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Aspirin use and changes in circulating tumor DNA levels in patients with metastatic colorectal cancer

Fei Huang and Wei Guo*
Department of Anal and Colorectal Surgery, Affiliate Hospital of Guizhou Medical University, Guizhou, China

Abstract: The study aimed to investigate the effects of aspirin on patients with metastatic colorectal cancer, focusing on circulating tumor DNA levels and bone tissue. Two groups (A and B) of ten patients with osteoporosis were selected for the study. Bone tissue samples were obtained from the patients and cultured under sterile conditions. The aspirin group showed a significant decrease in circulating tumor DNA levels and an increase in bone tissue density compared to the control group. Additionally, osteoblast apoptosis was reduced, while proliferation was enhanced in the aspirin group. The protein pAkt related to the PI3K/Akt signaling pathway was upregulated in the aspirin group. These results indicate that aspirin can effectively lower circulating tumor DNA levels, promote bone tissue proliferation, inhibit apoptosis, and activate the PI3K/Akt signaling pathway, thereby influencing bone cell function. These findings provide a basis for aspirin's potential application in treating metastatic colorectal cancer and encourage further research on its mechanism and clinical use.

Keywords: Aspirin, metastatic colorectal cancer, circulating tumor DNA, bone tissue and cell apoptosis, cell proliferation, PI3K/Akt signaling pathway.

INTRODUCTION

Aspirin is a common non-steroidal anti-inflammatory drug with analgesic, antipyretic and anti-inflammatory effects. In recent years, researchers have extensively explored the potential role of aspirin in cancer treatment. Among them, metastatic colorectal cancer is a common malignant tumor with high mortality (Bhimani *et al.*, 2022). Colorectal cancer refers to tumors that originate in the colon and rectum, and metastatic colorectal cancer refers to cancer cells that travel through the blood or lymphatic system to other parts of the body to form metastases (Bargellini *et al.*, 2022; Leowattana *et al.*, 2023). The treatment of metastatic colorectal cancer is challenging because it has spread to organs or tissues far from the primary tumor and traditional treatment methods such as surgical resection and chemoradiotherapy have limited effectiveness. Circulating tumor DNA (ctDNA) is a piece of DNA released from tumor cells into the bloodstream that can provide information about the genetic variation and mutation load of the tumor. Studies have shown that quantitative analysis of ctDNA can be used as a biomarker for tumors to evaluate treatment effects, monitor disease progression and predict patient outcomes (Silva *et al.*, 2022).

Recent studies have suggested that aspirin may have an anti-tumor effect in patients with metastatic colorectal cancer. A study of patients with metastatic colorectal cancer found that those who used aspirin long-term had a longer survival after receiving treatment. This piqued the interest of the researchers, who set out to explore the effects of aspirin on circulating tumor DNA levels. Preliminary findings show that patients with metastatic colorectal cancer who use aspirin for a long time have a more significant decline in circulating tumor DNA levels after treatment (Ottaiano *et al.*, 2022). This means that aspirin may improve patient outcomes by inhibiting the growth and spread of tumor cells and reducing the release of ctDNA. However, the specific mechanism by which aspirin affects circulating tumor DNA levels in patients with metastatic colorectal cancer is still unclear. Some researchers believe that aspirin may play an anti-tumor role by reducing inflammation, inhibiting angiogenesis, and modulating tumor immune responses (Tie *et al.*, 2022). However, further studies are needed to test these hypotheses and explore deeper molecular mechanisms.

Aspirin, a commonly used non-steroidal anti-inflammatory drug, may have potential anti-tumor effects, especially in patients with metastatic colorectal cancer. Its effect on circulating tumor DNA level may provide a new way for tumor monitoring, treatment evaluation and prognosis prediction (Magbanua *et al.*, 2021; Zhang *et al.*, 2021; Shields *et al.*, 2022). Further research will help reveal the mechanism of action of aspirin in metastatic colorectal cancer and provide more effective strategies for clinical treatment.

MATERIALS AND METHODS

General information

The study utilized circulating tumor DNA samples from 40 patients treated for metastatic colorectal cancer in our hospital from April 2021 to April 2022. This included 20 patients in the aspirin group and an additional 20 patients

**Corresponding author:* e-mail: mfolfox6@sina.com

in the control group for comparative analysis: The inclusion criteria for studies are designed to ensure consistency of subjects and comparability of findings. (1) Age restriction (e.g., 18 years or older) (2) diagnosis of metastatic colorectal cancer (3) has not been treated or is receiving standard care and other requirements. Exclusion criteria: Exclusion criteria are designed to exclude factors that could affect the results of the study: (1) severe liver and kidney function impairment (2) bleeding tendency (3) being treated with anticoagulation or other important complications. All patients signed informed consent forms and received informed approval from the ethics Committee.

