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LncRNA *H19* interacts with S-adenosylhomocysteine hydrolase to regulate *LINE-1* Methylation in human lung-derived cells exposed to Benzo[*a*]pyrene

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HIGHLIGHTS

- Carcinogen BaP was studied on three types of human lung-derived cells in vitro.
- BaP treatment enhances the interaction of *H19* and SAHH.
- Suppression of *H19* exaggerates SAHH protein expression and activity exposed to BaP.
- *H19*/SAHH regulates *LINE-1* methylation exposed to BaP.

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ABSTRACT

Benzo [*a*]pyrene (BaP) have been demonstrated to induce lung cancer risk in humans and many different animal models, with aberrant gene methylation as one of the epigenetic errors; however, the molecular mechanisms remain unclear. Here, we used three types of human lung-derived cells with BaP exposure as a model and attempted to investigate the long non-coding RNA (lncRNA) *H19*/S-adenosylhomocysteine hydrolase (SAHH) pathway that regulates gene methylation in vitro exposure to BaP. Results showed that compared to the controls, BaP-treated cells *H19* expressions were increased in a dose- and time-dependent manner, whereas SAHH protein expressions were decreased. Indeed, *H19* binds to and attenuates SAHH expressions and activity, and this interaction will be enhanced by BaP. However, suppression of *H19* exaggerates SAHH protein expression and activity exposed to BaP. Although BaP-treated cells *H19* single knockdown expectedly increased long interspersed nuclear elements-1 (*LINE-1*) methylation and inhibited benzo [*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) -DNA adducts formation with altering SAHH protein expressions and activity, the double knockdown restored methylation to the control level and exacerbated BPDE-DNA adducts formation. Overall, our results uncover a *H19*/SAHH circuit involving gene-methylation alterations by carcinogen BaP.

1. Introduction

Benzo [*a*]pyrene (BaP) is a main member of polycyclic aromatic hydrocarbons (PAHs), and is a potent carcinogen as confirmed in humans and many different animal models (Cai et al., 2012; Kerleyhamilton et al., 2012; Gao et al., 2011). In 2010, the carcinogenicity of BaP was reappraised and upgraded to a class I known human carcinogen by the International Agency for Research on Cancer. BaP and its metabolite, benzo [*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), can cause DNA damage (Tavakoly Sany et al., 2015; Møller and Loft, 2010) and various types of cancer (Basu,

Abbreviations: PAHs, Polycyclic aromatic hydrocarbons; BaP, Benzo[*a*]pyrene; lncRNA, long noncoding RNA; SAHH, S-adenosylhomocysteine hydrolase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; DNMT, DNA methyltransferase; FISH, fluorescence in situ hybridisation; RIP, RNA Binding Protein Immunoprecipitation.

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2010). Cancer is a complex disease arising from both genetic and epigenetic errors (Lewandowska and Bartoszek, 2011; Parsons, 2003). Moreover, aberrant gene methylation caused by the carcinogenic potential of BaP may play an important role in BaP carcinogenesis, which is one of the epigenetic changes in human lung cancer (Herbstman et al., 2012).

Long non-coding RNAs (lncRNAs) are defined as transcripts that are more than 200 nucleotides in length and without proof of protein coding potential. During the past decade, lncRNAs have come to the forefront of research interest in many fields. Recent discoveries have marked lncRNAs as new important players in the regulation of various cellular processes including cell cycle, cell differentiation, nuclear reprogramming, and epigenetics (Chung et al., 2011; Wilusz et al., 2009). *H19*, an imprinted long non-coding RNA, functions as a ribonucleoprotein by synergizing with other proteins in tumorigenesis (Betancur, 2016; Mazar et al., 2014). Recently, some scholars have found that *H19* binds to S-adenosylhomocysteine hydrolase (SAHH), the only known enzyme that catalyses the hydrolysis of S-adenosylhomocysteine (SAH) to homocysteine and adenosine, and inhibits SAHH function both in vivo and in vitro (Zhou et al., 2015; Oksana et al., 2013). Furthermore, it has been claimed that the interaction prevents SAHH from hydrolysing SAH that blocks gene methylation at numerous genomic loci, which differs from what has been described before for other lncRNAs.

Therefore, we attempted to investigate the *H19*/SAHH pathway that regulates gene methylation in vitro exposure to BaP. Long interspersed nuclear elements-1 (*LINE-1*), representing a family of non-long terminal repeat retroposons, are interspersed all over the genomic DNA and account for approximately 20% of the human genome (Saito et al., 2010; Babushok and Kazazian, 2007). The level of *LINE-1* methylation is regarded as a proxy of genome-wide methylation for its high frequency in the genome (Zelic et al., 2015).

In the current study, we used three types of human lung-derived cells with BaP exposure as a model and pyrosequencing to detect *LINE-1* methylation levels, and we hope to reveal the molecular regulation mechanism of *H19* on gene methylation alterations by carcinogen BaP.

