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Research Article
5-fluorouracil Synergized with Raloxifene and Cytosine β-D-arabinofuranoside to Combat Colorectal Cancers *in vitro* via Controlling Lipolysis

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Abstract

Background: Colorectal cancers (CRCs) are the 3rd leading mortality cause in the states. Raloxifene (RX) was recently approved for cancer prevention. Therefore, 5-flurouracil (FU), a DNA blocker, stimulates apoptotic cascade in CRC cells. Unfortunately, many of the therapies that use FU and RX are likely to become ineffective due to drug resistance. Therefore, providing cytosine-β-D-arabinoside (CYT), an S-phase specific chemotherapeutic drug, may be of great support. Lipases are principally elaborated in energy metabolism and cancer aggressiveness. Human colorectal cells (HCT 116 and Caco-2) were cultivated in their proper conditions. **Materials and Methods:** These cells were seeded to perform cell proliferation assay using MTT upon RX, FU and CYT combinations. Moreover, cells were proceeded for measuring lipase expression in the supernatant using appropriate lipase assay kit. **Results:** This study observed that RX alone has the most effective cytotoxicity against Caco-2 cells, scoring a very low IC_{50} equal 19.8 μM. Intriguingly, the triple therapy of RX+FU+CYT was the most effective against HCT 116 cells at 100 μM which kills approximately 90% of the cells and scoring a very low IC_{50} equal 38.4 μM. **Conclusion:** This study concluded that the synergistic effect of the triple therapy in the aggressive HCT 116 cells has the potential to kill those cells by inhibiting lipase activity. Killing colorectal cancer cells using FU combinations.

Key words: HCT 116 cells, Caco-2 cells, 5-fluorouracil, raloxifene, cytosine β-D-arabinofuranoside, lipases

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colorectal cancers (CRCs) are malignancies that start in either colon, colon cancer or rectum, rectal cancer. There are two main types of polyps; adenomatous polyps (adenomas) which occasionally change into malignancy; therefore, these adenomas is pre-cancerous and hyperplastic polyps which are more common but overall they are not pre-cancerous[1]. In 2016, American Cancer Society's estimates 95,270 new cases of CRCs about 1 in 21 (4.7%) for men and 1 in 23 (4.4%) for women. It is the 3rd leading cause of mortality in the states when both sexes are measured distinctly and the 2nd leading cause when both sexes are combined. It is estimated to cause approximately 49,190 deaths throughout 2016[2].

Egypt was entirely lacking incidence rates of CRCs internationally until the outputs given from multicenter hospital offices that could not be used for releasing the occurring rates[3-7]. Upto 1999, the only available rates are those from a cancer center in one region in Nile delta[8,9]. Occurrence rates upto 2007 were issued in volumes IX and X in five zones[10,11]. The published rates from that registry are 96.5 and 132.6/100,000 males and 97.3 and 122.1/100,000 females.

Raloxifene (RX) is a 2nd generation Selective Estrogen Receptor Modulators (SERM) sanctioned by the Food and Drug Administration (FDA) for the osteoporosis and the invasive breast cancer preventions[12]. Contemporary clinical trials presented that raloxifene expressively reduced the breast cancer occurrence in high-risk women by a lower effect compared to tamoxifen (38 versus 50%, respectively)[13].

Intriguingly, in contrast to tamoxifen and raloxifene does not cause endometrial proliferation[14]. Therefore, to enhance the cytotoxic effect on cancer with keeping its effect against endometrial proliferation and novel effective combinations are recommended.

The CRCs also fluctuate in their first response to 5-flurouracil (FU). The major tricky in the CRC chemoprevention is owing to those cells that are in residence in the G0-phase where they are less susceptible to routine chemotherapy. To overcome this phenomenon, researchers struggled to recruit the reentry of these cells into the cell cycle using a way to control tumor progression[15]. Mechanistically, FU is well-known to block DNA synthesis by interfering with thymidylate synthase which is controlled by cell cycle elements[16]. The anti-cancer effectiveness of FU is owing to the stimulation of the apoptotic cascade of Bax, relative to bcl-2 and bcl-xL in CRC cells[17,18]. Unfortunately, many of the therapies that use FU alone or in combination with other agents are likely to become ineffective due to drug resistance.

