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The Influence of 5-Fluorouracil on Drug Transporters is a Dose-Dependent Effect Mediated by Altered Expression of miRNAs

Sherien M. El-Daly [1,2*], Shaimaa A. Gouhar [1], Mahmoud T. Abo-elfadl [2,3]

[1] *Medical Biochemistry Department, Medicine and Clinical Studies Research Institute, National Research Centre, Dokki 12622, Cairo, Egypt.*

[2] *Cancer Biology and Genetics Laboratory, Centre of Excellence for Advanced Sciences, National Research Centre, Cairo, Egypt.*

[3] *Biochemistry Department, Biotechnology Research Institute, National Research Centre, Cairo, Egypt.*

Abstract

ATP-binding cassette (ABC) transporters are crucial in transporting nutrients into cells and expelling xenobiotics out of cells to ensure cell viability. Several investigations have shown interactions between drug transporters and anti-cancer drugs. Overexpression of drug transporters is a primary factor leading to multi-drug resistance (MDR), which may facilitate the efflux of many chemotherapeutics from cancer cells, hence reducing intracellular drug concentration. In the present study, we aimed to delineate the dose-dependent effect of one of the most applied chemotherapeutic drugs, 5-fluorouracil (5-FU), on the expression of drug transporters in colorectal cancer. We investigated the effect of different doses of 5-FU on the expression of a panel of ABC transporters, and we also evaluated the differential expression of miRNAs involved in drug resistance. Our study revealed that a low dose of 5-FU can upregulate ABCC2, ABCG2, ABCB1, and ABCC5 transporters significantly. However, by increasing the dose of 5-FU, the expression of these drug transporters decreased dynamically. The same pattern of expression was also detected in miRNAs, where the lowest dose of 5-Fu was responsible for the highest elevation in miR-27a, miR-31, and miR-200c levels, and by increasing the dose of 5-FU, this elevation significantly decreased. Our results suggest that a low dose of 5-FU may promote the development of drug resistance in colorectal cancer by enhancing drug transporters, stressing the need of adopting a chemotherapeutic dose that has an acceptable cytotoxic impact while also being effective in reducing the activity of drug transporters

Keywords: 5-Fluouracil ; colorectal cancer ; ABCC2 ; ABCG2 ; CYP3A4; miR-27a ; miR-31; miR-200c

1. Introduction

Colorectal cancer (CRC) is the second-deadliest and fourth-most-diagnosed disease worldwide, responsible for 11% of all cancer cases (Ferlay et al., 2020). Despite declining overall death rates, CRC is becoming more common in people aged 20 to 49 in numerous populations, including Egyptians (Rawla et al., 2019). CRC has no clear age preference in Egypt; however, research suggests that young patients have a higher risk of the disease, which increases its complexity and effect (Elhadidy & Haydara, 2022).

Therapy with 5-fluorouracil (5-FU) has been shown to reduce mortality in a variety of malignancies, with CRC having the most significant efficacy (Vodenkova et al., 2020). The first-line treatment for CRC is still 5-FU. Although 5-FU debulks the tumor mass, relapse and drug resistance following chemotherapy seems to be severe issue for CRC management (Cho et al., 2020).

Malignant cells gain resistance to a wide number of physiologically and structurally unrelated chemotherapeutic agents after being exposed to one for a long time. This type of resistance is called multi-drug resistance (MDR). Drug transport is a carefully controlled procedure controlled by the ATP-binding cassette (ABC) transporter family of proteins (Vasiliou et al., 2009). MDR in cancerous cells can reduce the effectiveness of chemotherapy. The primary mechanism involved in conferring MDR is the upregulation of several members of the ABC

*Corresponding author; Sherien M. El-Daly, e-mail: sm.el-daly@nrc.sci.eg.; sherien_eldaly@yahoo.com.

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transporters family, specifically ABCB1, ABCC1, ABCC2, and ABCG2 (Leslie et al., 2005). This can enhance drug efflux from tumor cells, lowering intracellular drug concentration and leading to a therapeutic failure (Mendoza et al., 2007; Sun et al., 2012).

The ABC transporter family is classified into seven subfamilies according to sequence similarity and structural organization. P-glycoprotein or multi-drug resistance protein 1 (MRP1)/ABCB1, and breast cancer resistance protein (BCRP)/ABCG2 comprise the majority of identified MDR in humans. Such proteins are capable of recognizing and transporting a wide range of chemically different drugs (Dean et al., 2001; Juliano & Ling, 1976). In CRC, completely nonresponsive cases often have upregulated ABC transporters (Hlavata et al., 2012). Several ABC transporters have been found to increase resistance to 5-FU and its active derivative metabolites-based CRC treatments, either directly or indirectly (Blondy et al., 2020; Pratt et al., 2005; Xie et al., 2017).

