The Role of SIRT5 and p53 Proteins in the Sensitivity of Colon Cancer Cells to Chemotherapeutic Agent 5-Fluorouracil

Ozlem Ekremoglu (✉ oeek1983@hotmail.com)

Ankara University Faculty of Pharmacy: Ankara Universitesi Eczacilik Fakultesi

Asli Koc

Department of Biochemistry, Faculty of Pharmacy, University of Ankara, Ankara, Turkey

<https://orcid.org/0000-0002-4679-9079>

Research Article

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Abstract

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), a chemotherapeutic agent used in the treatment of colon cancer, and also 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 expressions whether p53 dependent or independent manner. p53 protein expression is activated by nutlin3a in HCT-116 p53 +/+ cells and HCT-116 p53 -/- cells were used as counterpart cells that did not express p53. According to our MTT assay results, we showed that these modulators have significant effects on the sensitivity of both cells to 5-FU. In p53+/+ cells, Resveratrol (RSV) and Nutlin3a and in p53 -/- cells, Suramin was found to be more effective. These differences were evaluated together with the effect of 5-FU on the SIRT5-FOXO3a-Bim axis in p53 +/+ and p53 -/- cells. SIRT5 is known to deactivate FOXO3a which plays a role in the induction of apoptosis via Bim. 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 p53 independently. These results may help the discovery of new markers in colon cancer treatment.

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 and its role in cancer has been the least studied to date [3]. It was shown that mRNA expression of SIRT5 was increased in 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]. FOXO3a is a member of the FOXO subfamily of forkhead transcription factors and mediates many cellular processes [7]. 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 inhibit desuccinylase activity and induces deacetylase activity of SIRT5 [12]. Since SIRT5 has many roles in several pathologic diseases, targeting SIRT5 can be important therapeutic approach.

In this study, we investigated the effects of 5-FU and modulators of SIRT5 and p53 on the expression of SIRT5, p53, FOXO3a and Bim proteins in HCT p53+/+ and p53-/- colon cancer cells and 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 proliferation of colon cancer cells in p53 dependent or independent manner.

Material And Methods

Chemicals

Suramin sodium salt was purchased from Cayman Chemical. Resveratrol, Nutlin3a, glycine, PBS, thiazole blue tetrazolium bromide (MTT) and 5-fluorouracil was purchased from Sigma. Bradford reagent was from Thermo Scientific. Anti-SIRT5, anti-Foxo3a, anti-Bim, Anti-p53 and anti-beta actin antibodies and secondary anti-mouse and anti-rabbit antibodies were purchased from CST. 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 penicilin, 100 µg/mL streptomycin, 2mM 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 (0.01-100µM), resveratrol (0.01-200µM), nutlin3a (0.01-20µM) and 5FU (0.01-20µM) and they were incubated for 72h. 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 (0.01-20µM) and at the same time RSV (50µM) or suramin (100µM) or Nutlin3a (10µM) was added to the all wells except negative control group and incubated for 72h and cell proliferation was determined by MTT assay [13]. Cells containing equal concentrations of DMSO were used as a negative control. After incubation, MTT (5mg/ml) was dissolved in PBS and was added into the wells. Following 4h incubation

at 37°C, culture medium and MTT solution was discarded and DMSO (100µl) 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 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 100V for 1h., membranes were incubated with anti- SIRT5, anti- FOXO3a, anti-p53 and anti-Bim antibodies overnight at 4°C [14]. 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. [Band density was analyzed by using Image J software](#)

