

Effect of Gemcitabine on HOTAIR Expression Level in H596 and H1944 Cell Lines [†]

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Abstract: Genetic alterations affect carcinogenesis, and recent studies demonstrated that long non-coding RNAs (lncRNAs) have critical roles during this process. Hox transcript antisense intergenic RNA (HOTAIR) is a lncRNA molecule that affects proliferation, survival, migration, genomic stability, and drug resistance of cancer cells. The purpose of this study is to examine the possible effects of Gemcitabine treatment on HOTAIR expression level in non-small cell lung cancer (NSCLC) cell lines, H1944 and H596.

Keywords: lung cancer; lncRNA; HOTAIR; Gemcitabine

1. Introduction

Lung cancer is one of the most common cancer types in the world, and its morbidity and mortality rates continue to rise [1]. Lung cancer is divided into two groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for 85–90% of lung cancer and is one of the most deadly cancers with poor prognosis [2].

Long noncoding RNA (lncRNA) is a subgroup of non-coding RNAs with a variety of lengths (≥ 200 bp) and has become a new area of research in cancer [3]. Numerous studies have shown the pivotal role of lncRNAs in regulation of gene expression at the transcription, post-transcription, and epigenetic levels to induce cancer proliferation, invasion, cell differentiation, apoptosis, and drug resistance. Currently, the relation between lncRNAs and carcinogenesis is not clear [4].

Among different types of lncRNA molecules, Hox transcript antisense intergenic RNA (HOTAIR) is the most studied lncRNA. Overexpression of HOTAIR is associated with tumorigenesis and multiple cancer types including lung cancer. HOTAIR has important roles in carcinogenesis, such as proliferation, survival, migration, genomic stability, and drug resistance. Recent studies have shown that expression of HOTAIR is upregulated in lung cancer, and overexpression of HOTAIR is correlated with metastasis and poor prognosis [5].

Gemcitabine is a pyrimidine nucleoside antimetabolite, which is widely used in treatment of advanced NSCLC patients [6]. Gemcitabine significantly inhibits tumor growth in lung adenocarcinoma [7]. In a recent study, it was reported that Gemcitabine induced HOTAIR expression and promoted the self-renewal capacity, proliferation and migration of PANC-1 cancer stem cells [8].

In this current study, HOTAIR expression level was increased after treatment of Gemcitabine on H596 and H1944 NSCLC cell lines. This result can suggest a novel therapeutic approach for treatment of NSCLC patients in the future.

2. Material and Methods

A549, H358, H1944 and H596 NSCLC cell lines were obtained from American Tissue and Cell Collection (ATCC, Manassas, VA, USA). A549 was cultivated in F12-K media supplemented with 10% fetal bovine serum (Wisent, Saint-Jean-Baptiste, QC, Canada) and H358, H596, H1944 were cultivated in RPMI media supplemented with 10% fetal bovine serum (Wisent, Saint-Jean-Baptiste, QC, Canada) at 37 °C in a humidified incubator with 5%CO₂. Gemcitabine (purity ≥ 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in DMSO, the final concentration on DMSO was 0.1% in all experiments.

Basal HOTAIR gene expression level was analysed in all NSCLC cells by qRT-PCR. Cell Proliferation Assay was performed only in H1944 and H596 cells. These cells were seeded in a 96-well plate and incubated for 24 h. Cells were treated with different concentrations of Gemcitabine for 48 h. After treatment, WST-1 reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to each well. Then, absorbance (420–480 nm) was measured using MultiScan GO Microplate Spectrophotometer. The half maximal inhibitory concentration (IC₅₀) values of Gemcitabine was calculated using GraphPad Prism software program.

In order to analyse the effect of Gemcitabine on HOTAIR expression, H596 and H1944 cell lines were treated with Gemcitabine at their IC₅₀ values for 48 h. Control groups were treated with DMSO. After treatment, total RNA was isolated from these cells by PureLink RNA isolation kit (Invitrogen, Carlsbad, CA, USA). The total RNA concentration and purity values were determined by using the MultiScan GO Microplate Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). iTaq™ Universal SYBR® Green One-Step kit (Bio-Rad, Hercules, CA, USA) was used to analyse the expression levels of the HOTAIR gene by qRT-PCR. The GAPDH gene was used for normalization. Statistical analysis was performed using the GraphPad Prism.

3. Results

Basal expression levels of HOTAIR gene were evaluated in NSCLC cell lines by qRT-PCR. In this step, HOTAIR expression levels were found to be low in A549 and H1944 cells and high in H358 and H596 cells (Figure 1).

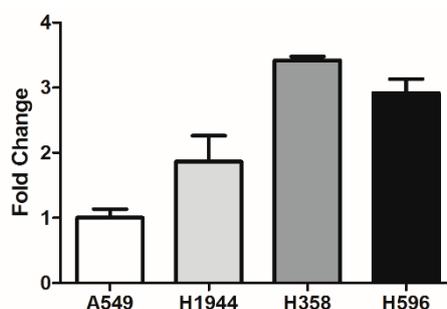


Figure 1. Analysis of basal HOTAIR gene expression levels in NSCLC cells by qRT-PCR.

In this study, the effect of Gemcitabine on cell proliferation in H1944 and H596 cell lines was studied using WST-1 cell proliferation assay. The IC₅₀ value of Gemcitabine on H1944 cell line was 1.2 µM, whereas the IC₅₀ value of Gemcitabine on a H596 was 4.3 µM. After treatment of H1944 and H596 cells with Gemcitabine, HOTAIR expression levels were analysed by qRT-PCR (Figure 2).

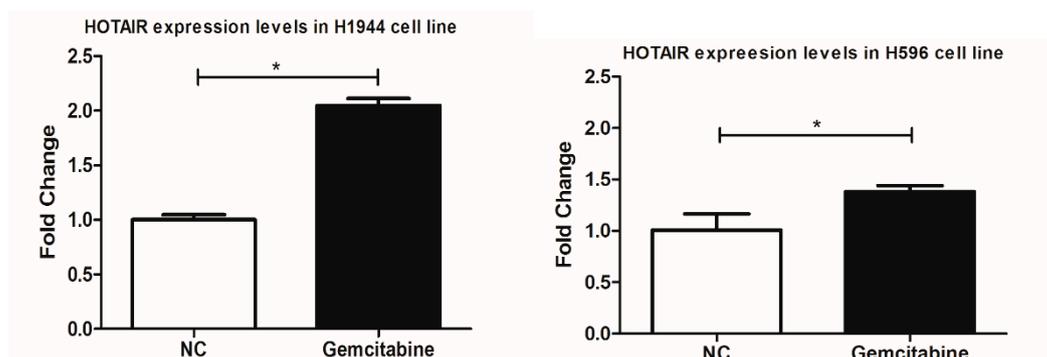


Figure 2. Analysis of HOTAIR gene expression in Gemcitabine treated H1944 and H596 cell lines by qRT-PCR (NC: negative control, *: $p < 0.05$).

4. Discussion

Lung cancer is the leading cause of cancer-related mortality worldwide. Both epigenetic and genetic changes contribute to the initiation, development and metastasis of lung cancer. Recent studies have begun to support the notion that lncRNAs function as new crucial regulators of diverse biological processes, and play crucial roles in tumorigenesis [4].

In conclusion, this study demonstrated that HOTAIR expression is induced by Gemcitabine in H1944 and H596 cell lines. This data suggests that HOTAIR may act as a tumor promoter in NSCLC cells by inhibiting drug sensitivity.

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