Although various mechanisms of MDR have been suggested, such as drug transporter dysregulation, defects of apoptosis and autophagy, modifications of drug metabolism and drug targets, disruption of redox homeostasis, the real mechanisms of MDR in cancer cells and the cross-talk among these different mechanisms and how they are regulated remain unclear (An et al., 2017). MicroRNAs (miRNAs) appear to be essential participants in the regulation of MDR. MiRNAs are small single sequence non-coding RNA that function by regulating the expression of their target genes post-transcriptionally through RNA interference mechanism. MiRNAs are involved in regulating many biological pathways, including proliferation, differentiation, cell cycle, and apoptosis (Gouhar et al., 2022, Winkle et al., 2021; El-Daly et al., 2020b). Various studies have also shown that miRNAs control the expression of several members of the drug transporter family. Consequently, the alteration in the expression of miRNAs is linked with the development of MDR and chemoresistance (Blower et al., 2008; Schmittgen et al., 2008; Zheng et al., 2010).

Although, tumors resistance to chemotherapy is common, probably as a result of drug transporter upregulation. It's still not clear if drug resistance develops in vitro at clinically relevant dosages of chemotherapeutic agents and whether the intensity of drug resistance can be linked to increased expression levels of specific drug transporters in a temporally and causative manner. To address these points, we used HT29 cells as a model for CRC to be exposed to different doses of 5-FU, and the expression of various drug transporters was evaluated. Also, the differential expression of miRNA related to MDR was investigated. The findings of our study suggest that a low dose of 5-FU may promote the development of drug resistance through the detected overexpression of drug transporters, stressing the need of adopting a chemotherapeutic dose that has an acceptable cytotoxic impact while also being effective in lowering the activity of drug transporters.

2. Materials and Methods

2.1. Chemicals

5-fluorouracil (5-FU) was purchased directly from Sigma-Aldrich (St. Louis, USA). 5-FU was dissolved in dimethyl sulfoxide (DMSO) in the dark at 1000-fold higher concentrations than the final cell test concentration. In all assays, 5-Fu was diluted in the medium before being treated to cells. Gibco (Thermo Fisher Scientific, NY, USA) and Corning Inc. were the provider source for cell culture materials. All real-time PCR supplies were purchased from Qiagen.

2.2. Cell Culture

Human colon adenocarcinoma cell line HT29 cells were grown in RPMI 1640 media supplemented with 10% fetal bovine serum, 2mM L-glutamine, and a penicillin/streptomycin cocktail. Cells were incubated at 37°C, 5% CO2 in a humidified environment. Adherent cells were maintained in monolayer culture and were regularly passaged every 3-4 days with Trypsin-EDTA 0.25%.

2.3. Cytotoxicity by MTT

The cytotoxicity of 5-FU was assessed in the HT29 cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, which relies on the reduction of tetrazolium salt to formazan crystals by active mitochondrial dehydrogenases (Hansen et al., 1989, Batran et al., 2022). First, the cytotoxicity of various dosages of 5-FU was examined, and the best doses to be employed in subsequent trials were determined. HT29 cells were seeded at a density of $1x10^4$ in a 96-well plate for 24h incubation. Cells were subsequently treated for 48 and 72 hours with varying concentrations of 5- FU (doses ranged from 1.56 µM to 100 µM). After 48 or 72 hours of treatment, MTT was added to all wells and incubated for 4 hours. The resulting formazan crystals were solubilized with 10% SDS/0.01M HCl. An absorbance microplate reader was used to measure the absorbance at 570nm and a reference wavelength of 690nm.

Cell viability following treatment with different doses of 5-FU was calculated according to the equation; Viability % = Absorbance of treated cells / Absorbance of control cells * 100 (Gamal-Eldeen et al., 2017, Yan & Caldwell, 2004). Depending on the results obtained from the MTT assay, three moderate toxic doses of 5-FU were chosen to be applied for all further experiments; 50 µM, 25 µM, and 12.5 µM.

2.4. RNA Extraction, Reverse Transcription, and RT-PCR.

HT29 cells were seeded at a density of 5×10^5 cells/ well in T75 cell culture flasks. Cells were individually treated with the different doses of 5-FU (50 uM, 25 uM, or 12.5 uM) and incubated for 48 h in a humidified 5% CO2 incubator at 37 °C. Following the incubation, cells were harvested, and total RNAs, including small sequence RNAs (miRNAs), were extracted and purified from treated- and untreated HT29 cells using Qiagen's miRNeasy Mini Kit (Hilden, Germany) according to the instructions of the manufacturer. Real-time PCR was performed using SYBR Green PCR reagents on a Light Cycler Agilent Mx3000P. For gene expression analysis, β-actin was set as the internal control, and for miRNA expression analysis, RNU6 was the internal control. Cycling conditions applied were optimized following our previous work (El-Daly et al., 2019a,b). The cycle threshold values were detected, and fold changes were estimated with the $2^{-\Delta\Delta Ct}$ analysis approach. Primers for genes and miRNAs used or RT-PCR were purchased from Qiagen's customized catalog. The primers used are as follows; ABCC2 (Cat#QT00056294), ABCB1 (Cat# QT00081928), ABCG2(Cat# QT00073206), ABCC5 (Cat# QT00049959), and CYP3A4 (Cat# QT00024969), β-actin (Cat# QT00095431) and the primer for miRNAs was miR-27a (Cat# MS00003241), miR-31(Cat# MS00003290), miR-200c (Cat# MS00003752), and the internal control RNU6 (Cat# MS00033740).

