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The Oncogenic lncRNA *HOTAIR* is not Expressed in the ATRT-MYC-derived Cell Line BT12

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ABSTRACT

Atypical teratoid Rhabdoid tumor (ATRT) is a highly aggressive pediatric tumor of the brain and spinal cord and is one of the most lethal neoplasms of early neonatal life, with a mortality rate exceeding 75%. Almost 95% of ATRT carry a biallelic SMARCB1 gene mutation. ATRT-MYC subgroup is distinguished by elevated levels of HOX Transcript Antisense Intergenic RNA, or HOTAIR, long non-coding RNA (LncRNA). We hypothesize that HOTAIR may play an epigenetic role in ATRT onset of tumorigenesis. In this study, we investigated the existence of potential interaction between the SMARCB1 and the HOTAIR in the non-tumor HEK-293 line, harboring a wild-type SMARCB1. First, the levels of HOTAIR in the BT-12 cell line were assessed using quantitative real-time PCR (qPCR) relative to the non-tumor HEK-293 line, harboring a wild-type SMARCB1, as a calibrator. In contrast to previous reports, our results showed that the HOTAIR expression level was below the detection limit of the qPCR assay, with a quantification cycle (Cq) of ≥ 40 cycles in the BT-12 cell line. Silencing of HOTAIR in HEK-293 cells using antisense oligonucleotides resulted in a significant 32% reduction in wound healing compared to mock-transfected cells.

Keywords

Atypical teratoid/rhabdoid tumor, Pediatric tumors, Brain, Spinal cord.

Introduction

Atypical teratoid/rhabdoid tumor (ATRT) is a rare aggressive pediatric malignancy that affect the brain and spinal cord with a relative 5-year survival rate of only 32.2% [1,2]. ATRTs have a very simple cancer genome, with the predominant, and frequently the only, recurrent molecular hallmark, being a biallelic mutation of the *SMARCB1*, which encodes a core subunit protein of the SWI/SNF chromatin-remodeling complex [3,4].

The simple genome however contradicts the molecular heterogeneity shown in ATRT cohorts [5]. ATRTs comprise three molecular subtypes that differ in their global level of gene expression, as well as variable degrees of DNA methylation and, are found in distinct anatomical locations [6]. The three main molecular subgroups of ATRTs, for which a consensus was reached to name them ATRT-TYR, ATRT-SHH, and ATRT-MYC [7].

SMARCB1, also named *INI1* or *SNF5* [8], is a tumor suppressor gene located on chromosome 22q11.2 [9]. The initiation and progression of aggressive cancers caused by *SMARCB1* loss may occur epigenetically via disturbance of transcriptional regulation rather than destabilizing the genome through the accumulation of mutations [10].

BT-12 is a ATRT cell line that has been allocated to MYC subgroup, that, generally, express elevated levels of *HOTAIR* [5]. *HOTAIR* lncRNA interacts with SMARCB1 subunit protein in clear cell renal cell carcinoma (ccRCC) [11].

Long noncoding RNAs (lncRNAs) are transcripts of 200 nucleotides (nt) or more lacking an open reading frame (ORF) that have been

reported to regulate transcription via recruitment of chromatin modifiers or bridging distal enhancer elements to gene promoters [12,13]. HOX transcript antisense RNA (*HOTAIR*) is a lncRNA with 6 exons and more than 2000 nucleotides [14]. *HOTAIR* is an oncogenic factor and plays a key role in the initiation, progression, and promotion of malignancy in different types of cancer [15]. Transcriptome analysis of 20 pediatric ATRT using nanoString platform revealed high expression of *HOTAIR* [16].

In this study, we investigated whether a possible link exists between *SMARCB1* loss in BT-12 ATRT cell line and the reported high *HOTAIR* expression in ATRT and the potential role of *HOTAIR* as an epigenetic regulator between loss of function of *SMARCB1* and the onset of tumorigenesis. We used real-time quantitative PCR (qPCR) to assess the expression level of *HOTAIR* in the authenticated ATRT-derived cell line, BT-12, compared to the non-tumor human line HEK293 hosting a wildtype *SMARCB1*. We also used customized small interfering RNAs (siRNAs) to silence *HOTAIR* in HEK-293 in order to explore the influence of such knock down on *SMARCB1* mRNA expression in this cell line or whether the loss of SMARCB1 might have influenced the expression of *HOTAIR*. We document that the *HOTAIR* expression level in BT-12 is below the qPCR limit of detection. This result suggests that *HOTAIR* plays no oncogenic role in the BT-12 cell line.

Methods

Materials

Roswell Park Memorial Institute (RPMI), Dulbecco's Modified Eagle's Medium (DMEM), and Phosphate Buffered Saline (PBS) were purchased from Lonza Bioscience, Absolute Ethanol was purchased from Honeywell, DEPC-treated water and Nuclease free water were purchased from Thermo Fisher Scientific, Chloroform was purchased from Carl Roth.