Statistical analysis

IBM SPSS Statistics 20 program was used for statistical analyse. 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 $\pm$ 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 (0.01-100µM), resveratrol (0.01-200 µM), nutlin3a (0.01-20 µM) and 5-FU (0.01-20 µM) alone and incubated for 72h. We found that IC50 values of RSV is 53.52 ± 7.162 µM in HCT-116 p53 -/- cells (Fig. 1A) and 48.51 ± 2.25 µM in HCT-116 p53 +/+ cells (Fig. 2A). IC50 values of 5-FU was found as 5.806 ± 0.3414 µM in HCT-116 p53 -/- cells (Fig. 1G) and 2.703 ± 0.2 µM were determined in HCT-116 p53+/+ cells (Fig. 2G). Suramin significantly inhibited viability of HCT-116 p53 +/+ and p53 -/- cells only at 100 µM ($p < 0.01$) (Fig. 1B and fig. 2B). Nutlin3a only at 20µM significantly inhibited cell viability of HCT-116 p53 -/- cells (Fig. 1C) and IC50 value is 2.84 ± 0.20 µM in HCT-116 p53+/+ cells (Fig. 2C). IC50 value of RSV (50µM) and 5-FU (0.01-20µM) combination was determined as 2.587 ± 0.512 µM in HCT-116 p53 -/- cells (Fig. 1D) and 0.024 ± 0.002 µM in HCT-116 p53 +/+ cells (Fig. 2D). IC50 value of suramin (100µM) and 5-FU (0.01-20µM) combination is 0.1894 ± 0.027 µM in HCT-116 p53 -/- cells (Fig. 1E) and 0.077 ± 0.01 µM in HCT-116 p53 +/+ cells (Fig. 2E).

IC50 value of nutlin3a (10 μ M) and 5-FU (0.01-20 μ M) combinations was calculated as $0.758 \pm 0.093 \mu$ M in HCT-116 p53 $-/-$ cells (Fig. 1F) and 0.0095 ± 0.002 were determined in HCT-116 p53 $+/+$ cells (Fig. 2F).

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 protein expressions in HCT-116 p53 $+/+$ and HCT-116 p53 $-/-$ cells, 5-FU, RSV, Suramin and Nutlin3a were treated to cells and incubated for 72h. 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 (Fig. 3A and fig. 4A).

While suramin decreased SIRT5 expression significantly in HCT-116 p53 $-/-$ cells ($p < 0.05$), it didn't change SIRT5 expression significantly in HCT-116 p53 $+/+$ cells. (Fig. 3A and fig. 4A). RSV and Nutlin3a exhibit any significant effect on SIRT5 protein expression in both cells (Fig. 3A and fig. 4A). 5-FU decreased FOXO3a protein expression significantly in HCT-116 p53 $+/+$ and HCT-116 p53 $-/-$ cells ($p < 0.01$) significantly (Fig. 3B and 3C). Suramin and Nutlin3a didn't change Foxo3a expression significantly in both cell lines (Fig. 3B and 4B). RSV, decreased FOXO3a protein expression significantly in HCT-116 p53 $-/-$ cells ($p < 0.01$) (Fig. 3B). 5-FU ($p < 0.01$), RSV ($p < 0.05$), Suramin ($p < 0.001$) and Nutlin3a ($p < 0.001$) increased significantly p53 protein expression in HCT-116 p53 $+/+$ cells (Fig. 4C). 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 (Fig. 3D and fig. 4D). While Suramin ($p < 0.01$) and Nutlin3a ($p < 0.05$) increased Bim protein expression significantly in HCT $+/+$ cells (Fig. 4D), they didn't increase Bim protein expression significantly in HCT-116 p53 $-/-$ cells (Fig. 3D).

