International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.201.208

Research Article
Interplay of USP47 and COX2 in Regulating Tumor Microenvironment in the Progression of Colon Cancer

[1]Youhua Wu, [1]Shuyu Wang, [1]Tianxiang Zhang, [2]Pengbin Zhang and [1]Yunfei Cao

[1]Department of Anesthesiology, Beilun District People's Hospital, 315800 Ningbo, Zhejiang, China
[2]Department of General Surgery, Beilun District People's Hospital, 315800 Ningbo, Zhejiang, China

Abstract

Background and Objective: The functional role of USP47 has been reported in many cancer types, but its role in colon cancer (CC) and its correlation with COX2 have not been reported so far. In this present investigation, the azoxymethane (AZ)/dextran sulphate sodium (DSS) mixture induces both early and late phases of colon cancer in mice, examines USP47 and COX2 in the development of CC and tries to examine whether both are correlated. **Materials and Methods:** The mice exhibited the early and advanced phases of CC after receiving a single injection of AZ, followed by either 3 or 7 injections of DSS at 5-day intervals. Histology, immunohistochemistry, *in-situ* hybridization and western blotting methods are employed to understand the expression of USP47 and COX2 and their association with CC progression. **Results:** The histology reveals cells with modest variations and the development of cellular clusters in early CC. The advanced phases of CC are characterised by the presence of many aggregations of undifferentiated cells that give rise to various foci. The levels of USP47 and COX2 exhibit a small rise in the early phases of CC, but their expression undergoes a significant exponential increase in the later stages of CC. On the other hand, the expression of COX2 in tissue where USP47 has been silenced is increased to a certain extent during the early stages of CC. However, it is suddenly reduced during the later stages of CC. **Conclusion:** Overall, current study data confirm that silencing of USP47 had a positive effect in inhibiting the progression of CC and also hampers the expression of COX2 at the final phase of CC.

Key words: USP47, COX2, colon cancer, mice study, histology, dextran sulfate sodium, inflammation

Citation: Wu, Y., S. Wang, T. Zhang, P. Zhang and Y. Cao, 2024. Interplay of USP47 and COX2 in regulating tumor microenvironment in the progression of colon cancer. Int. J. Pharmacol., 20: 201-208.

Corresponding Author: Yunfei Cao, Department of Anesthesiology, Beilun District People's Hospital, 315800 Ningbo, Zhejiang China
Tel/Fax: +86-0574-86776023

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Colon cancer (CC) is a prominent kind of cancer that ranks second and third in terms of cancer-related mortality worldwide[1,2]. Another key factor to note about CC is that it commonly results in malignant tumors with high deaths and incidence rates[3]. The common cause of CC is mostly linked to poor dietary habits, genetic mutations and induced inflammation[4]. The changes in genetic and epigenetic level alter the tissue microenvironment and that are evident with tumour development, invasion and metastasis[5]. Tumour progression and the interplay of its tissue microenvironment are considered important features to be investigated along with the genes responsible for carcinogenesis[6].

Early diagnosis of CC helps to improve the patient survival rate but when it progresses to advanced stages due to its aggressive and metastatic nature, the survival rate rapidly declines[7]. Ubiquitin-Specific Protease 47 (USP47) can deubiquitinate specific substrates like MAPK, E-cadherin, DNA polymerase β, SNAIL, β-catenin, β-Trcp, YAP and katanin-p60 thereby affect its stability, activity or localizations and regulate the cellular activity[8,9]. Recent research with USP47 confirms that in the absence of USP47 the CC proliferative, migrative and tumorigenicity properties are highly inhibited[10]. However, the interaction between USP47 and inflammatory markers like COX2 needs to be correlated to a better understanding of the function of USP47 in the advancement of CC.

The COX2 has a role in inflammation, cellular transformation, proliferation, tumour growth, cellular metastasis and tissue invasion and that promotes tumor development and progression[11,12]. The COX2 also has a role in angiogenesis in which increased expression of COX2 promotes VEGF overexpression[13]. Overexpression of COX2 is particularly associated with the gastrointestinal tract and implies its aggressive nature[14]. This study was planned to assess the expression of USP47 and COX2 in the development of CC and try to examine whether both are correlated.

MATERIALS AND METHODS

Study area: The current investigation was conducted at the Beilun District People's Hospital from June to August 2023.

