The role of SIRT5 and p53 proteins in the sensitivity of colon cancer cells to chemotherapeutic agent 5-Fluorouracil

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Abstract
Background In the treatment of colorectal cancer, it is important to develop drug combinations that will increase the effectiveness of chemotherapy and to determine the molecular targets of the drugs. Therefore, combined therapies that can increase the sensitivity of 5-Fluorouracil (5-FU) and the molecular pathways involved in this process are important in the treatment of the disease. Here we examined the SIRT5 (Resveratrol and Suramin) and p53 (Nutlin3a) modulators alone or in combination with 5-FU on the proliferation of colon cancer cells and effect of 5-FU on the SIRT5 and FOXO3a protein expressions whether p53 dependent or independent manner.

Methods and results According to our MTT assay results, Resveratrol (RSV), Nutlin3a and Suramin was found to be more effective in HCT-116 p53+/+ cells and these differences were evaluated together with the effect of 5-FU on the SIRT5, FOXO3a and Bim protein expressions in HCT-116 p53 +/+ and HCT-116 p53 −/− cells. SIRT5 is known to deacetylate FOXO3a which plays roles in the induction of apoptosis via Bim protein. Our western blot experiment results showed that while Suramin decreased SIRT5 and RSV decreased FOXO3a protein expressions significantly in HCT-116 p53 −/− cells, 5-FU decreased significantly SIRT5 and FOXO3a protein expressions in a p53 independent manner.

Conclusions In this study, the effect of 5-FU on SIRT5 and FOXO3a proteins was determined for the first time in HCT-116 p53 +/+ and HCT-116 p53 −/− cells. These results may help the discovery of new markers in colon cancer treatment.

Keywords Colon cancer · SIRT5 · 5-FU · FOXO3a · p53

Introduction
Studies on cancer which is one of the leading causes of human diseases and deaths have shown that it is caused by the disruption of the tight regulation in cellular processes. The molecular mechanisms underlying many types of cancer continue to be investigated. There is a great necessity to find new agents to be used in treatment or to be clarified their effects on the pathways that play roles in the cancer development process [1]. Although there have been new treatment options for colon cancer in recent years, the 5-year survival rate of the patients is very low due to the decrease in sensitivity to chemotherapy and the high tendency for recurrence. In this respect, the identification of new biomarkers related to colon cancer is very important in terms of further determining their clinical features [2].

SIRT5 is a mitochondrial circulatory protein belonging to the sirtuin family [3]. It was shown that mRNA expression of SIRT5 was detected in many types of tumors [4]. p53 is a nuclear transcription factor and activates many genes involved in the induction of cell cycle and apoptosis [5]. There are limited studies in the literature on the relationship between SIRT5 and p53 protein [6]. Lieber et al. showed that a decrease in SIRT5 expression is correlated with hyperacetylation of hepatic p53 in rats with chronic alcohol consumption [7]. Suramin (SIRT5 modulator) increases the DNA binding activity of p53 in NIH-3T3 fibroblast cells [8]. Wang et al. reported that SIRT5 has an effect on FOXO3 deacetylation in lung epithelial cells induced by cigarette smoke extract [9]. It has been reported that FOXO3 binds directly to p53 and provides the stabilization and activation of p53 dependent apoptosis [10]. Conversely, it has also been reported that p53 inhibits FOXO3 transcriptional activity and induces FOXO3 degradation under oxidative stress.
conditions [11]. Suramin has been shown to inhibit SIRT5 and Resveratrol inhibits desuccinilayse activity and induces deacetylase activity of SIRT5 [12]. Since SIRT5 has many roles in several pathologic diseases, targeting SIRT5 can be important therapeutic approach.

p53, tumor suppressor protein, has proapoptotic functions and 50% of the human cancers also 50–70% of colorectal cancers contain p53 mutation and thereby targeting the p53 pathway for the study of cancer therapeutics is an important strategy [13]. Besides, defective p53 mitigates the sensitivity of the cells to the chemotherapeutic drugs. It is an important advantage that chemical agents used in anticancer therapy exhibit antiproliferative effects in both p53 wild type and mutant p53 cells. p53 signal transduction pathway can be blocked by increased expression of MDM2. Inhibition of p53-MDM2 interaction and restitution of p53 activity is an important approach for cancer treatment. Nutlin3 is a MDM2 antagonist and activation of the p53 pathway through inhibition of p53-MDM2 interaction plays an important role in the apoptosis of cancer cells [13, 21].