2.5. Flow cytometry for ABCC2 after treatment with 5-FU

Treated and untreated HT29 cells ($1x10^6$) were rinsed individually in phosphate-buffered saline supplemented with 0.5 % BSA and 2mM EDTA, then incubated for 30 minutes at 4°C in the dark with polyclonal anti-ABCC2-PE-conjugated antibody (1:50, Abcam, Cambridge, MA, USA). To remove the unbound antibody, cells were washed with washing buffer (PBS containing 2mM EDTA). The fluorescence signal was then estimated using a FAC Scan flow cytometer.

2.6. Immunocytochemistry of ABCG2 in HT29 cells after treatment with 5-FU

Immunocytochemistry staining procedures were applied following the procedures described before (El-Daly et al., 2020a, Abo-Zeid et al., 2013). The HT29 cells were cultured in cell-chamber slides (SPL Life Sciences Co., Korea) at a dilution of 1×10^4 cells / well in a volume of 250 µL. The 5-FU was diluted in each well to reach the desired concentrations; 50 µM, 25 µM, and 12.5 µM. After 48 hrs incubation period, the media were discarded, and the cells were fixed with ice-chilled methanol. The fixed cells were permeabilized for 10 min with phosphate buffer saline (PBS) (Merck KGaA, Darmstadt, Germany) containing 0.1% Triton X-100 (Merck KGaA, Darmstadt, Germany). The cells were then blocked for one hour with PBS containing 10% fetal bovine serum. The blocked cells were then incubated with the 1ry antibody; Recombinant Anti-BCRP/ABCG2 antibody [EPR21122] (ab229193) (Abcam plc, Cambridge, UK) at dilution 1/100 in PBS containing 1% FBS at 4ºC overnight in a humidified chamber. On the second day, the cells were washed extensively with PBS three times, 5 min each. The 2ry antibody, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) (Abcam plc, Cambridge, UK) was added at dilution 1/500 for one hour at room temperature. Another round of extensive wash with PBS was conducted and left for complete air drying. The Mounting Medium with DAPI - Aqueous, Fluoroshield (ab104139) (Abcam plc, Cambridge, UK) was used as a counterstain and mounting medium for 10 min. The slide was visualized under a fluorescent microscope Axio Imager Z2, (Carl-Zeiss-Promenade, Jena, Germany).

2.7. Statistical Analysis

To assess the significant differences between the treated and control cells, a one-way ANOVA statistical analysis was applied, followed by a post hoc test. All experimental assays were carried out in triplicates and independently. The mean and standard deviation are used to represent the data, and differences with p values less than 0.05 were deemed statistically significant.

3. Results

3.1. Cell viability following 5-FU treatment

The MTT test was applied to assess the cytotoxic impact of 5-FU on HT29 cell viability. The percent of the surviving treated cells compared with the untreated cells is resembled in (Fig. 1). After 48h and 72h from the treatment with increasing doses of 5-FU (1.56-100µM), cell growth was modestly inhibited in a dose-dependent manner, with no observed change between the two-time points 48h or 72 h. Based on the results of the MTT assay, moderate toxic doses of 5-FU (50 µM, 25 µM, and 12.5 µM) were selected to be applied in all following experiments. These doses are routinely applied in clinics.

3.2. The Expression of drug transporters and CYP3A4

To delineate the effects of various 5-FU doses on the expression of the major drug transporters (ABCC2 (MRP2), p-glycoprotein (ABCB1), ABCG2, and ABCC5), as well as the expression of CYP3A4 gene, HT29 cells were treated individually with the three doses of 5-FU (50 µM, 25 µM, and 12.5 µM). Our findings revealed that the low dose of 5-FU (12.5 µM) upregulated the expression of all investigated ABC transporter genes significantly compared to control untreated cells. The highest expression of transporters was observed in cells treated with the

lowest dose of 5-FU (12.5 µM) compared to all other groups.

This significant increase in the ABC transporters expressing genes was declined by increasing the concentration of 5-FU introduced to cells (25 µM and 50 µM,) and this decrease in the expression was significant compared to the low dose (12.5 µM 5-FU) (Fig. 2). Concerning the expression of CYP3A4, it was upregulated in 12.5 µM 5-FU treated cells and declined in the case of higher doses, although not statistically significant (Fig. 3).

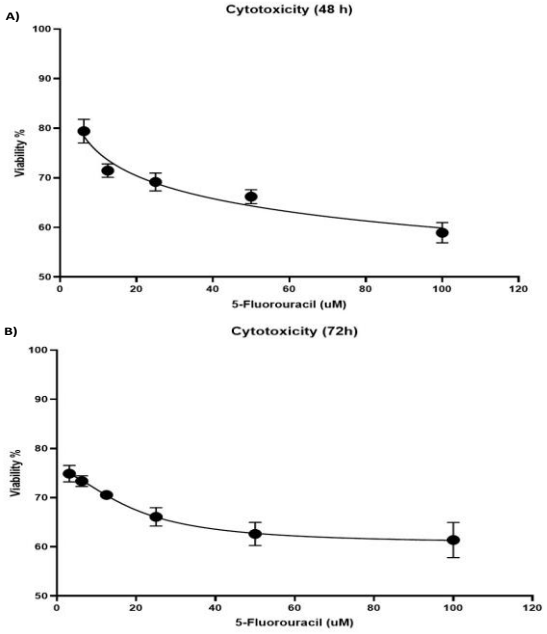

Figure 1: Effects of 5-FU on the viability of HT29 cells. Cell survival was determined by MTT assay after 48h (A) and 72h (B) of treatment. Cells were treated in a range of 1.56-100µM. Data are presented as mean ± S.D of three independent experiments.