Mice model: A total of 16 female C57BL/6 mice (8 weeks) were obtained from Jackson's Laboratory in Beijing, China. They were kept in a sterile setting for 15 days to adapt to their new surroundings. Following the experimental procedures, the animals were kept in a 12 hrs dark/light cycle and maintained a room temperature with a relative humidity of 55%. The animals are freely allowed to intake their food as well as tap water. To initiate CC in mice at both the early stage (n = 8) and advanced stage, a single subcutaneous injection of azoxymethane (AZ) (Santa Cruz Biotechnology, California, USA) (11 mg kg^{-1} b.wt.) was administered. On the fifth day of the AZ injection, dextran sulphate sodium (DSS) (4 % w/v) (Sigma-Aldrich, Shanghai, China) was delivered into the mice's bodies through the drinking water. Repeatedly, the same dose of DSS was given for every 5th day and like those three repeated doses for initial phases of CC-developing mice and seven repeated doses for advanced-stage developing mice were given. At the end of 3rd dose and 7th dose of DSS intake, the mice were sacrificed by decapitation. From the sacrificed mice, the colon portion of the mice was carefully dissected and kept for further experimental analysis.

Ethical consideration: The animal procedures and protocol were approved by the Institutional Animal Care Committee of the host institution (Reg. No. 2022/CO/0269).

Histology and immunohistochemistry: Following animal scarifies the colon samples shown with visible polyps are carefully dissected from the rest of the tissue and fixation for 2 days at room temperature in 10% formalin. The fixation aids in the preservation of the original tissue structure; the tissues are then dehydrated with a steady rise in isopropyl alcohol (Sigma-Aldrich, Shanghai, China). The tissues were then subjected to xylene (Sigma-Aldrich, Shanghai, China) treatment for 45 min which aids in tissue clearance and following that wax was allowed to impregnate the tissue. The tissues were ultimately encased in a wax block and secured in a microtome to create tiny slices measuring 6 µm in size. The thin tissue sections obtained in the slide were processed and finally stained with haematoxylin and eosin for visualization. In immunohistochemistry, the thin slices (6 µm in size) are dewaxed and then treated with a boiling sodium citrate solution (0.01 M, pH 6.0) (Sigma-Aldrich, Shanghai, China) for 20 min to retrieve the antigens. After being rinsed with 1X TBST (Sigma-Aldrich, Shanghai, China), the slides undergo a blocking phase to inhibit the non-specific binding of antibodies. This involves treating the sections with a 4% bovine serum albumin (BSA) solution in TBST buffer (Sigma-Aldrich, Shanghai, China) for 1 hr at 37°C. The main antibodies, anti-USP47 antibody (Abcam, ab3465; diluted 1:250) or anti-COX2 antibody (Abcam, ab19898; diluted 1:250) (Thermo Fisher, Shanghai, China), were mixed with 4% BSA and placed on top of the tissue slices for a duration of 6 hrs at 4°C. After undergoing thorough washing 3 times with a

solution of 1X TBST, the slides were next exposed to secondary antibodies that are conjugated with horseradish peroxidase (HRP) (Thermo Fisher, Shanghai, China). This exposure takes place for a duration of 2 hrs at 4°C. Following the washing step, the brown-coloured signals are obtained by using a 3,3'-diaminobenzidine (DAB) substrate (Sigma-Aldrich, Shanghai, China).

***In situ* hybridization:** For *in situ* hybridization, thin sections (6 µm size) were initially fixed on slides and subjected to dewaxing. Following rehydration, the sections undergo antigen retrieval by being immersed in preheated 50 mM of Tris solution containing 20 µg mL^{-1} proteinase K (ACRO Biosystems China, Beijing, China) for 15 min at a temperature of 37°C. The slices were immersed in a hybridization buffer solution and incubated at 37°C for 5 hrs. Subsequently, miR-122 probes (Exiqon, double-DIG-labelled LNA probes) were applied to the section and allowed to incubate at a concentration of 18 nM, coupled with denaturation buffer, at 56°C for 5 hrs. To ensure control, the U6snRNA probe (Exiqon, 3'-DIG-labelled LAN probe) was used at a concentration of 12 nM. After incubation, the non-specific binding of the probe is washed out using saline sodium citrate (SSC) buffer (Sigma-Aldrich, Shanghai, China). Before proceeding with anti-DIG HRP antibody (Roche; 1:3000; 6 hrs, 4°C) the slides were treated with blocking solution (3% Fetal calf serum and 0.1% Tween-20 in B1 solution) (Thermo Fisher, Shanghai, China) for 1 hr at room temperature. After incubation with the antibody, the slides were washed and the signals were generated by the DAB kit (Sigma-Aldrich, Shanghai, China).

