Original Article

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Noscapine Induces Apoptosis and Inhibits Invasion in Caco-2 Colon Cancer Cells *via* Overexpression of miR-218 As Well as Down-Regulation of lncRNA CCAT2, MYC, and GLI1

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Abstract

Background: There are growing evidences related with critical role of non-coding RNAs (lncRNAs), oncogenes, and microRNAs (miRNAs) in pathogenesis and metastatic capacity of Colorectal Cancer (CRC). This study aimed to elucidate the pro-apoptotic and anti-metastatic effect of Noscapine as alkaloid compound in addition its effect on CCAT2, miR-218-5p, MYC, and GLI in Caco-2 CRC cells.

Methods: Caco-2 cells were exposed to increasing concentrations of Noscapine (10–100 μM). The cytotoxicity, DNA damage, apoptosis recruitment, and cell cycle analysis were examined using MTT assay, comet assay, and fluorometric analysis. IC_{50} measured by GraphPad Prism software was used for anti-cancer analysis. To assess cell invasion, a scratch assay was performed. The quantitative changes in the expression of CCAT2, miR-218-5p, and MYC, GLI1 were evaluated by qRT-PCR.

Results: The MTT assay showed the dose and time dependent attenuation of Caco-2 cells survival rate compared to the control. Comet assay demonstrated significant DNA damage in treated cells. The flow cytometry indicated cell cycle arrest at the G0/G1 phase and late apoptotic and necrotic cell increment. The scratch assay confirmed impaired migration, with markedly reduced wound closure in IC50 treated group. The qRT-PCR showed that in contrast to remarkable up-regulation of miRNA 218 (*p<0.05), the level of CCAT2 (*p<0.05), MYC (**p<0.01) and GLI1 (**p<0.01) was significantly diminished in Noscapine treated cells.

Conclusion: Noscapine exhibited anti-proliferative and anti-migratory effects in Caco-2 CRC cells, which are associated with altered expression of *CCAT2*, *miR-218*, *MYC*, and *GLI1*.

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Introduction

Colorectal Cancer (CRC) is the third most common cancer and the second leading cause of cancer-related mortality in the world, with approximately 153,020 new cases annually in the United States [1]. The common methods of CRC treatment are surgery, chemotherapy, cryosurgery, radiation therapy, immunotherapy and targeted therapy which determine the rate of disease progression and the probability of survival of patients after treatment [2]. The accumulation of genetic and epigenetic alterations is a key driver of CRC development [3]. Regarding CRC, there are two main challenges for the effectiveness of treatment; the suppression of cell proliferation and cell cycle arrest, as well as the prevention of metastasis signaling cascade [1]. Studies have shown that malfunctioning of some genes and signaling pathways related to cell division and proliferation causes cancers such as colon cancer [4].

lncRNAs (long non-coding RNAs) play critical roles in regulating CRC invasion through processes such as transcription, splicing, RNA degradation, and translational control [1,5]. Among these regulatory factors, CCAT2 (lncRNA colon cancer- associated transcript-2) as one of the new members of the lncRNA family, has been located in the category of sense

lncRNAs and plays a crucial role in carcinogenesis and the regulation of programmed cell death [6]. CCAT2 directly interacts with RNA binding proteins and transcription factors and plays a fundamental role in maintaining chromosome instability [7]. CCAT2 is also an Epithelial Mesenchymal Transition (EMT) enhancer and promotes cell proliferation through down regulation of E-cadherin. This lncRNA was identified for the first time in CRC with high levels [8]. In addition, its high expression has also been observed in ovarian and prostate cancer metastasis [9].

Previous studies have shown that the up-regulation of *CCAT2* increases the invasion of colon cancer cells and has a regulatory effect on the expression of its downstream target gene, including *MYC*, which is associated with the risk of colon carcinoma [10]. *MYC* is one of the regulatory proto-oncogene genes which its over expression disrupts aspects of the regulatory metabolism of cell proliferation [11]. It has been reported that *MYC* is involved in therapeutic resistance in various cancer types and its over-expression induced oncogenic stress including oxidative, metabolic and replicative impact [12]. The expression of *MYC* is controlled by a wide range of coding RNAs, including miRNAs and lncRNAs. If the expression of this gene is not properly controlled, its oncogenic level disrupts aspects of the regulatory metabolism of cell proliferation [11]. *MYC* up-regulation leads to an increment in the downstream targets of the gene-mediated metastasis [12]. Following the aforementioned regulatory effect, it exhibited a positive relationship between CCAT2 and MYC in the form of a similar enhancing activity [13]. Therefore, deregulation of *MYC* by its upstream targets constructively contributes to the development of cancers by increasing the expression of other genes as mediators of metastasis, therefore *MYC* can be considered as a potential target for anticancer drugs [14].

The previous findings explored various miRNAs that act as tumor suppressor (miR-137, miR-143, miR-342) or even oncogenes (miR-21, miR-155, miR-499) in CRC [15]. It mentioned that the disruption of some signaling pathways such as the hedgehog cascade can cause uncontrolled proliferation in cells and lead to various cancers, including colon cancer [16]. If the hedgehog pathway is inhibited, the GLI1 acts as the terminal transcriptional effector of the Hedgehog signaling pathway and enters the nucleus as a transcriptional repressor [17]. GLI1 functions as the terminal transcriptional effector and final output of the Hedgehog (Hh) signaling pathway [18]. Dysregulation of the Hedgehog pathway can lead to aberrant activation of GLI1, allowing it to function as a transcriptional activator under pathological conditions [19]. Abnormal activation of GLI1 has been associated with enhanced proliferation, survival, angiogenesis, metabolic reprogramming, metastasis, and chemoresistance in various cancers [17]. To date, many miRNAs have been identified with regulatory roles in the expression pathway of various genes [20]. One of these miRNAs that plays a prominent role in regulating the expression of the *GLI1* gene is miR 218 [21,22]. According to the findings, it was shown that miR-218 suppresses the expression of the *GLI1* gene, prevents its excessive expression in the cell and prevents tumor formation [23]. As the Hedgehog-GLI1 signaling cascade exert an essential function in CRC chemoresistance, the suppression of *GLI1* can be proposed as therapeutic candidate against CRC chemoresistance [24].