Cell culture

ATRT cell line, BT-12, was a gift from Peter J. Houghton. The human embryonic kidney cell line, HEK-293, was purchased from The American Type Culture Collection. BT-12 cells were grown in RPMI medium and supplemented with 10% fetal bovine serum (FBS, Gibco). HEK-293 cells were cultivated in a DMEM medium supplemented with 5% FBS. For both lines were supplemented with 1% penicillin/streptomycin (Gibco). The cell lines were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO2.

Total RNA extraction and quantification

Total RNA was extracted from both BT-12 and HEK-293 cell lines using the TRIzol reagent (Thermo Fisher Scientific), according to the manufacturer's instructions and was quantified using a Qubit® RNA BR (Broad Range) assay kit according to the manufacturer's instructions (Thermo Fisher Scientific).

Reverse transcription and real-time PCR

cDNA was synthesized using a SuperScript® III First-Strand Synthesis System kit (Thermo Fisher Scientific). Quantitative real-time PCR (qPCR) analysis was performed in triplicate using TaqMan Fast Advanced PCR Master Mix in customized assays (The assay ID for *SMARCB1* primers: Hs00992516_m1, for *HOTAIR*: Hs03296631_m1, and for *GAPDH*: Hs00266705_g1) on a StepOnePlus™ Real-Time PCR System according to the manufacturer's protocol (Thermo Fisher Scientific). The exact binding sites of the primers and TaqMan probes are shown in Supplementary Figure 1. The PCR program was as follows: denaturation at 95°C for 2 minutes and 40 cycles of amplification consisting of denaturation at 95 °C for 10 seconds, annealing and extending at 60°C for 20 seconds. The Human *GAPDH* level was used as an internal control. Relative expression was assessed using the $2^{-\Delta\Delta}$ method [17].

HOTAIR knockdown in HEK-293 cell line

Small interfering RNAs (siRNAs) specifically targeting *HOTAIR* (siHOTAIR), siRNA specific for GAPDH (siGAPDH), and its negative control (siNC), were purchased from Thermo Fisher Scientific. All these siRNAs were transfected into the HEK-293 cell line using Lipofectamine RNAiMAX reagent (Thermo Scientific), referring to the instructions of the manufacturer. The assay ID for siHOTAIR: n272221.

Wound Healing Assay

HEK-293 cells were cultured in the 6-well plate and a straight line was drawn with a 10-µL sterile pipette tip. The repair was observed under a microscope at 0 and 24 hours. The wound area (scratch area) was calculated using an online tool, https://www.sketchandcalc.com/.

The rate of wound healing was calculated according to the formula:

$$Rate\ of\ wound\ healing = \frac{wound\ area\ at\ time\ 0\ hour - wound\ area\ at\ time\ 24\ hours}{wound\ area\ at\ time\ 0\ hour} \times 100$$

Results

qPCR results for HOTAIR expression levels in the BT-12 cell line.

The qPCR experiments revealed two unexpected results. First, in BT-12 the *SMARCB1* is expressed on the mRNA level albeit at a very low concentration. Second, compared to HEK-293, the *HOTAIR* level is below the detection level in contrast to the reported upregulation in the ATRT-MYC subgroup (Figure 1).

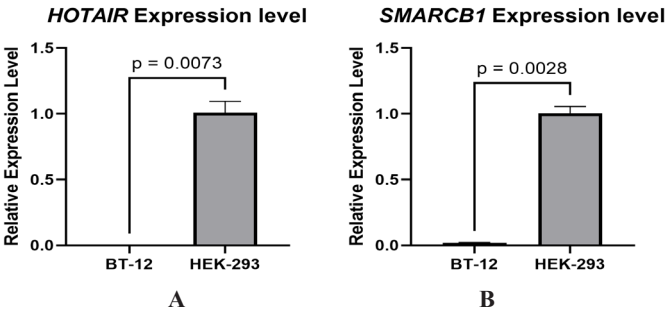

Figure 1: *SMARCB1,* and *HOTAIR* Fold gene expression in BT-12 relative to HEK-293 (calibrator).

Silencing of HOTAIR in HEK-293 cells.

Calculations using the comparative (Cq) method show successful *HOTAIR* siRNA treatment of HEK-293 relative to HEK-293 treated by negative control siRNA (non-targeting siRNA control) with a knockdown efficiency of 88%, approximately (Figure 2).