Western blotting: The proteins were isolated from the specimens taken from the colon and then analysed for protein content using the Lowry technique. Equal amounts of protein samples (60 µg/well) were put onto the 12% SDS-PAGE gel (Sigma-Aldrich, Shanghai, China) and divided by a voltage of 50V. The proteins were effectively moved from the gel to the PVDF membrane utilising the semi-dry technique. The membrane is blocked using a 4% BSA solution for 2 hrs at ambient temperature. After that, it was incubated with either the primary antibody anti-USP47 antibody (Abcam, ab3465; diluted 1:400) or the anti-COX2 antibody (Abcam, ab19898; diluted 1:400) (Thermo Fisher, Shanghai, China) at 4°C for 6 hrs. Following the rinse of the membrane with 1X TBST buffer (Sigma-Aldrich, Shanghai, China), the membrane is subjected to a 2 hrs incubation at 37°C with appropriate secondary antibodies that are conjugated with HRP (Thermo Fisher, Shanghai, China). Once the signals have been thoroughly washed to remove any unwanted substances, such as the secondary antibody that specifically targets the main antibody, they are then processed using the ECL chemiluminescence kit (Thermo Fisher, Shanghai, China) to produce a visible signal. The acquired signals were quantified via the use of ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) and the outcomes were recorded utilising the gel recording system (Bio-Rad Laboratories Co., Ltd., Shanghai, China). The β-actin was used for control and the ultimate outcomes were standardised to the control protein.

Statistical analysis: To attain significance levels, the investigations were replicated a minimum of 3 times or more and the outcomes were represented by the Mean±Standard Deviation (SD). For comparing multiple data groups ANOVA was performed using *post hoc* Tukey's t-test. The findings were deemed statistically trustworthy at $p<0.005$.

RESULTS

Inducing the early and late phases of CC: In mice, the AZ/DSS combination was employed to develop the early and late phases of CC. As explained in materials and methods, a single AZ injection followed by three or seven doses of DSS results in the development of early and late phases of CC respectively. To confirm the pathological changes associated with the early and late phases of CC development, histology was performed and compared along with the control colon tissue (Fig. 1a-c). In control colon tissue, the cells appear in uniform size without any abnormalities (Fig.1a), but in the early phases of CC cytologic atypia is common along with the loss of cellular polarity (Fig. 1b). Also, small loci start to appear along the tissue surface (Fig. 2b), similarly in a late phase of CC, multiple loci with more proliferated enlarged cells are observed throughout the tissue surface (Fig. 1c).

USP47 expression regulates COX2 in the early and late phases of CC: To analyse the levels of USP47 in the early and late phases of CC, immunohistochemistry was performed and it was compared with COX2 expression to correlate it (Fig. 2a-f). The USP47 shows profound expression in control colon tissue (Fig. 2a) and its expression was upregulated in the initial CC condition which is evident with a high-intensity signal (Fig. 2b). Also, USP47 expression was observed almost in all cells of the initial phase of CC tissue with strong signals, particularly around the boundary of loci (Fig. 2b). Surprisingly, USP47 was extremely downregulated through the tissue surface of the advanced stage of CC with signals remaining