One of the most effective therapeutic strategies against CRC is chemotherapy based on natural product [25]. The substantial documents verified that natural therapeutic agents can overwhelm CRC through apoptosis induction and p38-MAPK (Mitogen-activated protein kinases) inhibition [26]. Noscapine is a benzylisoquinoline alkaloid from various species of the papaveraceae family, which has non-addictive potential and low toxicity [27]. In recent years, it has been proven that Noscapine is an anti-mitotic agent that act as a microtubule inhibitor in the cell cycle and causes polyploidy and damage to the chromosomal spindle in cancer cells [28]. Also, it proved that Noscapine reduces pro-inflammatory factors such as interleukin- 6 without disturbance in the macrophage's lifespan [25].

Based on previous evidence, CCAT2 and MYC have been reported to exhibit coordinated expression patterns in colorectal cancer and are associated with tumor progression and cell proliferation [10]. In parallel, miR-218 and GLI1 have been implicated in cancer cell growth and migration and are functionally linked to the Hedgehog/GLI signaling context [23]. Therefore, these molecules have been selected as expression markers representing proliferation- and migration-related molecular changes in CRC cells. Caco-2 cells are widely used as an *in vitro* model of colorectal cancer due to their high proliferative capacity, ability to form tight junctions, and spontaneous differentiation into enterocyte-like cells, making them suitable for studies of intestinal drug absorption and transport [29]. The present study evaluated whether Noscapine treatment is associated with expression changes in selected proliferation- and migration-related markers in Caco-2 cells.

Materials and Methods

Cell culture

The Caco-2 cell line was prepared from the Iranian Genetic and Biological Resources Center (IBRC). Cells were maintained in 25 cm^2 flasks in DMEM (Dulbecco's Modified Eagle Medium) culture medium (Sigma, USA) supplemented with 10% FCS (Fetal Calf Serum) (Gibco, Germany), 1% penicillin-streptomycin (Gibco, Germany). Until the logarithmic phase of cell growth, Caco-2 cells maintained in an incubator (Memmert, Germany) with a temperature of 37°*C* with 5% CO_2 and 95% humidity.

Cell viability assay

The Caco-2 cells were exposed to different concentrations of Noscapine (Sigma, USA) (10, 25, 50 and

100 μM) for 24, 48 and 72 *hr*. The concentration range of 10–100 μM Noscapine was selected based on previously reported *in vitro* studies and was used to determine the IC_{50} value in Caco-2 cells [8,30]. Sham exposed cells was Caco-2 cells treated with Noscapine solvent (DMSO). After treatment, 20 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye (5 *mg/ml* dissolved in PBS; Phosphate-Buffered Saline), was added to each well. The plate was incubated for 4 *hr* in darkness. After removing the well contents, 100 μl of DMSO (Dimethyl sulfoxide; Merck, Germany) was added to each well to dissolve the Formazan in alive cells. The absorbance of each well was immediately read at 570 *nm* using an ELISA reader. Next, the concentration that reduced 50% of cell survival [50% inhibitory concentration (IC_{50})] was determined.

Inhibition (%) = [1 - (treated/control)] × 100

Viability (%) = 100 – inhibition (%)

Comet assay

Briefly, after treatment with the IC_{50} concentration of Noscapine for 48 *hr*, approximately 1×10^4 cells from both control and treated groups were harvested and resuspended in 1 *ml* of cold PBS. The cell suspension was mixed with 1% low-melting-point agarose (Sigma-Aldrich, USA) at a ratio of 1:10 (v/v) and immediately layered onto pre-coated microscope slides with a base layer of 1% normal-melting-point agarose. The slides were covered with coverslips and allowed to solidify at 4°*C* for 20 *min*.

The cover slips were then removed and the slides were immersed in a lysis buffer (2.5 *M* NaCl, 100 *mM* Na_2EDTA 10 *mM* Tris-HCl, 1% (w/v) sodium sarcosinate, pH=10; 1% triton X-100 and 10% DMSO add just before use) and kept at 4°*C* in dark for 60 *min*. Then the slides were placed in the alkaline electrophoresis buffer (200 *mM* Na 2 EDTA, 10 *M* NaOH) for 20 *min*, followed by horizontal electrophoresis at 25 *V*, 300 *mA* for 30 *min*. After neutralization (0.4 M Tris-HCl, pH=7.5), DNA was stained with 50 μl of ethidium bromide (20 $\mu g/ml$) and visualized using a fluorescence microscope (Olympus IX70, Japan) equipped with a 520 *nm* excitation filter and a 590 *nm* emission filter. For quantitative analysis, at least 50 randomly selected cells per slide were analyzed, and three independent experiments were performed. DNA damage was quantified using the percentage of DNA in the comet tail (% tail DNA) as the primary parameter. Image analysis was performed using ImageJ software. Representative fluorescence images of comet structures from control and Noscapine-treated cells were recorded to illustrate DNA damage patterns.

Flow cytometry analysis

To analyze the rate of apoptotic cells exposed to Noscapine, Annexin V/PI kit was used. First 10^5 Caco-2 cells were cultivated in 6 well plate. Then, after treatment with Noscapine (IC_{50} value, 48 *hr*), the treated and untreated cells were harvested by centrifugation at 800 *rpm* for 5 *min* and rinsed using cold PBS. For apoptosis quantification, 3 μl Annexin V-FITC (Vazyme, China) were added to cells in addition to 100 μl binding buffer. It was incubated for 15 *min* in the dark and analysis performed using FACS calibrator (Beckman, USA). In order to cell cycle analysis, Caco-2 cells were seeded in 6 well plate at density of 10^5 cell/well. The cells exposed to IC_{50} concentration of Noscapine for 48 *hr*, while untreated Caco-2 cells served as control. After the treatment period, the cells harvested by centrifugation, washed with PBS, fix with ethanol and treated with PI (Sigma, USA) mastermix including 40 μl PI, 10 μl RNase A and 450 μl PBS. Then, cell cycle evaluated using FACS flow cytometer (Beckman, USA).