Cytosine-β-D-arabinoside (CYT) is an S-phase specific key chemotherapeutic drug with verified clinical value. It is mainly used in leukemia therapy[19]. Recently, it was widely used in comparatively resistant solid cancers[20]. Ovarian cancer management via CYT has been exposed to be of therapeutic importance[21].

Lipases are principally convoluted in energy metabolism. Since, the primary account of lipases in the 19th century, several other lipolytic enzymes have been pronounced[22]. Especially, phospholipases are elaborated in multiple cancer-relevant signaling networks and have been revealed to be linked with cancer growth and development. Stratagems for the development of specific inhibitors for these lipases have been developed and owing to lipases with high expression in cancerous cells can be deliberated as possible targets for cancer response[23].

Overall, all of these previous observations encouraged to design the first study in which the anti-cancer effect of FU against colorectal cancer cell lines (HCT 116 and Caco-2) was ameliorated when combined with raloxifene and/or cytosine β-D-arabinofuranoside. The synergistic effects between the combinatorial therapies are likely to become effective against CRC resistance via targeting lipase and inhibiting its expression.

MATERIALS AND METHODS

Cell cultivation: Human colorectal cell lines (HCT 116 and Caco-2 cells) were purchased from American Type Culture Collection (ATCC). Caco-2 cells were propagated in the proper conditions (at 37°C and 5% CO_2) and maintained in Roswell Park Memorial Institute medium (RPMI-1640) with 1% L-glutamine (St Louis, MO, USA) and supplemented with 10% fetal calf serum for growth and 1% penicillin/streptomycin (Wexford, Ireland). Meanwhile, HCT 116 cells were cultured and propagated in a Complete-Dulbecco's Modified Eagle Medium (C-DMEM). When the cells are approximately 80% confluent and they were sub-cultured using trypsin-EDTA (Lonza, USA).

Cell line authentication: Determined the authenticity of HCT 116 and Caco-2 cells used in this study according to the ATCC method as cell lines are prone to change by cross-contamination or infection. This routine authentication process had three steps. (1) Assessed cell morphology (cell size, shape and performance) of both cell lines in order to confirm the health of these cells before the experiments. The early signs of bacterial and/or fungal contamination were also

detected in this way, (2) The HCT 116 and Caco-2 cell passaging were performed in their log growth phase in order to ensure the use of healthy cells by evaluating their proliferation. Growth curve analysis was done to determine population doubling times and (3) Mycoplasma contamination was detected using PCR-based approach for the detection and identification of multiple mycoplasma strains.

Cell proliferation assay: The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrasodium bromide (MTT, Sigma) is based on the conversion of MTT into formazan crystals by living cells which determines mitochondrial activity[24]. The mitochondrial activities of the 5-fluorouracil (5-FU), raloxifene (RX) and cytosine β-D-arabinofuranoside (CYT) individually or combined were measured by MTT assay using HCT 116 and Caco-2 cells. Briefly, the cells were cultured in 96-well plates at a density of 1×10^4 cells per well. About 0, 20, 40, 60, 80 and 100 μM of the chemotherapies were added per well in RPMI-1640 (Caco-2 cells) and DMEM (HCT 116 cells). Also, media without drug was added as a control. After 24 h incubation, MTT dissolved in PBS was added to each well at a final concentration of 5 mg mL^{-1} and the samples were incubated at 37°C for 4 h. Water-insoluble crystals of formazan that formed during MTT cleavage in actively metabolizing cells were then dissolved in dimethyl sulfoxide (DMSO). Absorbance was measured at 455 nm using a microplate reader (Model 500; BIORed Instrument Inc., USA). The mitochondrial activity (%) was calculated and compared with the control.

Half inhibitory concentration (IC_{50}) and fold change: The half maximal inhibitory concentrations (IC_{50}) values, the concentrations inhibit 50% of cell viability were obtained by plotting the percentages of cell viability versus the concentrations of the sample using polynomial concentration-response curve fitting models (OriginPro 8 software). Finally, the fold change of the combinatorial chemotherapies; RX+FU, RX+CYT and RX+FU+CYT versus the individual doses of FU, RX and CYT in HCT 116 and Caco-2 cells were measured as well.