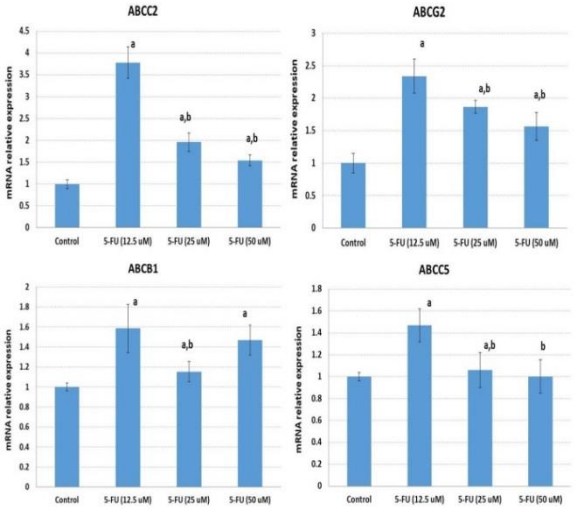

Figure 2: Relative mRNA expression of the drug transporter genes (ABCC2, ABCG2, ABCB1, and ABCC5) after treatment with different doses of 5-FU. Data are represented as mean ± S.D of three independent experiments. a Significance to Control, b Significance to 5-Fu (12.5 uM)..

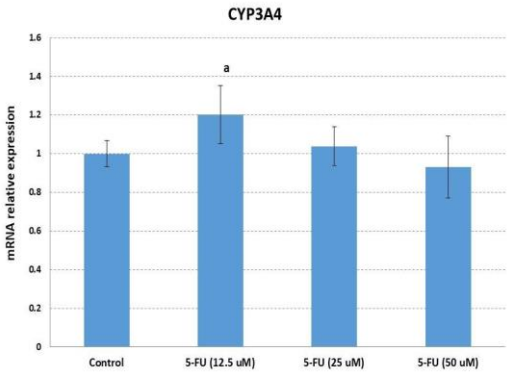

Figure 3: Relative expression of the drug-metabolizing enzyme CYP3A4 after treatment with different doses of 5-FU. Data are represented as mean ± S.D of three independent experiments. a Significance to Control.

3.3. The differential expression of MDR-related miRNAs following 5-Fu treatment

To investigate the effect of different doses of 5-FU on the expression of MDR-related miRNAs, HT29 cells were treated with the three doses of 5-FU (50 µM, 25 µM, and 12.5 µM) for 48h, and the expression of miR-27a, miR-31, and miR-200c was evaluated by RT-PCR. Our data revealed that treatment with 5-FU downregulated their expression significantly dose-dependent. The expression of miR-31 was the most downregulated among miRNAs following 5-FU treatment (Fig. 4).

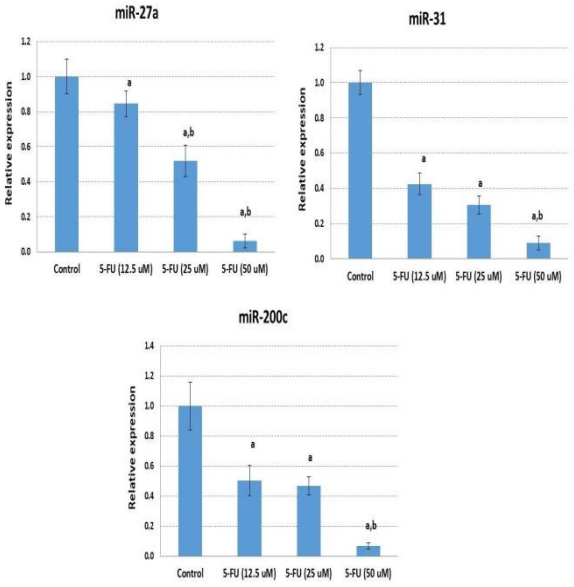

Figure 4: Relative Expression of MDR-related miRNAs following treatment with 5-FU. Data are represented as mean ± S.D of three independent experiments. [a] Significance to Control , [b] Significance to 5-Fu (12.5 µM)

4.4. ABCC2 (MRP2) representing HT29 cells by flow cytometry

We further investigated the dose-dependent effect of 5-FU on ABCC2, the drug transporter that showed the most significant alteration following 5-FU

treatment. The flow cytometric analysis was conducted to evaluate the percentage of ABCC2 representing cells in treated cells and control. The results revealed that the fraction of cells positively stained with ABCC2 was dramatically enhanced following treatment with a lower dose of 5-FU (12.5 µM). On the other hand, increasing the dose of 5-FU (25 µM and 50 µM) was able to significantly lower the positively stained cells (Fig. 5). These findings validate the prior ABCC2 mRNA results.

