Original Article

Aspirin induced long non coding RNA suppresses colon cancer growth

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Background: To study the effect of long non-coding RNAs (lncRNA) on aspirin-treated colon cancer cells.

Methods: To study the changes of lncRNA in aspirin-treated colon cells by microarray analysis, real-time quantitative PCR (qPCR) was used to verify the expression of selected lncRNA and mRNA. The effects of aspirin on the proliferation and metastasis of the siRNA transfected cells and control colon cancer cells were detected by Cell Counting Kit-8 (CCK-8) and Transwell experiments. Bioinformatics tools were employed to analyze the potential function of lncRNA.

Results: Compared with the control group, aspirin inhibited the proliferation and metastasis of colon cancer cells. Microarray analysis showed that a total of 10,568 lncRNAs and 22,126 mRNAs were noticeably expressed in the aspirin-treated group (≥1.5-fold, $P<0.05$). The qPCR results showed that lncRNA and mRNA expressions were consistent with microarray analysis. The analysis of the co-expression network profiles of 58 lncRNA and 101 mRNA differential genes showed a total of 158 nodes and 791 connections. Analysis of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways revealed that a variety of lncRNAs (NEAT1, LOC152578) were involved in the inhibition mechanism of aspirin in colon cancer.

Conclusions: lncRNA NEAT1 and LOC152578 are involved in the inhibition of tumor cell growth and metastasis by aspirin. The results of these analyses will help us further understand the mechanism of action of aspirin and the roles of lncRNAs in the prevention and treatment of colon cancer.

Keywords: Colon cancer (CRC); aspirin; lncRNA

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Introduction

Colorectal cancer (CRC) is the third most common cancer in the world, and the most common type is colon adenocarcinoma (CA) (1). It was estimated that approximately 1 in 18 people would eventually develop CRC in their lifetime, and 40% of whom would die within five years of diagnosis, mainly due to late diagnosis (2,3). The development of the CRC is a slow process, and it takes several years to invade and metastasize from atypical hyperplasia, polyps, adenomas, and CA (4,5). Chronic inflammation is one of the causes of CRC (6). Aspirin is a classic non-steroidal anti-inflammatory drug (NSAID)

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that has been used in a wide range of conditions, including fever, pain. and inflammatory diseases (7). Recent studies show that long-term and low-dose aspirin can reduce the incidence of cancer, delay the malignant transformation process, reduce the risk of tumor metastasis, and cancer mortality (8-14). Although the beneficial aspects of aspirin for cancer patients have been widely recognized, the mechanism of its effect remains unclear. Previous studies have confirmed that aspirin's anticancer effect is attributed to the inhibition of COX-2, which is upregulated in a variety of cancer cells (15,16). It is worth noting that there is increasing evidence that aspirin may also exert anticancer effects in a COX-independent manner. Long non-coding RNA (lncRNA) is an RNA molecule that is about 200 nucleotides long and does not translate protein functions. lncRNAs are similar to mRNAs in that they are usually transcribed by RNA polymerase II, 5' end-capped, 3' polyadenylation, and splicing multiple exons by classical genome splicing motifs (17-19). lncRNA has been shown to participate in a variety of biological processes (BP) such as transcription, translation, splicing, intracellular and extracellular transport, and has been implicated in a variety of diseases. lncRNA can interact with proteins, DNA, and RNA, to participate in all levels of gene regulation, including epigenetics, transcription, and post-transcriptional regulation (17,20-22). Many lncRNAs have been shown to play critical roles in the development of various diseases or identified as important biomarkers in diagnosis and treatment (23,24). At the same time, genome-wide association analysis of various tumor samples has revealed many lncRNAs associated with multiple types of cancers, including CRC (25). Previous studies have shown that multiple lncRNAs are involved in the occurrence and development of colon cancer. Some lncRNAs, such as CCAL, CASC11, CCAT2, H19, and HOTAIR (26-28), were overexpressed in CRC tissues and cells. Patients with high expression of these lncRNAs have a higher incidence of distant metastases and a lower survival rate. Although the molecular mechanism of aspirin's anti-cancer effect has been studied extensively, the involvement of many cellular components (CC), such as long non-coding RNAs, is not fully understood. This study attempts to answer these questions by looking into the expression differences of lncRNA and mRNA in aspirin-treated colon cancer cells. We present the following article in accordance with the MDAR checklist (available at http://dx.doi.org/10.21037/tcr-20-2248).

Methods

Cell culture and aspirin treatment

Human CRC cell lines HCT116, SW620, and DLD1 were purchased from the American Type Culture Collection (ATCC, China), and cultured in DMEM (Hyclone, Logan, Utah, USA) (Carlsbad, California, USA) containing 100 IU/mL penicillin and 100 μg/mL streptomycin. For all studies, colon cells were incubated at 37 ℃, 5% CO_2, and 90–95% of relative humidity. Aspirin was purchased from Sigma (Sigma-Aldrich, St. Louis, Missouri, USA), and a 1 M stock solution (dissolved in ethanol) was prepared.

Cell migration assay

For the detection of cell migration capacity, a 24-well chamber with 8 μm wells was used. First, 1×10^5 cells were seeded in DMEM medium in the upper chamber (excluding serum). 700 μL medium containing 10% Fetal Bovine Serum (FBS) was then added to the lower chamber. After 24 hours of incubation at 37 ℃, the cells in the upper chamber were carefully removed with a cotton swab. Cells that passed through the membrane were fixed with methanol and then stained with 0.5% crystal violet. For quantification, cell counts were performed under a microscope at ×100 magnification from eight randomly selected fields. Transiently transfect hNEAT1 and LOC152578 siRNA into colon cancer cell line SW620 and HCT116. After 48 hours, the effect of siRNA on the migration ability of colon cancer cells was verified.

Microarray analysis

Gene microarray analysis was performed on colon cells treated with aspirin to detect differentially expressed lncRNA and mRNA. Approximately 10,568 lncRNAs and 22,126 coding transcripts were detected by the Arraystar Human lncRNA Microarray V3. The cell preparation and microarray hybridization were performed using an Agilent Gene Expression Hybridization Kit (Agilent Technologies, Santa Clara, California, USA). The array was then scanned using an Agilent microarray scanner and analyzed using The GeneSpring v.13.1 software (Agilent Technologies).