Scratch assay

To assess cell invasion, a scratch assay was performed. Caco-2 cells were seeded in 6-well plates and cultured until 90% confluency. Mitomycin C as DNA synthesis inhibitor at concentration of 5 $\mu g/ml$ added to each well and incubated for 2 *hr* at 37°*C* (Mouritzen and Jenssen 2018) [2]. After aspiration of mitomyxin C, a uniform linear scratch was created across the cell monolayer using a sterile 200 μl pipette tip. Detached cells were carefully removed by rinsing with PBS, and the wells were then replenished with serum-free medium containing IC_{50} concentration of Noscapine. Images of the wound area were captured using an inverted microscope (Labomed® 400, USA). Wound closure was analyzed by measuring the scratch width at each time point using ImageJ software. The percentage of wound closure was calculated using the following formula:

Relative Wound Closure (%) = [(Initial width − Width at time X) / Initial width] × 100

This calculation provided a quantitative measure of the cell migration rate.

Gene expression analysis by qRT-PCR

Total RNA extraction was performed with RNX Plus Synaclon Bioscience, Karaj, Iran, according to the manufacturer's instructions, and isolated RNA concentration was determined using Nanodrop. To isolate RNA from genomic DNA contamination, 1000 *ng* of RNA was treated with DNase. After the removal of genomic DNA, the RNA preparation was reverse transcribed to synthesize complementary DNA (cDNA) using the BIOFACT kit, South Korea. To quantify the expression levels of lncRNA *CCAT2*, *MYC*, and *GLI1* genes in Caco-2 colon cancer cells, qRT-PCR) was conducted. Also, 1 μg of total RNA from each sample was reverse transcribed using a Stem miRNA 1st Strand cDNA Synthesis kit (Iran), according to the manufacturer's instructions. miR-218-5p was reverse transcribed using a stem-loop specific reverse transcription primer, while SNORD47 was reverse transcribed using the universal reverse transcription primer provided in the kit. Subsequently, quantitative gene

expression analysis was performed with SYBR Premix Ex Taq (Ampliqon, Denmark). Analysis of relative gene expressions was independently performed three times and each sample was verified in triplicate. To verify the effectiveness of DNase treatment and exclude genomic DNA contamination, no-reverse transcription (no-RT) control reactions were included for each RNA sample. No amplification was detected in no-RT controls, confirming the absence of genomic DNA contamination.

The relative expression levels of RNA were calculated using Ct values and the level of target gene expression ($2^{-\Delta\Delta Ct}$) was normalized concerning the GAPDH housekeeping reference for GLI, MYC, and CCAT2; in contrast, SNORD-47 was considered the reference for miR-218. Melt curve analysis was performed at the end of each qRT-PCR run to confirm amplification specificity. All reactions showed a single distinct peak, indicating specific amplification without primer-dimer formation. The primer sequence for *CCAT2* gene was as follows: forward primer: 5'-AAG AGGGAGGTATCAACAGAGAC-3', and reverse: 5'-TTTGGACGACGCCTTCATTTC-3'. For *MYC* gene: forward primer was 5'-CACATCAGCA CAACTACG-3' and reverse primer was 5'-GTTCGCCTCTTGA CATTC-3'. For *GLI1* gene: forward primer, 5´- CCC AAT CAC AAG TCA GGT TCC T-3´ and reverse, 3´-CCT ATG TGA AGC CCT ATT TGC C-5´. For *GAPDH* gene, the forward primer was 5´-ACCTTG GAAATAAATGGGAAG-3´ and the reverse primer was 5´-CTTCTGTGTTGCTGTAGTT GC-3´. The primers used for miR-218-5p is as follows: forward primer, 5´- TTG GGC TTG ATC TAA C -3´; snord-47 forward primer, 5´- TC ACT GTA AAA CCG TTC-3´ and universal reverse primer, 3´-GAGCAGGGTCCGA GGT-5´. AlleleID6, GeneRunner, Oligo6, and mfold programs were used for miRNA design.

Statistical analysis

Data analysis was performed using two-way ANOVA, one-way ANOVA, and T-tests through GraphPad Prism 8 software. Quantitative variables are presented as means±SD based on triplicate experiments. A p-value less than 0.05 was regarded as the threshold for significance in the tests.

Results

Cell survival assay

The MTT assay findings showed that the vital activity of Noscapine treated colon cancer cells decreased during the treatment period (24 to 72 *hr*), as compared with control (untreated cells). As shown in figure 1, with exposure to Noscapine, the survival rate of CRC cells diminished significantly in a dose dependent manner (***p<0.001). However, DMSO exposed cells as sham group exerted no significant effect on CRC cells. The results showed that the average inhibitory concentration of Noscapine was 50 μM after 48-*hr* treatment.

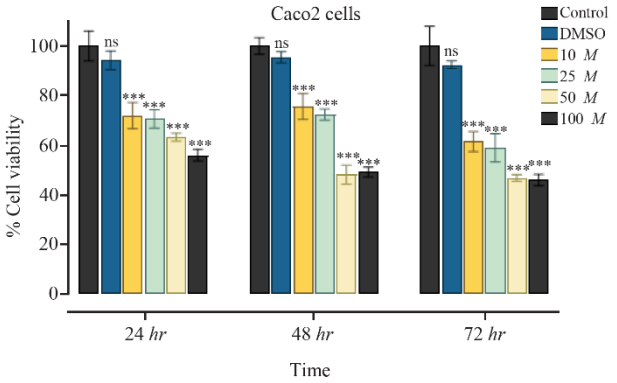

Figure 1. The effect of Noscapine on the survival rate of Caco-2 colon cancer cells in the presence of Noscapine at concentrations of (10, 25, 50, 100 μM), and DMSO after 24, 48 and 72 *hr* compared to control. Data was performed in triplicate and represented as mean ± SD. (***) indicates a significant difference in level (p<0.001).