Lipase activity: One million HCT 116 and Caco-2 cells were seeded and incubated at CO_2 incubator overnight. After 24 h incubation, 0, 20, 40, 60, 80 and 100 μM of the chemotherapies (5-FU, RX and CYT) were added per well in RPMI-1640 (Caco-2 cells) and DMEM (HCT 116 cells). After 24 h incubation, cells were harvested by trypsin/EDTA and washed by cold PBS. Then, CRC cells were resuspended in 100 μL of assay buffer and centrifuged for 5 min at 4°C at top speed to remove any insoluble material. Supernatant was collected and then lipase levels were assessed using the lipase assay kit from spectrum diagnostics.

Statistical analysis: All assays were repeated three times. Comparisons between groups versus controls were made using a two-tailed Student's t-test and values of $p<0.05$ were considered statistically significant using SPSS (version 17). Moreover, the IC_{50s} were made using a polynomial fitting of the OriginPro 8 program.

RESULTS

FU synergized with RX and CYT to significantly induce HCT 116 cell death: The cytotoxicity of RX, FU, RX+FU, CYT, RX+CYT and RX+FU+CYT were investigated against human colorectal HCT 116 cell line at different concentrations (0, 20, 40, 60, 80 and 100 μM) using MTT method. Data and images illustrated in Fig. 1a and c shows the percentage of viability of HCT 116 cells after 24 h incubation from the treatments versus control. The results revealed a significant dose dependent decrease ($p<0.05$) in cell viability of HCT 116 cells upon FU, CYT and RX+FU treatments with a high significant dose dependent decrease ($p<0.01$) in cell viability of HCT 116 cells upon individual RX as well as combinatorial RX+CYT and RX+FU+CYT therapies. Intriguingly, the triple therapy of RX+FU+CYT was the most effective against HCT 116 cells at 100 μM which kills approximately 90% of the cells, scoring a very low IC_{50} equal 38.4 μM and the highest fold change of 2.6 times over CYT and FU (Table 1). Furthermore, RX and RX+CYT had IC_{50s} equal 28.7 and 82.4 μM, respectively. However, the IC_{50} of the RX alone was lower than a little bit that of the triple therapy (RX+FU+CYT) on HCT 116 cells and the triple therapy was more powerful at 100 μM (Fig. 1a, c, Table 1). On the contrary, FU, CYT and RX+FU had a non-detectable IC_{50} (i.e., >100 μM) in HCT 116 cell line as illustrated in Table 1.

RX alone induces Caco-2 cell death more effectively than the combinatorial therapy: The cytotoxicity of RX, FU, RX+FU, CYT, RX+CYT and RX+FU+CYT were investigated against human colorectal Caco-2 cell line at different concentrations (0, 20, 40, 60, 80 and 100 μM) using MTT method. Data and

