

# p53 contributes to the chemotherapeutic drug doxorubicin-induced cell death in colorectal cancer cell line HCT116

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**Abstract:** Doxorubicin is a commonly used chemotherapy drug for cancer treatment, but its effectiveness varies across different cancer types. p53 is a key factor involved in cell death induced by therapeutic agents. It can be upregulated by Doxorubicin and have a function of apoptosis. To have a further study of the mechanism between p53 and Doxorubicin, we investigated whether p53 play a role in the doxorubicin-induced cell death in the colorectal cancer line HCT116. Our finding revealed that p53 was upregulated in HCT116 cells when treated with doxorubicin, and knockdown of p53 decreased the sensitivity of HCT116 cells to doxorubicin. These results suggest that p53 plays an important role in doxorubicin-induced cell death in HCT116 cells, which could contribute to more effective treatment approaches.

## 1. Introduction

Cancer is one of the most prevalent diseases worldwide with a relatively high mortality rate (Torre, Siegel et al. 2016). Chemotherapy is a commonly employed method for cancer treatment, and there are various types of chemotherapeutic drugs available (Peng, Darko et al. 2017). Doxorubicin, an anthracycline-type chemotherapeutic drug, is widely used in clinic to treat several types of cancer, including Hodgkin's lymphoma, ovarian malignancies, and various forms of leukemia (Carvalho, Santos et al. 2009). It functions by intercalating into DNA base pairs, causing the unwinding of the DNA helix. This action inhibits the activity of topoisomerase II and interferes with DNA synthesis, ultimately leading to apoptosis (Marei, Althani et al. 2021). However, the precise mechanism underlying these effects are not yet fully understood, and it has been suggested that different types of cancer exhibit varying sensitivity for doxorubicin (Khan, Saleh et al. 2018, Sritharan and Sivalingam 2021).

p53 is a transcription factor that becomes activated in response to various forms of cellular stress, including DNA damage (Miyashita, Krajewski et al. 1994). Once activated, p53 transcriptionally upregulates genes involved in apoptosis (Aubrey, Kelly et al. 2018). In this study, we investigated whether p53 is involved in doxorubicin-induced cell death in HCT116 cells. We found that p53 is upregulated in HCT116 cells treated with doxorubicin, and that down-regulation of p53 decreases the its sensitivity to doxorubicin.

## 2. Materials and method

### 2.1 Cell line

The human colorectal carcinoma cell line HCT116 was originally obtained from Dr. Bert Vogelstein (John Hopkins University). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% fetal bovine serum (FBS), 100U/ml penicillin and 100U/ml streptomycin. The HCT116 cells were cultured in a humidified incubator at 37°C with 5% CO<sub>2</sub>.

### 2.2 Reagents

Doxorubicin (Dox) was purchased from Sigma-Aldrich. Antibodies for p53, and GAPDH were from Cell Signaling Technology.

### 2.3 Western blotting

Cell lysates were prepared using the lysis buffer, and the protein samples were resolved by SDS-PAGE and then transfer onto a PVDF membrane. The membrane was blocked with 5% skim milk and probed with the indicated antibodies.

### 2.4 Cell survival analysis

$5 \times 10^3$ - $1 \times 10^4$  of HCT116 treated with indicated siRNAs were seeded in 12-well plates and cultured overnight. Th cells were then washed with PBS and counted every 24 hours.

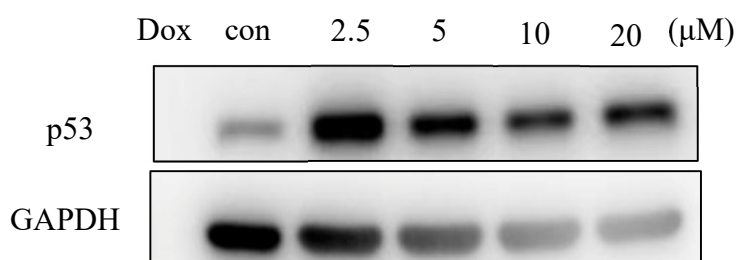
## 2.5 MTT assay

Approximately  $1 \times 10^3$ - $1 \times 10^4$  HCTT116 cells were seeded into 96-well plates and cultured overnight. Subsequently, different concentrations of doxorubicin were added to the cells which were then incubated for 48 hours. Following the incubation period, the media were removed, and MTT solution was applied to cells. The absorbance was then measured at the wavelength of 490nm using a microplate reader (Multiskan GO, Thermo Fisher Scientific).