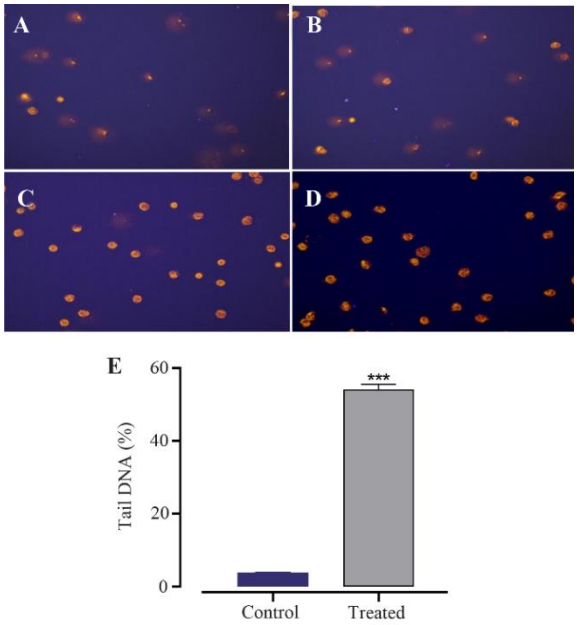

Figure 2. Upper panel: Single-cell gel electrophoresis (comet assay) of control and treated cells. Control or untreated cells (A & B). Noscapine treated cells (C&D). Lower panel: Percentage of the comet in control and Noscapine treated groups. Only cells exposed to Noscapine (IC_{50}) showed a significant difference at the level of (p<0.05) (E).

Accordingly, the dosage of Noscapine at 50 μM was selected for further assays as IC_{50} (Figure 1).

Comet assay

Comparing the results of control and treated cells, the cells treated with Noscapine indicated DNA break in large pieces and the comet formation. In addition, significant elevation (*p<0.05) in comet percentage was observed in Noscapine treated group (IC_{50}) (Figure 2).

Cell cycle assessment

The flow cytometry was used to evaluate cell cycle in CRC cells untreated and treated with Noscapine (IC_{50}). Cell cycle analysis exhibited that Noscapine

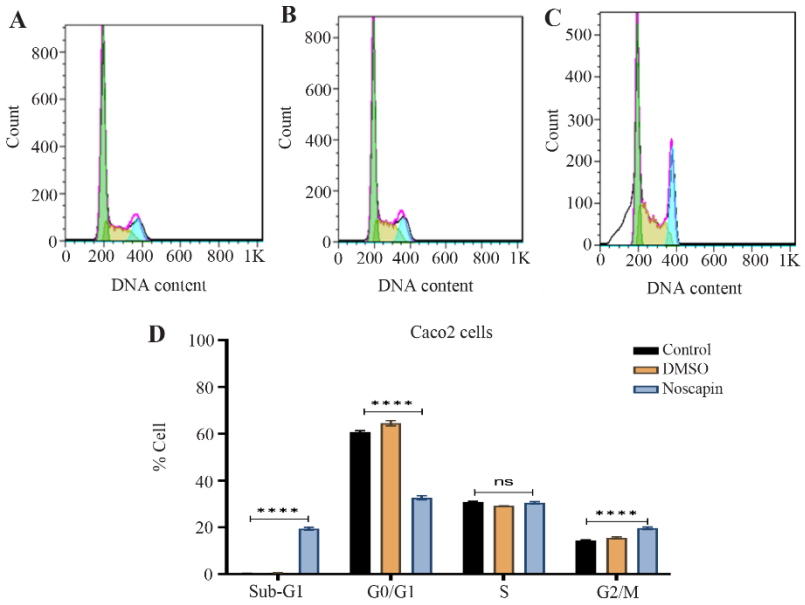

Figure 3. Noscapine at IC50 value could control cell cycle promotion through increment of sub-G1 population and arrest in cell cycle at G0/G1 stage. A) Control; B) Sham exposed (DMSO); C) Noscapine (IC_{50}). D) Data was performed in triplicate and represented as mean ± SD. (****) indicates a significant difference in level ($p<0.0001$).

