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-H2AX) 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 cell 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).

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. 2** The expression of cell viability on AGS pcDNA, CCAT2 G and T cell lines treated with oxaliplatin and AZD6738 after 48h post-therapy using flow cytometry.

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

**Fig. 3** Apoptosis assessment post-therapy with 10µM Oxaliplatin and 5µM AZD-6738 for 24-72h using MTS assay.

4. The evaluation of protein expression using Western Blot 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).

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

6. The evaluation of protein expression using Western Blot. In this section, DNA damage proteins (ATR, p-ATR, p-H2AX) were investigated 48h post-therapy with 10µM Oxaliplatin and 5µM AZD-6738.

**Fig. 7** Protein expression of DNA damage proteins after treatment with 10µM Oxaliplatin and 5µM AZD-6738.

**References**