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جامعة الملك سعود
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Senolytics: Novel Therapeutic Agents To Target Chemotherapy-Induced Senescence in Breast Cancer Cell Lines

Abdulrahman Alanazi, Faisal Alotaibi, Ali Alhoshani, Khalid Alhazzani, Homood M. As Sobeai, and Moureq Alotaibi

INTRODUCTION

- Breast cancer is the most diagnosed form of malignancies among women worldwide.[1]
- Saudi Arabia is no exception; breast cancer is the top cancer diagnosed in Saudi Women. [2,3]
- Resistance to chemotherapeutic agents is a central problem in cancer treatment.[4]
- Tumors develop resistance to chemotherapeutic agents by DNA damaging throughout course of treatment lead to developing growth arrest (senescence).[5]
- Eradicating senescent cells or pushing them toward irreversible cell death states such as apoptosis by emerging Senolytics would be a novel strategy to enhance therapeutic outcomes.[6]

OBJECTIVES

- The overall aim of the research project is to evaluate the ability of senolytics (venetoclax and navitoclax) in abolishing breast cancer cells induced into senescence by chemotherapeutic agents.
- To fulfill this aim, the following objectives have been executed:
  1. To determine the capacity of venetoclax and navitoclax to eliminate doxorubicin-induced senescent cells in MCF7, 4T1, and E0771.
  2. To investigate the molecular mechanisms by which venetoclax and navitoclax selectively target doxorubicin-induced senescent cells.

METHODS

- Breast cancer cell lines (4T1, MCF-7, and E0771) were treated with doxorubicin to induce senescence in-vitro, and then exposed to BCL-2 inhibitors (venetoclax and navitoclax) as senolytics.
- Senescence was confirmed via SA-β- galactosidase and C12-FDG staining.[7]
- The percentage of apoptotic cells was determined by Annexin V/7AAD assay.[8]
- expression of senescence-related genes (*TP53* and *CDKN1A* [P21]) was investigated using qRT-PCR.[9]

RESULTS

- The peak of senescent cell subpopulation was overserved on day 3 after doxorubicin treatment in the three cell lines. (**Figure 1**)
- 4T1 had the highest senescent cell subpopulation relative to the other cell lines, MCF7 and E0771 after doxorubicin treatment (**Figure 1B**).

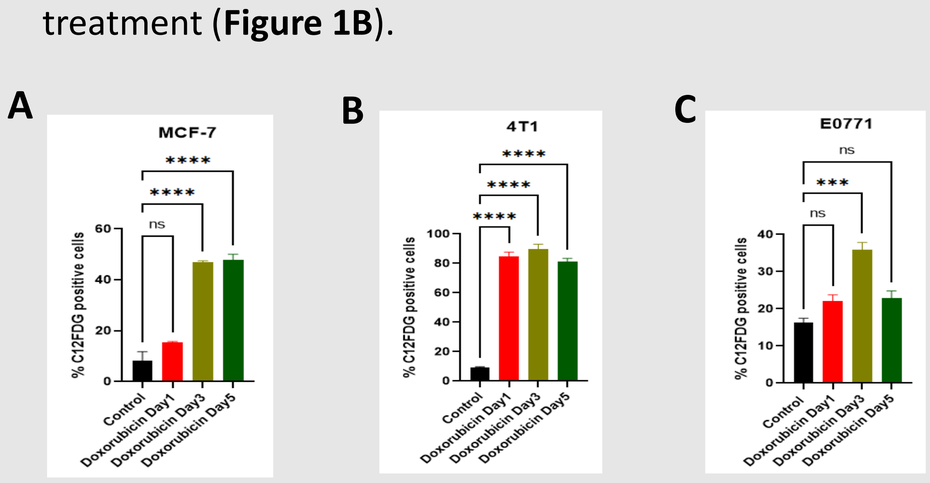

Figure 1. senescence induction by doxorubicin in breast cancer cell lines. A) MCF7, B) 4T1, and C) E0771

RESULTS (CONT.)

