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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, although its effectiveness varies across different cancer types. p53 is a key factor involved in cell death induced by therapeutic agents, and it can be upregulated by doxorubicin, exhibiting a function of apoptosis. To further investigate the mechanism between p53 and doxorubicin, this study explored whether p53 plays a role in doxorubicin-induced cell death in the colorectal cancer line HCT116. The findings revealed that p53 was upregulated in HCT116 cells when treated with doxorubicin, and the 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, potentially contributing to more effective treatment approaches.

Keywords: p53; Doxorubicin; Knockdown of p53

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1. Introduction

Cancer is one of the most prevalent diseases worldwide, with a relatively high mortality rate [1]. Chemotherapy is a commonly employed method for cancer treatment, with various types of chemotherapeutic drugs available [2]. Doxorubicin, an anthracycline-type chemotherapeutic drug, is widely used in clinics to treat several types of cancer, including Hodgkin's lymphoma, ovarian malignancies, and various forms of leukemia [3]. 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 [4]. However, the precise mechanism underlying these effects is not yet fully understood, and it has been suggested that different types of cancer exhibit varying sensitivity to doxorubicin [5,6].

p53 is a transcription factor that becomes activated in response to various forms of cellular stress, including DNA damage [7]. Once activated, p53 transcriptionally upregulates genes involved in apoptosis [8]. This study investigated whether p53 is involved in doxorubicin-induced cell death in HCT116 cells. It was found that p53

was upregulated in HCT116 cells treated with doxorubicin, and down-regulation of p53 decreased 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), 100 U/mL penicillin, and 100 U/mL streptomycin. HCT116 cells were cultured in a humidified incubator at 37°C with 5% CO_2.

2.2. Reagents

Doxorubicin (Dox) was purchased from Sigma-Aldrich. Antibodies for p53 and glyceraldehyde 3- phosphate dehydrogenase (GAPDH) were obtained from Cell Signaling Technology.

2.3. Western blotting

Cell lysates were prepared using lysis buffer, and the protein samples were resolved by sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% skim milk and probed with the indicated antibodies.

2.4. Cell survival analysis

Approximately 5×10^3–1×10^4 of HCT116 treated with indicated siRNAs were seeded in 12-well plates and cultured overnight. The cells were then washed with phosphate-buffered saline (PBS) and counted every 24 hours.

2.5. MTT assay

Approximately 1×10^3–1×10^4 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 the cells. The absorbance was then measured at the wavelength of 490 nm 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, the expression of p53 in HCT116 cells treated with various concentrations of doxorubicin was examined using Western blotting analysis. The results demonstrated that 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 μM. This result indicates that the level of p53 was increased in response to doxorubicin treatment.

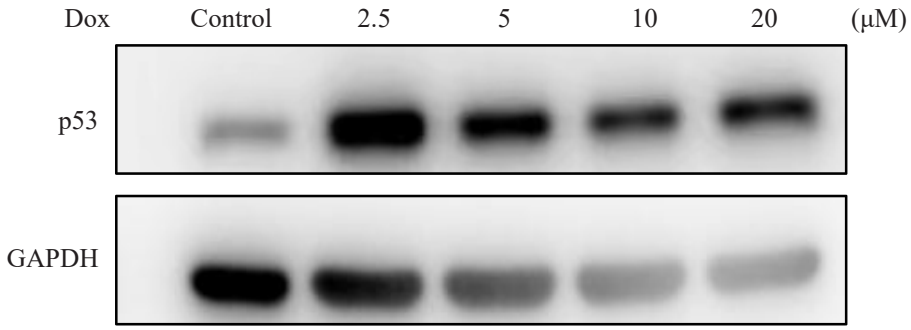

Figure 1. p53 expression in response to doxorubicin (Dox) treatment in HCT116 cells. The cells were treated with varying concentrations of Dox (2.5, 5, 10, 20 μM) for 24 hours and then analyzed by Western blotting using the indication antibodies

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

The effect of p53 knockdown was then examined on cell growth. Two specific small interfering RNA (siRNAs) were employed, and both effectively reduced the expression of p53 (**Figure 2**). These cells were subsequently cultured for 4 days and cell counts were performed 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.