Discussion

Colorectal cancer is the third deadliest cancer in the world and many people die every year in the world due to this disease. Chemotherapy takes an important place among colon cancer treatment options and 5-FU is widely used as a chemotherapeutic agent [15]. 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 [16]. A decrease in the response rate can be encountered in case of prolonged use of 5-FU [17]. For these reasons, combined therapies that can increase the sensitivity of cells against 5-FU and the molecular pathways involved in this process are important in the treatment of the disease. Our results show that HCT-116 p53 $+/+$ cells are more sensitive to 5-FU ($IC_{50} = 2.703 \pm 0.2$) to HCT-116 p53 $-/-$ cells ($IC_{50}=5.806 \pm 0.34$) (Figure 1G and figure 2G). These results are consistent with the studies done before with 5-FU [18]. We demonstrated first time that chemotherapeutic agent 5-FU decrease SIRT5 and FOXO3a protein expressions significantly and p53 independently ($p < 0.05$) (Fig 3A, 3B, 4A, 4B). SIRT5 is a member of the sirtuin family whose role in cancer has been least studied. It has dual roles in cancer as tumor suppressor or tumor promoter depending cell conditions, tumor stage and tissue of origin [12,19]. 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 [20,21]. 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 reducing the expression of Nrf2 and downstream targets [22]. SIRT5 overexpression was reported in Waldenstrom'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 [23-25]. Upregulation of SIRT5 was observed in hepatocellular carcinoma cell lines and cancerous tissues and association between poor survival and increase in SIRT5 expression was shown [26]. 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 [27]. SIRT5-mediated Lactate Dehydrogenase B (LDHB) deacetylation has been shown to trigger autophagy and tumorigenesis in colorectal cancer [28]. 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 [29,30]. It was shown that FOXO3a and β -catenin was accumulated in nucleus and this is correlated with metastasis of colon cancer [31]. In acute myeloid leukemia patients which have somatic mutations, there is a linkage between lower overall survival and high FOXO3 expression [32]. Also, in glioblastoma and pancreatic ductal adenocarcinoma progression, it was detected the high FOXO3a expression [33,34]. But besides that there are many studies that indicate the tumor suppressor roles of FOXO3a proteins [35,36]. Acetylation of FOXO has apoptosis promoting effect via activating the proapoptotic 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]. 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 and showed that these modulators have significant effects on 5-FU sensitivity of colon cancer cells. While we determined that RSV and Nutlin3a are more effective in p53+/+ cells than p53-/- cells (Fig. 1D and 1F and fig. 2D and 2F), suramin is more effective in p53 -/- cells when compared to p53 +/+ cells (Fig. 1E and 2E). In our study, in order to explain why the increase in the sensitivity of cells to 5-FU, we investigated the effects of these modulators and 5-FU on SIRT5 and p53 protein expressions as well as FOXO3a and Bim expressions. In this study, we examined the effect of 5-FU on the SIRT5-FOXO3a-Bim axis p53 dependent and independently. Nutlin3a has been shown that to induce p53 expression [40]. For this purpose, while activation of the p53 pathway was provided by nutlin3a in HCT-116 p53 +/+ cells, HCT-116 p53 -/- cells took part in the study as counterpart cells that did not express p53. 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 [41]. Lieber et al. showed that the decrease in SIRT1 and SIRT5 expressions caused posttranslational modification or hyperacetylation of PGC1- α and p53 [7]. We found that SIRT5 modulator RSV increased the sensitivity of HCT-116 p53 +/+ cells to 5-FU much more than HCT-116 p53 -/- cells (Figure 1D and figure 2D). Our results of western blot experiments indicate that RSV hasn't any significant effect on the SIRT5 protein expression in both cell lines (Fig. 3A and fig. 3B) We found that RSV increase SIRT5 protein expression 1.34 fold (0.634 ± 0.08 to 0.851 ± 0.180) in HCT 116 p53+/+ cells and increase 1.17 fold (1.00 ± 0.2 to 1.179 ± 0.38) HCT 116 p53-/- cells but it is not statistically significant. According to the previous studies, it has been found that deacetylase activity of SIRT5 is activated and desuccinylase activity is inhibited by resveratrol [42] and SIRT5 enzyme activity is decreased by suramin and increased by resveratrol [43]. Besides these studies, it has been shown that SIRT5 protein expression is not altered by resveratrol in H295R human adrenal cell lines [44]. We also found that RSV decreased FOXO3a protein expression in HCT-116 p53 -/- cells significantly (Fig. 3B), but it didn't have any significant effect on the FOXO3a protein in HCT-116 p53 +/+ cells (Fig. 4B). So this difference may be due to p53 and FOXO3a protein expression difference. 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. According to Yu et al, when HCT-116 cells were treated with 15 μ M RSV and 0.5 μ M 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 efficiency of RSV which is used in colon cancer treatment [45]. 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 μ M) and 50 μ M RSV. In our study and we found that HCT-116 p53 +/+ ($IC_{50} = 48.51 \pm 2.25$) cells are more sensitive to resveratrol than HCT-116 p53 -/- cells ($IC_{50} = 53.52 \pm 7.162$). Dermani et al reported that apoptosis and cell cycle arrest is induced by resveratrol [46]. In 3DC, resveratrol induce PDE4 activity and luminal cavity formation and induced apoptosis [47]. Resveratrol inhibited colon cancer cell invasion and metastasis by Wnt/ β -catenin signal pathway [48-53]. In this study, we aimed to investigate the effect of SIRT5 modulators on the sensitivity of HCT-116 colon cancer cells to 5-FU. We found that RSV (50 μ M) decrease the IC_{50} of HCT-116 p53 -/- cells from 5.80 ± 0.34 to 2.58 ± 0.512 and decrease the IC_{50} of HCT-116 p53 +/+ cells from 2.703 ± 0.2 to 0.024 ± 0.002 . There are no studies in the literature investigating the effects of SIRT5 and p53 modulators on the sensitivity of colon cancer cells to 5-FU and examining the effect of 5-FU, SIRT5 and p53 modulators on the SIRT5, p53, FOXO3a and Bim protein expressions in both p53 +/+ and p53 -/- colon cancer cells. This is the first study to examine changes in SIRT5 protein expression as a result of p53 activation by nutlin3a and to be used p53 -/- cells as a counterpart cell line. For this purpose, both cells were incubated with nutlin3a for 72h. According to our results, nutlin-3a increased p53 protein expression in p53 +/+ cells compared to the control group (Nutlin-3a free cells) (Fig. 4C). When the effect of nutlin-3a on the expression of SIRT5, FOXO, Bim proteins was investigated, it was found that nutlin-3a significantly increase Bim protein in only p53 +/+ cells (Fig. 4D) and it has not been detected any significant effect on the SIRT5 or FOXO3a expression in p53 +/+ and p53 -/- colon cancer cells. Similar to our results, the increase in nutlin-3a-mediated Bim protein expression found p53 dependent [54]. Renault et al compared FOXO3a protein levels in p53 +/+ and p53 -/- mouse embryonic