Real-time qRT-PCR assay

Total RNA was extracted from samples using TRIzol

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Table 1 Primers and siRNA sequences

Gene name	Sense (5'-3')	Antisense (3'-5')
GAPDH	GACCTGACCTGCCGTCTA	AGGAGTGGGTGTCGCTGT
SCARNA13	TCTGTAGTCTTGGAGCCG	TCTTACTGTTGGCGGATA
PKI55	GGATCATAATTCAACCCAT	TTACCCTTGCCTTACCAC
LOC100288432	TGCCCTACAACACCAACC	CATTCACTTCCCTCTGCTT
LOC100128191	ATTGAAGGGCATCATAGC	CTGGGTGACAGAGCGAGA
HSP90AB4P	AGATGTGGGTTCCGATGA	TCGGCTTGGTCTGGTTTA
LOC152578	ACGAAGGTGGTAACAGAG	TTAAGCCAAGAAGTGAGG
TRIM49	CCCCTGTGCATGAACTAC	GGTTTATCTGCTCGGTTG
RFTN2	TTCGGATGGCTTCTGACA	GCGGCTGGCTTTCTTATC
AKAP14	GTGGAAGAGGAGCGAAAC	AGAAACAACAATGGGTGC
OR1A1	ATCGGTAACCATCCCTAA	CAAGACCCAGCAATAAGC
CYB5B	GAAGAGGTTCTGCTGGAA	AGGTCACTCGGATGGATA
S100Z	AGAATCTTCCACCGCTAT	AGGTCCTGCACTATCTTATC
CXorf66	TGTCTGAGCGATGATGCG	TTCTGTGCTGGATTGTGC
HBEGF	TGTCTGAGCGATGATGCG	TTCTGTGCTGGATTGTGC
CCDC36	ATTCAGAAACCCTATCAGC	CAAAGGAGGAGGAAATAA
ZNF699	AGGATGTGGCTGTGGACT	AAAGATGCCCTGGATAAG
hNEAT1-998 siRNA	UGGUAAUGGUGGAGGAAGATT	UCUUCCUCCACCAUUACCATT
hNEAT1-3558 siRNA	GGGAAGUAGUCUCGGGUAUTT	AUACCCGAGACUACUUCCCTT
hNEAT1-2352 siRNA	GUGAGAAGUUGCUUAGAAATT	UUUCUAAGCAACUUCGUUCTT
hLINC01618-133 siRNA	GAACGAAGGUGGUAACAGATT	UCUGUUACCACCUUCGUUCTT
hLINC01618-120 siRNA	GGUUAAGCAGGGAGAACGATT	UCGUUCUCCCUGCUUAACCTT
hLINC01618-182 siRNA	GAGAGUGAUUCAUCCUAAATT	UUUAGGAUGAAUCACUCUCTT

(Invitrogen, Carlsbad, California, USA). Detection of relative gene expression by SYBR Green PCR Mix (BIORESEARCHER, Beijing, China) and LightCycler 96 Real-Time System.

The qRT-PCR was performed on (Roche LC96). The thermal cycling program was 95 ℃ for 5 min, followed by 40 cycles of denaturation at 95 ℃ for 10 s, and amplification at 60 ℃ for 1 min. Melting curve analysis was performed by progressive heating from 65 to 95 ℃. The relative expression level of lncRNA or mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method and standardized for GAPDH. The primer sequences are summarized in *Table 1*. Data represent the average of three independent experiments.

Identification of differentially expressed genes (DEGs)

Probes with more than one gene and empty probes were discarded according to the annotation platform of each expression profile. We then extracted the expression profiles of lncRNA and mRNA, respectively. Three different aspirin-treated colon cells were compared with control. Finally, P value and log2(FC) value of the two expression profiles were calculated, and a paired *t*-test was used to recognize DEGs. Statistically significant DEGs were

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defined as |log2(FC)|<log2(1.5) and P<0.05.

siRNA transfect and cell counting kit-8 (CCK8)

The hNEAT1 and LOC152578 siRNA [Sangon Biotech, (Shanghai) Co., Ltd.] transfected cells and control cells were seeded in 96 well plates and cultured for 0, 24, 48 and 72 h, respectively. In order to transiently transfect siRNA into colon cancer cell line SW620, we first identified the putative promoter region of the target gene, then selected the reporter gene and the corresponding reporter gene analysis method, and finally inserted the promoter into the upstream of the reporter gene of the appropriate vector. After 48 hours, the effect of siRNA on the migration ability of colon cancer cells was verified. 10 μL CCK8 was added to each well and cultured at 37 ℃ and 5% CO_2. The optical density (OD) value was measured at 450 nm. The proliferation activity of SW620 cells was detected at 0, 24, 48 and 72 h after siRNA overexpression (*Table 1*).

Construction of a co-expression network with GO and KEGG analysis

In order to identify the interaction between differentially expressed lncRNA and mRNA, a co-expression network was constructed using Cytoscape software, with validated lncRNA and related mRNA based on correlation analysis. Pearson's correlation coefficient was no less than 0.9. Pathway analysis was used to study important signaling pathways for DEGs. GO analysis was used to investigate the biological effects of aberrantly expressed mRNAs in three aspects: BP, molecular functions (MF), and CC.

Statistical analyses

All data are represented as mean ± SD. Graphpad Prism 5.0 (San Diego, California, USA) was used for statistical analysis. The *t*-test was used to analyze the differences between the control and aspirin-treated data in this study. Pearson's correlation analysis is used to detect the relationship between lncRNA and mRNA. P<0.01 is used as a threshold to define a GO term/pathway that is significantly enriched.