In our study, we determined the effects of SIRT5 modulators in both HCT-116 p53 +/+ and HCT-116 p53 −/− cells. We aimed to compare the effects of these modulators alone or in combination with 5-FU on the cell viability in both p53 +/+ and p53 −/− cells. In p53 +/+ cells, we used Nutlin3a for stimulation of p53 protein expression. We also investigated the effects of 5-FU and modulators of SIRT5 and p53 on the expression of SIRT5, p53, FOXO3a and Bim proteins in HCT-116 p53 +/+ and HCT-116 p53 −/− colon cancer cells and the effects of these modulators on the sensitivity of colon cancer cells to 5-FU. This study is the first to examine the effects of SIRT5 and p53 modulators alone or in combination with 5-FU on the proliferation of colon cancer cells and effect of these modulators and 5-FU on SIRT5 and FOXO3a protein expressions in p53 dependent or independent manner.

Materials and methods

Chemicals

Suramin sodium salt was purchased from Cayman Chemical. Resveratrol, Nutlin3a, glycine, PBS, thiazole blue tetrazolium bromide (MTT) and 5-Fluorouracil were purchased from Sigma. Bradford reagent was from Thermo Scientific. Anti-SIRT5 (8782S), anti-Foxo3a (2497S), anti-Bim (2933S), Anti-p53 (9282S) and anti-Beta actin (4970S) antibodies and secondary anti-rabbit antibodies (7074S) were purchased from Cell Signalling Technology (CST). All antibody solutions were diluted 1:1000 in PBS. Nuclear extraction kit was purchased from Active Motif.

Cell culture

HCT-116 p53 +/+ ve HCT-116 p53 −/− colon cancer cell line was a kindly gift from Bert Vogelstein. HCT-116 p53 +/+ ve HCT-116 p53 −/− colon cancer cells were grown in RPMI 1640 medium which was supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, %10 heat inactivated fetal bovine serum. Cells were incubated in an incubator (5% CO₂–95% O₂, 37 °C). Cells were trypsinized for detachment when they were 80% confluent.

Cell viability

To determine the effect of Suramin, Resveratrol, Nutlin3a and 5-FU on the viability of cells, firstly stock solutions were prepared in DMSO and further dilutions were made in cell culture medium. HCT-116 p53 +/+ ve HCT p53 −/− colon cancer cells (5000 cells/well) were seeded to 96 well plate and after overnight incubation, cells were treated with Suramin (0.01–100 µM), Resveratrol (0.01–200 µM), Nutlin3a (0.01–20 µM) and 5-FU (0.01–20 µM) and they were incubated for 72 h. To determine whether SIRT5 and p53 modulators increase or not the sensitivity of the cells to 5-FU, cells were treated with increasing concentrations of 5-FU (0.01–20 µM) and at the same time RSV (50 µM) or suramin (100 µM) or Nutlin3a (10 µM) was added to the all wells except negative control group and incubated for 72 h and cell proliferation was determined by MTT assay [14]. Cells containing equal concentrations of DMSO were used as a negative control. After incubation, MTT (5 mg/ml) was dissolved in PBS and was added into the wells. Following 4 h incubation at 37 °C, culture medium and MTT solution was discarded and DMSO (100 µl) was added to the wells and absorbance at 550 nm was measured on a spectrophotometer. Absorbance of the cells which were used as control group was considered as 100% cell viability. IC50 values were determined by using GraphPad Prism 7.0 Software.