4.5. Fluorescence immunostaining of ABCG2

To validate the dose-dependent effect of 5-FU on another essential drug transporter that showed to be significantly deregulated, we evaluated the protein expression of ABCG2, which plays a vital role in MDR against chemotherapy following treatment with different doses of 5-FU. We used the immunocytochemistry technique where HT29 cells were stained with an antibody against ABCG2, and the Nuclei were visualized using Hoechst 33342 fluorescent. According to the staining images, the cytoplasmic Expression of ABCG2 in cells treated with the lower dose of 5-FU (12.5 µM) was significantly strong compared to control cells and also compared to the other treatment doses. The cell-staining intensity decreased by increasing the 5-FU dose, indicating lower protein expression of ABCG2 (Fig. 6,7).

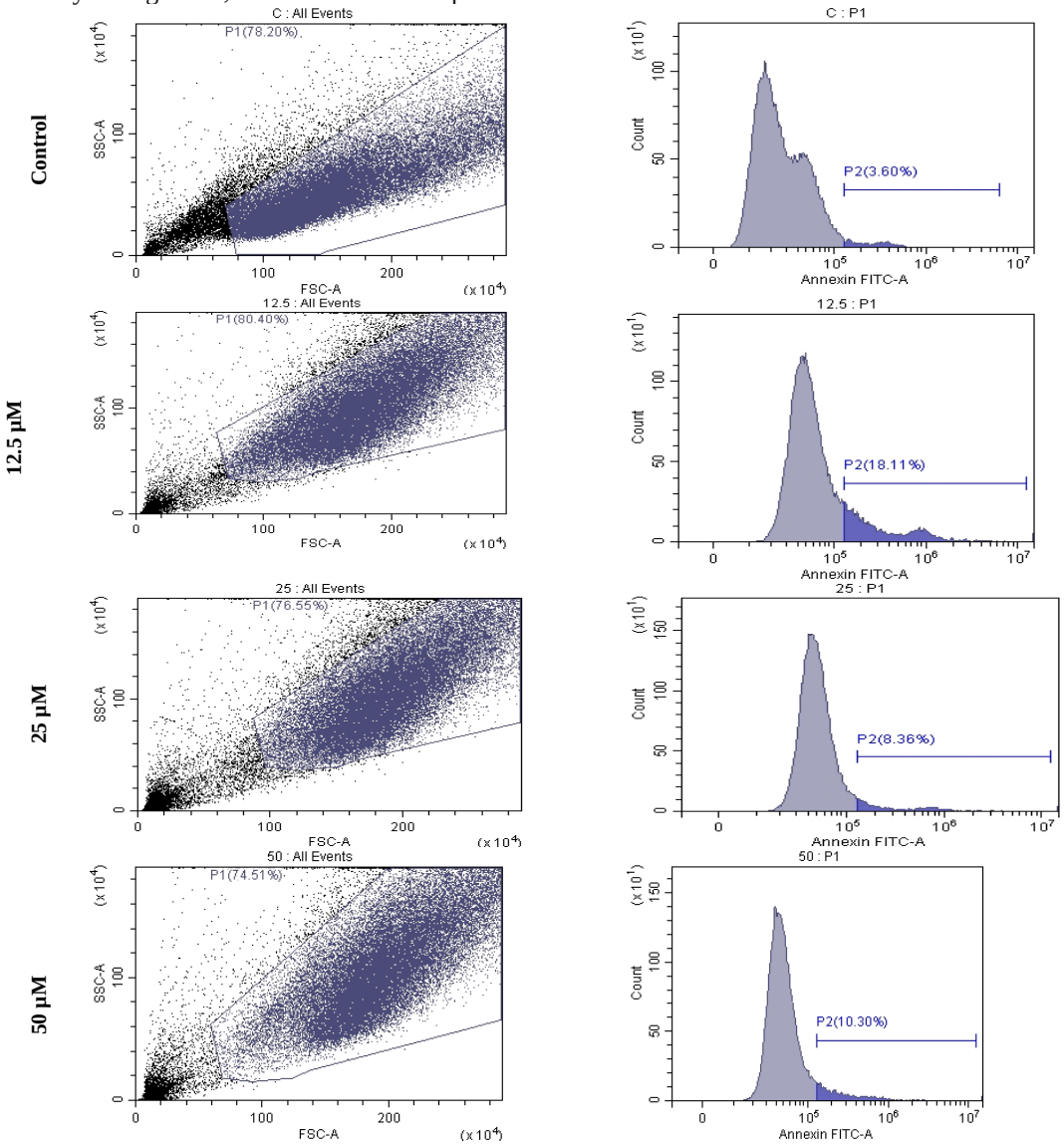

Figure 5: Flow cytometry analysis of of HT29 cells expressing ABCC2 (MRP2) following treatment with different doses of 5-FU.

