TARGETING APOPTOSIS IN BREAST CANCER TO AVOID RESISTANCE TO CHEMOTHERAPY

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Abstract

Despite advances in cancer research, breast cancer remains one of the leading causes of death among women. In particular, triple negative breast cancer (TNBC) is the most aggressive subtype with the worst prognosis, as it lacks specific treatments. In many cases, it is resistant to the chemotherapy drugs used for its treatment. For this reason, many research groups are focused on finding alternative therapies for this type of tumor. In this line, the main objective of this work has been to evaluate drugs that can reverse acquired resistance to chemotherapy drugs. To this end, we have generated a cisplatin-resistant model from a TNBC cell line, MDA-MB-231, obtaining the MDA-MB-231R line. This line showed an alteration in the expression of proteins of the BCL-2 family proteins, involved in apoptosis processes, so we decided to evaluate the action of the inhibitor, Obatoclax, on this resistant model. Our results show that Obatoclax can inhibit cell proliferation and the capacity of invasion and migration, while increasing apoptosis in the resistant model, showing no statistically significant differences with the parental lines. BET protein inhibitors (BETi) have shown an effect on the population of cancer stem cells in TNBC, which are largely responsible for these tumors. Thus, after evaluating the effect of Obatoclax individually, its combination with BETi, JQ1, was assessed. The combination of both drugs showed a potent synergistic effect on the TNBC cell line with acquired resistance to chemotherapy, so it could be an alternative to reverse the intrinsic resistance. For this reason, we also decided to evaluate the action of a new generation compound that acts on BET proteins, PROTAC ARV-825. This compound is capable of inhibiting cell proliferation at low doses and shows an effect on proteins of the BCL-2 family. In addition, it was shown to have a greater effect on resistant TNBC cells. In summary, the use of a combined BCL-2 and BET protein inhibition strategy could be a good therapeutic alternative to treat tumors with acquired resistance in TNBC.

Introduction

Triple negative breast cancer is one of the leading causes of death among women, as it is one of the most aggressive and with the worst prognosis. TNBC lacks specific treatments and resistance to chemotherapy is common. The treatments are usually focused on inhibiting proteins or inhibiting apoptosis, as these are some of the main ways that allow the development of the tumor. Currently, new compounds have emerged, PROTACs, and are demonstrating great effectiveness, since they are capable of degrading the main target proteins that have been identified.

Results

TNBC cells with acquired resistance to cisplatin have altered expression levels of BCL-2 family proteins

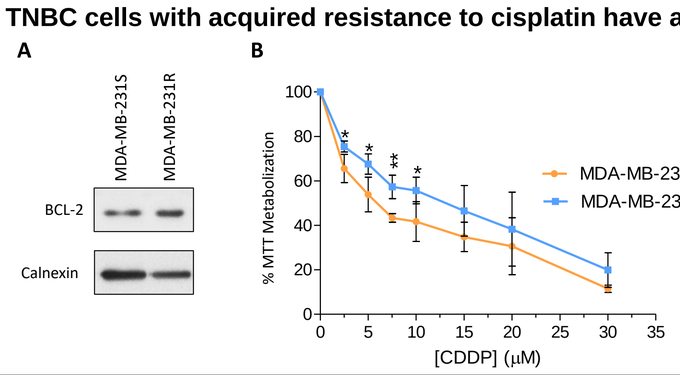

Figure 1. Characterization of the cisplatin-resistant model. (A) Analysis of BCL-2 expression in MDA-MB-231S and MDA-MB-231R by Western Blot, using calnexin as load control **(B)** Dose-response curves at different concentrations of cisplatin (µM) in MDA-MB-231S and MDA-MB-231R after 72 hours of treatment. **(C)** Analysis of the expression of MCL-1 and BCL-xL in MDA-MB-231S and MDA-MB-231R by Western Blot, using calnexin as a charge control. Representation of the mean ± standard deviation (n=3). Student's test: *p<0.05.