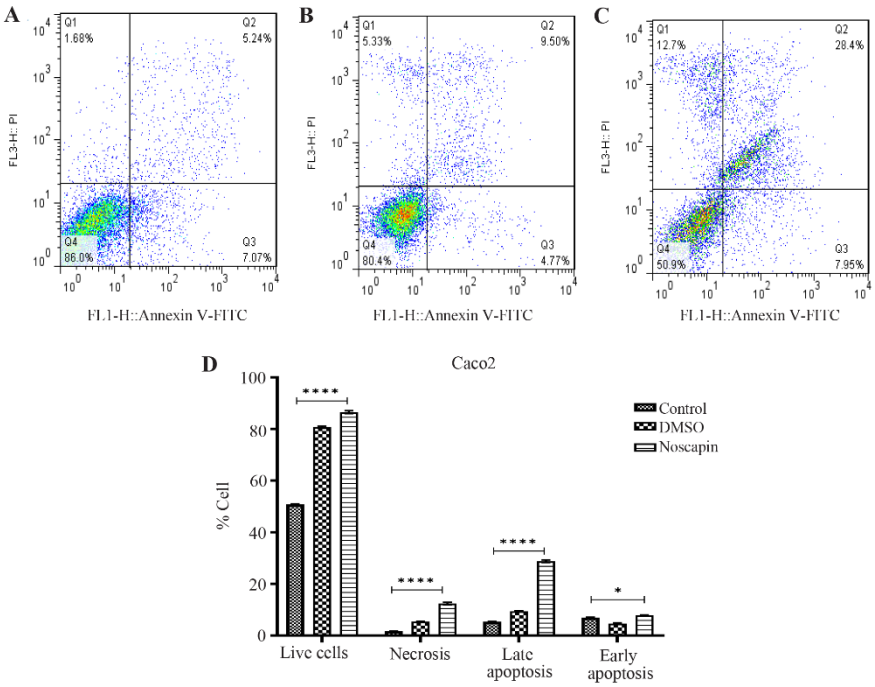

Figure 4. Apoptosis detection using Annexin-V –PI double staining on Caco-2 cancer cells. A) Control B) Sham exposed cells (DMSO). C) Noscapine Caco-2 treated cells. Q1 (necrotic cells), Q2 (late apoptotic cells), Q3 (early apoptotic cells), and Q4 (live cells). D) Quantitative analysis of alive, early and late apoptosis as well as necrosis rate in sham and Noscapine treated cells as compared with control. Statistical significance was determined through a two-way ANOVA analysis of variance and Dunnett's multiple comparison test. Data are presented as mean±SD (*$p<0.05$, ***$p<0.0001$ *vs.* control).

induced sub-G1 proportion and G0/G1 arrest indicating disrupted cell cycle progression. A remarkable elevation in the proportion of sub-G1 (1 to 20%) has been indicated in Noscapine treated cells compared with control and sham exposed groups (Noscapine solvent: DMSO). Further, apoptosis-associated sub-G1 accumulation demonstrated the pro-apoptosis effect of Noscapine and its capacity to control cell progression (Figure 3).

Apoptosis quantitative assay

Annexin V-PI double staining revealed that Noscapine (IC_{50} value) induced more late apoptosis and necro-

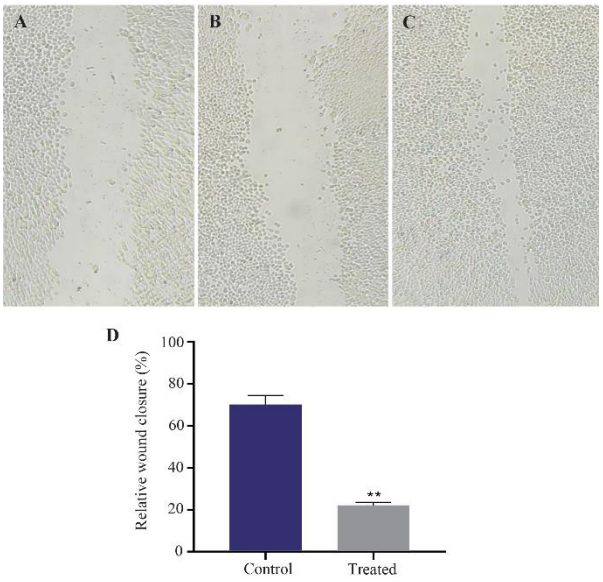

Figure 5. Scratch assay of Caco-2 in cancer cells. A) Initial wound at 0 *hr*. B) Cells treated with the IC_{50} dose of Noscapine after 48 *hr*. C) Untreated group at 48 *hr*, showing substantial wound closure. D) Quantitative ImageJ analysis of relative wound closure at 48 *hr*, demonstrating ~63.8% closure in controls versus only ~26.8% in the treated group. Data are presented as mean ± SD (**p<0.01 control).

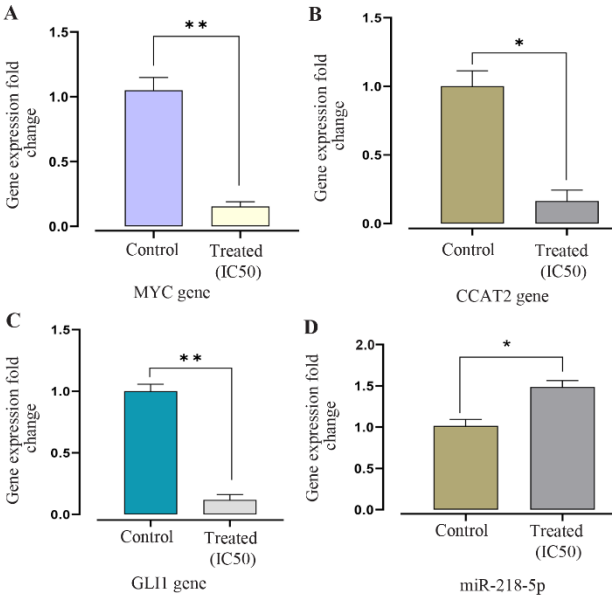

Figure 6. Fold changes in *CCAT2*, *miR-218-5p*, and *MYC*, *GLI1* relative expression using GAPDH and SNORD as housekeeping genes. The calculation of gene expression fold changes was performed using 2-ΔΔCt method. A-C) Changes in *CCAT2*, *MYC*, and *GLI1* expression in control and Caco-2 cells treated with Noscapine (IC_{50}) indicated a significant difference in level (*p<0.05, **p<0.01, **p<0.01; respectively) D). Changes in *miR-218-5p* gene expression in control and Noscapine treated CRC cells indicated a significant upregulation as compared with control (*p<0.05)

sis as compared with control. As indicated in figure 3, Noscapine elevated late apoptosis proportion from 5.24 to 28.4%, while necrotic percentage increased from 1.68 to 12.7%. Flow cytometry data elucidated late apoptosis as well as necrosis in Noscapine-CRC treated cells (Figure 4).

