A Combination of Vitamin C with DNA methylation inhibitor Decitabine Preserves the Colon Immunogenicity and Overcomes the Chemo-resistance

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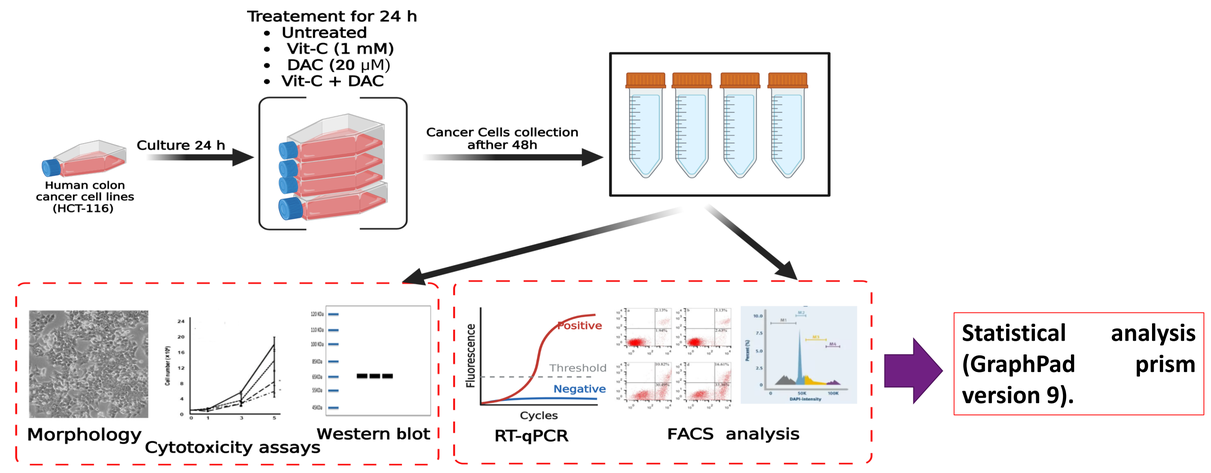
Introduction:

Decitabine (DAC) is an anti-cancer hypomethylating drug used to activate silenced genes by promoter demethylation. In a previous study, we have shown in lung cancer cells that DAC induced the expression of the *New York esophageal squamous cell carcinoma* (**NY-ESO-1**), a highly immunogenic cancer testis antigen known to induce both humoral and cellular immune responses (1). This effect would consequently enhance tumor immunogenicity and stimulate anti-tumor immune cells response. However, it has also been shown that DAC would increase Programmed Death Ligand-1 (**PD-L1**) expression in tumor cells leading to resistance to cancer therapy. L-ascorbic acid or Vitamin C (Vit-C), has emerged as a novel epigenetic regulator of the DNA demethylation and histone modifications (2). It has been shown that Vit-C is capable of downregulating transcription factors involved in the regulation of PD-L1 expression. This would result in a reinvigoration of immune cells activity.

Project Goals:

Our major aim is to investigate whether vit-C could improve the effect of DAC by reducing PD-L1 and enhance the NY-ESO-1 expression. Therefore, in this study, we aim to determine the effect of a combination of Vit-C and DAC in colorectal cancer (CRC) cells.

Project description



Findings

Cytotoxicity assays :