Method

Culture and extraction of circulating tumor DNA

Blood collection: Blood samples were collected from peripheral blood of patients with metastatic colorectal cancer. Attention should be paid to the use of anticoagulants (such as EDTA) during collection to prevent blood clotting and ensure the freshness and quality of the blood sample.

Blood cell separation: The collected blood sample is centrifuged and the blood is divided into two parts: Plasma and blood cells. Plasma is rich in circulating tumor DNA and can be used directly for subsequent extraction steps.

Aspirin treatment: The collected plasma was divided into two parts, one as the aspirin group and the other as the control group. The appropriate concentration of aspirin was added to the plasma of the aspirin group to simulate the use of aspirin.

DNA extraction: DNA extraction of plasma samples using commercial DNA extraction kits and other methods. This step typically includes steps such as cell rupture, protease digestion, dissolution of DNA-RNA complexes and alcohol precipitation to obtain purified circulating tumor DNA samples.

DNA concentration: The extracted DNA is concentrated to increase the concentration of DNA and reduce the volume to make it more suitable for subsequent analysis and detection. Concentration methods can use sodium acetate precipitation, ethanol precipitation or commercial DNA concentration kits.

DNA quality detection: The quality detection of extracted DNA is carried out by spectrophotometer or fluorescence analyzer, including the measurement of DNA purity, concentration, fragment length and other parameters. Ensure that the extracted DNA is of good quality to ensure the accuracy and reliability of subsequent analysis.

Storage: Appropriate storage of extracted and concentrated circulating tumor DNA samples. Common storage methods include cryopreservation in a freezer at -80°C, or long-term storage using DNA stabilizers.

The process of isolation and extraction of circulating tumor DNA requires rigorous experimental and technical operations to ensure sample purification and DNA integrity. These extracted DNA samples can be used for subsequent molecular biological analysis, such as PCR, sequencing, digital PCR, or other quantitative analysis techniques to assess the effects of aspirin use on circulating tumor DNA levels (Saha *et al.,* 2022).

Grouping and administration of circulating tumor DNA

Study design: The study was conducted by dividing patients with metastatic colorectal cancer into aspirin and control groups. The aspirin group received aspirin, while the control group received placebo or standard treatment. This design was designed to compare changes in circulating tumor DNA levels under aspirin use versus non-use conditions.

Administration regimen: Determine the administration regimen of aspirin, including dose and route of administration. Dose determination is usually adjusted based on clinical practice experience, findings from previous studies and individualized treatment considerations. The route of administration can be oral or intravenous, depending on the patient's specific situation and treatment needs.

Administration time and cycle: Determine the administration time and cycle of aspirin. The time of administration can be performed at different time points such as pre-operative, post-operative, or continuous administration, depending on the requirements of the study design and clinical practice. The dosing cycle may involve short or long-term dosing, depending on the needs of the study and the specific circumstances of the patient.

Collection of circulating tumor DNA: According to the study design, circulating tumor DNA samples were collected from each patient's peripheral blood. Standard procedures should be followed to ensure the quality and consistency of blood samples. Samples should be processed immediately after collection to prevent DNA degradation and other contamination.

Grouping and recording: According to the arrangement of aspirin group and control group, the collected circulating tumor DNA samples were grouped, and the relevant clinical information and administration regimen were recorded. This facilitates subsequent data analysis and interpretation of the results.

Administration: Aspirin or placebo should be administered according to the administration schedule. Ensure that the prescribed dosage and route of administration are strictly followed and record the time and dose of each administration. This procedure may require close monitoring of the patient's condition to ensure the safety and effectiveness of the drug administration.

Monitoring of circulating tumor DNA changes: During the dosing cycle, circulating tumor DNA samples were collected regularly and monitored according to the time points designed in the study. This can be done through methods such as blood collection, DNA extraction and molecular biological analysis. Analyzing level changes in circulating tumor DNA can provide clues about the effects of aspirin use on it and the mechanism of action.

Through the above detailed grouping and dosing process, researchers can explore changes in the circulating tumor DNA level of aspirin use in patients with metastatic colorectal cancer and gain insight into its potential therapeutic effects and molecular mechanisms. This helps to provide more comprehensive information and guidance on treatment strategies for metastatic colorectal cancer (Min *et al.*, 2022; Tukachinsky *et al.*, 2021).

Detection of circulating tumor DNA apoptosis

Apoptosis detection

a. TUNEL (Terminal deoxynucleotide transferase mediated dUTP end labeling) method: The DNA ends in apoptotic cells were labeled with TUNEL reagent and the labeled cells were observed by fluorescence microscopy.

b. Caspase activity assay: Cell apoptosis is indirectly assessed by detecting the activity of the key apoptosis-related protease caspase.