Scratch assay

The wound healing assay revealed that the Noscapine markedly suppressed cell migration after 48 *hr* treatment. Quantitative ImageJ analysis showed that relative wound closure after 48 *hr* was 63.8% in untreated cells, compared to only 26.8% in cells treated with the IC_{50} dose of Noscapine. These results confirmed that the Noscapine significantly impairs the migratory capacity of Caco-2 cancer cells (Figure 5).

Gene expression analysis

Findings showed that the expression of LncRNA of *CCAT2* (*p<0.05), *MYC* (**p<0.01), and *GLI1* (**p< 0.01) significantly decreased compared to the control. On the other hand, the up-regulation of miR-218-5p (*p<0.05) was observed in Noscapine treated cells. Gene expression analysis showed that in Caco-2 treated cells with considering the diminish in transcriptional level of LncRNA of *CCAT2*, *MYC* and *GLI1*, Noscapine has an inhibitory effect on cell proliferation and progression of Caco-2 cells (Figure 6).

Discussion

In CRC, the genetic and epigenetic changes together, promotes uncontrolled cell proliferation and metastasis potential [25]. Despite advancement in drug development in CRC, chemotherapeutic resistance mainly due to genetic mutations and epigenetic modifications has been considered as critical challenge in treatment. On the basis of findings, the low-risk and minimal side effects of phytochemicals candidate them in chemotherapy based on natural products for the treatment and control of CRC tumor growth to suppress cancer cells *via* the ceRNA network [8,31]. It has been demonstrated natural products that inhibit cell survival pathways while selectively inducing death in cancer cells are considered promising therapeutic candidates. Numerous studies have demonstrated that Noscapine exerts cytotoxic, genotoxic, and apoptosis-inducing effects in various cancer cell types [32]. The present study aimed to investigate the anti-cancer effect of Noscapine on CRC cell line Caco-2 by evaluating the quantitative changes in the expression of *LncRNA CCAT2*, *miR-218-5p*, *GLI1* and *MYC* genes.

In this study it is evident that increasing the concentrations of the Noscapine led to the inhibition of cell proliferation in the Caco-2 cancer cell line. This inhibition occurred in a time- and concentration-dependent manner. The results also demonstrate that Noscapine (at concentrations of 50 μM) reduced cell viability decreased to 48.15% within 48 *hr* and it is consistent with previous studies reporting. Quisbert-Valenzuela & Calaf, showed that Noscapine has demonstrated antiproliferative activity across multiple cancer cell models. In MCF-7 breast cancer cells, Noscapine at IC_{50}

concentration induced apoptosis after 24 *hr* of exposure [33]. Similarly, Noscapine treatment of HL60 leukemia cells resulted in an IC_{50} value of 19.5 μM after 72 *hr* and in FM3A mouse breast carcinoma cells, an IC_{50} of approximately 50 μM was reported following 48 *hr* of treatment [8]. These findings support the selection of the Noscapine concentration range used in the present study and indicate that its anti-proliferative effects have been observed across different cancer cell types. Flow cytometric analysis also demonstrated an increased proportion of apoptotic cells in the treated groups and accumulation of cells in the G0/G1 phase, indicating inhibition of cell cycle progression. These findings suggest that Noscapine suppresses colorectal cancer cell growth by simultaneously promoting apoptotic pathways and restricting proliferative capacity [11].

The alkaline comet assay was used in this study to evaluate the genotoxicity of Noscapine on Caco-2 cancer cell line at IC_{50} concentrations. Our results showed a significant increase in DNA damage following Noscapine treatment. DNA fragmentation is defining features of apoptotic cell death [30]. In the other hand, the scratch assay results showed that Noscapine suppressed cell migration in Caco-2 cancer cell line. The suppression of cell migration is crucial in regard to cancer metastasis, as it indicates that Noscapine may be able to prevent cancer cells from spreading to different parts of the body. Consistent with previous reports, Noscapine has been shown to exert anti-cancer effects through both interference with metastatic signaling pathways and induction of apoptotic processes. These observations are in agreement with our findings in Caco-2 cells, where Noscapine treatment was associated with apoptosis induction and reduced migratory capacity Noscapine suppressed [30,34].

The expression levels of *CCAT2*, *GLI1*, and *MYC* were reduced by approximately 4-fold in Caco-2 cancer cells when compared to the control group while the expression of *miR-218-5p* increased by approximately 1.5-fold. Our data are consistent with previous studies conducted by Nulamuga *et al* indicated anti-cancer potential of Noscapine [28]. The *c-MYC* gene serves as an oncogene and has a role in multiple cellular processes, including differentiation, proliferation, apoptosis, and sensitivity to therapy. Previous studies have reported that upregulation of *CCAT2* promotes colon cancer cell invasion and is associated with increased expression of its downstream-related oncogene MYC, which is linked to colorectal tumor progression. In the present study, Noscapine-treated Caco-2 cells exhibited reduced expression of both *CCAT2* and *MYC*, suggesting that Noscapine exposure is associated with downregulation of genes implicated in proliferative and invasive signaling pathways [10]. Similar to this type of research, especially in the field of studies related to liver tumor progression, it stated that with the progression of tumors located in the liver region, the up-regulation of *CCAT2* also leads to an increment in *MYC* gene expression ($p<0.05$) [35]. Also, a significant correlation between the relative expression of *MYC* and *CCAT2* in MCF-7 breast cancer cell line was proved by Sarrafzadeh *et al* in 2017. According to the data analysis, they found that the expression of these two genes increased in MCF-7 cell line as well as cell proliferation ($p<0.001$) [36].

miR-218-5p and GLI1 have been implicated in signaling pathways related to tumor growth and invasion. Previous studies have shown that miR-218 may negatively regulate *GLI1* expression in certain cancer contexts [23]. Consistent with these reports, Noscapine-treated Caco-2 cells demonstrated increased *miR-218-5p* expression accompanied by reduced *GLI1* expression. These observations suggest that Noscapine exposure is associated with modulation of genes involved in proliferation- and migration-related signaling pathways, although causal interactions cannot be inferred from the present data. Based on the study, the downstream target of miR-218 (*GLI1* gene) under normal conditions is suppressed by miR-218 which prevents tumor formation [23]. In contrast, under tumor development conditions, the high expression of *miR-218* can be induced tumor formation and uncontrolled cell division. Thus, the expression of *miR-218* and *GLI1* is considered as a suitable diagnostic markers to identify the tumor in its early stages [22]. Previously, it proved that *GLI1* down-regulated by *miR-218* overexpression which confirmed the tumor suppressor efficacy of miR 218 through GLI1 inhibition against prostate cancer [37].