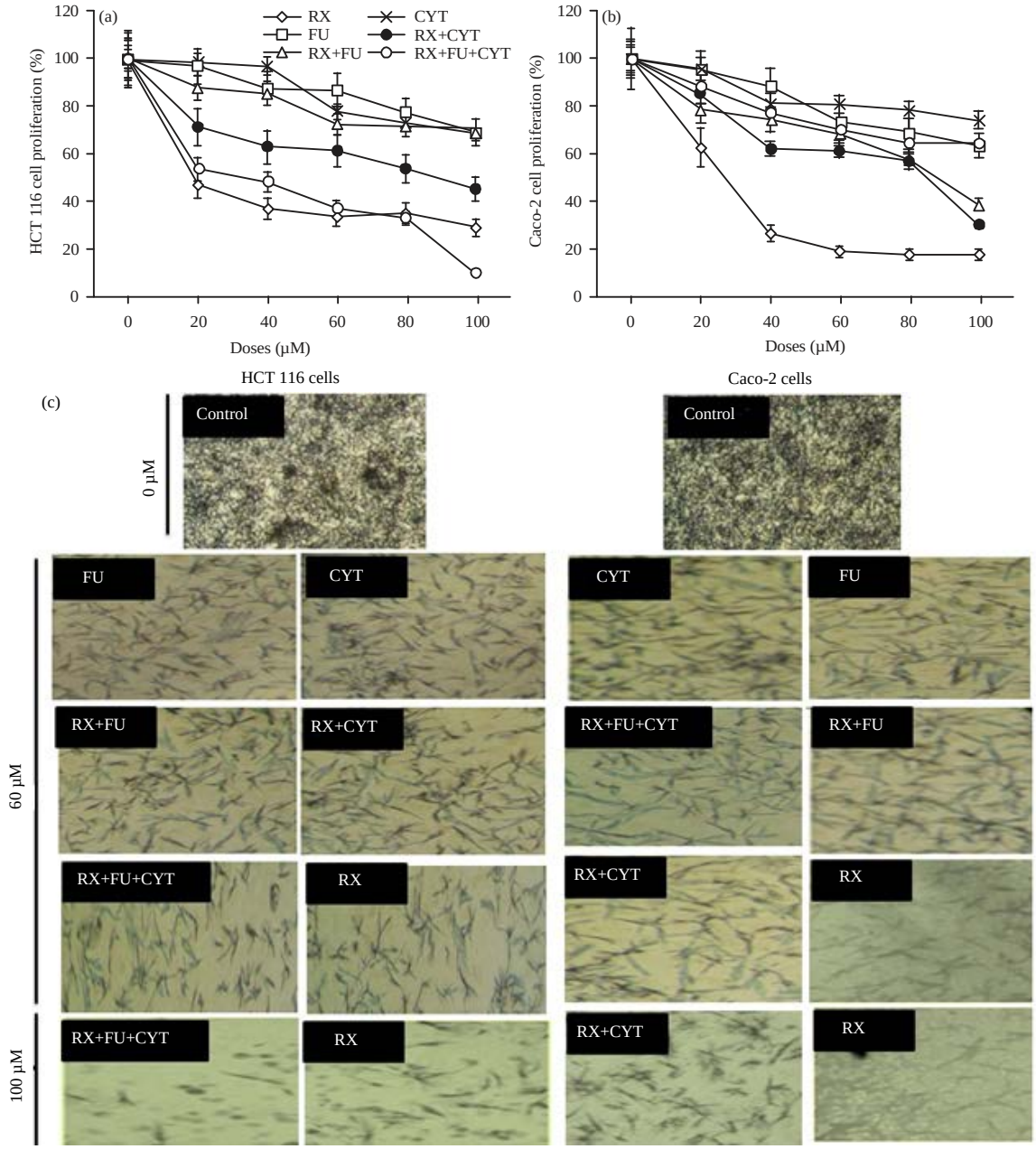

Fig. 1(a-c): Cytotoxicity assay and crystal imaging, (a) MTT-mediated cell death of HCT 116, (b) Caco-2 cell lines (n = 3) and (c) Their water-insoluble crystals of formazan that formed during MTT cleavage in actively metabolizing HCT 116 and Caco-2 cells at 60 and 100 μM of the individual and the combinatorial therapeutic regimens of FU, RX and CYT after 24 h incubation

images in Fig. 1b and c illustrated the percentage of viability of Caco-2 cells after 24 h incubation from the treatments versus control. The results revealed a significant dose dependent decrease ($p<0.05$) in cell viability of Caco-2 cells upon FU, CYT and RX+FU+CYT therapies with a high significant dose dependent decrease ($p<0.01$) in cell viability of Caco-2 cells upon individual RX as well as combinatorial RX+FU and RX+CYT treatments. Intriguingly, on the contrary of HCT 116, the triple therapy of RX+FU+CYT had an undetectable IC_{50} (i.e., >100 μM) in Caco-2 cell line (Fig. 1b, c, Table 1).

The RX alone has the most effective cytotoxicity against Caco-2 cells at all doses and kills approximately 82.5% of the cells, scoring a very low IC_{50} equal 19.8 μM. Furthermore, the