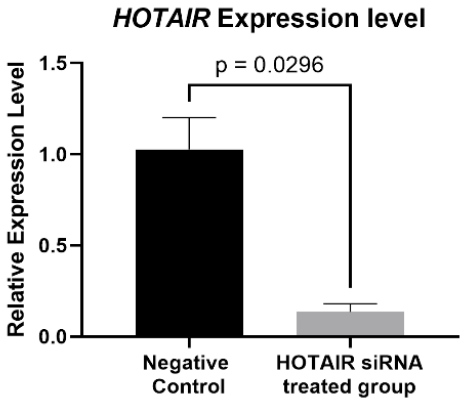

Figure 2: *HOTAIR* Fold gene expression in HEK-293 treated by *HOTAIR* siRNA relative to HEK-293 transfected with a negative control siRNA (calibrator).

Wound healing assay

The rate of wound healing i.e., % Closure of the wound in the siHOTAIR, siNC groups were 54%, and 79% respectively (see Figure 3,4). The results indicate that *HOTAIR* knockdown in HEK-293 cells decreased cell migration rate by 32% compared to the negative control group.

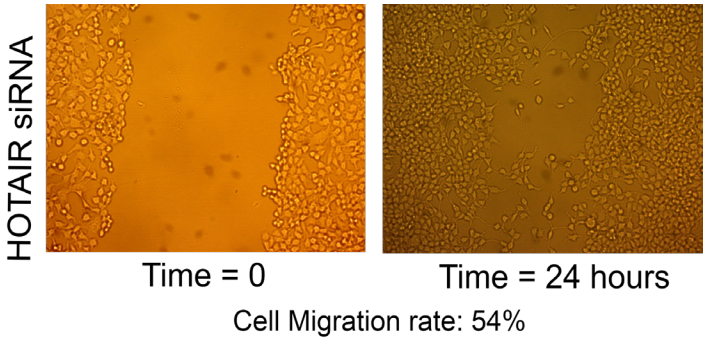

Figure 3: Negative control experiment using non targeting siRNA in wound healing (scratch) assay for HEK-293 cells.

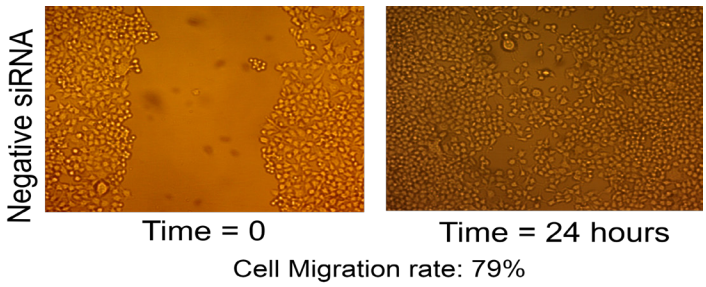

Figure 4: Positive control experiment using GAPDH siRNA in wound healing (scratch) assay for HEK-293 cells.

Discussion

The simple genomes of ATRT tumors imply the involvement of an epigenetic dimension of regulation accomplished by chromatin condensation, DNA methylation at promoter CpG islands and/or noncoding RNAs.

SMARCB1 forms a core subunit of the SWI/SNF chromatin remodeling complex, which is a critical regulator of human embryonic development. This subunit is frequently described as a tumor suppressor that abolishes super-enhancers controlling hESC lineage determination [18]. The nature of this interaction, however, is not clearly understood. The reported upregulation of lncRNAs in ATRT encouraged us to hypothesize that noncoding RNAs, such as *HOTAIR*, might mediate the SMARCB1-super-enhancer interaction. This suppressive level of regulation antagonizes the oncogenic MYC protein, also reported to be regulated by SMARCB1 [19]. Thus, the loss of SMARCB1 function can drive malignancy in ATRT.

Our qPCR results showed that *SMARCB1* is low level of mRNA expressed in BT-12. This comes in agreement with an earlier observation by (Biegel et al.) who reported the expression of *SMARCB1* in three out of seven primary rhabdoid tumors [20].

The oligonucleotides of the *SMARCB1* assay used in this study (Hs00992516_m1; ThermoFisher Scientific) target the 5'-region of the gene with the probe spanning exons 1 and 2. Thus, in BT-12, at least the initial part of *SMARCB1* mRNA is being transcribed or carries a nonsense mutation that apparently did not affect oligo binding.

Chakravadhanula et al. studied the expression of the *HOX* and *HOTAIR* genes in pediatric tumors (20 ATRTs, 10 ependymomoas, 10 medulloblastomas, 6 glioblastoma multiforme, and 9 juvenile pilocytic astrocytomas (JPAs) using nanoString technology. They report high expression level of the *HOXC* and *HOTAIR* genes [16]. Our qPCR results in BT-12, however, contradict this finding; in this cell line, *HOTAIR* expression was below the detection limits of the assay.