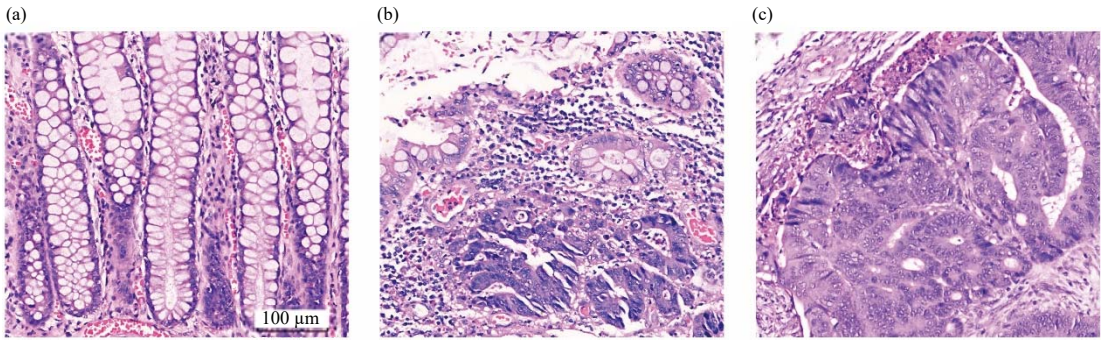

Fig. 1(a-c): Histological variation of initial and advanced stage of colon cancer, (a) Control colon tissue with compact and regularly packed cells, the nucleus size is small and uniform throughout the tissue structure, (b) Mice developed with initial stage of colon cancer show a heterogenous population of cells (cytologic atypia) along with losing cellular polarity and (c) Mice developed with advanced stage of colon cancer represent the formation of multiple loci with enlarged cellular structure
Scale bar 100 µm

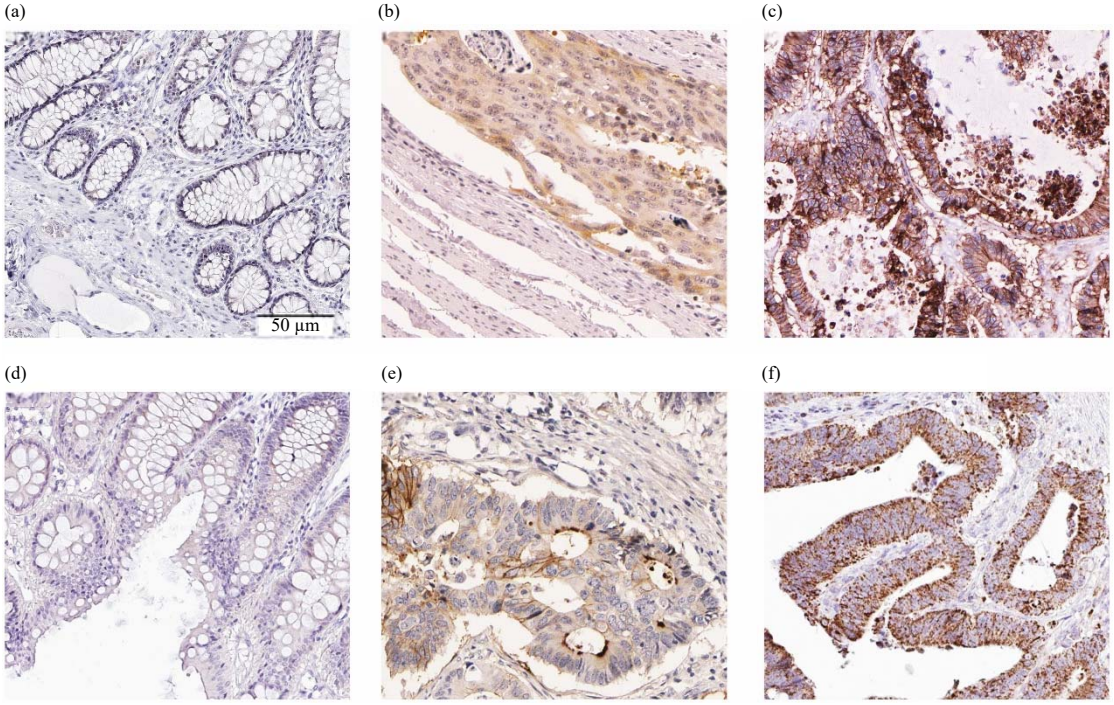

Fig. 2(a-f): USP47 and COX2 expression in initial and advanced stages of colon cancer tissue layers, (a) Slight expression of USP47 in control colon tissue, (b) In relative to control tissue, USP47 is highly expressed in the initial phases of colon cancer, (c) USP47 is overexpressed in advanced stages of colon cancer when compared with the control tissue, (d) Lack of COX2 expression in control colon tissue, (e) COX2 expression is upregulated and observed only in restricted cells in initial colon cancer and (f) COX2 is sharply upregulated in almost every cell of the advanced stage of colon cancer
Scale bar 50 µm

observed only in the boundary of some loci (Fig. 2c). The COX2, a marker for cancer stem cells is not detectable in control colon tissue (Fig. 2d) but it shows very limited expression in initial CC tissue (Fig. 2e). Notably, COX2 overexpression was observed throughout the tissue surface in the late phases of CC (Fig. 2f).