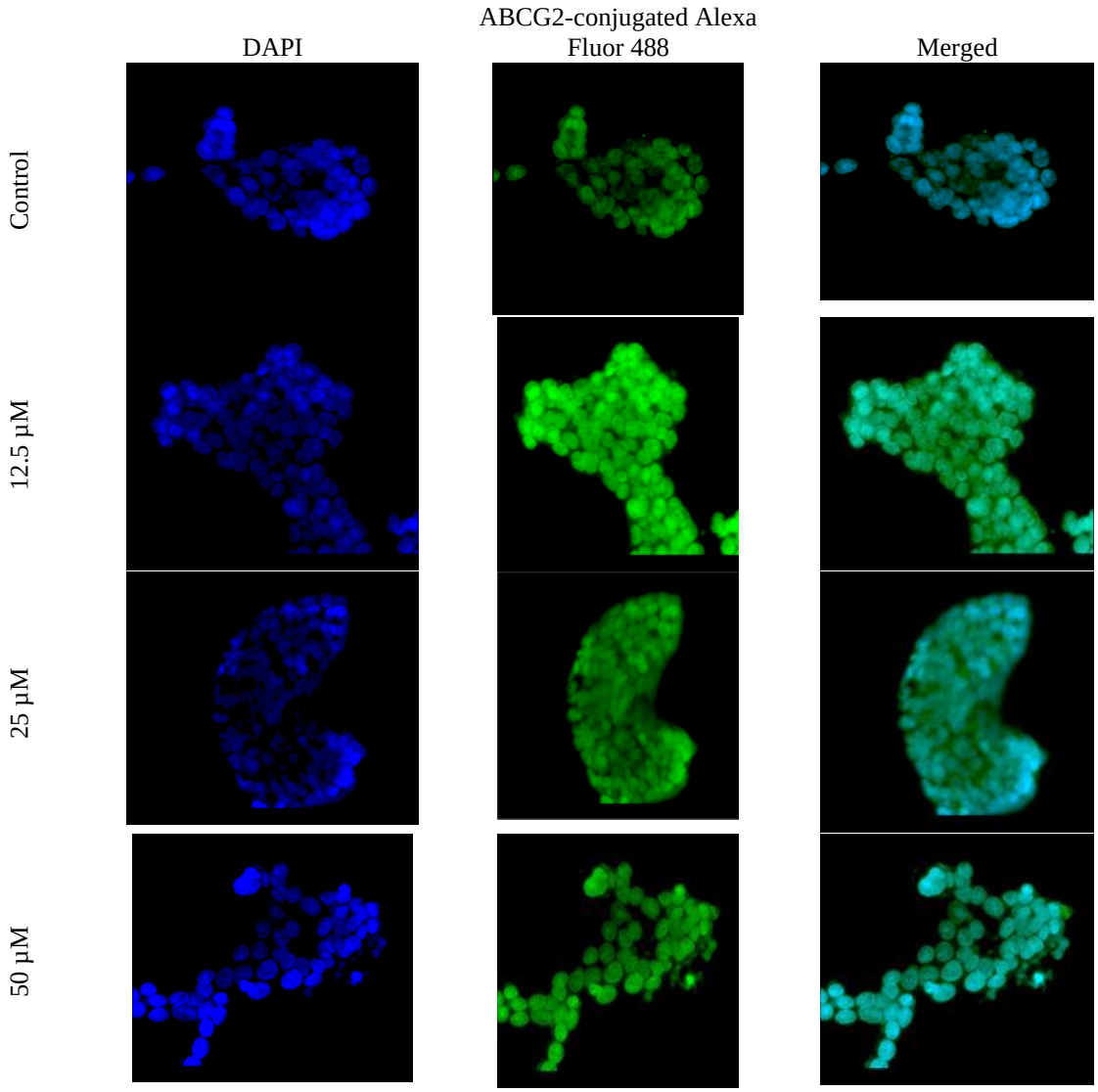

Figure 6: Fluorescence immunostaining of ABCG2 protein expression in HT29 cell line at different 5-FU concentrations. Maximum protein expression intensity is shown at 12.5 µM followed by the 25 µM concentration and finally at the 50 µM concentration compared to the control. Scale bar 200 µm, (X40).

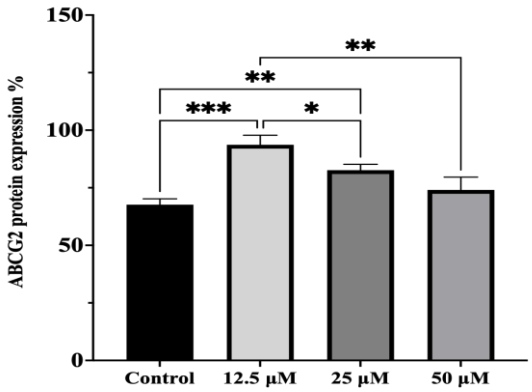

Figure 7: ABCG2 protein expression % in the HT29 cell line at different 5-FU concentrations. The 12.5 µM concentration showed a significant increase in the protein expression compared to all other groups. The 25 µM concentration showed a significant increase in the protein expression compared to the control group only. The data were calculated on basis of 1000 cells/ group. The data are expressed as mean ± SD (n = 3). * p< 0.05, ** p< 0.01, *** p< 0.001.