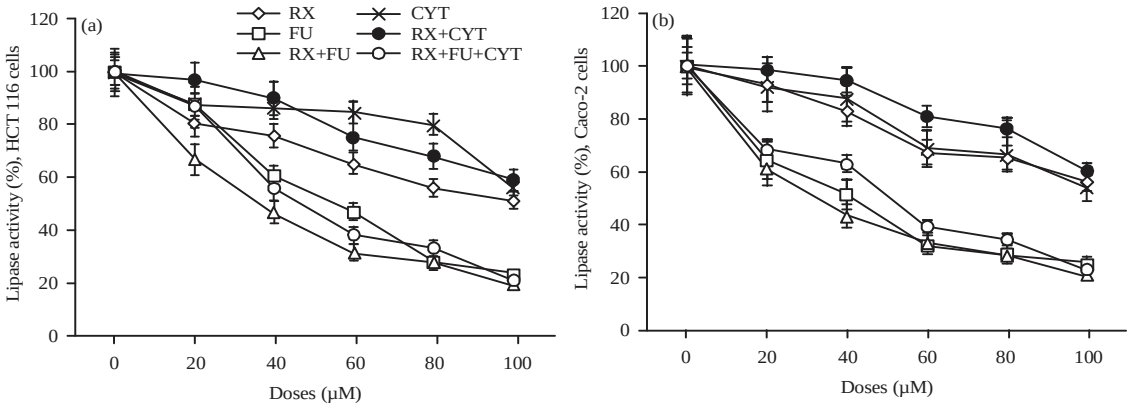

Fig. 2(a-b): Lipase activity. Cells were seeded and a wide range of doses (0-100 µM) of the individual and the combinatorial therapeutic regimens of FU, RX and CYT were added, (a) Lipase levels in HCT 116 and (b) Caco-2 cell lines were measured in the supernatant using the lipase assay kit after treatment

Table 1: Cytotoxicity IC_{50} of the individual and combinatorial regimens of FU, RX and CYT in HCT 116 and Caco-2 cell lines

Drugs	HCT 116 cells	Fold change in IC_{50}	p-value	Caco-2 cells	Fold change in IC_{50}	p-value
FU	>100	-	-	>100	-	-
RX	28.7	-	-	19.8	-	-
RX+FU	>100	1	>0.05	76.1	1.31	<0.05
CYT	>100	-	-	>100	-	-
RX+CYT	82.4	1.21	<0.05	64.1	1.56	<0.05
RX+FU+CYT	38.4	2.60	<0.01	>100	1	>0.05

Cells were treated for 24 h, IC_{50} for three experiments is shown, fold change was calculated by dividing the IC_{50} of the individual compound (FU, RX and CYT) by that of the respective combinatorial regimen (RX+FU, RX+CYT and RX+FU+CYT), p-values show statistically significant differences between mean IC_{50} of the combinatorial regimen and their individual counterparts, FU: 5-flurouracil, RX: Raloxifene, CYT: Cytosine β-D-arabinofuranoside and IC_{50}: Half maximal inhibitory concentration of cell growth

Table 2: Lipase IC_{50} of the individual and combinatorial regimens of FU, RX and CYT in HCT 116 and Caco-2 cell lines

Drugs	HCT 116 cells	Caco-2 cells
RX	>100	>100
FU	55.3	37.6
RX+FU	37.2	34.8
CYT	>100	>100
RX+CYT	>100	>100
RX+FU+CYT	50.5	49.2

Cells were treated for 24 h, Lipase levels were measured after treatment, IC_{50} for three e xperiments is shown upon treatment of the individual compound (FU, RX and CYT) by that of the respective combinatorial regimen (RX+FU, RX+CYT and RX+FU+CYT), p-values show statistically significant differences between mean IC_{50} of the combinatorial regimen and their individual counterparts, FU: 5-flurouracil, RX: Raloxifene, CYT: Cytosine β-D-arabinofuranoside and IC_{50}: Half maximal inhibitory concentration of lipase levels

IC_{50s} of RX+FU and RX+CYT were 76.1 and 74.1 µM, respectively. On the contrary, the same as the triple therapy FU and CYT had non-detectable IC_{50s} (i.e., >100 µM) in Caco-2 cell line (Table 1).

Individual and combination therapies of FU specifically inhibit lipase activity in both HCT 116 and Caco-2 cells: Human colorectal cancer cells, HCT 116 and Caco-2 were treated for 24 h with different concentrations of RX, FU, RX+FU, CYT, RX+CYT and RX+FU+CYT. Lipase levels were investigated using the lipase assay kits. Data in Fig. 2a and b illustrates the change percentage of lipase expression in HCT 116 and Caco-2 cells upon therapy versus control. The results revealed a significant dose dependent inhibition ($p<0.05$) in lipase levels of the human colorectal cancer cells upon RX, CYT and RX+CYT therapies while, there was a high significant dose dependent inhibition ($p<0.01$) in lipase levels of the human colorectal cancer cells upon the individual and combinatorial therapies of FU (i.e., FU, RX+FU and RX+FU+CYT). Intriguingly, FU, RX+FU and RX+FU+CYT had high IC_{50s} equal 55.3, 37.2 and 50.5 µM in HCT 116 cells and 37.6, 34.8 and 49.2 µM in Caco-2 cells, respectively as illustrated in Table 2.