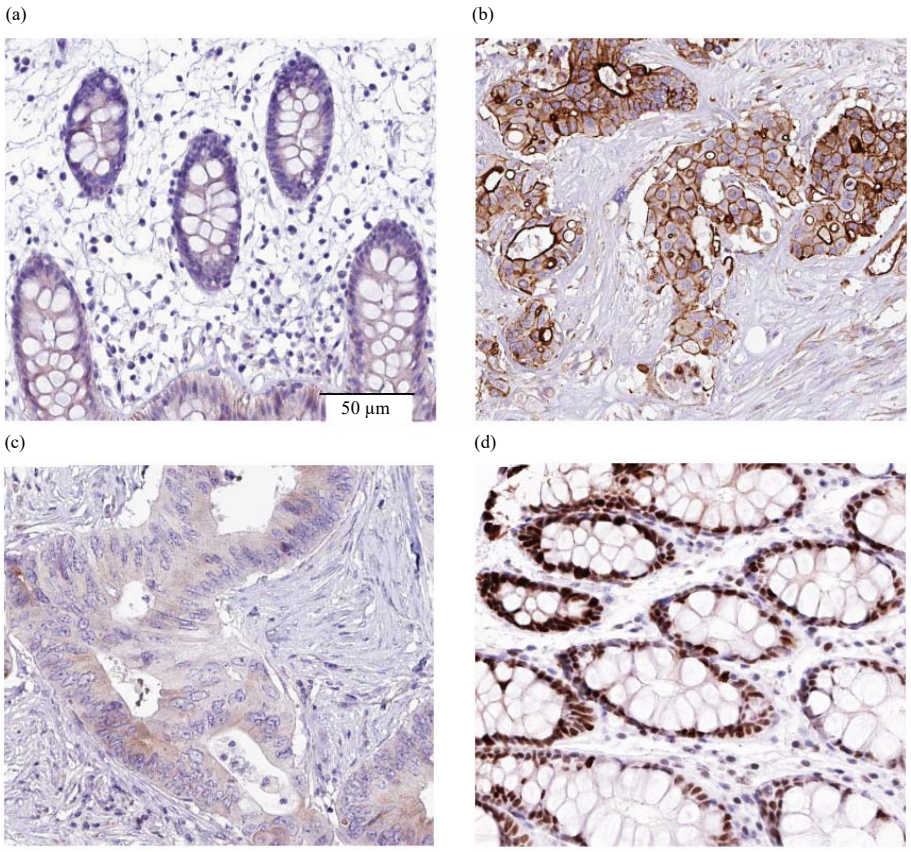

Fig. 3(a-d): Assessment of silenced USP47 in tumor progression using *in-situ* hybridization technique, (a) Silenced USP47 show very limited expression in control colon tissue, (b) Silenced USP47 expression is positively upregulated in the initial phase of colon cancer, (c) Silenced USP47 expression is diminished in advanced stages of colon cancer and (d) U6 snRNA expression in control colon tissue acts as a positive control