Western blot analysis

SIRT5, FOXO3a, p53 and Bim protein expressions was determined by western blot analysis. For this purpose, HCT-116 p53 +/+ and HCT-116 p53 −/− cells were lysed by complete lysis buffer (Active Motif) containing 1 mM DTT, Lysis Buffer AM1 and Protease inhibitor cocktail according to manufacturer instructions and total protein extracts were prepared according to the nuclear extract kit (Active Motif). According to the Bradford method, protein concentrations of the lysates were determined and lysates containing equal amount of protein were loaded on to the SDS polyacrylamide gel electrophoresis. After transfer to the PVDF
membrane using 100 V for 1 h, membranes were incubated with anti-SIRT5, anti-FOXO3a, anti-p53 and anti-Bim antibodies overnight at 4 °C [15]. Next day, membranes were incubated with appropriate secondary antibodies at room temperature for 1 h then visualized on Licor Odyssey Fc Imaging System using ECL reagent (Cell Signalling). Band density was analyzed by using Image J software.

**Statistical analysis**

IBM SPSS Statistics 20 program was used for statistical analysis. To determine the significance between the groups, One way Anova variance analysis test was performed and Tukey post hoc test was used. Data were expressed as mean ± SD and p < 0.05 was considered as significant.

**Results**

**Effect of SIRT5 and p53 modulators alone or in combination with 5-FU on the viability of HCT-116 p53 +/- and HCT-116 p53 −/− cells**

To determine the IC50 values of 5-FU, Suramin, Nutlin3a and RSV, HCT-116 p53 +/+ and HCT-116 p53 −/− cells were incubated with these modulators alone or in combination and MTT assay was performed. For this purpose, firstly cells were treated with different concentrations of Suramin (0.01–100 µM), RSV (0.01–200 µM), Nutlin3a (0.01–20 µM) and 5-FU (0.01–20 µM) alone and incubated for 72 h.

We found that IC50 values of RSV is 53.52 ± 7.162 µM in HCT-116 p53 −/− cells (Fig. 1D) and 48.51 ± 2.25 µM in HCT-116 p53 +/+ cells (Fig. 1C). So HCT-116 p53 +/+ and p53 −/− cells were treated with 50 µM RSV which close to the IC50 value in combined treatment with 5-FU. IC50 values of 5-FU was found as 5.80 ± 0.34 µM in HCT-116 p53 −/− cells (Fig. 1B) and 2.7 ± 0.2 µM were determined in HCT-116 p53 +/- cells (Fig. 1A). Suramin significantly inhibited viability of HCT-116 p53 +/+ and p53 −/− cells only at 100 µM (p < 0.01) (Fig. 1E, F). So HCT-116 p53 +/+ and p53 −/− cells were treated with 100 µM Suramin which caused the inhibition of cell proliferation significantly in combined treatment with 5-FU. Nutlin3a only at 20µM significantly inhibited cell viability of HCT-116 p53 −/− cells (p < 0.001) (Fig. 1H) and IC50 value is 2.84 ± 0.20 µM in HCT-116 p53 +/+ cells (Fig. 1G). We used Nutlin3a for p53 activation and we used 10 µM according to the literature and also HCT-116 p53 +/+ and p53 −/− cells were treated with 10 µM Nutlin3a in combined treatment with 5-FU [16, 17].

IC50 value of RSV (50 µM) and 5-FU (0.01–20 µM) combination was determined as 2.58 ± 0.51 µM in HCT-116 p53 −/− cells (Fig. 2B) and 0.024 ± 0.002 µM in HCT-116 p53 +/+ cells (Fig. 2A). IC50 value of Suramin (100 µM) and 5-FU (0.01–20 µM) combination is 0.1894 ± 0.027 µM in HCT-116 p53 −/− cells (Fig. 2D) and 0.077 ± 0.01 µM in HCT-116 p53 +/+ cells (Fig. 2C). IC50 value of Nutlin3a (10 µM) and 5-FU (0.01–20 µM) combinations was calculated as 0.758 ± 0.093 µM in HCT-116 p53 −/− cells (Fig. 2F) and 0.0095 ± 0.002 were determined in HCT-116 p53 +/+ cells (Fig. 2E).

Coefficient of drug interaction (CDI) was calculated according to Zhao et al. [18]. For both HCT-116 p53 +/- and HCT-116 p53 −/− cells, CDI values of Suramin (100 µM) and 5-FU (2.5 µM for HCT-116 p53 +/- cells and 5 µM for HCT-116 p53 −/− cells) and Nutlin3a (10 µM) and 5-FU (2.5 µM for HCT-116 p53 +/- cells and 5 µM for HCT-116 p53 −/− cells) combinations were < 1 which shows the synergistic effect. Although CDI value of RSV (50 µM)-5-FU (2.5 µM for HCT-116 p53 +/- cells and 5 µM for HCT-116 p53 −/− cells) combination is < 1 in HCT-116 p53 +/- cells, it is > 1 which shows antagonistic effect in HCT-116 p53 −/− cells.