Results

Aspirin inhibits colon cancer cell proliferation and metastasis

The concentrations of aspirin used in this study ranged from 1 to 15 mM, based on past studies (29). The inhibitory effect of aspirin was determined by cell growth. By treating HCT116, DLD1, and SW620 cells with different concentrations of aspirin, aspirin inhibited colon cancer cell proliferation in a dose-dependent manner. When 50% of the cells were inhibited, the concentrations of aspirin were approximately 5, 5, and 3 mM, respectively (*Figure 1A*). The migration ability of the colon cells was also inhibited by the same concentrations (*Figure 1B,C*). These concentrations of aspirin were used in subsequent experiments for the three cell lines. Furthermore, when NEAT1 was knocked down, the cell proliferation rate decreased, indicating that NEAT1 could promote the proliferation of SW620 cell line. Aspirin has been reported to inhibit the growth of colon cancer cell line NEAT1. So, our experimental results are consistent with previous reports.

lncRNA and mRNA expression analysis in aspirin-treated colon cells

To compare the expression differences between lncRNA and mRNA in aspirin-treated colon cells, microarray analysis was employed to assess their expression levels. The expression profile heatmap was then generated with R language (*Figure 2A,B*). Next, we calculated the differentially expressed lncRNA and mRNA in three types of colon cells treated with aspirin, using |log2(FC)|<log2(1.5) and P<0.05 as the significance threshold. As shown by the volcanic maps, 58 significantly dysregulated lncRNAs were identified in the aspirin-treated group, with 28 being upregulated (red dots, *Figure 3A*) and 30 downregulated (green dots, *Figure 3A*). In comparison, 101 mRNAs were found to be significantly dysregulated, with 56 being upregulated (red dots, *Figure 3B*) and 45 downregulated (green dots, *Figure 3B*). Forty lncRNAs and 40 mRNAs with the most significant differential expressions are summarized in *Table 2* and *Table 3*, respectively.

Validation of the microarray data using qRT-PCR

Ten mRNAs and 6 lncRNAs were randomly selected for qRT-PCR analysis to verify the results of microarray analysis. Consistent with the microarray analysis results, 5 of the selected mRNAs (TRIM49, RFTN2, AKAP14, OR1A1 and CYB5B) of the aspirin-treated group were shown to be upregulated, while the other five mRNAs (S100Z, CXor66, HBEGF, CCDC36 and ZNF699) downregulated. Furthermore, qRT-PCR analysis showed that lncRNA

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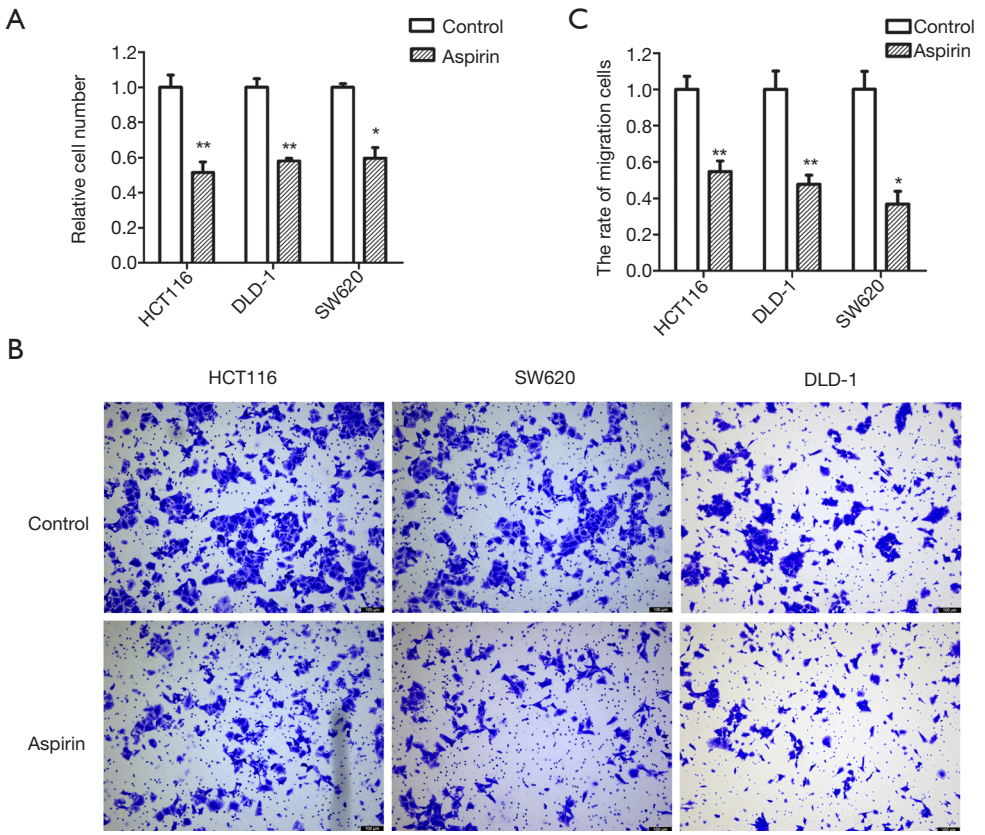

Figure 1 Aspirin inhibits colon cancer cell proliferation and metastasis. (A) The IC50 of aspirin on HCT116, SW620, and DLD1 cell proliferation were 5, 5, and 3 mM, respectively. (B) Three cell lines were treated with aspirin at the same concentrations for migration assays. Representative images show the inhibition of migration by aspirin in three colon cell lines. (C) Statistical results of migration assays. *P<0.05, **P<0.01 (Dyeing method: 0.5% Crystal violet; dyeing scale: ×100).

SCARNA13, PKI55, LOC100288432 and LOC100128191 were upregulated, while lncRNAs HSP90AB4P and LOC152578 downregulated (*Figure 4*), confirming the validity of the microarray results. Both analyses provided compelling evidence that these lncRNAs and mRNAs may be involved in the pathogenesis of aspirin-treated colon cancer.

GO term enrichment analysis

GO term enrichment analysis results were presented with a bubble chart (*Figure 5*). DEGs were significantly enriched in GO cell components (CC), such as mitochondrial part, organelle envelope, mitochondrial membrane, and mitochondrial envelope. For MF, the DEGs were enriched in RNA binding and phosphatase inhibitor activity. In addition, BP analysis also displayed that the DEGs enriched in detection of stimulus involved in sensory perception (*Figure 5*).