Scale bar 50 µm

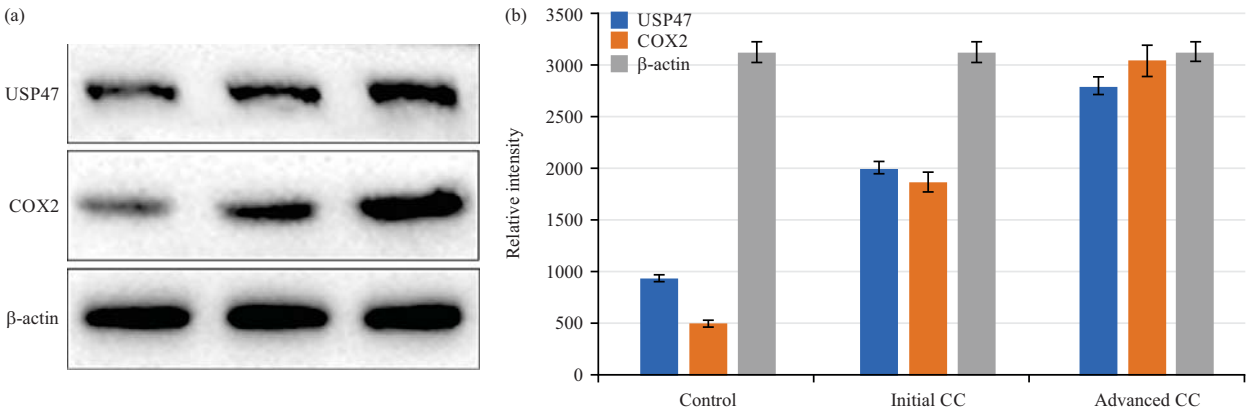

Fig. 4(a-b): Expression analysis of (a) USP47, COX2 and β-actin protein using western blotting and (b) Band intensity of USP47, COX2 and β-actin are analyzed using ImageJ software and plotted using a bar diagram

USP47 is 3.2 times highly overexpressed in the initial phase of colon cancer when compared with the control tissue but 2.4 times more likely downregulated in an advanced stage of colon cancer when compared it to the control tissue, COX2 shows no signal in control tissue but it is 7.6 times upregulated in advanced phase colon cancer when compared with the initial phase of colon cancer, Lane 1 represents control colon tissue, Lane 2 represents the initial phase of colon cancer, Lane 3 shows advanced stages of colon cancer and "CC" represents colon cancer

Silencing of USP47 in different phases of CC: To assess the role of silenced USP47 in CC progression, the expression of silenced USP47 was tracked in various phases of CC using a specific probe and investigated through *in-situ* hybridization technique as described in materials and method (Fig. 3a-d). In control colon tissue, the silenced USP47 shows only restricted expression and is observed only in a limited number of cells (Fig. 3a). As the CC progresses to the initial stage, silenced USP47 is highly overexpressed in almost every cell of the initial stage colon tissue surface (Fig. 3b). On the other hand, its expression diminished as the complexity of CC advanced and it is mostly observed only at the loci boundary of the advanced phase of CC (Fig. 3c). A U6 snRNA probe was used as a positive control and it displayed a signal in the colon tissue of the control group (Fig. 3d).

Assessing the expression of USP47 and COX2 via western blotting: Western blotting assessments were conducted to determine the precise degree of overexpression or downregulation of USP47 and COX2 in various phases of CC. The results of these experiments are shown and described in Fig. 4(a-b). The expression of USP47 normally appears in a predominate level in control colon tissue and its level increased to a fold of 3.2 times in the initial phase of CC. In contrast, USP47 depletion was observed at a level of 2.4 times in the advanced phase of colon tissue when compared with the control tissue (Fig. 4a-b). The COX2 a cancer stem cell marker shows only a slight signal in control colon tissue and also shows limited expression in the initial phase of CC. But perhaps COX2 expressed 7.6 times higher expression in advanced phases of CC tissue than in primary colon cancer tissue.

DISCUSSION

The USP47 is an mRNA-binding protein that regulates many proteins and predominantly contributes to the tumor-suppressive role in many cancer types. In addition to that, current studies with USP47 in CC confirmed similar findings. Initially, USP47 is 3.2 times upregulated in initial CC but downregulated to 2.4 times in advanced colon stages when compared with control tissue. Even though the expression of USP47 in an advanced phase of CC is downregulated its expression is observed in the boundary of loci representing the extreme abnormality of those cells. The results represent it had control over the initial stage of CC because the COX2 expression is also under control in the initial phase of colon development. But once the USP47 is downregulated in an advanced phase of CC the COX2 reaches maximum expression and thereby USP47 has a direct link in regulating COX2 expression.

In this research, the well-proven combination of AZ/DSS was used to induce CC in a mice model[15]. Following a single dose of AZ, a tumor-inducing agent and with a subsequent dose of DSS which was adjusted to induce the severity of colitis to form successfully the initial and advanced phase of CC. Histopathology results show cytologic atypia initiates in the initial phase of CC and progresses with the formation of enlarged cells with round nuclei which denote the advancement of histopathological condition[16].