## 3. Results

### 3.1 Doxorubicin induces upregulation of p53 in HCT116 cells

To determine whether p53 responds to doxorubicin treatment, we examined the expression of p53 in HCT116 cells treated with various concentrations of doxorubicin using western blotting analysis. The results demonstrated the level of p53 increased significantly compared to the level of GAPDH in untreated cells (Figure 1). Remarkably, p53 can be upregulated even at a doxorubicin concentration as low as  $2.5 \mu\text{M}$ . This result indicates that the level of p53 was increased in response to doxorubicin treatment.

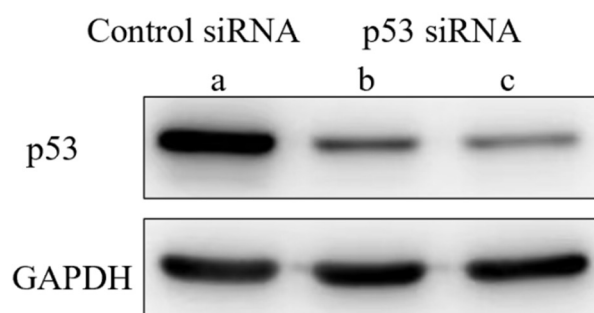


**Fig 1. p53 expression in response to doxorubicin (Dox) treatment in HCT116 cells.** The cells were treated with varying concentrations of Dox (2.5, 5, 10,  $20 \mu\text{M}$ ) for 24 hours and then analyzed by Western Blotting using the indication antibodies.

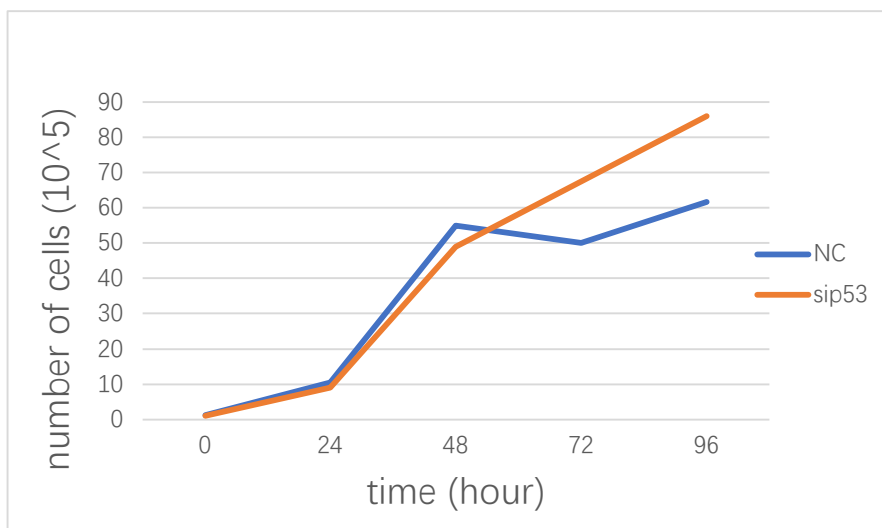
### 3.2 Knockdown of p53 minimally affects the growth of HCT116 cells in the first 48 hours

We then examined the effect of p53 knockdown on cell growth. Two specific siRNAs were employed, and both effectively reduced the expression of p53 (Figure 2). We

subsequently cultured these cells for 4 days and performed cell count every 24 hours. As shown in Figure 3, the growth rate of the p53 knockdown cells was slightly slower than that of the control cells for the first 48 hours. However, p53 knockdown cells appeared to grow much faster than the control cells after 48 hours. These results suggested that while p53 knockdown does not affect short-term cell growth, it may promote long-term cell growth.



**Fig 2. p53 knock down by siRNAs in HCT116 cells.** The HCT116 cells, transfected with the specified siRNAs, were analyzed by western blotting-using indicating antibodies. Control siRNA (a), p53 siRNA (b, c)