Co-expression analysis and construction of lncRNA-mRNA network

To analyze the co-expression profile of lncRNA and mRNA, colon cells treated with aspirin were determined for each possible lncRNA-mRNA pair in the expression data. First, the lncRNAs or mRNAs which were differentially expressed were extracted. By using Pearson correlation coefficient (PCC), we find the correlation expression of lncRNA and mRNA PCC >0.9 and P<0.01 was set as the threshold. A total of 169 DEGs (58 lncRNA and 101 mRNA) was filtered into the DEGs PPI network complex, containing 158 nodes and 791 edges. lncRNA-mRNA co-expression network was visualized by Cytoscape software (*Figure 6*).

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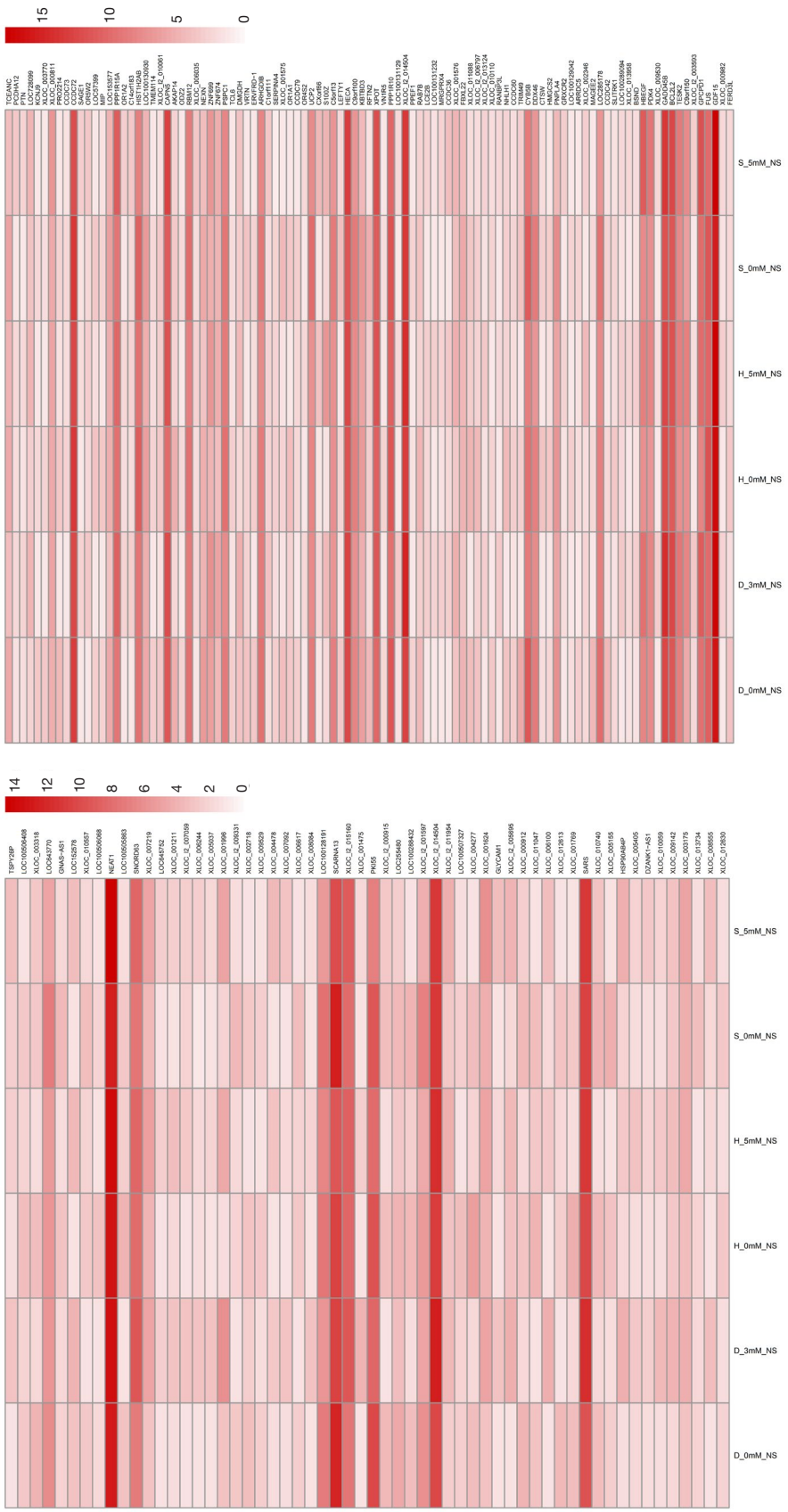

Figure 2 Heat maps of significantly differentially expressed lncRNAs (A) and mRNAs (B) induced by aspirin. The LucRNA (mRNA) name is located on the right following. Red represents high relative expression, and white represents low relative expression; $|\log2(FC)| < \log2(1.5)$ are fold changes in the corresponding spectrum.

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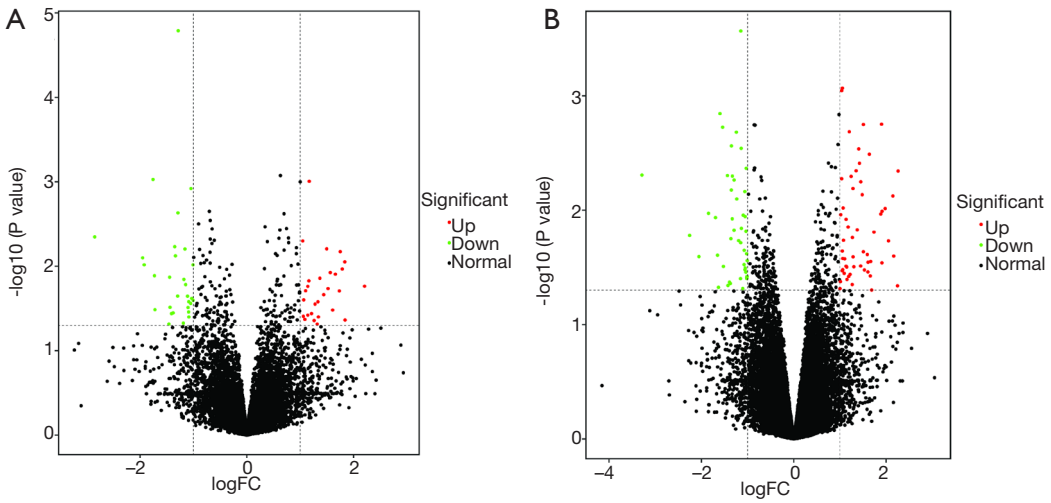

