The novel Saquinavir-derivative AD-12 activates the ER stress and triggers *parthanatos*, a caspase-independent programmed death, in colorectal cancer cells

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INTRODUCTION

The continuous evolution and adaptation of tumours to conventional treatments drive the urgent search for novel anti-cancer agents. In this scenario, drug repurposing is revolutionizing oncology by reducing the high costs and long timelines of *de novo* development [1]. A promising strategy involves targeting protein homeostasis: the high metabolic demand of malignant cells induces proteotoxic stress, which sensitizes them to proteasome inhibitors. In this context, several HIV protease inhibitors (PIs), originally approved for AIDS, are currently under investigation for their pleotropic anti-tumor activity [2]. Colorectal cancer (CRC) represents an ideal target for this approach, as it remains a leading cause of mortality often characterized by late-stage diagnosis and resistance to standard therapies [3]. Within this framework, we present the anti-proliferative activity of AD-12, a Saquinavir derivative, in 2D CRC models. While 2D monolayers cannot fully replicate in vivo architecture, investigating AD-12 in these models is a fundamental prerequisite to establish its cytotoxicity and mechanism of action [4]. Our results demonstrate that AD-12 induces G1 phase cell cycle arrest and parthanatos, a non-conventional form of programmed cell death, providing a solid baseline for subsequent 3D implementation.

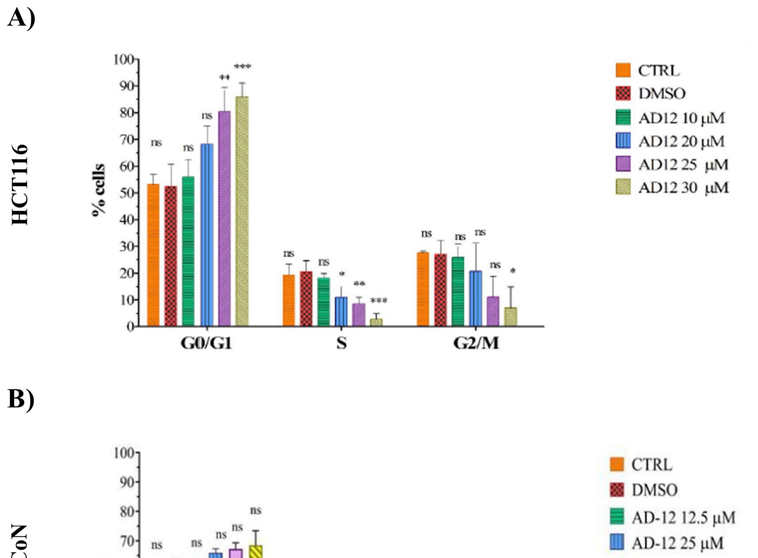
METHODS

- ***Cell culture and treatments***

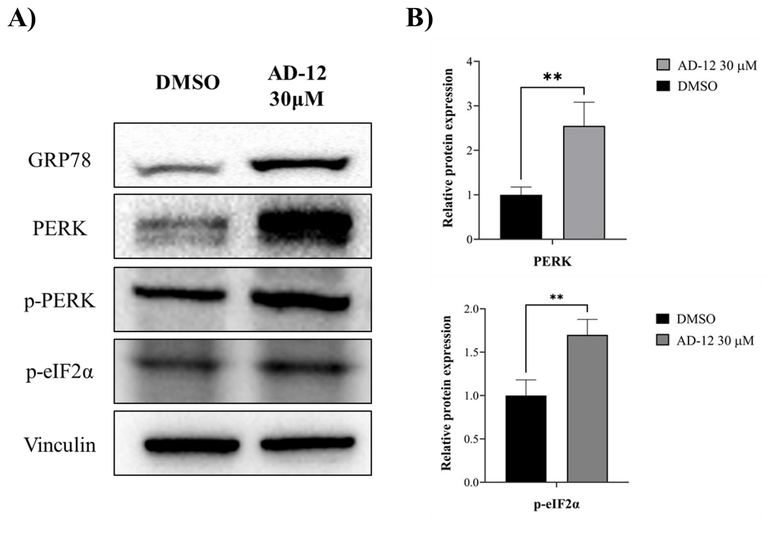
The human colon cancer cell line HCT116 was cultured in McCoy's Medium supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamine 200 mM, 0,1% penicillin/streptomycin 10000 U/mL. CCD-841 CoN are human colonocytes cultured in Dulbecco's Modified Eagle Medium (DMEM-Gibco®) low glucose (1 g/L) supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamine 200 mM, 0.1% penicillin/streptomycin 10000 U/mL. All the compounds were solubilized in DMSO as 50 mM stock solutions and the working solutions were freshly prepared by appropriate dilution in cell culture medium.