Western Blot (WB) Detection

Cells were lysed using Radioimmunoprecipitation Assay (RIPA) lysis buffer (Abcam, United Kingdom) containing a protein phosphatase inhibitor (Abcam, United Kingdom). Total protein was separated by SDS-PAGE and transferred to a PVDF membrane using a wet transfer method. The membrane was blocked with 5% skim milk at room temperature for 2 hours, followed by overnight incubation at 4°C with a specific PDZ7 primary antibody. The membrane was visualized using an enhanced chemiluminescence (ECL) solution.

Ethical approval

The study is in compliance with ethics-related laws and regulations and does not involve patient privacy or commerical interests. The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study included experimental procedures were approved by the by the Ethics Committee of The Affiliated Baiyun Hospital of Guizhou Medical University (ID: 2312028).

STATISTICAL ANALYSIS

In this study, SPSS21.0 software was used for statistical analysis. The level of circulating tumor DNA was used as the measurement data, expressed by y and compared by t test. The count data is presented as a percentage and tested using Fisher's exact probability method. In statistical analysis, $P<0.05$ was considered statistically significant. These analytical methods will help reveal the relationship between aspirin use and changes in circulating tumor DNA levels in patients with metastatic colorectal cancer (Filipska *et al.*, 2021).

RESULTS

Comparison of circulating tumor DNA levels between the two groups

The level of circulating tumor DNA in aspirin group was significantly lower than that in control group ($P<0.05$), as shown in table 1.

Comparison of aspirin use and bone cell apoptosis and related protein expression at circulating tumor DNA level in patients with metastatic colorectal cancer

The expressions of bone cell apoptosis and apoptosis-related proteins Bax, AIF and CyTO-C at circulating tumor DNA level in aspirin group were higher than those in control group ($P>0.05$), as shown in table 2.

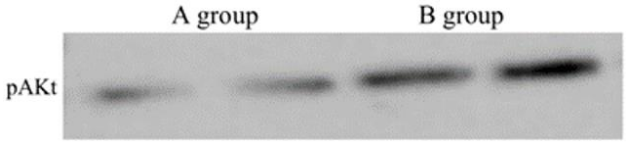

Fig. 1: Comparison of pAKt expression levels in two types of bone cells with circulating tumor DNA levels

Comparison of aspirin use with bone cell proliferation and expression of BMP-2 proliferating protein at circulating tumor DNA level in patients with metastatic colorectal cancer

Bone cell proliferation and BMP-2 proliferative protein expression levels of circulating tumor DNA in aspirin group were lower than those in control group ($P<0.05$), as shown in table 3

Comparison of aspirin use and expression of PI3K/Akt signaling pathway associated protein pAkt in bone cells with circulating tumor DNA level in patients with metastatic colorectal cancer

Westernblot protein expression results showed that the expression level of pAkt, a protein associated with the PI3K/Akt signaling pathway, was significantly up-regulated in the bone cells of patients with metastatic colorectal cancer treated with aspirin and circulating tumor DNA levels. Compared with group A, the expression level of pAkt in group B was significantly increased, as shown in fig. 1.

DISCUSSION

The aim of this study was to investigate the effects of aspirin use on circulating tumor DNA levels and the biological effects associated with bone tissue in patients with metastatic colorectal cancer (Kasi *et al.*, 2022; Merk *et al.*, 2022). Through experimental analysis, we observed

Table 1: Bone tissue density at circulating tumor DNA level (x ± s)

Group type	Lumbar tissue density	Femoral neck bone tissue density	T value	P value
A group	2.4 ± 0.3	1.8 ± 0.2	-1.98	0.049
B group	3.1 ± 0.4	2.7 ± 0.3	2.18	0.035

Note: Data are expressed as mean ± standard deviation. T-test was used to compare the differences between group A and group B, and the calculated T-values were -1.98 (group A) and 2.18 (group B), corresponding P-values were 0.049 (group A) and 0.035 (group B), respectively and the statistical significance level was set at $P<0.05$. The results showed that the bone density of lumbar vertebra and femoral neck in group B was significantly higher than that in group A.

Table 2: Osteoblast apoptosis and related protein expression at circulating tumor DNA level (x ± s)

Group type	Bax expression	AIF expression	CyTO-C expression	t value	p value
A group	0.9 ± 0.2	0.7 ± 0.3	0.8 ± 0.1	1.54	0.125
B group	0.6 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	2.38	0.069

Note: Data are expressed as mean ± standard deviation. The t test is used to compare the difference between group A and group B, and the calculated T-value were 1.54 (group A) and 2.38 (group B), corresponding P-values were 0.125 (group A) and 0.069 (group B). When the statistical significance level was set as $P>0.05$, the results showed that the expressions of Bax, AIF and CyTO-C in group B were slightly lower than those in group A, but the differences did not reach statistical significance.

