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Silencing of *HOTAIR* Induced Apoptosis in Human Colorectal Cancer Cells through Up-Regulation of *Bax* and Down-Regulation of *Bcl2*

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Abstract

HOX transcript antisense RNA (*HOTAIR*), as a long noncoding RNA (lncRNA) is a highly cited transcript modulating variety of signaling pathways such as cell growth and apoptosis. Altered expression of *HOTAIR* has been reported in human cancers, which contributes with cancer progression and metastasis. Increased expression level of *HOTAIR* has been observed in colorectal cancer (CRC). It seems that dysregulation of *HOTAIR* may inhibit the apoptosis. The present study was aimed to evaluate the effect of *HOTAIR* silencing on expression of apoptosis markers *Bax* and *Bcl2* using real-time polymerase chain reaction (PCR). The data showed that *HOTAIR* and *Bcl2* are highly expressed in CRC cells while the expression level of *Bax* is low. Following siRNA treatment, Blc2 was downregulated but *Bcl2* was upregulated. These findings suggest that *HOTAIR* silencing can promote apoptosis, and thus it can be considered as a promising strategy to kill cancer cells.

Keywords: Colorectal Cancer, Apoptosis, Long Noncoding RNA, *HOTAIR*, *Bax*, *Bcl2*

1. Background

Colorectal cancer (CRC) has been recorded as one of the global top 10 cancers allocating rank four after skin, lung, and breast (1, 2). In 2017, 1.8 million incident cases of CRC along with 896000 deaths were reported. CRC is more prevalent in men than women (1/26 for men vs. 1/40 for women) (3, 4). Family history of the disease, inflammatory bowel disease, obesity, and alcohol and tobacco consumption are well-documented risk factors of CRC (5-7). Although this type of cancer has mostly been observed in adults, CRC has been diagnosed at any age (8). Dysregulation of genes may affect the cellular behavior and promote tumorigenesis (9, 10). Long noncoding RNAs (lncRNAs) are lengthy non-protein coding transcripts which are actively involved in modulating critical cellular signaling pathways containing cell cycle, apoptosis, and differentiation (11, 12). Abnormal expression levels of lncRNAs have been described in different human tumors (13). HOX transcript antisense RNA (*HOTAIR*) is one of the well-studied lncRNAs which has been dysregulated in a variety of solid tumors (13, 14). The literature demonstrated that *HOTAIR* is associated with apoptosis. Increased expression of *HOTAIR* may suppress apoptosis (15, 16)]. Gene silencing through RNA interference (RNAi) strategy is recently suggested as the therapeutic method against cancer (17, 18). This is a non-invasive approach which can effectively target the critical pathways of cell proliferation, growth, or invasion (19).

2. Objectives

The present study aimed to investigate the effect of *HOTAIR* silencing in triggering apoptosis through targeting *Bax* and *Bcl2* in cellular model of CRC.

3. Methods

3.1. Cell Lines/Culture Condition

The cellular model of CRC Caco-2 was purchased from the national cell bank of the Pasteur Institute, Tehran, Iranian Biological Resource Center. Cells were cultured in Dul-

becco's Modified Eagle's Medium (DMEM) (Life Technologies, USA) containing 2mM Glutamine, 10% FBS, 1% penicillin, and 1% streptomycin (Life Technologies, USA). Cells were incubated at 37°C and atmosphere of 5% CO_2.

3.2. HOTAIR siRNA Transfection

The 3×10^4 cells were plated onto the 6-well plates and treated with 50 nM siRNA duplexes (siHOTAIR I, SASI Hs02 00380445) for 48h using Lipofectamine™ 2000 (Invitrogen, USA) according to the user guideline. Concentration of siRNA was set based on the concentration in our pilot research, and found this concentration more suitable for knocking out.MISSION® siRNA Universal Negative Control #1, SIGMA/SIC001) was used as negative control (20). The mock test was defined as cells treated with lipofectamine and without siRNA.

3.3. RNA Extraction and Real-Time Polymerase Chain Reaction (PCR)

Following 48h of treatment with siRNA, total RNA was extracted from transfected cells using Trizol reagent (Invitrogen, USA) based on manufacturer protocol. The RNAs were reverse transcribed to cDNA through Primer-Script one step RT-PCR kit (Biofact). The cDNA samples were amplified using the SYBR green kit (Biofact) during real-time PCR. The primers used in this study are listed in Table 1.Relative gene expression was calculated as $2^{-\Delta\Delta Ct}$.