- ***Cell Cycle Analysis by FACS***
- ***Western Blotting***
- ***DNA Fragmentation Analysis by Agarose Gel Electrophoresis***
- ***Immunofluorescence Analysis by Confocal Microscopy***

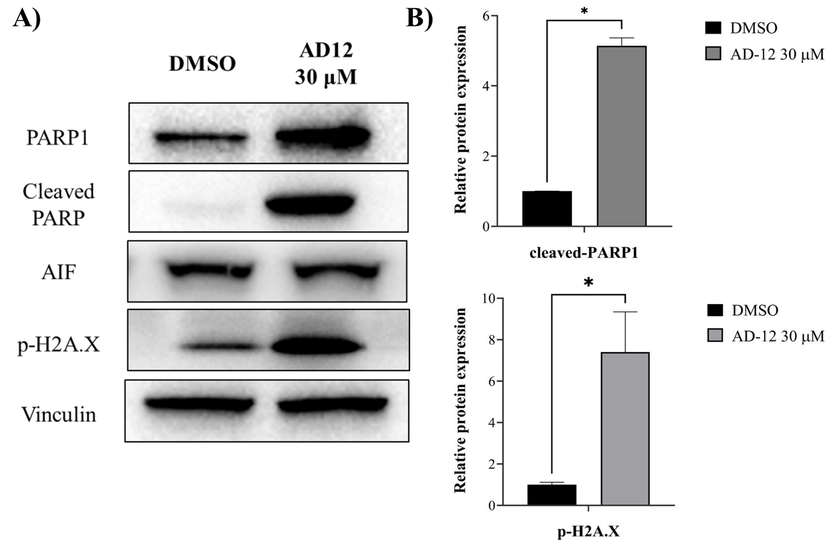
RESULTS



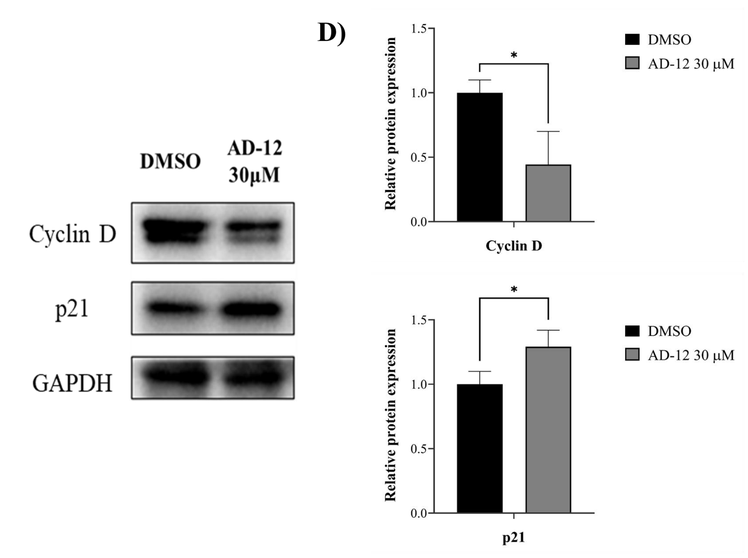
AD-12 selectively arrests the growth of HCT116 in the G1 phase. Cell cycle analysis by FACS of HCT116 (A) and CCD-841 CoN (B). (C) Representative western blotting and (D) densitometric analysis. GAPDH was used as the loading control. Significance calculated by one-way ANOVA, *** p < 0,05, ** p < 0.01, *** p < 0.001.**



AD-12 induces ER stress in HCT116. (A) Representative western blotting and (B) densitometric analysis. Vinculin was used as the loading control. Significance calculated by one-way ANOVA, **** p < 0.01.**



AD-12 triggers parthanatos and DNA damage in HCT116. (A) Representative western blotting and (B) densitometric analysis. Vinculin was used as the loading control. Significance calculated by one-way ANOVA, **** p < 0.01.**