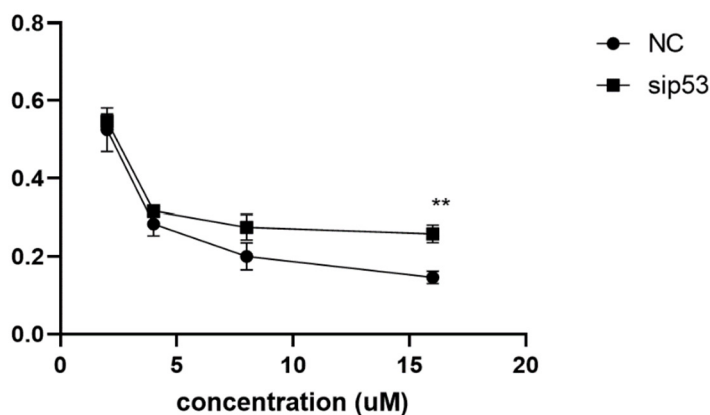


**Fig 3. The effect of p53 knocking down on HCT116 cells growths.** The HCT116 cells were transfected with the specific siRNAs, and seeded in a 12-well plate and cultured overnight. The cells numbers were counted every 24 hours

### 3.3 p53 knockdown reduces the sensitivity of HCT116 cells to doxorubicin

To examine whether the presence of p53 affect the sensitivity to doxorubicin, we treated both p53 knockdown cells and control HCT116 cells with different

concentrations of doxorubicin, and then conducted an MTT assay. As shown in Figure 4. The control HCT116 cells exhibited a lower cell survival rate compared to p53 knockdown cells across in all doxorubicin concentrations. The observation that p53 knockdown cells have a higher survival advantage when exposed to the same concentration of doxorubicin, indicated p53 plays an important role in doxorubicin-mediated cell death.



**Fig 4. The effect of knocking down of p53 on the sensitivity of HCT116 cells to doxorubicin.** The HCT116 cells, transfected with the indicated siRNAs. were seeded in a 96-well plate and cultured overnight. The cells were treated with different concentrations of doxorubicin followed by a MTT assay

### 3.4 Discussion

In this study, we investigated the role of p53 in doxorubicin-induced anti-cancer treatment. Our finding revealed a significant increase in the level of p53 following doxorubicin treatment, and notably, a reduction in p53 led to a decreased sensitivity of HCT116 cells to doxorubicin. These results provide compelling evidence that p53 plays an important role in doxorubicin-induced cell death, confirming many previous studies in this area.

Our results clearly demonstrated a significant increase in p53 levels following doxorubicin treatment. Doxorubicin induces DNA damage, subsequently causing

a complex cascade events that indirectly increase the level of p53 in the cells (Yeh, Chuang et al. 2004). Once p53 is stabilized and activated, it induces the expression of various types of proteins that influence the cellular process. For instance, p53 can upregulate BH3-only proteins such as BIM, NOXA, and PUMA(Thijssen, Diepstraten et al. 2021). These proteins can inhibit the pro-survival BCL2 family members resulting in apoptosis Although our study did not provide a precise mechanism for the role of p53 in inhibition of doxorubicin-induced cell growth (Thijssen, Diepstraten et al. 2021), we suspect that both p53-mediated cell cycle arrest and apoptosis are involved in this process. Further research is warranted to elucidate the intricate detail of the role of p53 in this process.

Interestingly, we found p53 was also involved in the growth of HCT116 cells in the absence of doxorubicin treatment. There was no significant difference in cell growth between the control and p53 knockdown cells within the first 48 hours after p53 was knocked down. However, the number of p53 knockdown cells increased dramatically in comparison with the control cells after 48 hours of siRNA treatment. The dramatic changes in the number of HCT116 cells indicated that knocking down of p53 can indirectly affect the growth of the cells, especially in the first 48 hours after p53 was knocked down by siRNA.

In Figure 3, it is evident that the number of normal cells surviving at 72 hours was unexpectedly lower than the number at 48 hours. While this discrepancy could be attributed to experimental variabilities, another possible explanation arises. The number of normal cells continued to increase after being seeded in the 12 well-plate, and possibly reaching to the peak between 24-48 hours. Consequently, there was not enough space and nutrition for them to grow. As a result, some cells might have died during that time, leading to a decrease in the number of surviving cells at 72 hours. Nevertheless, the experiment needs to be repeated to minimize potential errors.

In conclusion, it is clear from our result that doxorubicin can upregulate the level of p53 in HCT116 cells, and p53 knockdown affects the sensitivity of HCT116 cells to doxorubicin, suggesting that p53 plays an important role in doxorubicin-induced cell death in this cell type. Additionally, the impact of p53 on the growth of HCT116 cells requires further confirmation through repeated experiments. Also, further studies about the precise mechanism and correlation between doxorubicin and p53 can be held to reduce the side effect of chemotherapy.

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