DISCUSSION

Due to the global problems of colorectal cancer resistance and 5-flurouracil (FU) is likely to become ineffective due to drug resistance, this study has used two drugs in combination with FU; cytosine-β-D-arabinoside (CYT), an S-phase specific key chemotherapeutic drug and raloxifene (RX), 2nd generation Selective Estrogen Receptor Modulators (SERM)

approved by the Food and Drug Administration (FDA) for the osteoporosis and recently the invasive breast cancer preventions. In addition, it used a novel strategy to target lipase which is principally convoluted in energy metabolism and cancer aggressiveness.

The most universally known treatment for colorectal cancer (CRC) is chemotherapy yet to eliminate the issue of chemoresistance, new option strategies required in the treatment of CRC. Expression examples of various molecules in various signaling pathways in cancer-causing tissue and typical tissues help to comprehend their positive/negative part in carcinogenesis. This will help in picking potential chemopreventive agents[25].

The current study observed that raloxifene (RX) alone has the most effective cytotoxicity as compared to 5-flurouracil (FU) and cytosine-β-D-arabinoside (CYT) against human colorectal cancer Caco-2 cells at all doses and kills approximately 82.5% of the cells, scoring a very low IC_{50} equal 19.8 μM. On the other hand, RX had an IC_{50} equal 28.7 μM in HCT 116 cells. This study is the first one to assess the impact of RX on colorectal cancers however, this study obtained results came to agree with some previous studies approved the cytotoxic effect of RX against mammary, breast, cervical and lower reproductive tract cancers[26,27]. Raloxifene was found to restrain the growth of 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumors[28]. Before 2007, the main approved-indication of raloxifene was for avoidance and treatment of osteoporosis; however, the consequences of the investigation of tamoxifen and raloxifene (STAR) trial investigated that raloxifene is as powerful as tamoxifen in diminishing the danger of invasive breast cancer with decreased risk of fractures and stroke compared with tamoxifen[29,30]. Thus, raloxifene is likewise utilized as a part of the counteractive action of breast cancer in post-menopausal ladies with increased risk of developing the disease[31-33].

On the other hand, treatment of squamous cell carcinomas of the lower reproductive tract with either the complete ERa antagonist, fulvestrant (ICI 182,780) or the Selective Estrogen Receptor Modulator (SERM) and raloxifene was very productive in advancing tumor regression[26].

Inspection of the recurrence of lower reproductive tract tumors after treatment with RX showed that neoplastic illness recurs after cessation of treatment and regardless of exogenous estrogen in spite of the fact that it increased the recurrent of neoplastic disease. All recurrent cancers retained an active estrogen/ERa signaling pathway and were responsive to retreatment with RX. The study speculated that keeping mice on RX treatment prevented the high recurrence rates of cancer. This study bolsters the reason that SERMs for example, RX might be successful in treating HPV-related human cervical tumors however, their adequacy may require long haul treatment[27]. Thus, the hypothesis of the present study was to make combinatorial regimens of RX, FU and CYT to enhance the synergistic effect for killing human colorectal cancers (HCT 116 and Caco-2 cells).

In this study, making a combinatorial therapy of RX and FU increases the cytotoxic power against human colorectal cancer Caco-2 cells with a detectable IC_{50} equal 76.1 μM. The CRCs also fluctuate in their first response to 5-flurouracil (FU)[15] so, its combination with RX decreased this unfavorable fluctuation and the cytotoxic effect gradually decreased with a significant manner. Unfortunately, the major tricky in the CRC chemoprevention is owing to those cells that are in residence in the G0-phase where they are less susceptible to routine chemotherapy. To overcome this phenomenon, researchers struggled to recruit the reentry of these cells into the cell cycle using a way to control tumor progression[15]. Mechanistically, FU is well-known to block DNA synthesis by interfering with thymidylate synthase which is controlled by cell cycle elements[16]. The anti-cancer effectiveness of FU is owing to the stimulation of the apoptotic cascade of Bax, relative to bcl-2 or bcl-xL in CRC cells[17,18]. But, studied the mechanism of action from another point of view, depending on the bio-energetics deprivation of the colorectal cancer cells by inhibiting lipase activity which represents one of the main sources of producing energy required for cancer aggressiveness as illustrated in Fig. 3.