The Obatoclax inhibitor decreases the tumor initiation, invasion and migration capabilities of cisplatin-resistant TNBC cells.

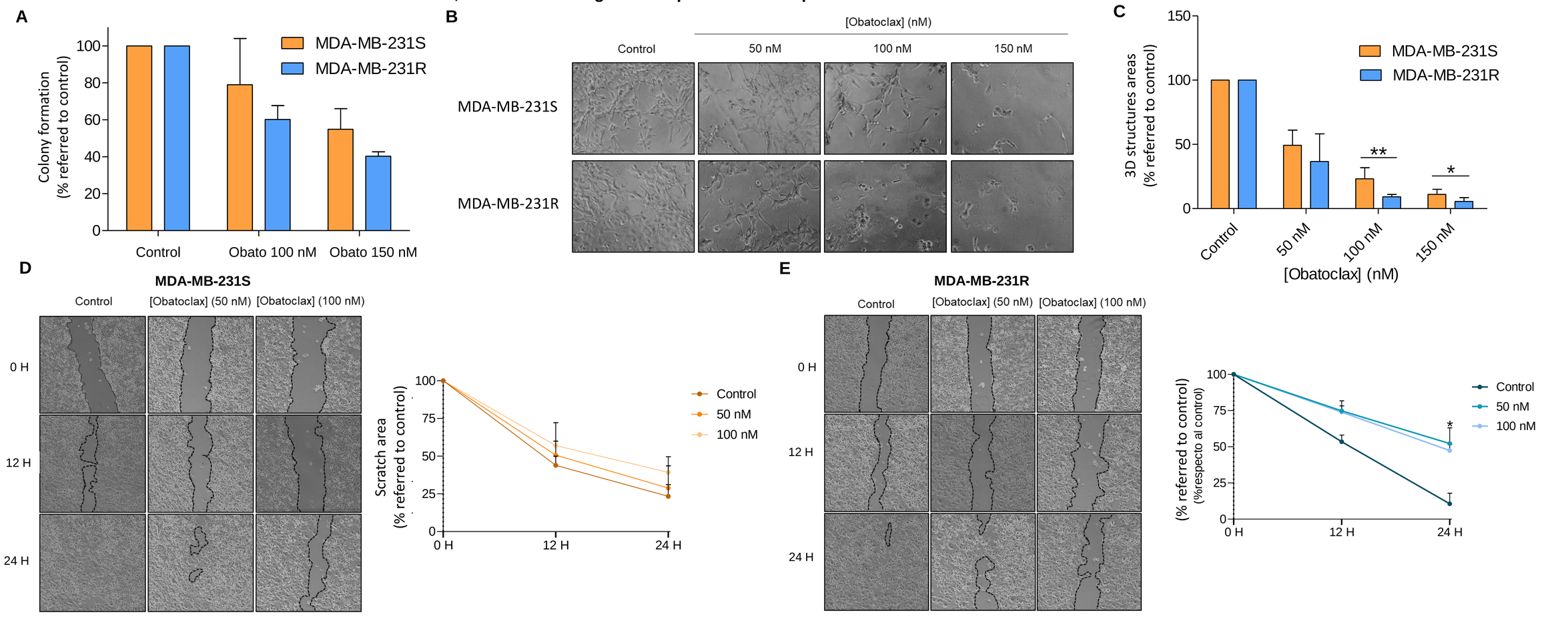

Figure 2. Evaluation of colony formation, invasion and migration in MDA-MB-231S and MDA-MB-231R. (A) Representation of the quantification of colonies stained with violet glass for each condition and cell line 10 days post-treatment Representation of the mean ± standard deviation (n=2). **(B)** Representative images of the invasion test in matrigel after 72 hours of treatment with 50, 100 and 150 nM Obatoclax. **(C)** Graphic representation of the quantification of the area of the 3D spheres formed in matrix after 72 hours of treatment with 50, 100 and 150 nM Obatoclax. Representation of the mean ± standard deviation (n=4). Student's test: *p<0.05, **p<0.01. **(D)** Representative images of the migration test and quantification of the scratch area at the beginning and after 12 and 24 hours of treatment with 50 and 100 nM Obatoclax in MDA-MB-231S. **(E)** Representative images of migration test and quantification of scratch area at baseline and after 12 and 24 hour treatment with 50 and 100 nM Obatoclax on MDA-MB-231R. Representation of the mean ± standard deviation (n=4). Student's test: *p<0.05.