**Effect of SIRT5 and p53 modulators and 5-FU on the expression of SIRT5, FOXO3a, p53 and Bim proteins in HCT-116 p53 +/- and HCT-116 p53 −/− cells**

To determine the effect of 5-FU, RSV, Suramin and Nutlin3a on the protein expression of SIRT5, FOXO3a, p53 and Bim in both HCT-116 p53 +/+ and HCT-116 p53 −/− cells, 5-FU (5.8 µM for HCT116 p53 −/− cells/2.7 µM for HCT-116 p53 +/+ cells), RSV (50 µM), Suramin (100 µM) and Nutlin3a (10 µM) were treated to cells and incubated for 72 h. Cells were treated with 50 µM RSV which close to the IC50 value and Suramin was applied to the cells at 100 µM since only this concentration inhibits cell viability significantly and cells were treated with 10 µM Nutlin3a for proper p53 activation according to literature [16, 17].

Then western blot experiments were performed. We found that 5-FU significantly decreased SIRT5 protein expressions in both HCT-116 p53 +/+ (p < 0.01) and HCT-116 p53 −/− (p < 0.05) cells (Figs. 3A, B, 4A and B). While Suramin decreased SIRT5 protein expression significantly in HCT-116 p53 −/− cells (p < 0.05), it didn’t change SIRT5 expression significantly in HCT-116 p53 +/- cells (Figs. 3A, B, 4A and B). 5-FU decreased FOXO3a protein expression significantly in HCT-116 p53 +/+ and HCT-116 p53 −/− cells (p < 0.01) significantly (Figs. 3A, B, 4A and B). Suramin and Nutlin3a didn’t change FOXO3a protein expression significantly in both cell lines (Figs. 3A, B, 4A and B). RSV decreased FOXO3a protein expression significantly in HCT-116 p53 −/− cells (p < 0.01) (Fig. 3A and B). 5-FU
Fig. 1 Effect of RSV, Nutlin3a, Suramin and 5-FU on the viability of HCT-116 p53 --/- and HCT-116 p53 +/- colon cancer cells. HCT-116 p53 --/- and HCT-116 p53 +/- colon cancer cells were incubated for RSV, Nutlin3a, Suramin and 5-FU for 72 h and MTT assay was performed. A 5-FU (0.5–20 µM) decreased cell viability in HCT-116 p53 +/- cells significantly. B 5-FU (1–20 µM) decreased cell viability in HCT-116 p53 --/- cells significantly. C RSV (20–200 µM) decreased cell viability in HCT-116 p53 +/- cells significantly. D RSV (0.1–200 µM) decreased viability of HCT-116 p53 --/- cells significantly. E Suramin at 100 µM concentration decreased cell viability in HCT-116 p53 +/- cells significantly. F Suramin at 100 µM concentration decreased cell viability in HCT-116 p53 --/- cells significantly. G Nutlin3a (0.5–20 µM) decreased cell viability in HCT-116 p53 +/- cells significantly. H Nutlin3a at 20 µM decreased cell viability in HCT-116 p53 --/- cells significantly (Significance between RSV, Nutlin3a, Suramin, 5-FU treated vs. untreated group was shown as *p < 0.05, **p < 0.01, ***p < 0.001).
Fig. 2 Effect of 5-FU and RSV combination, 5-FU and Suramin combination and 5-FU and Nutlin3a combination on the viability of HCT-116 p53 +/+ and HCT-116 p53 −/− colon cancer cells. HCT-116 p53 +/+ colon cancer cells were incubated for 5-FU and RSV combination, 5-FU and Suramin combination and Nutlin3a and 5-FU combination for 72 h and and MTT assay was performed. A 5-FU (0.01–20 µM) and RSV (50 µM) combination decreased cell viability significantly in HCT-116 p53 +/+ cells. B 5-FU (0.01–20 µM) combination decreased cell viability significantly in HCT-116 p53 −/− cells. C 5-FU (0.01–20 µM) and Suramin (100 µM) combination decreased cell viability significantly in HCT-116 p53 +/+ cells. D 5-FU (0.01–20 µM) and Suramin (100 µM) combination decreased cell viability significantly in HCT-116 p53 −/− cells. E 5-FU (0.01–20 µM) and Nutlin3a (10 µM) combination decreased cell viability in HCT-116 p53 +/+ cells significantly. F 5-FU (0.1–20 µM) and Nutlin3a (10 µM) combination decreased cell viability in HCT-116 p53 −/− cells significantly (Significance between RSV, Nutlin-3a, Suramin, 5-FU, 5-FU and RSV combination, 5-FU and suramin combination and 5-FU and Nutlin3a combination treated vs. untreated group was shown as *p < 0.05, **p < 0.01, ***p < 0.001)
**Fig. 3** A Effects of Suramin (100 µM), RSV (50 µM), Nutlin3a (10 µM) and 5-FU (5.8 µM) on the protein expression of SIRT5, FOXO3a, Bim and p53 in HCT-116 p53 −/− cells. HCT-116 p53 −/− colon cancer cells were incubated for RSV, Nutlin3a, Suramin and 5-FU for 72 h, total cell lysates were prepared and western blot analysis was performed. B Densitometry analysis of SIRT5, FOXO3a and Bim protein expressions. 5-FU and Suramin decreased SIRT5 protein expression significantly (p < 0.05). 5-FU and RSV decreased FOXO3a protein expression significantly (p < 0.01). 5-FU and RSV increased Bim protein expression significantly (p < 0.001 and p < 0.01 respectively). Significance between RSV, Nutlin-3a, Suramin, 5-FU treated vs. untreated group was shown as *p < 0.05, **p < 0.01, ***p < 0.001.