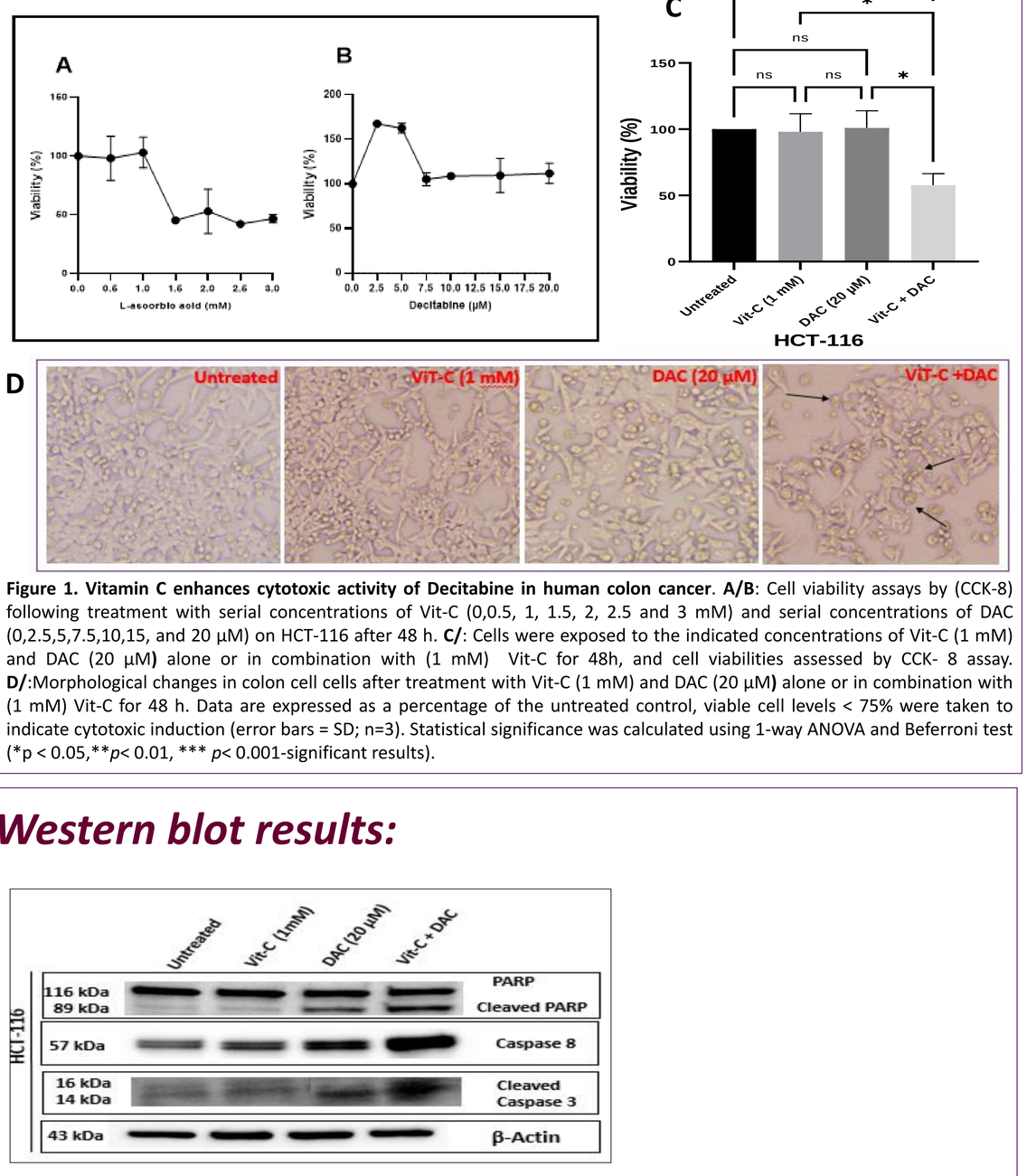


Figure 1. Vitamin C enhances cytotoxic activity of Decitabine in human colon cancer. **A/B**: Cell viability assays by (CCK-8) following treatment with serial concentrations of Vit-C (0,0.5, 1, 1.5, 2, 2.5 and 3 mM) and serial concentrations of DAC (0,2.5,5,7.5,10,15, and 20 µM) on HCT-116 after 48 h. **C/**: Cells were exposed to the indicated concentrations of Vit-C (1 mM) and DAC (20 µM**)** alone or in combination with (1 mM) Vit-C for 48h, and cell viabilities assessed by CCK- 8 assay. **D/**:Morphological changes in colon cell cells after treatment with Vit-C (1 mM) and DAC (20 µM**)** alone or in combination with (1 mM) Vit-C for 48 h. Data are expressed as a percentage of the untreated control, viable cell levels < 75% were taken to indicate cytotoxic induction (error bars = SD; n=3). Statistical significance was calculated using 1-way ANOVA and Beferroni test (*p < 0.05,**p< 0.01, *** p< 0.001-significant results).