Recent studies with USP47 showed that it fine-tunes the miRNA-145-5p and thereby contributes to the tumor suppressive activity of prostate cancer[17]. In this present investigation, it was observed that the silenced USP47 expression is correlated with the USP47 expression, as that the higher expression of silenced USP47in the initial stage of CC aids in downregulating the USP47 in an advanced stage of CC. The downregulation of USP47 on the other hand favours the overexpression of COX2 in advanced stages of CC. The CPEB mediates both translational repression as well as translational activation of many genes[18] and here, the USP47 inactivation affects many associated genes that may control tumour development.

In hepatocellular carcinoma, the differential expression of silenced USP47 is reported[19,20]. Through current studies, the differential expression pattern of silenced USP47 is systematically analysed and it provides an important understanding that the higher expression of silenced USP47in initial CC is downregulated after suppressing the tumour suppressor protein like USP47 in the latter stage of CC. Other than cancer, silenced USP47 contribute to the development of cardiovascular diseases, diabetes, immunological diseases and obesity[21,22] and it denotes silenced USP47higher expression in initial CC is associated with tumour enhancement as well as contributes to dysfunction of cellular function.

This investigation is restricted. The current study only examines limited factors, such as USP47 and COX2, in the development of CC. There are more factors to investigate, thus additional research will be conducted in the future. More study is required to investigate the processes behind CC-induced apoptosis. To successfully address this issue, further research activities focusing on possible long-term remedies are required. The aforementioned therapeutic goals have a significant influence on cognitive outcomes. As a result, researchers must prioritize the study of preventative measures and the use of effective apoptosis.

CONCLUSION

Overall, the investigation unveiled the function of suppressed USP47 in controlling USP47 throughout various stages of CC. The upregulation of USP47 in early CC is counteracted by the upregulation of miRNA-122. The expression of the cancer stem cell marker COX2 is controlled in a coordinated manner and relies on the expression of USP47.

SIGNIFICANCE STATEMENT

Among the many types of cancer, colon cancer (CC) is a significant form of the condition that ranks second and third in terms of fatality rates associated with cancer globally. During this experiment, the combination of azoxymethane and dextran sulphate sodium was shown to promote both the early and late stages of colon cancer in mice. The inquiry revealed the role of suppressed USP47 in managing USP47 throughout the different phases of CC. This was based on the findings that were gained from the research. The increase of miRNA-122 in early CC acts as a counterbalance to the upregulation of USP47 at this stage. It is dependent on the expression of USP47 that the expression of the cancer stem cell marker COX2 is regulated in a coordinated way when it comes to cancer stem cells.

ACKNOWLEDGMENT

This study was supported by Ningbo Health Science and Technology Plan, China (No: 2019Y47).