Few studies have mentioned the reasonable relationship amongst obesity and the risk of CRC. Adipose tissue incorporates lipoprotein lipase (LPL), the critical enzyme for intravascular catabolism of triglyceride (TG)-rich lipoproteins. The opposite association of both LPL and Fatty Acid Synthase (FAS) in tumors has been generally mentioned in various studies in their actions in visceral adipose tissue gathered from CRC patients and cell lines and this supports tumor development. Lipases are basically required in energy metabolism and the generation of second messengers. Thus, lipases with high expression in tumor cells can be considered as potential targets for cancer cells as illustrated in this HCT 116 cell line (Fig. 3)[22,23,34,35].

Some studies demonstrated a huge reduction in both LPL and FAS gene expression and activity in adipose tissue near to tumor injury. This outcome underlines the impact of the tumor micro-environment on lipid digestion showing a tumor-induced degeneration in the formation and

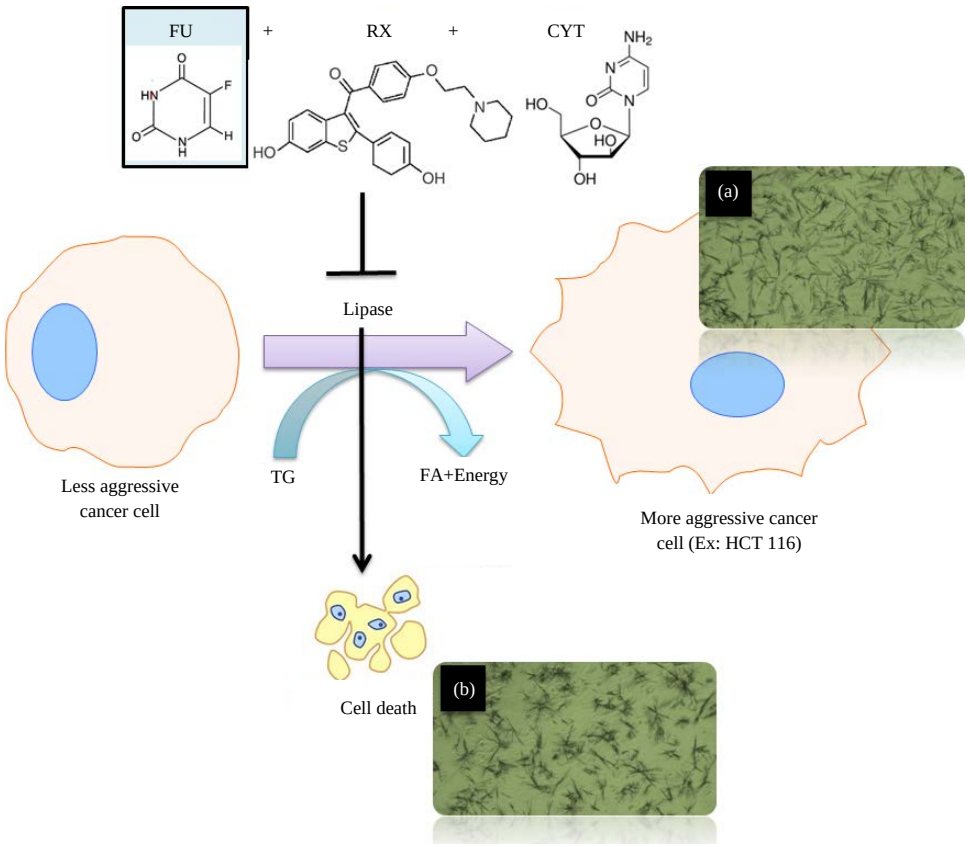

Fig. 3: Schematic diagram of the underlying mechanism behind the FU+RX+CYT-mediated cell death via lipase inhibition, basically, lipase is predominantly convoluted in energy metabolism for elaborating multiple cancer-relevant signaling networks, this is relevant to be linked with cancer aggressiveness, tested whether this therapeutic regimens have the power to inhibit lipase expressions in colorectal cancer cells and found that the synergetic effect between FU, RX and CYT (triple therapy) can successfully mediates HCT 116 cancer cell death via bio-energetics deprivation through lipase inhibition. Water-insoluble crystals of formazan that formed during MTT cleavage in actively metabolizing HCT 116 cells without treatment (a) After treatment with the triple combinatorial regimen and (b) At the IC_{50} (38.4 μM) for 24 h

lipid-storing ability of adipose tissue in CRC[36]. Deregulated lipid metabolism seems to improve colorectal malignancy. Phospholipases A2 (PLA2) catabolizes phospholipid to create lysophospholipids and its over expression has been found in colorectal adenomas from familial adenomatous polyposis to increase the levels of lysophosphatidylcholine and phosphatidylcholine plasmalogen in colorectal cancer when contrasted with normal tissues[37,38]. Phospholipids are critical not just in the development of the cytoplasmic membrane and membranes of various organelles but additionally in the control of numerous cellular processes such as gene transcription, cell signaling, cell survival and proliferation. In any case, the molecular mechanisms underlying the deregulations of phospholipid metabolism remains not fully understood and should be further investigated[39].