3.4. Statistical Analyses

Data were analyzed using a one-way analysis of variance with Newman-Keuls correction for multiple comparisons using the GraphPad Prism version 6 (GraphPad Software, USA). The nonparametric tests were used if normal distribution was not met. P-value less than 0.05 was considered as a statistically significant difference.

4. Results

As illustrated in Figure 1*HOTAIR* is strongly expressed in normal culture of Caco-2 cells before siRNA treatment. However, its expression level has been significantly downregulated since the *HOTAIR*-specific siRNA was added into the culture medium. The fold-change was 0.06 ± 0.011 (P-value < 0.001).

In the same way, the expression level of *Bcl2* was high in normal culture of Caco-2 and downregulated after 48 h of treatment with *HOTAIR*-specific siRNA. The corresponding fold-change was 0.26 ± 0.042 (P-value < 0.001) (Figure 2). Conversely, the expression level of *Bax* was weak in Caco-2 cells and significantly increased as the cells were treated with *HOTAIR*-siRNA. The calculated fold-change was 4.6 ± 0.031 (P-value < 0.001) (Figure 3).

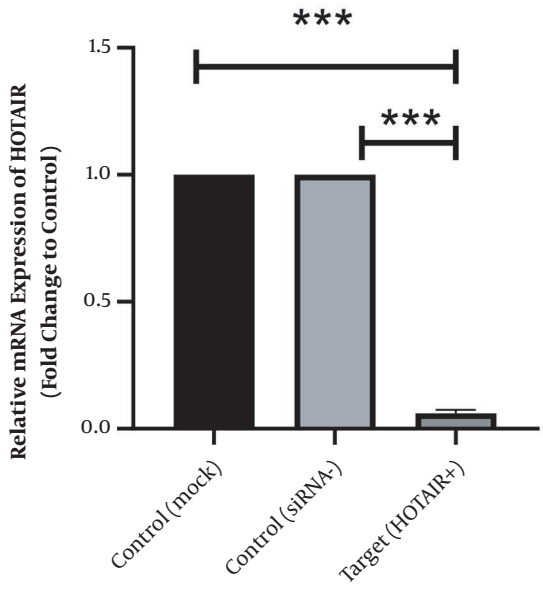

Figure 1. Effects of *HOTAIR* specific siRNA on suppressing *HOTAIR* transcript after treatment with 50 ng siRNA after 48 hours of treatment. Data expressed as mean ± standard deviation (the stars *** show the P-value less than 0.001 compared to the control group).

5. Discussion

The present study showed the effects of *HOTAIR* silencing on the induction of apoptosis. Two well-known modulators of apoptosis, *Bax*, and *Bcl2*, were traced. Data showed Increased expression of *Bax* and decreased expression of *Bcl2*. Previously studies indicated that inhibition of *HOTAIR* can promotes apoptosis through different signaling pathways. However, the underlying mechanism is not well-understood in CRC. The *Bax* protein, which is a pro-apoptosis factor, activates apoptosis, while the *Bcl2* is an anti-apoptotic protein. The *Bax*/*Bcl2* ratio acts as a controller which decides whether cells turn on apoptosis or not. At the lower levels of ratio, the apoptosis is suppressed (21). Similarly, previous studies showed the high expression of *Bcl2* in different cancers, which made them resistant to chemo- or radio-therapy (22). This is the reason that the oncoprotein *Bcl2* has been proposed as therapeutic target for cancer through apoptosis induction (23). We also found that *Bax* had low level of expression, and its expression increased around 4-fold following siRNA treatment. The low expression of this gene was previously reported in colorectal and lung cancers (24, 25). It has been demonstrated that *Bax* permeabilizes the mitochondrial membrane to release cytochrome c and eventually apoptosis (26). Therefore, agents that activate *Bax* can be considered as promising anticancer drug. Several drugs have

Table 1. Used Primers in This Study

Target Gene	Sequence Primer	Amplicon Length
HOTAIR	F':AGGCCCTGCCTTCTGCCT	147 bp
	R:TGCTCTCTTACCCCCACGGA	
BAX	F':TTCATCCAGGATCGAGCAGG	103 bp
	R:TGAGACACTCGCTCAGCTTC	
BCL2	F:GAGGATTGTGGCCTTCTTTG	143 bp
	R:CGTTATCCTGGATCCAGGTG	
GAPDH	F:GTGAACCATGAGAAGTATGACAAC	123bp
	R:CATGAGTCCTTCCACGATACC	