**Fig. 4** A Effects of Suramin (100 µM), RSV (50 µM), Nutlin3a (10 µM) and 5-FU (2.7 µM) on the protein expression of SIRT5, FOXO3a, Bim and p53 in HCT-116 p53 +/+ cells. HCT-116 p53 +/+ colon cancer cells were incubated for RSV, Nutlin3a, Suramin and 5-FU for 72 h, total cell lysates were prepared and western blot analysis was performed. B Densitometry analysis of SIRT5, FOXO3a, p53 and Bim protein expressions. 5-FU decreased SIRT5 and FOXO3a protein expression significantly (p < 0.01). 5-FU (p < 0.01), RSV (p < 0.05), Suramin (p < 0.001) and Nutlin3a (p < 0.001) increased p53 protein expression in HCT-116 p53 +/+ cells significantly. RSV (p < 0.05), Suramin (p < 0.01), Nutlin3a (p < 0.05) and 5-FU (p < 0.001) increased Bim protein expression significantly. Significance between RSV, Nutlin-3a, Suramin, 5-FU, treated vs. untreated group was shown as *p < 0.05, **p < 0.01, ***p < 0.001.
(p < 0.01), RSV (p < 0.05), Suramin (p < 0.001) and Nutlin3a (p < 0.001) increased significantly p53 protein expression in HCT-116 p53 +/+ cells (Fig. 4A and B). 5-FU (p < 0.001) and RSV (p < 0.01 and p < 0.05 respectively) increased significantly Bim protein expression in HCT-116 p53 −/− and HCT-116 +/+ cells (Figs. 3A, B, 4A and B). While Suramin (p < 0.01) and Nutlin3a (p < 0.05) increased Bim protein expression significantly in HCT-116 p53 +/+ cells (Fig. 4A and B), they didn’t increase Bim protein expression significantly in HCT-116 p53 −/− cells (Fig. 3A and B).

