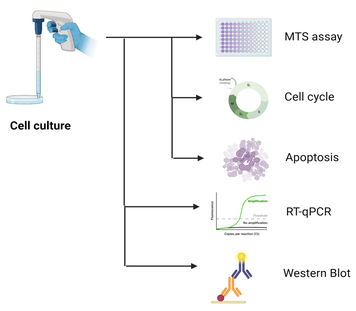
Hypothesis:

1. Cell viability assessment of both therapeutic agents on gastric cancer cell lines.
2. The evaluation of the cellular and molecular alterations occur post-therapy – CCAT2 expression level.
3. The evaluation of DNA damage 48h post-therapy.

Aim: To identify a potential therapeutical approach in gastric cancer based on CCAT2 implication.

Materials and Methods:

- Gastric cancer cell lines: AGS pcDNA, AGS CCAT2 G and AGS CCAT2 T;
- Treatment: AZD6738 and Oxaliplatin;
- Functional assays: MTS assay (to assess cell proliferation post-therapy), apoptosis (the activation of cell death after therapy) and cell cycle (provides information about G1, S, G2, M stages) using flow cytometry;
- The expression level of CCAT2 using RT-qPCR;
- Protein expression (ATR, p-ATR, p-HA2.X) through Western Blot.
- Data analysis: GraphPad Prism 9 software using student's t-test.



Results

1. The evaluation of CCAT2 expression level in gastric cancer cells lines, AGS pcDNA, CCAT2 T and G through RT-qPCR. A significant expression level was observed in AGS CCAT2 T and G cell lines compared to the control group.

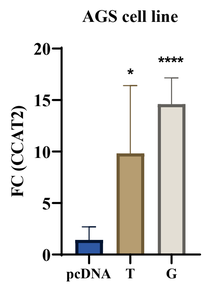


Fig. 1 The expression level of CCAT2 in gastric cancer cell lines (AGS pcDNA, CCAT2 T and AGS CCAT2 G) using RT-qPCR ($p^* < 0.05$, $p^{***} < 0.001$)