Figure 3 Volcano plots of differentially expressed genes. Scatter plot of lncRNA (A) and mRNA (B) expression variation between the aspirin-treated colon cells and control group. Red, upregulated (FC >1.5, P<0.05); green, downregulated (FC <1.5, P<0.05); black, non-differentially expressed.

Effects of NEAT1 and LOC152578 siRNA on proliferation and migration of colon cancer cell lines

In order to study the effects of NEAT1 and LOC152578 siRNA on the proliferation and migration of colon cancer cell lines, we carried out CCK8 and Transwell experiments. First, we verified the success of siRNA knockdown by PCR (*Figure 7A*). Next, we tested the effects of NEAT1 and LOC152578 knockdown on the proliferation of colon cancer cell lines. The results showed that NEAT1 could promote the proliferation of SW620 cells, while LOC gene knockout did not inhibit the proliferation of SW620 cells (*Figure 7B*). In addition, the metastatic ability of SW620 and HCT116 cells was inhibited by knockdown of NEAT1 and LOC (*Figure 7C*). Aspirin has been reported to inhibit the growth of colon cancer cell line NEAT1. Therefore, our experimental results are consistent with previous reports (*Figure 7D*).

Discussion

There has been a growing number of studies in recent years indicating that not only can aspirin prevent several types of cancer, but also reduce the incidence of cancer and significantly inhibit the growth and promote apoptosis of cancer cells. Studies have shown that taking aspirin regularly can significantly reduce the incidence of CRC, factors affecting its efficacy including the time and dose of aspirin as well as the genetic background of the patient (30,31). The antitumor effect of aspirin is mainly due to its inhibition on several major signaling pathways that promote cancer progression, such as COX/PGE2, PI3K/AKT/mTOR, NF-κB, WNT/β-catenin, and MAPK signaling pathways (32,33). lncRNA plays a key role in gene expression regulation at both the transcriptional and the post-transcriptional levels, leading to a wide range of biological processes, such as tumorigenesis, growth, and metastasis in different human diseases, including cancer (34-36).

In this study, we investigated the changes of lncRNA and mRNA expression profiles in response to aspirin treatment in different CRC cells (DLD1, SW620 and HCT116) through microarray analysis. By using bioinformatics methods to analyze the common differential genes, 58 lncRNAs and 101 mRNAs were found to be significantly dysregulated in aspirin-treated colon cells. Further analysis revealed that 28 of the 58 dysregulated lncRNAs were upregulated and 30 downregulated, in response to the aspirin treatment in CRC cells. In the microarray results, the expression of lncRNA NEAT1, LOC152578, GLYCAM1, and SARS was markedly downregulated, compared to their expressions in human colon cancer without aspirin treatment (37,38).

Traditionally a transcriptional regulator, NEAT1 (nuclear enriched abundant transcript 1) and the ribonucleoprotein complexes around NEAT 1 form the paraspeckles, a type of subnuclear body that is found adjacent to nuclear

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Table 2 Top 20 up and down expressed mRNAs in microarray analysis

Seq ID	P value	Log Fold change	Regulation
XLOC_001575	0.004553	2.2583499	up
TRIM49	0.045804	2.245447967	up
RFTN2	0.0252	2.165628467	up
PRO2214	0.007501	2.144754433	up
AKAP14	0.018551	2.052417583	up
OR1A1	0.009666	1.977090867	up
PNPLA4	0.010213	1.9107158	up
XLOC_011088	0.028823	1.9078093	up
LOC57399	0.00177	1.9007854	up
CYB5B	0.010799	1.884759667	up
LEFTY1	0.015506	1.7362161	up
MAGEE2	0.049958	1.684898467	up
VRTN	0.028117	1.666205867	up
CCDC42	0.037526	1.659008567	up
HMGCS2	0.00324	1.635138567	up
LOC285178	0.02851	1.609337333	up
NHLH1	0.034705	1.606518767	up
UCP2	0.024398	1.592529767	up
NEXN	0.033308	1.5285312	up
MIP	0.001781	1.5077345	up
S100Z	0.004927	–3.2854481	down
CXorf66	0.016625	–2.255098667	down
HBEGF	0.025477	–2.051823133	down
CCDC36	0.010654	–1.846675433	down
ZNF699	0.011622	–1.694414533	down
C9orf150	0.024717	–1.649728967	down
GPCPD1	0.047309	–1.631447	down
LOC728099	0.001431	–1.596140967	down
XLOC_l2_013124	0.001884	–1.5388562	down
DMGDH	0.031061	–1.5235226	down
PPP1R15A	0.004982	–1.436081667	down
XLOC_002346	0.045504	–1.424194267	down
GADD45B	0.042651	–1.393122	down
CAPN5	0.043676	–1.371299	down
GDF15	0.017752	–1.360741667	down
RANBP3L	0.006665	–1.352340433	down
MRGPRX4	0.002742	–1.3505622	down
LCE2B	0.011893	–1.331988933	down
LOC100131129	0.005041	–1.3289113	down
OR5W2	0.014999	–1.293592933	down

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Table 3 Top 20 up and down expressed lncRNAs in microarray analysis