Discussion

In the treatment of colorectal cancer, it is important to develop drug combinations that will increase the effectiveness of chemotherapy and to determine the molecular targets of the drugs [19]. For these reasons, combined therapies that can increase the sensitivity of cells against 5-FU and the molecular pathways involved in this process play important roles in cancer treatment. Our results show that HCT-116 p53 +/+ cells are more sensitive to 5-FU (IC50 = 2.703 ± 0.2) according to HCT-116 p53 −/− cells (IC50 = 5.806 ± 0.34) (Fig. 1A and B). These results are consistent with the studies done before with 5-FU [20]. We examined the effects of SIRT5 and p53 modulators when used alone or in combination with 5-FU on proliferation of colon cancer cells p53 dependent and independently. Since the p53 mutation is very common in colorectal cancer patients, we thought it could be useful in developing new treatment strategies. It is also the first study to examine the differences between SIRT5 and FOXO3a protein expressions in HCT-116 p53 +/+ and HCT-116 p53 −/− colon cancer cells and the effects of 5-FU, which is the first line therapy in colon cancer treatment, on these proteins. In this study, for the first time, the effects of Nutlin3a, which is a non-genotoxic p53 activator, on SIRT5 and FOXO3a proteins were also investigated. And thus, in cases of p53 activation, changes in SIRT5 and FOXO3a proteins were determined by comparing them with p53 −/− cells that do not express p53. The aim of this study is to shed light on new approaches in the treatment of colon cancer patients with both mutant and wild p53 genes. It has been known that the correlation between p53 mutations and 5-FU resistance and decrease in cell response to 5-FU and other chemotherapeutic drugs such as cisplatin, temozolomide, doxorubicin, and gemcitabine [24]. We examined the differences between the responses of p53 mutant and wild type cells to these modulators. Most genotoxic therapies such as radiotherapy and chemotherapeutic drugs induce the p53 pathway via DNA damage stimulation. Nevertheless these kind of cancer therapies can cause the formation of another secondary tumors in the body since the genotoxic effects on the normal tissues. Nutlin3 increases p53 activation via non genotoxic mechanism, it inhibits the p53-MDM2 interaction and leads to stabilization and activation of p53 pathway [13, 21]. The purpose of applying Nutlin3 in combination with 5-FU is to achieve the desired increase in p53 expression with low doses of 5-FU treatment in a non-genotoxic way. It has been reported that Nutlin-3 exhibit synergetic effects when combined with genotoxic drugs doxorubicin, chlorambucil, and fludarabine in B-cell chronic lymphocytic leukemia [22]. Jin et al. reported that combination of Nutlin3 with bortezomib leads to cell cycle arrest and induces the apoptosis of mantle cell lymphoma (MCL) cells [23]. Nutlin3 and Roscovitine and DRB which are CDK inhibitors have synergetic effects on the p53 activation in such cancer cells including melanoma, colon carcinoma, breast cancer, hepatocarcinoma [25]. For this purpose, both cells were incubated with Nutlin3a for 72 h. According to our results, Nutlin3a increased p53 protein expression in p53 +/+ cells compared to the control group (Nulin-3a free cells) (Fig. 4A and B).

Our results showed that these modulators have significant effects on 5-FU sensitivity of colon cancer cells. We determined that RSV, Suramin and Nutlin3a are more effective in HCT-116 p53 +/+ cells than HCT-116 p53 −/− cells (Fig. 2A-F). We demonstrated first time that chemotherapeutic agent 5-FU decrease SIRT5 and FOXO3a protein expressions significantly and p53 independently (Fig. 3A, B, 4A, B). SIRT5 is a member of the sirtuin family whose role in cancer has been least studied. Limited research studies revealed that it acts as a tumor suppressor or tumor promoter depending cell conditions, tumor stage and tissue of origin [12, 26]. Some studies indicate that possible tumor suppressor function of SIRT5. SIRT5 has been shown to be significantly downregulated in head and neck and hepatocellular carcinoma tissues [27, 28]. In addition, there are many studies showing that it is a tumor promoter. SIRT5 overexpression and its role in facilitating drug resistance has been indicated in human non-small cell lung cancer cells by increasing the expression of Nrf2 and downstream targets [29]. SIRT5 overexpression was reported in Waldenström’s macroglobulinemia (WM) CD19+ cells and colon cancer cell lines (HCT-116 and Lovo) and colorectal cancer tissues when compared to normal tissues. siRNA silencing of SIRT5 has been shown to inhibit cancer cell proliferation [30–32]. In another study, regarding the role of SIRT5 in colon cancer, it was shown that the combination of SIRT5 inhibitors with chemotherapeutic agents (cetuximab) exhibited a therapeutic approach in wild type Kras colorectal patients [33]. SIRT5-mediated Lactate Dehydrogenase B (LDHB) deacetylation has been shown to trigger autophagy and tumorigenesis in colorectal cancer [34].