5. Discussion

The chemotherapeutic drug 5-FU is one of the most widely used and essential anti-cancer medications for several malignancies, including colorectal cancer (Gustavsson et al., 2015). However, one of the challenges associated with the use of 5-FU is the development of resistance, which makes treating colorectal cancer with systemic chemotherapy complicated. The principles of drug resistance have been the subject of numerous studies. MDR is one of the most important processes through which cancer cells develop resistance to both traditional and innovative chemotherapy drugs, forming a formidable barrier in the treatment of malignancy (Majidinia et al., 2020). First, it was recognized that ATP-dependent cellular pumps were the source of chemotherapy resistance, but subsequent research has discovered that other mechanisms such as increased drug metabolism, limited drug entry, and deficient

apoptotic mechanisms are also implicated in this process (Penta et al., 2021). Several studies attempted to solve this issue and suggested multiple strategies, with several suggestions that increasing chemotherapy concentrations may overcome tumor cell acquired resistance by dosage intensification. In the clinic, increasing the chemotherapy dosage resulted in improved clinical outcomes, but at the expense of significant toxicity (Odaimi & Ajani, 1987; Selle et al., 2016). Other research revealed that administering extremely low doses of chemotherapy may overcome MDR by sensitizing cancer-related fibroblasts and decreasing P-glycoprotein levels (Emmenegger et al., 2011; Ma et al., 2017). In our study, we aimed to investigate whether different doses of 5-FU could change the response of cells regarding MDR. To fulfill this aim, we first identified the doses of 5-FU with moderate toxicity on colorectal cancer HT29 cells after 48h and 72h (treatment doses ranged from 1.56-100µM) using MTT assay. Based on the results of MTT assay, moderately toxic doses of 5-FU were calculated and selected to be applied in all subsequent experiments.

It is generally recognized that cancer cells may acquire treatment resistance by altering drug metabolic pathways. CYP3A4 is a member of the cytochrome p450 (CYP) enzyme superfamily, which is involved in chemoresistance (Donzelli et al., 2014). The ABC transporter P-glycoprotein (P-gp) and the drug-metabolizing enzyme CYP3A4 are identified as possible main obstacles to drug absorption (Mansoori et al., 2017; Wrighton et al., 2000). P-gp is a membrane-associated glycoprotein responsible for the efflux of toxins, medications, and cytotoxic drugs from cells (Kuo, 2009; Penta et al., 2021). Many researchers documented the upregulation of p-gp protein in chemo-resistant cancer cells. For example, adherent chemo-resistant SCLC and NSCLC cell lines have elevated expression of p-gp (Penta et al., 2021). In 5-FU resistant CRC cells, P-gp was also found to be overexpressed (Wang et al., 2018).

Another member of the ABC-transporter family is ABCC2 (MRP2), which effluxes different chemicals across extracellular and intracellular membranes, making cells highly drug-resistant (Zhan et al., 2013). Interestingly, various resistant human cancer cells overexpress MRP2, like ovarian cancer, hepatocellular carcinoma, bladder cancer, and colon cancer (Koike et al., 1997; Kool et al., 1997; Liedert et al., 2003; Materna et al., 2005). Cisplatin-resistant cells express high levels of ABCC2 in A549/DDP resistant cells (Zhan et al., 2013). In addition to the previously mentioned drug transporters, ABCG2 is another member that its elevated expression is reported to be related to increased levels of resistance to many chemotherapeutic drugs (Candeil et al., 2004; Theile et al., 2009).

To investigate the molecular mechanism by which the CRC cell line HT29 cells may acquire resistance to 5-FU by significantly deregulating the expression of drug transporters, we exposed HT29 cells to three different doses of 5-FU (50 µM, 25 µM, and 12.5 µM) and measured the mRNA levels of a panel of ABC transporter genes (ABCC2 (MRP2), ABCB1 (P-glycoprotein), ABCG2, and ABCC5 and also the expression of CYP3A4, all of which play important roles in MDR.

We found that the highest expression of ABC transporters was observed in cells treated with the lowest dose of 5-FU (12.5 µM) compared to all other doses. This significant increase in expression was reduced by increasing the concentration of 5-FU introduced to cells (25 µM and 50 µM). Our findings delineate that a low dose of 5-FU (12.5 µM) could initiate MDR by significantly upregulating a large panel of drug transporters. In terms of CYP3A4 expression, it was elevated in cells treated with the 12.5 µM dose and decreased in cells treated with greater doses, albeit not statistically significant.

In our study, we supported our findings through flow cytometric analysis of ABCC2. We evaluated the percentage of ABCC2 representing cells in treated and control cells. We found that the proportion of ABCC2 representing cells was significantly increased following treatment with the low dose of 5-FU (12.5 µM) as compared to all other groups. This elevation was found to be reduced by increasing the 5-FU doses introduced to cells (25 µM and 50 µM). Also, we evaluated the expression of the ABCG2 protein by immunostaining to confirm the RT-PCR results. The 12.5 µM dose showed a significant increase in the ABCG2 protein expression compared to all other doses, where the maximum protein expression intensity is detected at cells treated with the 12.5 µM followed by the 25 µM concentration, and finally the 50 µM concentration compared to control. These findings support prior findings of ABCC2 and ABCG2 mRNA expression, as well as the capacity of a low dose of 5-FU to generate MDR in HT29 cells.