2. Materials and methods

2.1. Cell culture

Three types of human lung-derived cells, including human bronchial epithelial cells (16HBE), human lung epithelial cells (BEAS-2B), and human embryonic lung fibroblasts (VA13), were maintained with growth medium (Alpha-Minimum Essential Medium Eagle, supplement with 1% penicillin/streptomycin and 10% fetal bovine serum) at 37 °C and 5% CO_2. All media and supplements used for cell cultures were obtained from Gibco (Gaithersburg, MD, USA).

2.2. Cell transfection

To prepare for transfection, cells were seeded in at a density of 1×10^5 cells per well in six-well plates (Corning, NY, USA). The expressions of *H19* or *SAHH* were inhibited by small interfering RNA (siRNA) in cells to constructions of *H19* or *SAHH* low-expression cell model. The siRNA duplexes and a nonsense control (siRNA-NC) were synthesised by Guangzhou RiboBio (Guangdong, China). The sequences of the oligonucleotides were as follows: 5′−CCCACAACAUGAAAGAAAUdTdT-3′ and 5′-dTdTGGGUGUUGUACUUUCUUUA-3′ for *H19*, and 5′-CAAGCTAACTGAGAAGCAAdTdT-3′ and 5′-dTdTGUUCGAUUGACUCUUCGUU-3′ for *SAHH*. The siRNA transfection was performed using lipofectamine-RNAi MAX (Invitrogen, Carlsbad, CA, USA) following the manufacturer's recommendations. After transfection, RNA or protein was extracted to analyse the relative expressions of *H19* and *SAHH*. Maximal inhibition of *H19* or *SAHH* expressions were observed after 30 h transfection and the effect was sustained up to 72 h after transfection. Thus, cells were treated with BaP at 30 h after transfection.

2.3. Treatment of cells

For the expression analysis of *H19* and SAHH protein, wild-type (WT) cells were exposed to BaP (98% purity, Sigma-Aldrich, St. Louis, MO, USA) at concentrations of 0, 1, 2, 4, 8, 16, and 32 μmol/L for 24 h, or exposed to 16 μmol/L BaP for 0, 1, 2, 4, 8, 12, and 24 h. Cells were treated with 0.1% dimethyl sulfoxide (DMSO) as a solvent control. Prior to the other analysis, four type cells (WT, siRNA-*H19*, siRNA-*H19* + siRNA-*SAHH*, and siRNA-NC) were exposed to 16 μmol/L BaP for 24 h. After treatment cells were harvested and transferred to a 1.5-mL tube. All experiments were independently performed for three times on different days and the average was used for comparison.

The dose and time in this study were selected based on our preliminary experiments, which suggested that cell viability decrease less than 15% and S-phase arrest are implicated in the BaP-induced DNA damage.

2.4. RNA extraction and RT-PCR

Total RNAs were extracted from cells by TRIzon reagent (CWbiotech, Beijing, China). cDNA was synthesised using Prime Script RT Master Mix (Perfect Real Time) (Takara, Dalian, China) in a 20-μL reaction containing 1000-ng total RNAs. Real time polymerase chain reaction (RT-PCR) was performed in a 20-μL reaction containing 2 μL cDNA using SYBR Premix Ex Taq II (Takara) and performed on Roche LC 480 system (Roche, Switzerland) by initial denaturation at 95 °C for 30 s, followed by 45 cycles of 5 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. Specificity was verified using melting curve analysis and agarose gel electrophoresis. The endogenous control (β-actin) was used to normalise quantification of the specific RNA targets. The initial RNA amount was calculated using the $2^{-\Delta\Delta Ct}$ method and each sample was performed in triplicate. The RT-PCR primers are listed in Table 1.

2.5. Western blot

Proteins were extracted from cells with radioimmunoprecipitation assay lysis buffer (CWbiotech) and quantified using BCA Protein Assay Kit (CWbiotech) by microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA). An equivalent amount of protein was loaded on 10% sodium dodecyl sulphate-

Table 1
Primer sequences of human mRNA and product size (bp).

Gene	Forward Primer	Reverse Primer	bp
β-actin	5′-TCCCTGGAGAAGAGCTACGA-3′	5′-AGCACTGTGTTGGCGTACAG-3′	194
H19	5′-CACACTCACGCACACTCGTA-3′	5′-CACCACCTCCCTCTTCTTCTT-3′	200
SAHH	5′-GACCGGTATCGGTTGAAGAA-3′	5′-TTCTTGGGCAGGAAATGAAC-3′	191
GAPDH	5′-GCACCGTCAAGGCTGAGAAC-3′	5′-TGGTGAAGACGCCAGTGGA-3′	138
LINE-1	5′-Bio-AAAATCAAAAAATTCCCTTTC-3′	5′-TTTTGAGTTAGGTGTGGGATATA-3′	152