In the literature, there is very few study about the relationship between SIRT5 and FOXO3a. SIRT5 has an effect on the deacetylation of FOXO3a in lung epithelial cells induced...
by cigarette smoke extract and deacetylation of FOXO3a by SIRT5 leads to prevention of lung epithelial cell apoptosis. FOXO3a is a transcription factor that induces gene expression involved in antioxidant defense and translocates to the nucleus upon deacetylation by SIRT5 [9]. Some studies indicated the relationship between FOXO3a and metastasis/bad prognosis in cancer [35]. It was shown that FOXO3a and β-catenin was accumulated in nucleus and this is correlated with metastasis of colon cancer [36]. Acetylation of FOXO3a has apoptosis promoting effect via activating the pro-apoptotic genes (Bim, p21, FASL6 etc.) and as a result of deacetylation of FOXO3a by SIRT1 leads to cell survival and inhibition of FOXO3a mediated apoptosis [37, 38]. According to our results, due to the reducing effect of 5-FU on SIRT5 expression, deacetylation of FOXO3a is alleviated and FOXO3a-dependent apoptotic pathways are induced. This idea is consistent with the stimulating effect of 5-FU on Bim protein. SIRT5 is known to deacetylate FOXO3a [9] and FOXO3a leads to the induction of apoptosis via Bim [39]. RSV and Suramin were used as SIRT5 modulators in this study. We examined the effects of these modulators on SIRT5 and FOXO3a protein expressions since FOXO3a is a target of SIRT5 and decacylated by SIRT5 directly. To determine how the possible differences which are caused by Suramin and Resveratrol on the SIRT5 expression effects FOXO3a and its target protein Bim, we examined the effect of Suramin and Resveratrol on the protein expressions of SIRT5, FOXO3a and Bim expressions. Also p53 is the target of SIRT5 protein, we investigated the effect of the possible alterations of SIRT5 made by Suramin and Resveratrol on the p53 expressions. And both cells were incubated with RSV (50 µM) and Suramin (100 µM) for 72 h it was shown that both Suramin and RSV increased p53 protein expressions in HCT-116 p53 +/- cells significantly (Fig. 4A and B).

Our results of western blot experiments indicate that RSV hasn’t any significant effect on the SIRT5 protein expression in both cell lines (Figs. 3A, B, 4A and B). In previous studies, RSV (20 µM) has been shown that reversed SIRT1-7 activity reduced by TNF-α in HUVECs [40]. According to the previous studies, it has been found that deacetylase activity of SIRT5 is activated and desuccinylase activity is inhibited by Resveratrol [12, 41] and SIRT5 enzyme activity is decreased by Suramin and increased by Resveratrol [42]. Besides these studies, it has been shown that SIRT5 protein expression is not altered by Resveratrol in H295R human adrenal cell lines [43]. We also found that RSV decreased FOXO3a protein expression in HCT-116 p53 +/- cells significantly (Fig. 3A and B), but it didn’t have any significant effect on the FOXO3a protein in HCT-116 p53 +/- cells (Fig. 4A and B). RSV in both cells increase the sensitivity of colon cancer cells to 5-FU. But increase in the sensitivity is much more in HCT-116 p53 +/- cells than HCT-116 p53 +/- cells. So this difference may be due to p53 and FOXO3a protein expression difference. According to Mohapatra et al. when HCT-116 cells were treated with 15 µM RSV and 0.5 µM 5-FU, cells was blocked at S phase, enhanced DNA damage and apoptosis was observed. Mohapatra et al. reported that 5-FU can increase the chemotherapy efficacy of RSV which is used in colon cancer treatment [44]. The difference of our study from these studies is to be performed all analysis both p53 +/- and p53 +/- cells to compare the effects due to differences in p53 expression and cells were treated with 5-FU at increasing concentration (0.01–20 µM) and 50 µM RSV. It has been shown that Resveratrol inhibited colon cancer cell invasion and metastasis by Wnt/β-catenin signal pathway [45].