Seq ID	P value	Log Fold change	Regulation
XLOC_004277	0.01726	2.205158	Up
SCARNA13	0.043459	1.840178	Up
XLOC_001769	0.008871	1.835909	Up
PKI55	0.010782	1.788771	Up
LOC100288432	0.006722	1.750393	Up
XLOC_005155	0.019629	1.733925	Up
XLOC_l2_000915	0.012424	1.658995	Up
XLOC_011047	0.033063	1.608756	Up
XLOC_l2_001597	0.011913	1.564671	Up
LOC100128191	0.018414	1.521939	Up
XLOC_012613	0.006236	1.495513	Up
XLOC_002718	0.021716	1.440106	Up
XLOC_000912	0.013868	1.368646	Up
XLOC_010740	0.039863	1.341683	Up
XLOC_003318	0.026375	1.333408	Up
LOC100505863	0.048456	1.318521	Up
XLOC_006617	0.02811	1.278153	Up
LOC643770	0.044026	1.260499	Up
GNAS-AS1	0.036415	1.212332	Up
XLOC_009529	0.000986	1.169137	Up
XLOC_001996	0.004501	–2.85207	Down
XLOC_006100	0.007955	–1.95364	Down
XLOC_l2_005695	0.009628	–1.9238	Down
XLOC_004478	0.00094	–1.75715	Down
HSP90AB4P	0.012944	–1.73137	Down
LOC152578	0.032715	–1.72143	Down
XLOC_001475	0.04838	–1.46048	Down
XLOC_010059	0.013521	–1.4465	Down
GLYCAM1	0.030637	–1.43861	Down
XLOC_001624	0.036424	–1.41323	Down
XLOC_008555	0.035774	–1.38151	Down
XLOC_001211	0.005873	–1.35059	Down
XLOC_007219	0.007546	–1.33812	Down
XLOC_007092	0.022619	–1.29642	Down
XLOC_l2_011954	0.002338	–1.29142	Down
LOC645752	1.62E-05	–1.28678	Down
XLOC_l2_014504	0.047263	–1.19057	Down
XLOC_005037	0.014354	–1.17846	Down
XLOC_l2_007059	0.006258	–1.1601	Down
NEAT1	0.01653	–1.14778	Down

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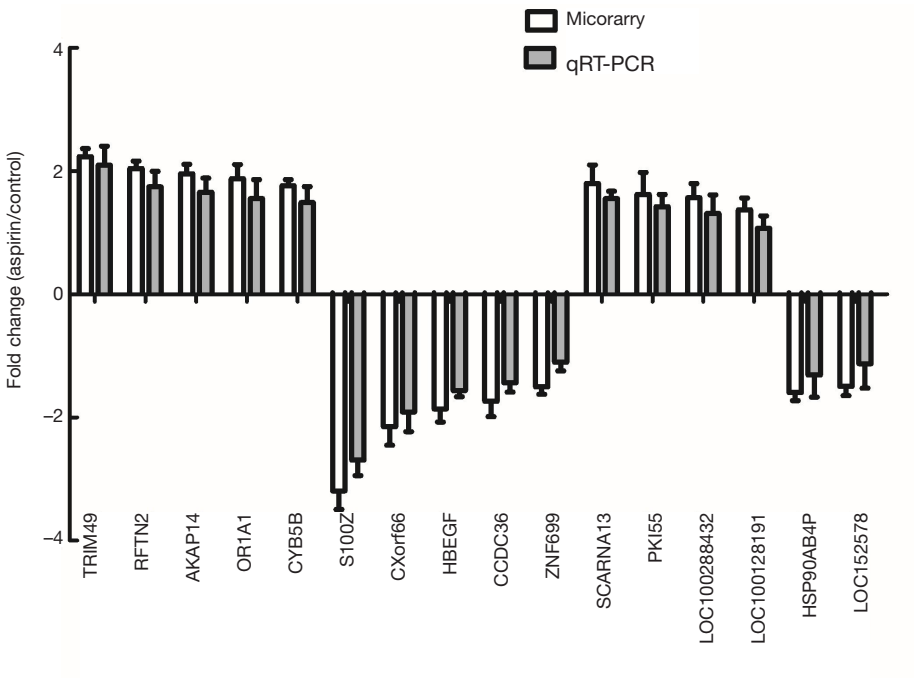

Figure 4 lncRNA and mRNA expression verified by qRT-PCR. Consistent with the microarray assay data, qRT-PCR results show that the expression levels of four lncRNAs (SCARNA13, PKI55, LOC100288432 and LOC100128191) and five mRNAs (TRIM49, RFTN2, AKAP14, OR1A1 and CYB5B) were upregulated in the aspirin-treated group when compared with the control. Two other lncRNAs (HSP90AB4P and LOC152578) and five other mRNAs (S100Z, CXor66, HBEGF, CCDC36 and ZNF699) were downregulated.

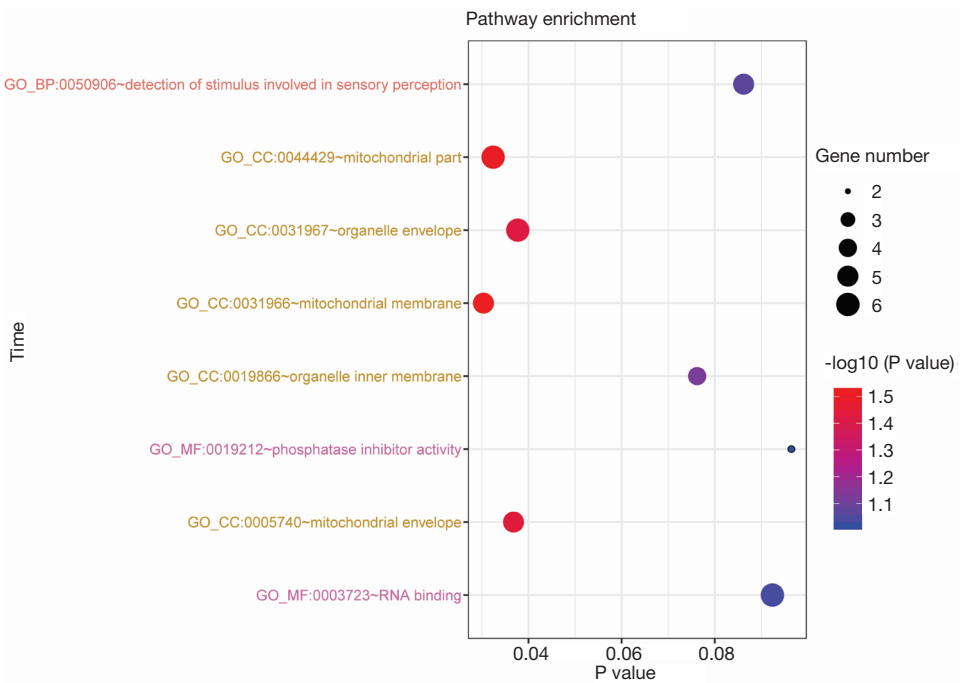

Figure 5 GO and KEGG significant enrichment analysis for DEGs in network. The color of the y-axis indicates the classification of GO terms. Red, biological process (BP); yellow, cellular component (CC); purple, molecular function (MF).