FACS Results:

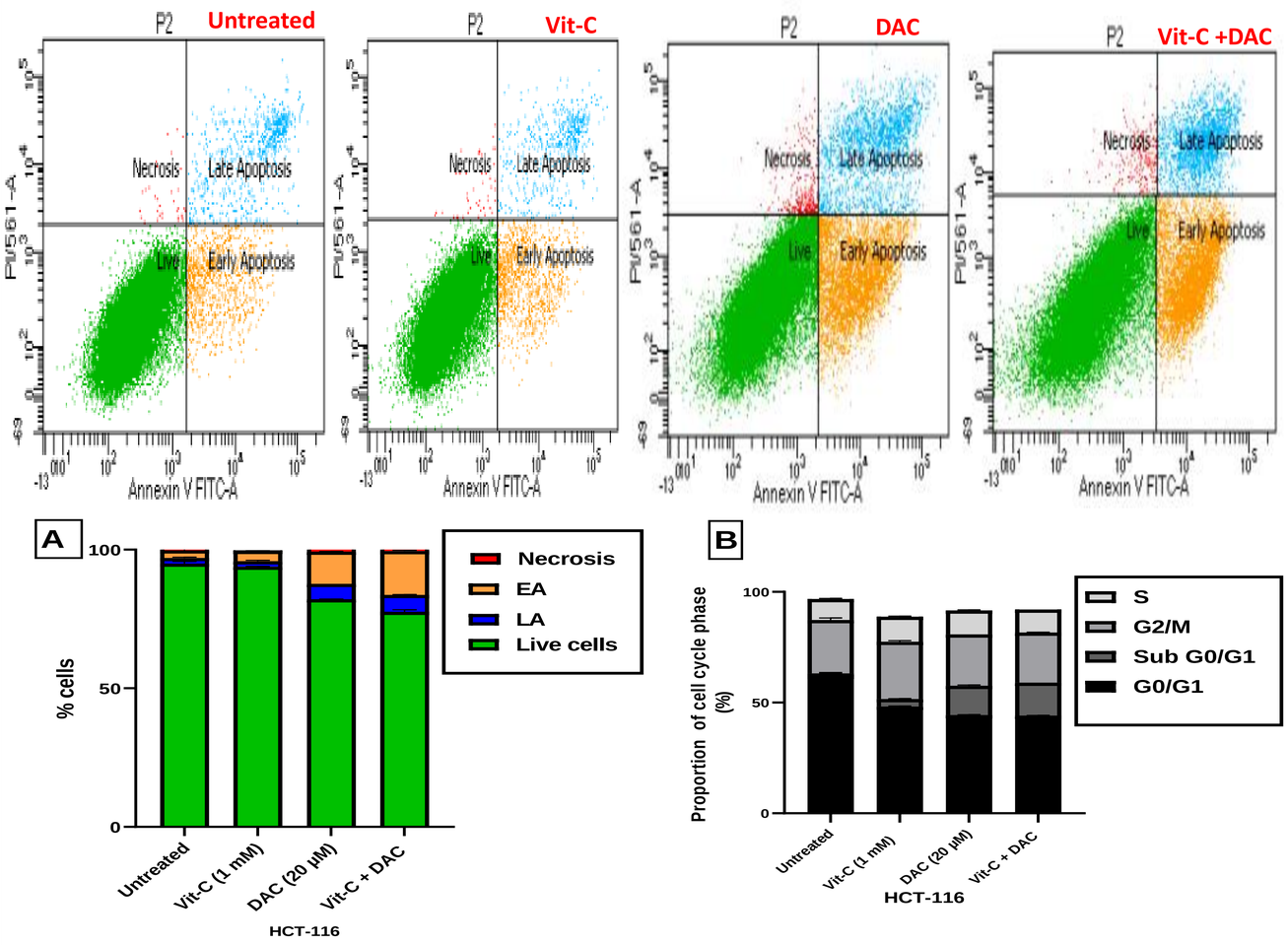


Figure 3. Combination of Vitamin C and Decitabine induced apoptosis after 48 h treatment. Annexin-V positive cells were detected by flow cytometry. **A/**Apoptosis analysis showing an increased percentage of apoptotic cells after combination treatment relative to either DAC or Vit-C alone. Cells were stained with annexin V-FITC and PI and were analyzed by flow cytometry. **The cell cycle distribution after Vitamin C, Decitabine and their combination treatment in human colon cancer**. **B/** 1 mM Vit-C caused no obvious change of cell cycle distribution; while 20 µM DAC alone or combined with 1 mM Vit-C could increase **Sub G0/G1** phase cell fraction after 48 h treatment. All the data are the average of two independent the experiments. Statistical significance was tested using two-way ANOVA Tukey's test. *p ≤ 0.05, **p< 0.01, *** p< 0.001-significant results. Error bars represent the standard deviation..