We used Nutlin3a to induce p53 expression and to test the effect of p53 induction on the expression of other proteins, we examine the effect of Nutlin3a on SIRT5, FOXO3a and Bim protein expressions. The effects of Nutlin3a on SIRT5, FOXO3a and Bim protein expressions were examined to determine whether activation of p53 by Nutlin3a was a p53-induced change on the expression of other proteins. At the same time to compare these effects with the cells which can’t express p53, we determine the effects of these modulators in p53 +/- cells. When the effect of Nutlin3a on the expression of SIRT5, FOXO, Bim proteins was investigated, it was found that Nutlin3a significantly increase Bim protein in only p53 +/- cells (Fig. 4A and B) and it has not been detected any significant effect on on the SIRT5 or FOXO3a expression in p53 +/- and p53 +/- colon cancer cells. Similar to our results, the increase in Nutlin3a-mediated Bim protein expression found p53 dependent [46].

The aim of the use of both a SIRT5 activator and inhibitor is to determine the effect of SIRT5 inhibition and activation on the cell proliferation and so the role of SIRT5 protein on the proliferation of colon cancer cells. At the same time, we aimed to investigate the role of SIRT5 protein whether it is p53 dependent or independent. But according to our study, of these modulators, Suramin decreased SIRT5 expression only in HCT-116 p53 +/- cells, while Resveratrol did not alter SIRT5 expression in HCT-116 p53 +/- or HCT-116 p53 +/- cells. Therefore, its effects on 5-FU treatment can be said to be due to a cause independent of SIRT5. In the previous studies, Suramin has been shown increases p53 protein levels, but fails to increase p21 mRNA levels or to activate the G ~ checkpoint. These data suggest that suramin induces growth arrest in NIH-3T3 cells by a mechanism that is independent of cellular p53 status [8]. RSV increased p53 and Bim protein expression significantly in HCT-116 p53 +/- cells (Fig. 4A and B) and increase Bim expression in HCT-116 p53 +/- cells significantly (Fig. 3A and B).

In the literature, there is no study that investigates the effect of Suramin on the proliferation of HCT-116 p53 +/- and p53 +/- colon cancer cells. Suramin has been shown
that inhibited SIRT5 NAD(+) -dependent deacetylase activity [12, 47]. We found that while Suramin hasn’t any statistically significant effect on SIRT5 expression in HCT-116 p53 +/+ cells (Fig. 4 A and B), and decreases SIRT5 protein expression significantly in HCT-116 p53 −/− cells (p < 0.05) (Fig. 3A and B). Suramin decreases FOXO3a in both cells but it is not significant (Figs. 3A, B, 4A and B). Suramin increases p53 and Bim protein expressions in HCT-116 p53 +/+ cells (Fig. 4A and B) and exhibits any significant effect on Bim protein expressions in HCT-116 p53 −/− cells (Fig. 3A and B).

There are limited studies in the literature on the relationship between SIRT5 and p53 protein. It has been shown that p53 cannot be deacetylated by sirtuins except SIRT1 [6]. Solomon et al. showed that SIRT5 can deacetylate cytochrome c but not p53 [48]. Lieber et al. showed that the decrease in SIRT1 and SIRT5 expressions caused post-translational modification or hyperacetylation of PGC1-α and p53 [7].

In conclusion, this study showed that 5-FU decreased SIRT5 and FOXO3a protein expressions in p53 independent manner for the first time. And SIRT5 and p53 modulators increase the sensitivity of colon cancer cell lines to 5-FU. The role of SIRT5 protein expression on the cell proliferation of colon cancer cell lines was explained. SIRT5 inhibition by Suramin leads the increase in the sensitivity of colon cancer cells to 5-FU in HCT-116 p53 −/− cells. Thus, it is important in terms of being a guide for new approaches for colon cancer treatment and new drugs that are planned to be developed.

Author contributions OE and AK performed all experiments, analyzed the experiments results, performed statistical analysis and wrote the manuscript. All experiments are designed and planned by AK.

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Data availability All data obtained in the study can be accessed if desired.

Declarations Conflict of interest The authors declare that they have no conflict of interest.

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