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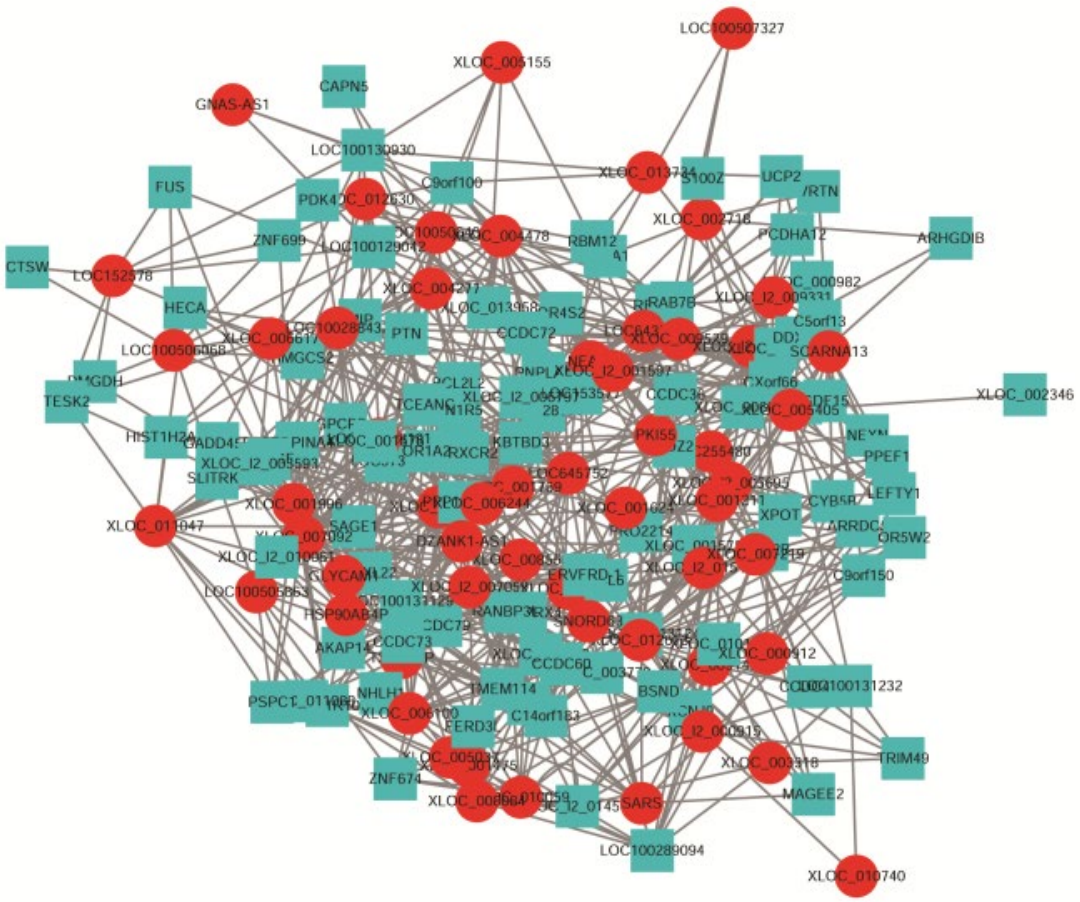

Figure 6 DEGs mRNA–lncRNA network complex and modular analysis. Using the Cytoscape software, a total of 159 DEGs (red circles represent lncRNA, turquoise squares represent mRNA) was filtered into the DEGs network complex.

speckles (38,39). Furthermore, recent studies observed that NEAT1 is upregulated in human CRC tissue and is associated with poor prognosis of CRC, suggesting a critical role in tumor invasion and metastasis. The fact that NEAT1 level is regulated by NF-κB and STAT3 downstream of the epidermal growth factor receptor (EGFR) signaling pathway makes NEAT1 and its upstream mediators interesting therapeutic targets in malignant tumors (40). Despite potential side effects, blocking NF-κB and STAT3 activity may represent a good approach to suppress tumors overexpressing NEAT1 (41). In chronic inflammatory diseases and cancer, NF-κB usually appears to be abnormally active and promotes disease and tumor progression by promoting inflammation, preventing differentiation, driving stem cell proliferation and inhibiting apoptosis (42-44). A large body of data supports that NF-κB activity dysregulation plays a critical role in intestinal tumorigenesis, the type of cancer most sensitive to aspirin treatment.