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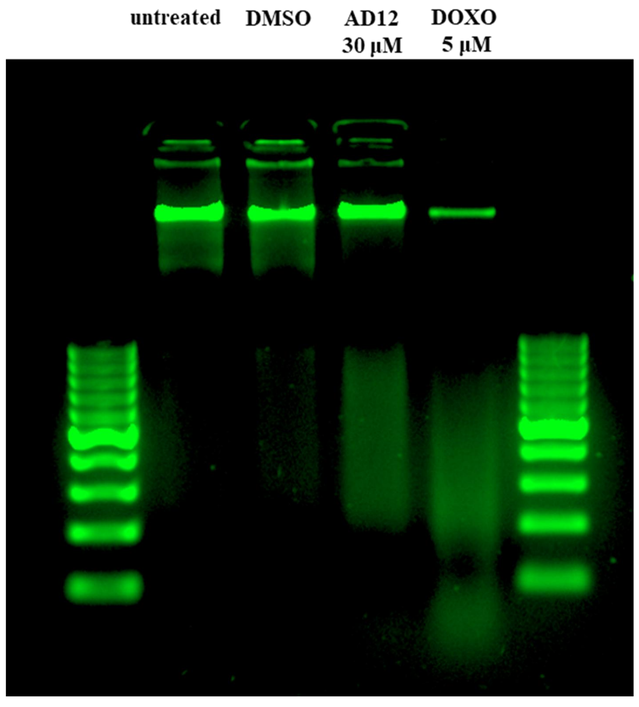
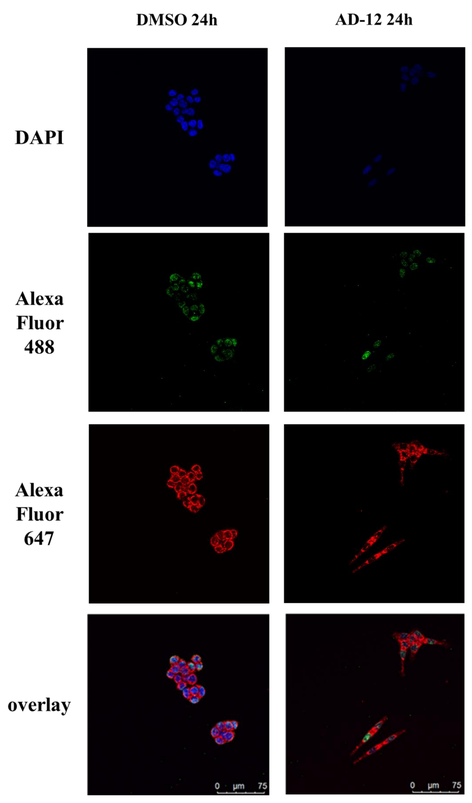


AD-12 promotes a hallmark of parthanatos in HCT116: large-scale DNA fragmentation. The DNA fragmentation was assessed by 2% agarose gel electrophoresis. Cells treated with doxorubicin hydrochloride were used as a positive control for apoptosis.



AD-12 induces AIF translocation in HCT116. AIF intracellular localization was assessed by immunofluorescence and confocal microscopy imaging. DAPI (blue) stains nuclei; p-H2A.X (green, Alexa Fluor-488) marks DNA damage; AIF (red, Alexa Fluor-647).

CONCLUSIONS

The results show that AD-12, at sub-IC50 concentrations, selectively induces G-1 phase arrest in HCT116, while sparing healthy CCD-841 CoN epithelial cells, via ER stress activation. This is likely caused by chaperone inhibition and protein misfolding, while being independent of proteasome inhibition and ROS production. At its IC50, AD-12 induces parthanatos, a non-conventional form of programmed cell death. These findings establish the essential mechanistic baseline for subsequent 3D implementation. Future studies will evaluate AD-12 in combination strategies to overcome chemoresistance in apoptosis-resistant tumors within more physiological environments.

REFERENCES

[1] Y. Xia, M. Sun, H. Huang, and W. L. Jin, *Signal Transduct. Target Ther.* **9**, 92 **(2024) ;** [2] D. Maksimovic-Ivanic et al., *Int. J. Cancer* **140**, 1713 **(2017);** [3] M. S. Hossain et al., *Cancers* **14**, 1732 **(2022);** [4] Z. Peng, X. Lv, H. Sun, L. Zhao, and S. Huang, *Mol. Cancer* **24**, 93 **(2025)**