Western blot results:

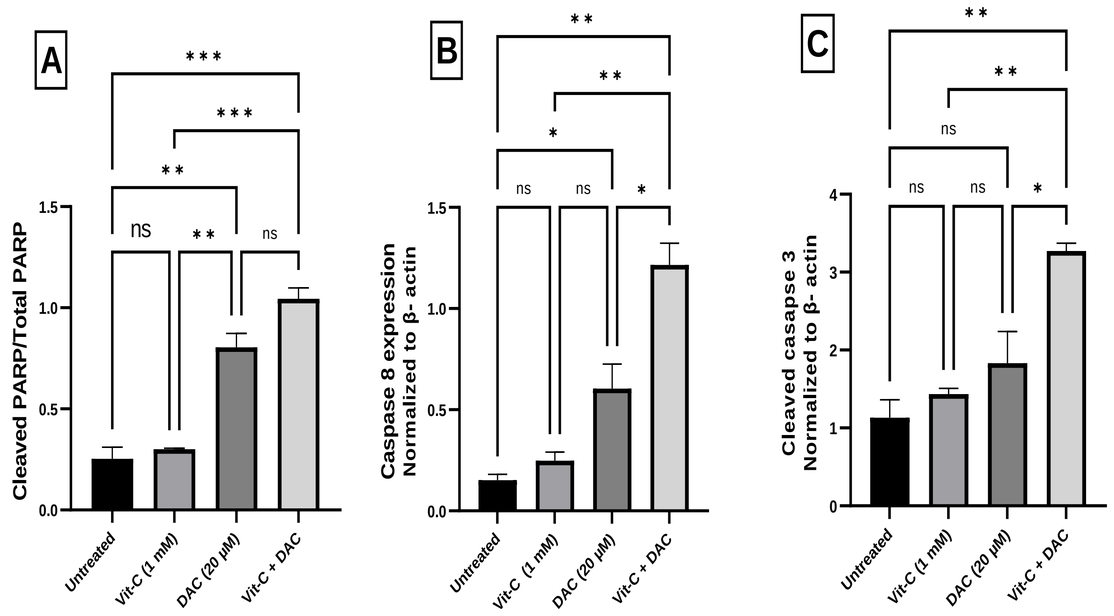


Figure2. Vitamin C and Decitabine combination therapy alters the expression levels of pro-apoptotic proteins in colon cancer cells. Whole cell extracts were prepared from cells treated with DAC, Vit-C and their combination for 48 h, and the expression levels of total PARP **(A)** caspase-8 **(B)** and cleaved caspase 3 **(C)** were determined by western blotting. β-actin was used as the internal control. Statistical significance was tested using one-way ANOVA Beferroni test. *p ≤ 0.05, ,**p< 0.01, *** p< 0.001-significant results. Error bars represent the standard deviation.

Western blot and RT-aPCR results:

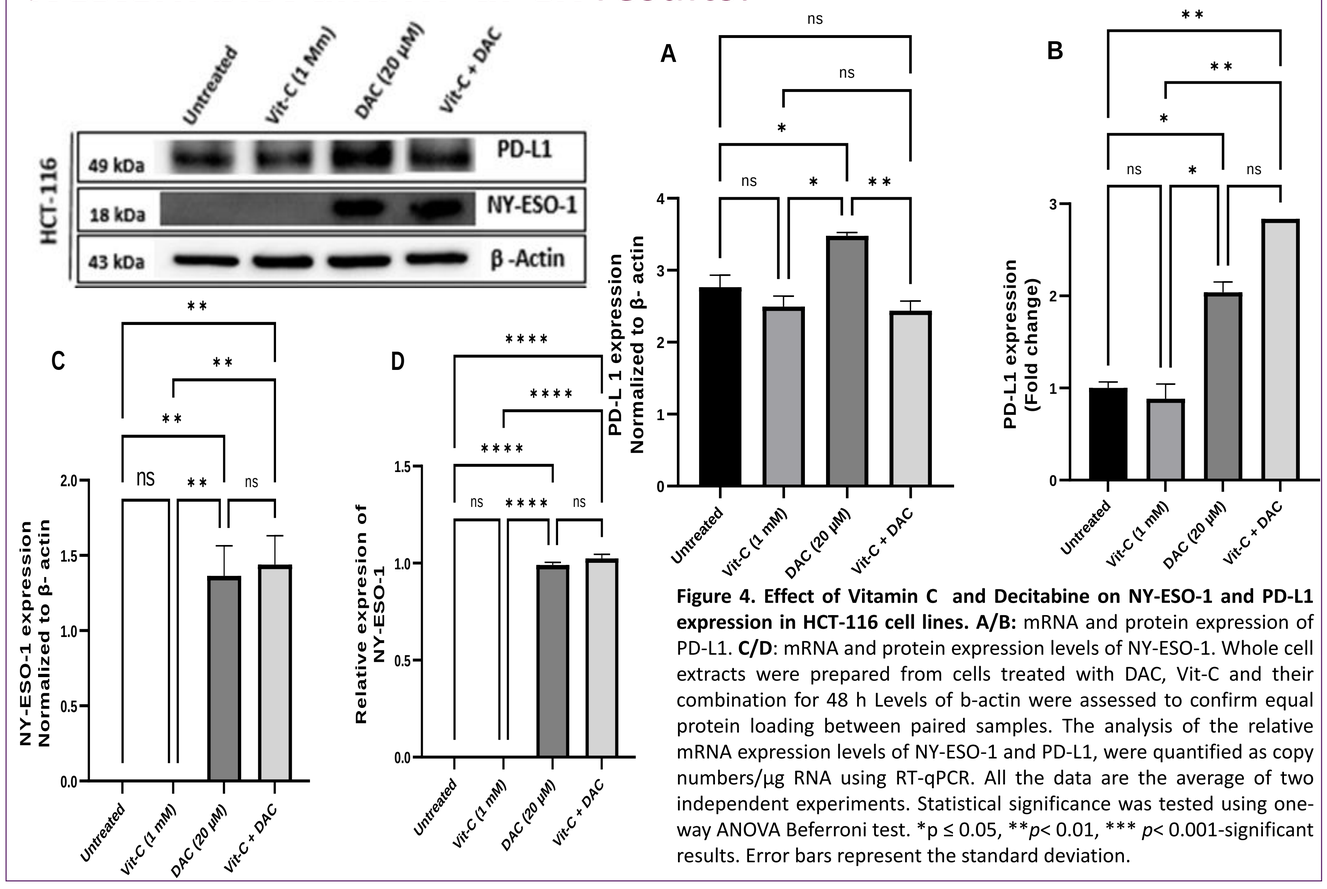
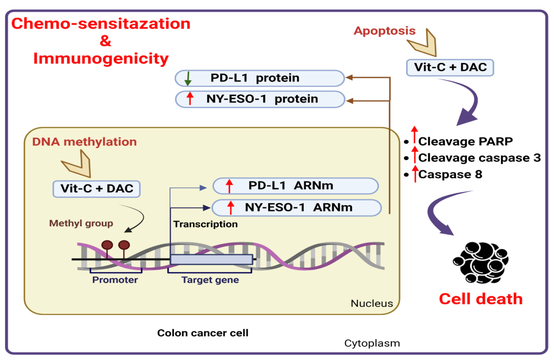


Figure 4. Effect of Vitamin C and Decitabine on NY-ESO-1 and PD-L1 expression in HCT-116 cell lines. A/B: mRNA and protein expression of PD-L1. **C/D**: mRNA and protein expression levels of NY-ESO-1. Whole cell extracts were prepared from cells treated with DAC, Vit-C and their combination for 48 h Levels of b-actin were assessed to confirm equal protein loading between paired samples. The analysis of the relative mRNA expression levels of NY-ESO-1 and PD-L1, were quantified as copy numbers/µg RNA using RT-qPCR. All the data are the average of two independent experiments. Statistical significance was tested using one-way ANOVA Beferroni test. *p ≤ 0.05, **p< 0.01, *** p< 0.001-significant results. Error bars represent the standard deviation.

Conclusions/Future Directions:



- The upregulation of PD-L1 by the chemotherapeutic drug DAC was prevented by a concomitant treatment with Vit-C
- Vit-C enhanced the cytotoxic effect of DAC and promoted cell apoptosis.
- We will investigate as future experiments the factors involved in the down regulation of PD-L1 expression.

Reference literature:

1.Raza, A., et al., *Unleashing the immune response to NY-ESO-1 cancer testis antigen as a potential target for cancer immunotherapy.* Journal of Translational Medicine, 2020. **18**(1): p. 1-11.

2. Mikkelsen, S.U., et al., *The role of vitamin C in epigenetic cancer therapy.* Free Radical Biology and Medicine, 2021. **170**: p. 179-193.

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