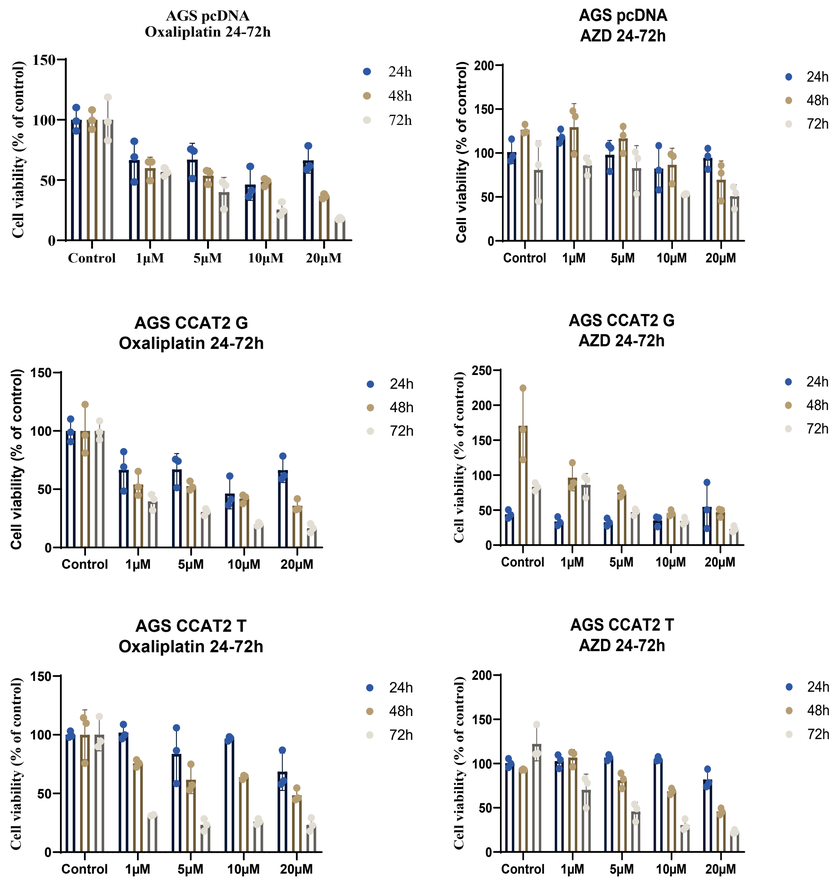


Fig. 2 The evaluation of cell viability on AGS pcDNA, CCAT2 G and T cell lines treated with oxaliplatin and AZD6738 for 24-72h using MTS assay.

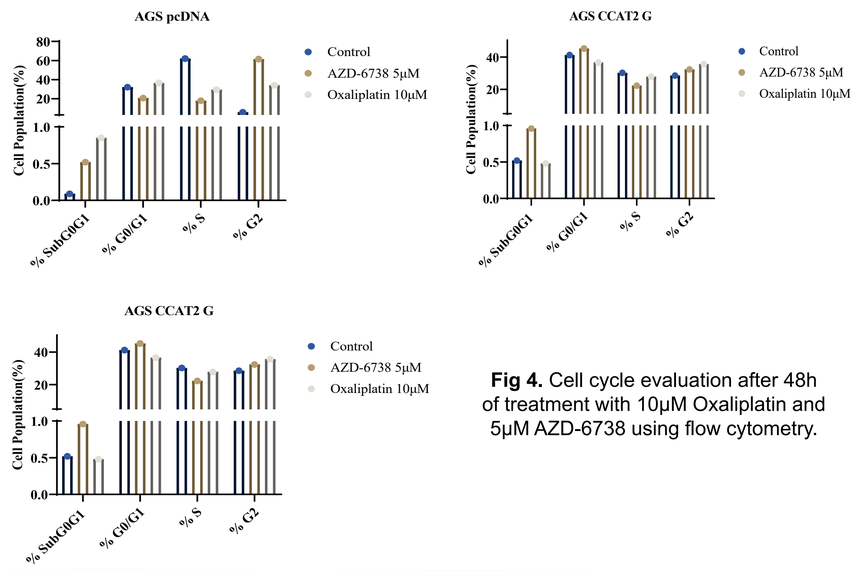


Fig 4. Cell cycle evaluation after 48h of treatment with 10μM Oxaliplatin and 5μM AZD-6738 using flow cytometry.

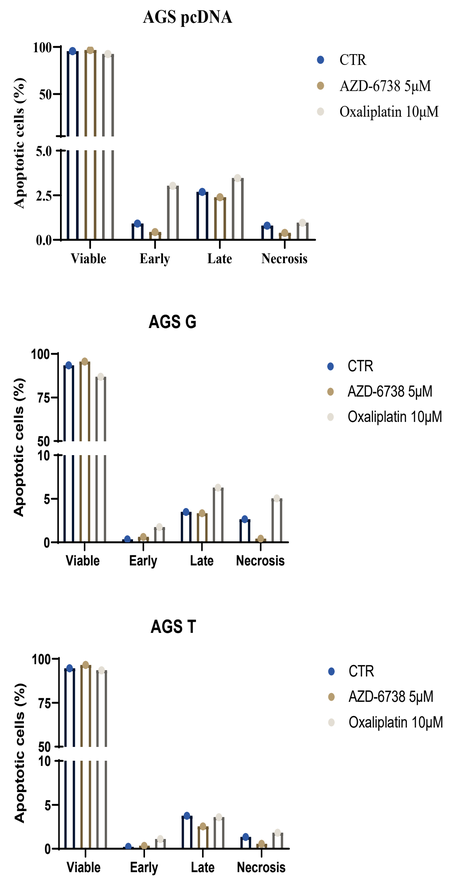


Fig 3. Apoptosis assessment post-therapy with 10μM Oxaliplatin and 5μM AZD-6738 after 48h using flow cytometry.