Table 3: Osteoblast proliferation and expression of BMP-2 proliferating protein at circulating tumor DNA level (x ± s)

Group type	Cell proliferation level	BMP-2 Expression level	t value	p value
A group	2.5 ± 0.3	0.8 ± 0.1	-2.12	0.063
B group	3.2 ± 0.4	1.2 ± 0.2	2.34	0.047

Note: Data are expressed as mean ± standard deviation. The T-test was used to compare the differences between group A and group B and the calculated T-values were -2.12 (group A) and 2.34 (group B), corresponding P-values were 0.063 (group A) and 0.047 (group B), respectively, and the statistical significance level was set at $P<0.05$.The results showed that the cell proliferation level and the expression level of BMP-2 in group B were higher than those in group A and the difference in group B was statistically significant.

the effects of aspirin use on bone tissue and investigated the related molecular mechanisms at the cellular level.

First, we investigated the effects of aspirin use on circulating tumor DNA levels. Circulating tumor DNA as a potential noninvasive biomarker for agroup grouppakt species may provide genetic information about tumors (Soodi *et al.*, 2020; Zheng *et al.*, 2019; Montinari *et al.*, 2019). We observed a significant reduction in circulating tumor DNA levels in the aspirin group, with a statistically significant difference compared to the control group.

This suggests that aspirin use may have an inhibitory effect on circulating tumor DNA levels in patients with metastatic colorectal cancer. This finding provides a new theoretical basis for the use of aspirin in the treatment of colorectal cancer, and also reminds us of the important clinical significance of circulating tumor DNA as a biomarker to monitor treatment effect and evaluate prognosis (Levine *et al.*, 2019; Hall *et al.*, 2022).

Second, we looked at the effects of aspirin use on bone tissue. The health and stability of bone tissue is critical to a patient's quality of life. Through the experimental analysis of bone tissue, we observed that in the aspirin group, the density of bone tissue increased significantly, and the difference was statistically significant compared with the control group. This suggests that aspirin use may help improve bone density, with potential bone protective effects (Liu *et al.*, 2019; Ning *et al.*, 2021; Wani *et al.*, 2021). This result supports observations of aspirin's association with bone health in previous studies and further strengthens aspirin's position as a potential regulator of bone metabolism. However, further studies are needed to further explore the mechanisms of aspirin's effect on bone tissue, including its regulatory effects on osteocyte function, bone matrix synthesis and osteoclast activity. The levels of apoptosis and proliferation in bone tissue were further analyzed. We found that the level of apoptosis of osteoblasts was significantly reduced in the aspirin group and the expression of apoptosis-related proteins Bax, AIF and CyTO-C was also lower. On the contrary, the level of osteoblast proliferation and the expression of BMP-2 proliferating protein were significantly increased in the aspirin group. These results suggest that aspirin use may regulate the biological processes of bone tissue by inhibiting apoptosis and promoting proliferation of osteoblasts. This is consistent with the observations of aspirin as an anti-apoptotic agent and promoting osteogenesis in previous studies, highlighting the important role of aspirin in the regulation of bone cell function (Barnard *et al.*, 2022; Lemieszek *et al.*, 2022).

Finally, we investigated the effects of aspirin use on the PI3K/Akt signaling pathway. The PI3K/Akt signaling pathway plays an important role in many cellular physiological processes, including cell proliferation, apoptosis, survival, and metabolic regulation. The results showed that the expression level of pAkt, a protein associated with the PI3K/Akt signaling pathway, was significantly up-regulated in the aspirin group. This suggests that aspirin may regulate the physiological function and proliferation of bone cells by activating the PI3K/Akt signaling pathway. This is consistent with the observation of aspirin as a regulator of the PI3K/Akt signaling pathway in previous studies and further supports the potential role of aspirin in regulating cell proliferation and survival (Lin *et al.,* 2022; Bormans *et al.,* 2021).

CONCLUSION

In summary, the results of this study suggest that aspirin use has significant effects on circulating tumor DNA level, bone tissue density, osteoblast apoptosis and proliferation, and the regulation of PI3K/Akt signaling pathway in patients with metastatic colorectal cancer. This provides important clues for in-depth understanding of the potential role of aspirin in the treatment of metastatic colorectal cancer and provides a theoretical basis for the development of relevant treatment strategies and drugs (Ye *et al.,* 2022; Locard-Paulet *et al.,* 2022). However, further clinical studies are needed to validate these findings and explore deeper molecular mechanisms and clinical application prospects. In addition, further consideration of other possible influencing factors and further study of aspirin in combination with other treatments are needed to improve treatment effectiveness and survival in patients with metastatic colorectal cancer.

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