Our study results were in accordance with the study by Yamasaki et al. (2011), where the drug transporter ABCC2 level was evaluated in patients who did or did not receive chemotherapy, including 5-FU, doxorubicin, and cisplatin. MRP2-positive cells were found to be more detected in patients who received chemotherapy. Moreover, The MRP2-positive patients had a poor prognosis (5-year survival rate, 25.6%) when compared to MRP2-negative patients (5-year survival rate 55.7%).

On the contrary, the study by Ma et al. (2017) reported that a low dose of 5-FU could recover MDR by repressing the expression of P-gp. This contradiction in results could be attributed to the difference in applied doses, and the type of cancer studied. Since in the same study by Ma et al. (2017), the effect of a low dose of 5-FU that reversed MDR by down-regulating P-gp was detected in the KB-3-1cell line (human mouth epidermal carcinoma cells)

but not in H460/Tax-R cell line (non-small lung carcinoma cells).

Recently, it has been discovered that miRNAs have a fundamental role in drug resistance to chemotherapy (Wu et al., 2018; Xie et al., 2016). Aberrant expression of miRNAs is strongly connected with the colorectal carcinogenesis process (El-Daly et al., 2019c), and their expression is potentially related to drug resistance in CRC (Akao et al., 2011; Kumazaki et al., 2013; Nakagawa et al., 2019). MiR-27a is an oncogene that initiates the occurrence and differentiation of different cancer types. Moreover, miR-27a and miR-31 can also act as chemotherapeutic drug resistance genes in multiple cancer types such as ovarian cancer, leukemia, pancreatic cancer, CRC, and adenocarcinoma (Ding et al., 2017; Korourian et al., 2017). MiR-31 is reported to be correlated with 5-FU resistance in CRC (Salem et al., 2021, Li et al., 2019).

In order to investigate the effect of different doses of 5-FU on miRNAs involved in MDR, we measured the expression of three miRNAs that are related to MDR in cancer (miR-27a, miR-31, and miR-200c). Our results showed that 5-FU significantly reduced the expression of miR-27a, miR-31, and miR-200c in a dose-dependent manner. In the same line of our results, Wang et al., (2010) reported that downregulated miR-31 sensitizes cancer cells to 5-FU at an early stage and affects cell migration and invasion in HCT-116 colon cancer cells. Also, it was previously reported that upregulation of miR-31 enhances resistance in colorectal tumors (Nakagawa et al., 2019). Regarding miR-27a, the downregulation of this miRNA was able to reduce treatment resistance in gastric cancer, as found by (Zhao et al., 2011). Meanwhile, an elevated level of miR-27a increased oxaliplatin resistance by initiating P-gp expression in gastric cancer (Ding et al., 2017). In contrast to these results, in Korourian et al., (2017) study, upregulation of miR-31 increased 5-FU sensitivity in gastric adenocarcinoma. MiR-27a enhanced the 5-FU effect in HCC cells by decreasing P-gp and β-catenin Expression (Ding et al., 2017). Also, transfection of miR-331-5p and miR-27a enhances the effeciency of doxorubicin in K562 chronic myelogenous leukemia cells by suppressing P-gp Expression (Feng et al., 2011). In melanomas, the ABC transporters ABCG2, ABCG5, and ABCB1, were reported to have an inverse expression with miR-200c and E-cadherin (Liu et al., 2012). This disparity in findings might be attributed to differences in miRNAs expression across the various cancer types.

Conclusion

In the present study, we primarily investigated the association between various doses of 5-FU and the induction of MDR in colorectal cancer as represented by the expression of a panel of drug transporters (ABCB1, ABCC2, ABCG2, ABCC5). We also investigated the involvement of miRNAs in this activity. Our results demonstrate that 5-Fu has a dose-dependent influence on the expression of several drug transporters, with the lowest dose of 5-FU (12.5μM) causing a significant elevation in the expression pattern. On the other hand, by increasing the doses of 5-FU, a significant reduction in gene and protein expression of these drug transporters was detected. This dynamic change was also detected in miRNAs expression, as 5-FU downregulated MDR-related miRNAs (miR-27a, miR-31, and miR-200c) in a dose-dependent manner.

These findings demonstrate that a low dosage of 5-FU may accelerate the development of drug resistance in colorectal cancer, emphasizing the necessity of using a chemotherapeutic dose with an acceptable cytotoxic impact and, at the same time, effectiveness in reducing the activity of drug transporters.

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Availability of data and material

All data generated or analyzed during this study are available from the authors upon reasonable request and with permission of the National Research Centre.

Competing interests

The authors declare that they have no competing interests

Consent for publication

All Authors mutually agree to forward this manuscript for submission. We confirm that the submitted manuscript has not been previously published in any language and is not submitted for publication elsewhere.

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