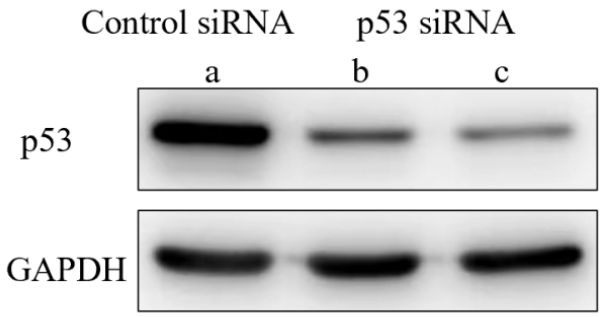

Figure 2. p53 knockdown by siRNAs in HCT116 cells. The HCT116 cells, transfected with the specified siRNAs, were analyzed by Western blotting using indicated antibodies. (a) Control siRNA; (b, c) p53 siRNA

Figure 3. The effect of p53 knockdown on HCT116 cells growths. The HCT116 cells were transfected with the specific siRNAs, seeded in a 12-well plate, and cultured overnight. The cell 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 affects the sensitivity to doxorubicin, both p53 knockdown cells and control HCT116 cells were treated with different concentrations of doxorubicin, and then an MTT assay was conducted. As shown in **Figure 4**, the control HCT116 cells exhibited a lower cell survival rate compared to p53 knockdown cells across all doxorubicin concentrations. The observation that p53 knockdown cells have a higher survival advantage when exposed to the same concentration of doxorubicin indicates that p53 plays an important role in doxorubicin-mediated cell death.

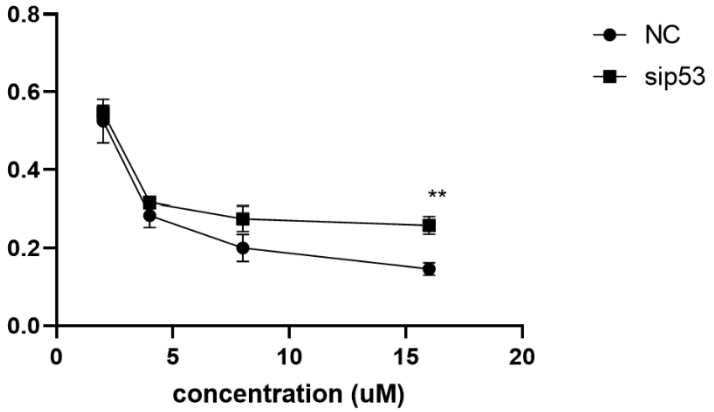

Figure 4. The effect of p53 knockdown on the sensitivity of HCT116 cells to doxorubicin. The HCT116 cells, transfected with the indicated siRNAs. were seeded in a 96-well plate and cultured overnight. The cells were treated with different concentrations of doxorubicin followed by an MTT assay

4. Discussion

This study investigated the role of p53 in doxorubicin-induced anti-cancer treatment. The findings revealed a significant increase in the level of p53 following doxorubicin treatment, and notably, a reduction in p53 led to 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.

The results clearly demonstrated a significant increase in p53 levels following doxorubicin treatment. Doxorubicin induces DNA damage, subsequently causing a complex cascade of events that indirectly increase the level of p53 in the cells [9]. Once p53 is stabilized and activated, it induces the expression of various types of proteins that influence cellular processes. For instance, p53 can upregulate B-cell lymphoma-2 (Bcl-2) homology 3 (BH3)-only proteins such as Bcl-2-like protein 11 (Bim), Phorbol-12-myristates-13-acetate-induced protein 1 (Noxa), and p53 upregulated modulator of apoptosis (Puma) [10]. These proteins can inhibit the pro-survival Bcl-2 family members, resulting in apoptosis. Although this study did not provide a precise mechanism for the role of p53 in the inhibition of doxorubicin-induced cell growth [10], it is suspected that both p53-mediated cell cycle arrest and apoptosis are involved in this process. Further research is warranted to elucidate the intricate details of the role of p53 in this process.

Interestingly, p53 was also found to be 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 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, possibly reaching its 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 the results 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 effects of chemotherapy.

Disclosure statement

The author declares no conflict of interest.

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