The present study focused on molecules previously associated with CRC cell proliferation and migration. *CCAT2* and *MYC* have been reported to exhibit coordinated expression patterns during tumor progression, while *miR-218* and *GLI1* are involved in signaling processes related to cancer cell growth and invasion. The observed expression changes following Noscapine treatment suggest that the cellular effects of Noscapine are accompanied by alterations in genes implicated in proliferation- and migration-related pathways. However, these findings represent expression-based associations and do not establish a direct mechanistic pathway.

Limitations

Finally, we acknowledge a few limitations in this study. While we demonstrated changes in *CCAT2*, *miR-218*, *MYC*, and *GLI1* expression, these changes are based on expression analyses and therefore indicate associations rather than direct regulatory relationships. Functional validation experiments, such as gain- and loss-of-function approaches, are required to determine causal interactions and underlying mechanisms. Future studies should address these aspects to further clarify the molecular pathways involved in the effects of Noscapine in colorectal cancer cells. Another limitation of this study is that all experiments were performed in a single CRC cell line (Caco-2). Therefore, the findings should be interpreted within the context of this specific

in vitro model and may not be directly generalizable to other CRC subtypes or *in vivo* conditions. Future studies using multiple CRC cell lines and animal models are required to validate and extend these findings.

Conclusion

According to the results, Noscapine exerts anti-CRC effect in Caco-2 cells used as the intestinal epithelial barrier *in vitro* model with high proliferative capacity mainly *via* apoptosis recruitment, cell cycle arrest and anti-invasive effect. The pro-apoptosis and anti-invasiveness effects of Noscapine verified mechanistically by down-regulation of *CCAT2* LncRNA as a target for the *MYC* gene expression involved in cell proliferation and metastasis and through down-regulation of *GLI* and increased *miR-218* level involved in cell cycle progression and apoptosis. For future research, Noscapine treatment on the molecular subtypes of CRC cell lines proposed to overcome an obstacle of introduction Noscapine as natural anti-CRC therapeutic agent and to achieve accurate mechanistic insights.

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Conflict of Interest

The authors declare that they have no competing interests.

References

1. Shin AE, Giancotti FG, Rustgi AK. Metastatic colorectal cancer: mechanisms and emerging therapeutics. Trends Pharmacol Sci 2023 Apr;44(4):222-236.
2. Vang Mouritzen M, Jenssen H. Optimized Scratch Assay for In Vitro Testing of Cell Migration with an Automated Optical Camera. J Vis Exp 2018 Aug 8;(138):57691.
3. Mattiuzzi C, Sanchis-Gomar F, Lippi G. Concise update on colorectal cancer epidemiology. Ann Transl Med 2019 Nov;7(21):609.
4. Ahadi A. Functional roles of lncRNAs in the pathogenesis and progression of cancer. Genes Dis 2020 Apr 21;8(4):424-437.
5. Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W. Emerging roles of lncRNA in cancer and therapeutic opportunities. Am J Cancer Res 2019 Jul 1;9(7):1354-1366.
6. Pirlog R, Drula R, Nutu A, Calin GA, Berindan-Neagoe I. The Roles of the Colon Cancer Associated Transcript 2 (CCAT2) Long Non-Coding RNA in Cancer: A Comprehensive Characterization of the Tumorigenic and Molecular Functions. Int J Mol Sci 2021 Nov 19;22(22):12491.
7. He P, Xiong G, Guo W, Jiang G, Li Y, Li H. Long non-coding RNA CCAT2 promotes prostate cancer cell proliferation and invasion by regulating the Wnt/β-catenin signaling pathway. Oncol Lett 2020 Oct;20(4):97.
8. Jayaraj RL, Beiram R, Azimullah S, M F NM, Ojha SK, Adem A, Jalal FY. Noscapine Prevents Rotenone-Induced Neurotoxicity: Involvement of Oxidative Stress, Neuroinflammation and Autophagy Pathways. Molecules 2021 Jul 30;26(15):4627.
9. Ma S, Wang W, Zhang D, Zhao G, Lu Z. Long non-coding RNA colon cancer-associated transcript 2: role and function in human cancers. Chin Med J (Engl) 2022 Dec 5;135(23):2785-2797.
10. Xie G, Huang Z, Liu Z, Lin Z, Ma L. NCPHLDA: a novel method for human lncRNA-disease association prediction based on network consistency projection. Mol Omics 2019 Dec 2;15(6):442-450.
11. Iaccarino I. lncRNAs and MYC: An Intricate Relationship. Int J Mol Sci 2017 Jul 12;18(7):1497.
12. Donati G, Amati B. MYC and therapy resistance in cancer: risks and opportunities. Mol Oncol 2022 Nov;16 (21):3828-3854.
13. Stasevich EM, Murashko MM, Zinevich LS, Demin DE, Schwartz AM. The Role of Non-Coding RNAs in the Regulation of the Proto-Oncogene MYC in Different Types of Cancer. Biomedicines 2021 Jul 30;9(8):921.
14. Ma L, Cao J, Liu L, Du Q, Li Z, Zou D, Bajic VB, Zhang Z. LncBook: a curated knowledgebase of human long non-coding RNAs. Nucleic Acids Res 2019 Jan 8;47 (D1):D128-D134.
15. Mehrgou A, Ebadollahi S, Seidi K, Ayoubi-Joshaghani MH, Ahmadieh Yazdi A, Zare P, et al. Roles of miRNAs in Colorectal Cancer: Therapeutic Implications and Clinical Opportunities. Adv Pharm Bull 2021 Feb;11(2): 233-247.
16. Wu X, Zhang Y, Chuang KH, Cai X, Ajaz H, Zheng X. The Drosophila Hedgehog receptor component Interference hedgehog (Ihog) mediates cell-cell interactions through trans-homophilic binding. J Biol Chem 2019 Aug 16;294(33):12339-12348.
17. Avery JT, Zhang R, Boohaker RJ. GLI1: A Therapeutic Target for Cancer. Front Oncol 2021 May 25;11:673154.
18. Wu F, Zhang Y, Sun B, McMahon AP, Wang Y. Hedgehog Signaling: From Basic Biology to Cancer Therapy. Cell Chem Biol 2017 Mar 16;24(3):252-280.
19. Peng R, Li B, Chen S, Shi Z, Yu L, Gao Y, et al, Wang H. Deleterious Rare Mutations of *GLI1* Dysregulate Sonic Hedgehog Signaling in Human Congenital Heart Disease. Front Cardiovasc Med 2022 Apr 4;9:798033.
20. Lu MY, Chtarbanova S. The role of micro RNAs (miRNAs) in the regulation of Drosophila melanogaster's innate immunity. Fly (Austin) 2022 Dec;16(1):382-396.
21. Kogo R, How C, Chaudary N, Bruce J, Shi W, Hill RP, et al. The microRNA-218~Survivin axis regulates migration, invasion, and lymph node metastasis in cervical cancer. Oncotarget 2015 Jan 20;6(2):1090-100.
22. Zhang J, Li S, Li Y, Liu H, Zhang Y, Zhang Q. miRNA-218 regulates the proliferation and apoptosis of cervical