REFERENCES

1. Dong, Y., M.H. Wei, J.G. Lu and C.Y. Bi, 2019. Retracted: Long non-coding RNA HULC interacts with miR-613 to regulate colon cancer growth and metastasis through targeting RTKN. Biomed. Pharmacother., 109: 2035-2042.
2. Huang, W.Y., C.L. Ho, C.C. Lee, C.W. Hsiao and C.C. Wu *et al*., 2017. Oral tegafur-uracil as metronomic therapy following intravenous FOLFOX for stage III colon cancer. PLoS ONE, Vol. 12. 10.1371/journal.pone.0174280.
3. Wang, Z.K., L. Yang, L.L. Wu, H. Mao, Y.H. Zhou, P.F. Zhang and G.H. Dai, 2018. Long non-coding RNA LINC00261 sensitizes human colon cancer cells to cisplatin therapy. Braz. J. Med. Biol. Res., Vol. 51. 10.1590/1414-431X20176793.
4. Mármol, I., C. Sánchez-de-Diego, A.P. Dieste, E. Cerrada and M.J.R. Yoldi, 2017. Colorectal carcinoma: A general overview and future perspectives in colorectal cancer. Int. J. Mol. Sci., Vol. 18. 10.3390/ijms18010197.
5. Schiavoni, G., L. Gabriele and F. Mattei, 2013. The tumor microenvironment: A pitch for multiple players. Front. Oncol., Vol. 3. 10.3389/fonc.2013.00090.
6. Swartz, M.A., N. Iida, E.W. Roberts, S. Sangaletti and M.H. Wong *et al*., 2012. Tumor microenvironment complexity: Emerging roles in cancer therapy. Cancer Res., 72: 2473-2480.
7. Siegel, R.L., K.D. Miller, A.G. Sauer, S.A. Fedewa and L.F. Butterly *et al*., 2020. Colorectal cancer statistics, 2020. CA: A Cancer J. Clinicians, 70: 145-164.
8. Lei, H., H.Z. Xu, H.Z. Shan, M. Liu and Y. Lu *et al*., 2021. Targeting USP47 overcomes tyrosine kinase inhibitor resistance and eradicates leukemia stem/progenitor cells in chronic myelogenous leukemia. Nat. Commun., Vol. 12. 10.1038/s41467-020-20259-0.
9. Ashton-Beaucage, D., C. Lemieux, C.M. Udell, M. Sahmi, S. Rochette and M. Therrien, 2016. The deubiquitinase USP47 stabilizes MAPK by counteracting the function of the N-end rule ligase POE/UBR4 in *Drosophila*. PLoS Biol., Vol. 14. 10.1371/journal.pbio.1002539.
10. Yu, L., L. Dong, Y. Wang, L. Liu and H. Long *et al*., 2019. Reversible regulation of SATB1 ubiquitination by USP47 and SMURF2 mediates colon cancer cell proliferation and tumor progression. Cancer Lett., 448: 40-51.
11. Roelofs, H.M.J., R.H.M. te Morsche, B.W.H. van Heumen, F.M. Nagengast and W.H.M. Peters, 2014. Over-expression of COX-2 mRNA in colorectal cancer. BMC Gastroenterol., Vol. 14. 10.1186/1471-230X-14-1.
12. Chandrasekharan, N.V., H. Dai, K.L.T. Roos, N.K. Evanson, J. Tomsik, T.S. Elton and D.L. Simmons, 2002. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: Cloning, structure and expression. Proc. Natl. Acad. Sci. USA., 99: 13926-13931.
13. Seno, H., M. Oshima, T.O. Ishikawa, H. Oshima and K. Takaku *et al*., 2002. Cyclooxygenase 2- and Prostaglandin E_2 Receptor EP_2-dependent angiogenesis in *Apc*$^{\Delta 716}$ mouse intestinal polyps. Cancer Res., 62: 506-511.
14. Fujimura, T., T. Ohta, K. Oyama, T. Miyashita and K. Miwa, 2006. Role of cyclooxygenase-2 in the carcinogenesis of gastrointestinal tract cancers: A review and report of personal experience World J. Gastroenterol., 12: 1336-1345.
15. Andújar, I., A. Martí-Rodrigo, R.M. Giner, J.L. Ríos and M.C. Recio, 2018. Shikonin prevents early phase inflammation associated with azoxymethane/dextran sulfate sodium-induced colon cancer and induces apoptosis in human colon cancer cells. Planta Med., 84: 674-683.
16. Fleming, M., S. Ravula, S.F. Tatishchev and H.L. Wang, 2012. Colorectal carcinoma: Pathologic aspects. J. Gastrointestinal Oncol., 3: 153-173.
17. Rajabi, F., W.Y. Liu-Bordes, M. Pinskaya, F. Dominika and G. Kratassiouk *et al*., 2020. CPEB1 orchestrates a fine-tuning of miR-145-5p tumor-suppressive activity on TWIST1 translation in prostate cancer cells. Oncotarget, 11: 4155-4168.

18. Minshall, N., J. Walker, M. Dale and N. Standart, 1999. Dual roles of p82, the clam CPEB homolog, in cytoplasmic polyadenylation and translational masking. RNA, 5: 27-38.
19. Ding, Y., J.L. Yan, A.N. Fang, W.F. Zhou and L. Huang, 2017. Circulating miRNAs as novel diagnostic biomarkers in hepatocellular carcinoma detection: A meta-analysis based on 24 articles. Oncotarget, 8: 66402-66413.
20. Hu, L., H. Kolibaba, S. Zhang, M. Cao and H. Niu *et al*., 2019. MicroRNA-204-5p inhibits ovarian cancer cell proliferation by down-regulating USP47. Cell Transplant., 28: 51S-58S.
21. Dai, M., L. Li and X. Qin, 2019. Clinical value of miRNA-122 in the diagnosis and prognosis of various types of cancer. Oncol. Lett., 17: 3919-3929.
22. Fulda, S. and S. Pervaiz, 2010. Apoptosis signaling in cancer stem cells. Int. J. Biochem. Cell Biol., 42: 31-38.