5. The evaluation of CCAT2 expression level in gastric cancer cells lines after 48h post-therapy with 10μM Oxaliplatin and 5μM AZD-6738. After post-therapy with both agents, the expression level of CCAT2 is significant overexpressed.

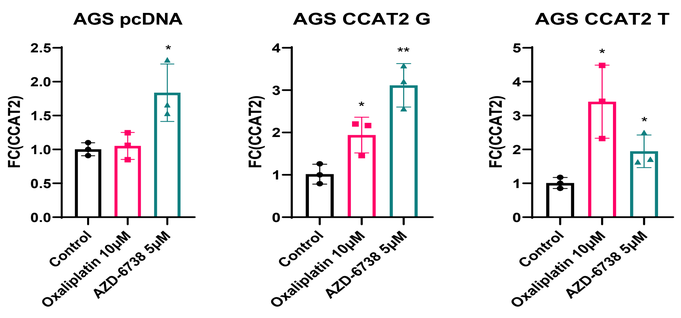


Fig. 5 The expression level of CCAT2 in gastric cancer cell lines (AGS pcDNA, CCAT2 G and CCAT2 T) using RT-qPCR ($p^* < 0.05$, $p^{**} < 0.01$)

6. The evaluation of protein expression using Western Blot. In this section, DNA damage proteins (ATR, p-ATR, p-H2A.X) were investigated 48h post-therapy with 10μM Oxaliplatin and 5μM AZD-6738.

1. pcDNA Control
2. pcDNA Oxaliplatin
3. pcDNA AZD-6738
4. CCAT2 G Control
5. CCAT2 G Oxaliplatin
6. CCAT2 G AZD-6738
7. CCAT2 T Control
8. CCAT2 T Oxaliplatin
9. CCAT2 T AZD-6738

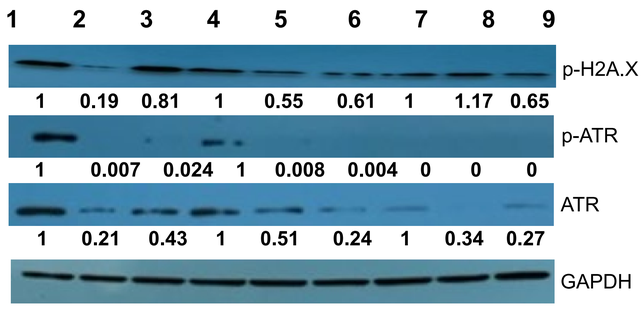


Fig. 6 Protein expression of DNA damage proteins after treatment with 10μM Oxaliplatin and 5μM AZD-6738 using Western Blot

Results:

In our study, we assessed the therapeutic potential of the ATR inhibitor AZD-6738 and the chemotherapy drug Oxaliplatin in gastric cancer cell lines. Cell viability was measured using the MTS assay at 24, 48, and 72 hours with various concentrations of 1uM, 10pM, and 20M. Results indicated that the effects of both agents were dependent on the dose and time. Cell viability analysis using flow cytometry 48 hours post-therapy revealed a decrease in viable cells, correlated with the activation of apoptosis. Also, the percentage of necrotic cells increased after therapy with Oxaliplatin compared to the control group. We also investigated cell cycle phases 48 hours post-therapy using flow cytometry, and while there were slight modifications in the differences between treated and control cells, the combination of Oxaliplatin and AZD-6738 demonstrated time and dose-dependent effects on cell viability and cell cycle distribution. Furthermore, we observed significant CCAT2 expression in AGS CCAT2 T and G cell lines compared to the control group, with treated gastric cancer cell lines showing altered CCAT2 expression levels and variations in protein expression related to DNA damage and repair.

Conclusion:

In conclusion, our findings suggest that the combination therapy targeting DNA repair pathways and cell division holds promise for improving outcomes in gastric cancer patients.

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References

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