fibroblasts (in the presence and absence of doxorubicin) that doxorubicin and nutlin-3a increases FOXO3a in p53 +/+ cells but it didn't change in p53 -/- cells (Renault et al., 2011). RSV and Suramin were used as SIRT5 modulators in this study. And both cells were incubated with RSV(50µM) and Suramin (100µM) for 72h it was shown that both suramin and RSV increased p53 protein expressions in HCT p53 +/+ cells (Figure 4C). In the previous studies, suramin has been shown increases p53 protein levels, but fails to increase p21 mRNA levels or to activate the G₁ checkpoint. These data suggest that suramin induces growth arrest in NIH-3T3 cells by a mechanism that is independent of cellular p53 status [8]. According to our results, RSV decreased FOXO3a protein expression significantly in HCT-116 p53-/- cells, but it didn't any significant effect in HCT-116 p53 +/+ cells (0.54 ± 0.05 to 0.35 ± 0.12) ($p < 0.01$) (Fig. 3B and fig. 4B). And RSV increase p53 and Bim protein expression significantly in HCT-116 p53+/+ cells (Fig. 4 C and 4D) and increase Bim expression in HCT-116 p53-/- cells significantly (Fig. 3D). In previous studies, RSV (20uM) has been shown that reversed SIRT1-7 activity reduced by TNF-α in HUVECs [55]. It has been reported that RSV promote nuclear translocation of FOXO3a and cause apoptosis in HeLa cells [56]. RSV inhibited PI3K-AKT signal transduction pathway, decrease Foxo protein expression and induce apoptosis in H2O2 treated FLS cells [57]. Resveratrol exhibits apoptotic effects via FOXO proteins [30]. It was reported that RSV inhibits orthotopic pancreatic tumor growing via FOXO activation [58]. Resveratrol activates FOXO in prostate cancer xenografts factor [59,60]. 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 [61,12]. We found that while suramin hasn't any statistically significant effect on SIRT5 expression in HCT-116 p53 +/+ cells. We found that suramin decreases SIRT5 protein expression from 0.63 ± 0.08 to 0.47 ± 0.14) but this decrease couldn't be found statistically significant (Fig. 4A), and decreases SIRT5 protein expression significantly in HCT-116 p53 -/- cells ($p < 0.05$) (Fig. 3 A). Suramin decreases FOXO3a in both cells but it is not significant (Fig. 3B and fig. 4B). Suramin increases p53 and Bim protein expressions in HCT-116 p53 +/+ cells (Fig. 4D) and exhibits any significant effect on Bim protein expressions in HCT-116 p53 -/- cells (Fig. 3D). Falcone et al. showed that suramin made colorectal cancer cells sensitive to 5-FU + LV [62].