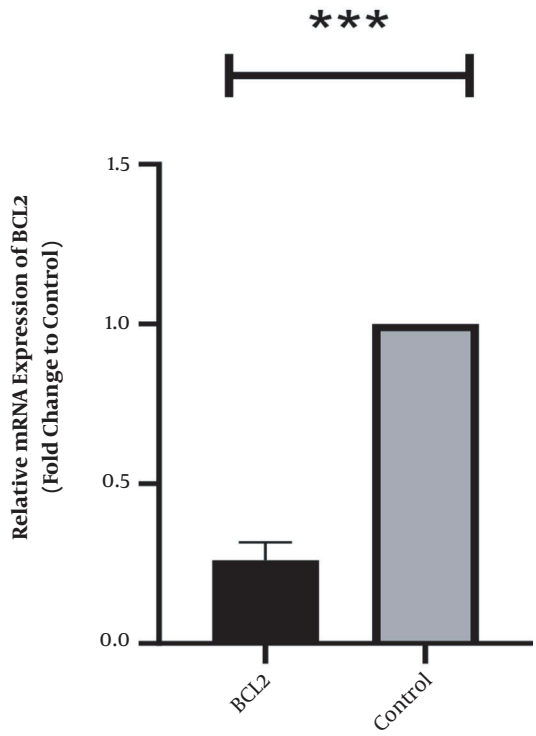

Figure 2. *HOTAIR* specific siRNA effects on suppressing *Bcl2* transcript after treatment with 50 ng *HOTAIR*-siRNA after 48 hours of treatment. Data expressed as mean ± standard deviation (the stars *** show the P-value less than 0.001 compared to the control group).

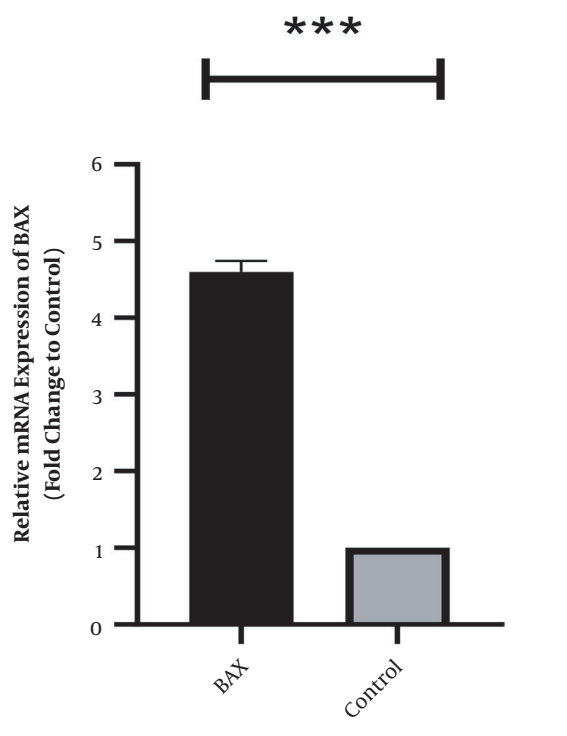

Figure 3. *HOTAIR* specific siRNA effects on suppressing *Bax* transcript after treatment with 50 ng *HOTAIR*-siRNA after 48 hours of treatment. Data expressed as mean ± standard deviation (*** P < 0.001 compared to control; the stars *** show the P-value less than 0.001 compared to the control group).

been confirmed or candidate to activate *Bax* directly or indirectly. Bortezomib, Ixabepilone, and Sorafenib are three examples of *Bax* activators (26). In conclusion, high level of *Bcl2* protein and low level of *Bax* can inhibit apoptosis in cancer cells, while low amount of *Bcl2* and high amount of *Bax* make cancer cells susceptible to the apoptosis. As we observed here, *HOTAIR* silencing can act as apoptosis inducer, which is partly due to the activation of *Bax* and inhibition of *Bcl2*. It would be ideal to consider the growth inhibitory effect of *HOTAIR* silencing on mouse models for CRC. Such a study might clarify whether *HOTAIR* silencing can provide a promising strategy to cure cancers. It is necessary to remind that apoptosis is only one among hundreds of molecular pathways under the control of *HOTAIR*. This lengthy noncoding transcript regulates variety of cellular pathways, including cell cycle, differentiation, and

growth (27). Therefore, *HOTAIR* silencing may inhibit the cancer progression through different pathways, which can be considered in future studies.

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Footnotes

Authors' Contribution: Study concept and design, R. D.; Analysis and interpretation of data, R. D., and M. T. and M. H.; Drafting of the manuscript, R. D., and M. T and M. H.; Critical revision of the manuscript for important intellectual content, M. T., M. H., and A. E.; Study supervision, M. T. and M. H.

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