cancer cells via targeting Gli3. Exp Ther Med 2018 Sep;16(3):2433-2441.

23. Dang S, Zhang R, Tian S, Hou P, Li G, Ji M. MicroRNA-218 inhibits the malignant phenotypes of glioma by modulating the TNC/AKT/AP-1/TGFβ1 feedback signaling loop. Int J Mol Med. 2021 Nov;48(5):205.
24. Po A, Citarella A, Catanzaro G, Besharat ZM, Trocchianesi S, Gianno F, et al. Hedgehog-GLI signalling promotes chemoresistance through the regulation of ABC transporters in colorectal cancer cells. Sci Rep 2020 Aug 19;10(1):13988.
25. Cohen R, Pudlarz T, Delattre JF, Colle R, André T. Molecular Targets for the Treatment of Metastatic Colorectal Cancer. Cancers (Basel) 2020 Aug 20;12(9):2350.
26. Han Z, Meng L, Huang X, Tan J, Liu W, Chen W, et al. Inhibition of p38 MAPK increases the sensitivity of 5-fluorouracil-resistant SW480 human colon cancer cells to noscapine. Oncol Lett 2022 Feb;23(2):52.
27. Gupta R, Soni D, Upadhayay S, Dhureja M, Kumar P. Impact of noscapine on halting the progression of pentylenetetrazole induced kindling epilepsy in mice. Clin Exp Pharmacol Physiol 2023 Dec;50(12):984-991.
28. Nulamuga B, Uzairu A, Babalola IT, Ibrahim MT, Umar AB. In silico analysis of noscapine compounds as antitumor agents targeting the tubulin receptor. J Taibah Univ Med Sci 2022 Aug 14;18(1):32-44.
29. Shinji S, Ogawa Y, Yamada T, Matsuda A, Uehara K, Yokoyama Y, et al. Morphological and functional analysis of colorectal cancer cell lines in 2D and 3D culture models. Sci Rep. 2025 Jan 24;15(1):3047.
30. Alassiri S, Alqarni A, Hosmani J, Alqahtani AM, Alshehri B, AlHamid NZ, et al. Noscapine Suppresses Metastasis in Oral Cancer by Targeting the CXCL-8/CXCR2 Chemokine Signaling Pathway. Natural Product Communications 2025 Jun;20(6): 1934578X251349289.
31. Siegel RL, Wagle NS, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. CA Cancer J Clin 2023 May-Jun;73(3):233-254.
32. Sung WJ, Hong J. Targeting lncRNAs of colorectal cancers with natural products. Front Pharmacol 2023 Jan 9;13:1050032.
33. Quisbert-Valenzuela EO, Calaf GM. Apoptotic effect of noscapine in breast cancer cell lines. Int J Oncol 2016 Jun;48(6):2666-74.
34. Tian X, Liu M, Huang X, Zhu Q, Liu W, Chen W, et al. Noscapine Induces Apoptosis in Human Colon Cancer Cells by Regulating Mitochondrial Damage and Warburg Effect via PTEN/PI3K/mTOR Signaling Pathway. Onco Targets Ther. 2020 Jun 11;13:5419-5428.
35. Sun Z, Liu J, Chen C, Zhou Q, Yang S, Wang G, et al. The Biological Effect and Clinical Application of Long Noncoding RNAs in Colorectal Cancer. Cell Physiol Biochem 2018;46(2):431-441.
36. Sarrafzadeh Sh, Geranpayeh L, Tasharrofi B, Soudyab M, Nikpayam E, Iranpour M, et al. Expression Study and Clinical Correlations of MYC and CCAT2 in Breast Cancer Patients. Iran Biomed J 2017 Sep;21(5):303-11.
37. Guan B, Mu L, Zhang L, Wang K, Tian J, Xu S, et al. MicroRNA-218 inhibits the migration, epithelial-mesenchymal transition and cancer stem cell properties of prostate cancer cells. Oncol Lett 2018 Aug;16(2):1821-1826.