The current study revealed that there is a significant dose dependent inhibition of lipase levels of the colorectal cancer cells upon FU, CYT and RX+CYT therapies while, there was a high significant dose dependent inhibition in lipase levels of those cells upon the individual and combinatorial therapies of FU. Intriguingly, FU, RX+FU and RX+FU+CYT had high IC_{50s} equal 55.3, 37.2 and 50.5 μM in HCT 116 cells and 37.6, 34.8 and 49.2 μM in Caco-2 cells, respectively. The FU is an anti-CRC agent has been demanded to have advantageous effects in human pancreatitis because of its ability to inhibit protein synthesis and secretion for example, lipases. Protein synthesis and secretion was significantly depressed in isolated pancreatic acini derived from rats with sodium taurocholate-induced pancreatitis[40]. On the contrary, in the response of the combination of FU and polyunsaturated fatty

acids (PUFAs) on gastric cancer cell line in relation to the ability of the cells to secrete tumor necrosis factor-α (TNF-α) and vascular endothelial growth factor (VEGF) and lipid metabolism-related factors lipoprotein lipase (LPL), peroxisome proliferator-activated-γ (PPAR-γ) and CCAAT enhancer-binding protein (C/EBP) it was found that cells produced a significant growth inhibitory action compared with either agent alone by inhibiting the production of TNF-α and VEGF and a simultaneous increase in the expression of LPL, PPAR-γ and C/EBP so, it was dedicated that PUFAs enhance the tumoricidal action of the FU by acting on anti-antigenic factors and enzymes involved in lipid metabolism[41].

This study results revealed a significant dose dependent decrease in cell viability of HCT 116 cells upon CYT treatment with a high significant dose dependent decrease in cell viability of HCT 116 cells upon combinatorial RX+CYT and RX+FU+CYT therapies. Intriguingly, the triple therapy of RX+FU+CYT was the most effective against HCT 116 cells at 100 μM which kills approximately 90% of the cells, scoring a very low IC_{50} equal 38.4 μM and the highest fold change of 2.6 times over CYT and FU. Furthermore, RX+CYT had IC_{50} equal 82.4 μM. However, the IC_{50} of the RX alone was lower than a little bit that of the triple therapy (RX+FU+CYT) on HCT 116 cells, the triple therapy was more powerful at 100 μM as illustrated in Fig. 1c. Intriguingly, on the contrary of HCT 116, the triple therapy of RX+FU+CYT had an undetectable IC_{50} in Caco-2 cell line. Furthermore, the IC_{50} of RX+CYT was 74.1 μM. Researchers reported that CYT is an S-phase specific major chemotherapeutic agent with proved clinical efficacy, mainly in acute non-lymphoblastic leukemia. Recent trials have attempted its use also in relatively resistant solid tumors[20]. Administration of CYT to ovarian cancer patients with small residual disease has been shown to be of therapeutic value[21]. The CYT at low doses has recently been shown to induce differentiated features in neuroblastoma cell lines[42]. The combined treatment of CYT and guanosine on melanoma cell lines represents a relatively chemotherapy for resistant malignancy. The results demonstrated a synergistic anti-proliferative effect of guanosine on these cells[43]. Intriguingly, lipase levels upon RX+FU+CYT treatment had a high IC_{50} equal 50.5 μM in HCT 116 cells and 49.2 μM in Caco-2 cells.

CONCLUSION

In summary, the synergistic effect of the triple therapy of RX+FU+CYT in the metastatic colorectal cancer HCT 116 cells has the potential to kill those cells by remarkably inhibiting lipases and in turn bio-energetically deprives them. On the other hand, RX alone has the most effective cytotoxicity against Caco-2 cells and induces mitochondrial cell death. These therapeutic strategies will help us to overcome the global resistance concern of the CRCs.

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