Recent studies have shown that aspirin inhibits the degradation of I-κB by blocking the activation of the NF-κB pathway to exert antitumor effects *in vitro* and in animal experiments (45). After being phosphorylated and activated by JAK, STAT3, a member of the STAT family of transcription factors, forms a dimer that is transferred from the cytoplasm to the nucleus, and combines with the promoter of the gene of interest and promotes its expression. In recent years, STAT3 has been shown to promote the occurrence and development of gastrointestinal malignant tumors by regulating the overexpression of Bcl-2, survivin, MMP, VEGF, and other proteins in cell proliferation and anti-apoptosis, tumor invasion and metastasis, and tumor angiogenesis (46). On the other hand, aspirin administration has been demonstrated to reduce the

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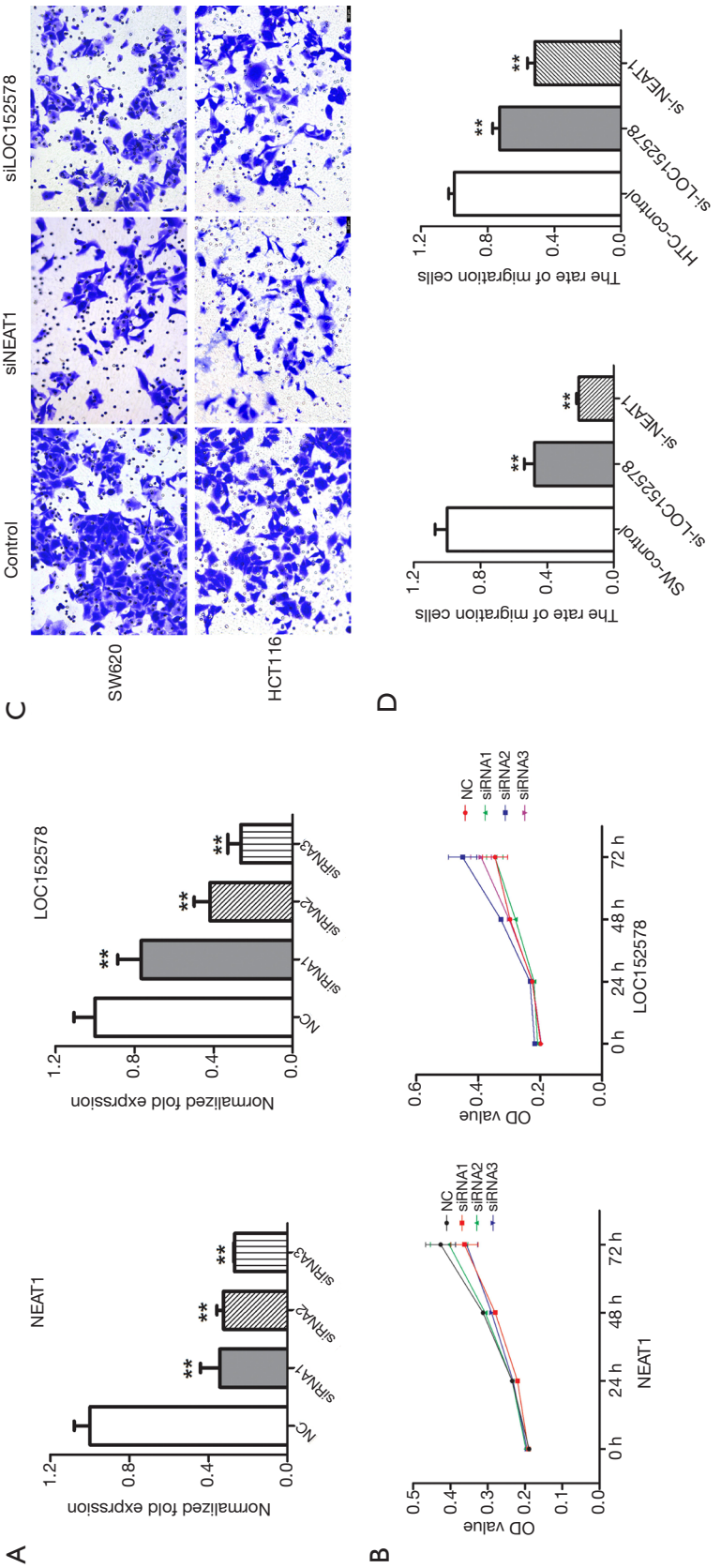

Figure 7 (A) RT-PCR shows siRNA experiment is successful; (B) in both proliferation experiments, knockdown of NEAT1 inhibited cell proliferation, while knockdown of LOC152578 did not; (C) the transfer capability of SW620 cells and HCT116 cells could be inhibited after knockdown of NEAT1 and LOC. (Staining method: crystal violet dyeing, ×200). (D) Statistical chart of Transwell results (**P<0.05).

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MMP-9 expression and inhibit EMT by blocking STAT3 phosphorylation in other tumors (47). However, the exact mechanism remains nebulous thus far. The findings from the current suggest that NEAT1 is a plausible downstream mediator for aspirin's anticancer effects.

Apart from NEAT1, a novel lncRNA, LOC152578, has also been reported recently for its association with CRC carcinogenesis and postoperative recurrence (37,48). However, its regulatory mechanism has not been reported. We therefore took special interests in this lncRNA and included it in our bioinformatic analyses to evaluate its potential as a biomarker or molecular therapeutic target for CRC. Our study observed downregulation of LOC152578 and upregulation of HMGCS2 in aspirin-treated colon cancer cells.

Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS2), a member of the HMG-CoA protein family, is a potential regulatory point in the pathway that converts acetyl-CoA to ketone bodies. However, the role of HMGCS2 in CRC is largely unknown. Recent bioinformatics analyses of TCGA data found that the median survival time of CRC patients with low HMGCS2 expression is significantly shorter than that of those with high HMGCS2 expression (49,50). Zou *et al.* reported in their most recent paper that HMGCS2 expression was significantly reduced in CRC and was negatively correlated with neovascularization density in CRC (51). Taken together, these findings suggest that HMGCS2, as a tumor suppressor gene, was not only downregulated in CRC but also associated with tumor differentiation. In this study, both LOC152578 and HMGCS2 responded to aspirin treatment in colon cancer cells, with the former being downregulated and the later upregulated, implying a negative correlation between these two RNA molecules. Further studies are needed to confirm if the two responds to aspirin treatment independently or two manifests of a common underlying mechanism.

In this study, we aimed to discover the major role of lncRNA in aspirin's inhibition of colon cancer growth, metastasis, and progression. However, it is worth noting that the mechanism of aspirin in tumor prevention and treatment is still unclear. In order to obtain maximum anti-cancer benefits, future research needs to focus on accurate lncRNA molecules and reasonable selection and dosage.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-20-2248). The authors have no conflicts of interest to declare.

Ethical Statement: 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.

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