- No additive apoptotic effect of both, venetoclax and navitoclax, were seen when they combined with doxorubicin compared with doxorubicin alone in MCF7 **(Figure 2A)**.
- Navitoclax significantly induced apoptosis when combined with doxorubicin relative to doxorubicin alone in 4T1 (Figure 2B).
- Venetoclax and navitoclax significantly increased apoptotic cell subpopulations when they combined with doxorubicin relative to doxorubicin alone in E0771 **(Figure 2C)**.

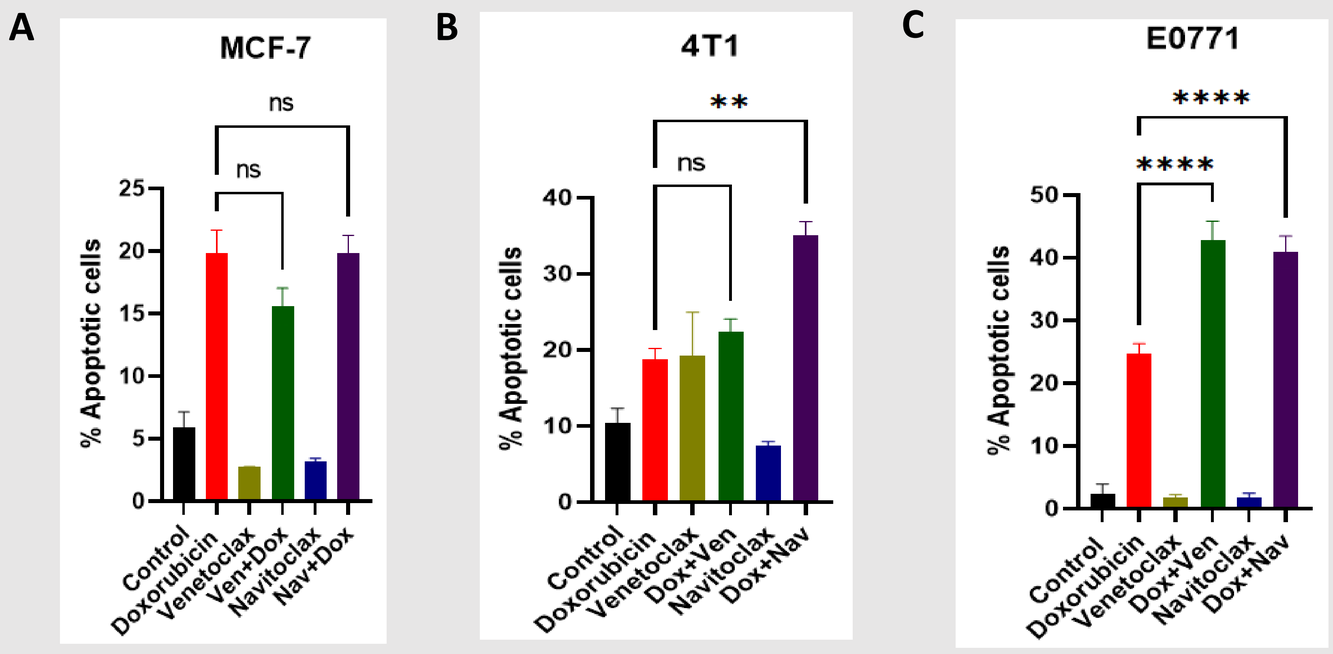

Figure 2. The apoptotic effect of senolytics (venetoclax and navitoclax) on doxorubicin-induced senescent breast cancer cells. A) MCF7, B) 4T1, and C) E0771

- *TP53* was downregulated in the experimental groups treated with the combination senolytics and doxorubicin compared with doxorubicin-treated groups in MCF7 and 4T1. However, such downregulation did not reach the statistical significance. both cell lines when compared with doxorubicin alone (**Figure 3A and 3B)**.
- The combination venetoclax and doxorubicin reduced *CDKN1A* (P21) gene expression relative to doxorubicin alone in 4T1 (**Figure 3D)**.
- Navitoclax inhibited *CDKN1A* (P21) gene expression when combined with doxorubicin relative to doxorubicin alone in MCF7 and 4T1 (**Figure 3C and 3D)**. Statistically significant reduction in the gene expression was seen in 4T1.