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 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.

Declarations

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Conflict of interest

Authors confirm that no declaration of interest

Availability of data and material

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

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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Figures

Figure 1

Effect of RSV, Nutlin3a, Suramin, 5-FU, 5-FU and RSV combination, 5-FU and suramin combination and 5-FU and Nutlin3a combination on the viability of HCT-116 p53^{-/-} colon cancer cells. HCT-116 p53^{-/-} colon cancer cells were incubated for RSV, Nutlin3a, Suramin, 5-FU, 5-FU and RSV combination, 5-FU and Suramin combination and Nutlin3a and 5-FU combination for 72h and MTT assay was performed. (A) RSV (0.1-200 μM) decreased viability of HCT-116 p53^{-/-} cells significantly (B) Suramin at 100 μM

concentration decreased cell viability significantly ($p < 0.01$). (C) Nutlin3a at 20 μM decreased cell viability significantly (D) 5-FU (1-20 μM) decreased cell viability significantly (E) 5-FU (0.01-20 μM) and RSV(50 μM) combination decreased cell viability significantly. (F) 5-FU(0.1-20 μM) and Suramin (100 μM) combination decreased cell viability significantly. (G) 5-FU (0.1-20 μM) and Nutlin3a (10 μM) combination decreased cell viability 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 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Figure 2

Effect of RSV, Nutlin3a, Suramin, 5-FU, 5-FU and RSV combination, 5-FU and suramin combination and 5-FU and Nutlin3a combination on viability of HCT-116 p53 +/+ colon cancer cells. HCT-116 p53+/+ colon cancer cells were incubated for RSV, Nutlin-3a, Suramin, 5-FU, 5-FU and RSV combination, 5-FU and suramin combination and Nutlin3a and 5-FU combination for 72h and and MTT assay was performed. (A) RSV (20-200 μM) decreased viability of HCT-116 p53 +/+ cells significantly (B) Suramin at 100 μM concentration decreased cell viability significantly (C) Nutlin-3a (0.5-20 μM) decreased cell viability significantly (D) 5-FU (1-20 μM) decreased cell viability significantly (E) 5-FU (0.5-20 μM) and RSV(50 μM) combination decreased cell viability significantly. (F) 5-FU (0.5-20 μM) and suramin (100 μM) combination decreased cell viability significantly. (G) 5-FU (0.1-20 μM) and nutlin3a (10 μM) combination decreased cell viability 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 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

HCT p53 -/-

Figure 3

Effect of Suramin, RSV, Nutlin-3a and 5-FU on the protein expression of SIRT5, FOXO3a, Bim and p53. HCT-116 p53 -/- colon cancer cells were incubated for RSV, Nutlin-3a, Suramin, 5-FU, 5- for 72h and total cell lysates were prepared and western blot analysis was performed. (A) 5-FU and suramin decreased SIRT5 protein expression significantly ($p < 0.05$). (B) 5-FU and RSV decreased FOXO3a protein expression significantly ($p < 0.01$) (C) p53 protein expression in HCT 116 p53 -/- cells. (D) RSV and 5-FU increased Bim protein expression significantly ($p < 0.01$ and $0 < 0.001$ respectively). 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 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure 4

Effect of Suramin, RSV, Nutlin-3a and 5-FU on the protein expression of SIRT5, FOXO3a, Bim and p53. HCT-116 p53 +/+ colon cancer cells were incubated for RSV, Nutlin-3a, Suramin, 5-FU, 5- for 72h and total cell lysates were prepared and western blot analysis was performed. (A) 5-FU decreased SIRT5 protein expression significantly ($p < 0.01$). (B) 5-FU decreased FOXO3a protein expression significantly ($p < 0.01$) (C) 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. (D) 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, 5-FU and RSV combination, 5-FU and suramin combination and 5-FU and nutlin3a combination treated vs untreated group was shown as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).