Exposure to Obatoclax increases apoptosis in TNBC cells.

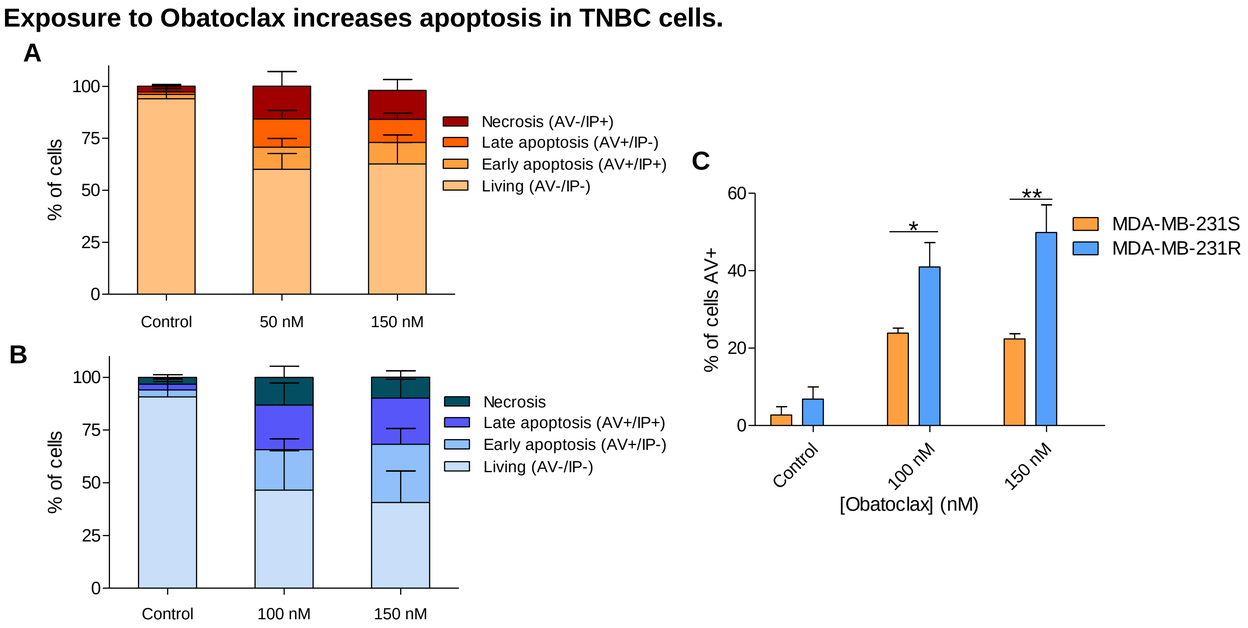

Figure 3. Evaluation of cell death in MDA-MB-231S and MDA-MB-231R after exposure to 100 nM and 150 nM for 72 hours. (A) Graphic representation of apoptotic, necrotic and live cells in MDA-MB-231S lines after 72 hours of treatment with 100 and 150 nM Obatoclax. **(B)** Graphical representation of apoptotic, necrotic and live cells in the MDA-MB-231S lines after 72 hours of treatment with 100 and 150 nM Obatoclax. **(C)** Graphical representation of apoptotic cells in MDA-MB-231S and MDA-MB-231R after 72 hours of treatment Representation of the mean ± standard deviation (n=4) Student's test: *p<0.05, **p<0.01.

The combination of Obatoclax with JQ1 exerts a synergistic effect on TNBC lines resistant to chemotherapy.