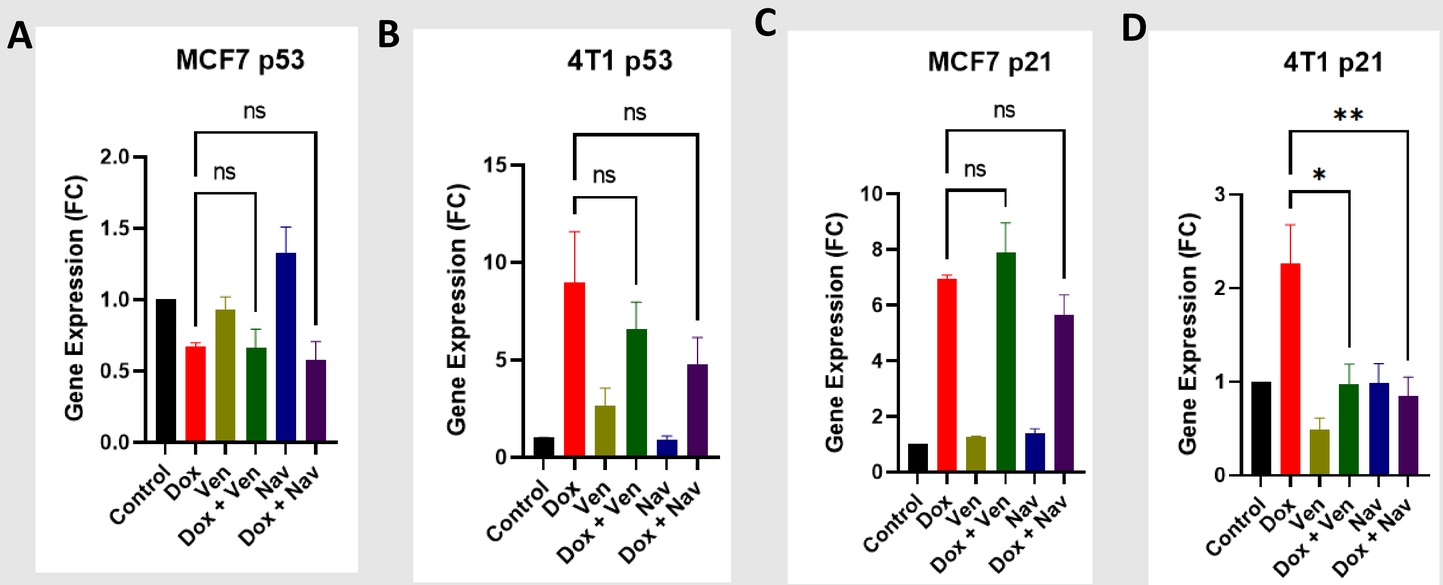

Figure 2. The impact of senolytics (venetoclax and navitoclax) when combined with doxorubicin on *TP53* and *CDKN1A* [P21] gene expression. A) *TP53* gene expression in MCF7, B) *TP53* gene expression in 4T1, C) *CDKN1A* [P21] gene expression in MCF7, and D) *CDKN1A* [P21] gene expression in 4T1

CONCLOSIONS

- These results suggest that use of senolytics following chemotherapy may enhance tumor cells killing by targeting chemotherapy-induced senescent cell subpopulation, which ultimately may improve therapeutic outcomes and delay disease recurrence.
- 4T1 and E0771 are appropriate cell line models to investigate senolytic effects on doxorubicin-induced senescent breast cancer cells.
- Navitoclax has stronger senolytic properties relative to venetoclax against doxorubicin-induced senescent breast cancer cells.

REFERENCES

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