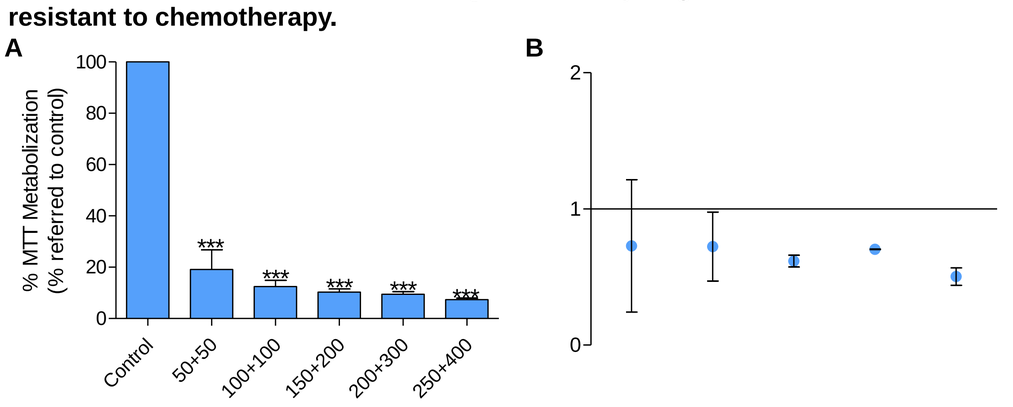

Figure 4. Evaluation of the cellular viability of MDA-MB-231R after combined treatment with different concentrations of JQ1 and Obatoclax for 72 hours. (A) Graphical representation of the dose-response curve at different concentrations of JQ1 and Obatoclax (nM) in MDA-MB-231R after 72 hours of treatment. **(B)** Graphical representation of the CIs obtained using the *Calcusyn* program for MDA-MB-231R after 72 hours of treatment with different concentrations of JQ1 and Obatoclax. Representation of the mean ± standard deviation (n=3). Student's test: ***p<0.001.

ARV-825 treatment has a more potent effect on TNBC cells than JQ1 and Obatoclax combination therapy.

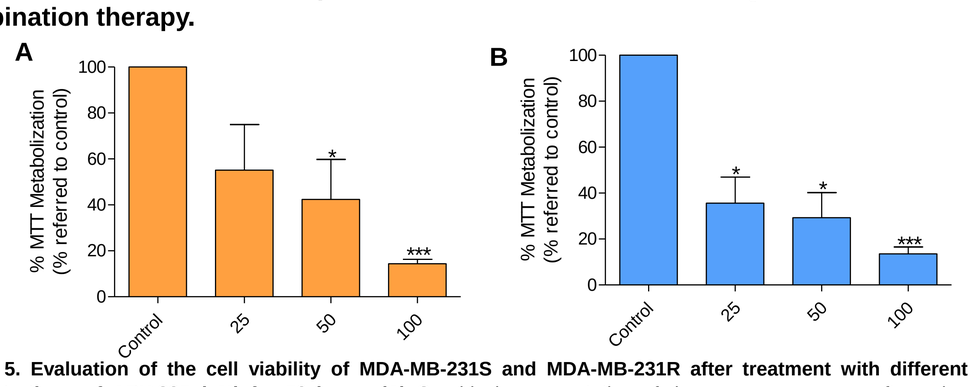

Figure 5. Evaluation of the cell viability of MDA-MB-231S and MDA-MB-231R after treatment with different concentrations of ARV-825 (nM) for 72 hours (A) Graphical representation of dose-response curves after using different concentrations of ARV-825 (nM) in MDA-MB-231S. **(B)** Graphical display of dose-response curves after using different concentrations of ARV-825 (nM) in MDA-MB-231R. Representation of the mean ± standard deviation (n=3). Student's test: *p<0.05, ***p<0.001.

Conclusion

The use of Obatoclax as single agent or combined with JQ1, as well as AresistanceRV-825, could be a good therapeutic alternative in the treatment of CMTN with acquired to cisplatin.