The BT-12 cell line has been allocated to the ATRT-MYC molecular subgroup of rhabdoid tumors by several authors [5,6]. Members of this subgroup typically exhibit high expression levels of MYC, lack tyrosinase signature, and show an elevated *HOX* gene expression. This could align with our results that BT-12 also lacks the *HOTAIR* that is typically upregulated in ATRT-MYC.

Our study however, does not negate the possibility of *HOTAIR* interacting with *SMARCB1* in other cell lines allocated to ATRT-MYC or in other tumors in general. For example, (Sumida et al.) performed RNA immunoprecipitation (RIP) assays in renal cell carcinoma and found that *HOTAIR* binds to both SMARCB1 and ARID1A [21].

siRNA-mediated knockdown of *HOTAIR* in HEK-293 cells reduced cell migration rate by 32% compared to the negative control

siRNA group, the reason is that *HOTAIR* causes dysregulation in the expression of proteins involved in diverse cellular components, including the cytoskeleton and the respiratory chain. (Zheng et al.) did functional studies on vimentin (VIM), a key protein involved in maintaining the integrity of the cytoskeleton and cell shape, revealed that *HOTAIR* exerts its effects on migration and invasion of HeLa cells, at least in part, through the regulation of VIM expression [22]. Also, *HOTAIR* can promote cell migration and invasion by regulating Megakaryoblastic leukemia 1 (*MKL1*) via inhibition of *miR206* expression in HeLa cells [23].

References

1. Ginn KF, Gajjar A. Atypical teratoid rhabdoid tumour: current therapy and future directions. Frontiers in oncology. 2012; 2: 114.
2. Rorke LB, Packer RJ, Biegel JA. Central nervous system atypical teratoid/rhabdoid tumours of infancy and childhood: definition of an entity. Journal of neurosurgery. 1996; 85: 56-65.
3. Frühwald MC, Biegel JA, Bourdeaut, F, et al. Atypical teratoid/rhabdoid tumours—current concepts, advances in biology, and potential future therapies. Neuro-oncology. 2016; 18: 764-778.
4. Margol AS, Judkins AR. Pathology and diagnosis of SMARCB1-deficient tumors. Cancer genetics. 2014; 207: 358-364.
5. Ho B, Johann PD, Grabovska Y, et al. Molecular subgrouping of atypical teratoid/rhabdoid tumours—a reinvestigation and current consensus. Neuro-oncology. 2020; 22: 613-624.
6. Torchia J, Picard D, Lafay-Cousin L, et al. Molecular subgroups of atypical teratoid rhabdoid tumours in children: an integrated genomic and clinicopathological analysis. The lancet oncology. 2015; 16: 569-582.
7. Upadhyaya SA, Robinson GW, Onar-Thomas A, et al. Relevance of molecular groups in children with newly diagnosed atypical teratoid rhabdoid tumour: results from prospective St. Jude multi-institutional trials. Clinical Cancer Research. 2021; 27: 2879-2889.
8. Pulice JL, Kadoch C. Composition and function of mammalian SWI/SNF chromatin remodelling complexes in human disease. In Cold Spring Harbor symposia on quantitative biology. 2016; 81: 53-60.
9. Roberts C, Biegel J. The role of SMARCB1/INI1 in the development of rhabdoid tumours. Cancer biology & therapy. 2009; 8: 412-416.
10. Kim KH, Roberts CW. Mechanisms by which SMARCB1 loss drives rhabdoid tumour growth. Cancer genetics. 2014; 207: 365-372.
11. Imai-Sumida M, Dasgupta P, Kulkarni P, et al. Genistein represses HOTAIR/chromatin remodeling pathways to suppress kidney cancer. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology. 2020; 54: 53.
12. Cao J. The functional role of long non-coding RNAs and epigenetics. Biological procedures online. 2014; 16: 1-13.
13. Werner MS, Ruthenburg AJ. Nuclear fractionation reveals thousands of chromatin-tethered noncoding RNAs adjacent to active genes. Cell reports. 2015; 12: 1089-1098.
14. Rinn JL, Kertesz M, Wang JK, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell. 2007; 129: 1311-1323.
15. Huang L, Liao LM, Liu AW, et al. Overexpression of long noncoding RNA HOTAIR predicts a poor prognosis in patients with cervical cancer. Archives of gynecology and obstetrics. 2014; 290: 717-723.
16. Chakravadhanula M, Ozols VV, Hampton CN, et al. Expression of the HOX genes and HOTAIR in atypical teratoid rhabdoid tumors and other pediatric brain tumors. Cancer Genetics. 2014; 207: 425-428.
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods (San Diego, Calif.). 2001; 25: 402-408.
18. Lee F Langer, James M Ward, Trevor K Archer. Tumor suppressor SMARCB1 suppresses super-enhancers to govern hESC lineage determination ELife. 2019; 8: e45672.
19. Weissmiller AM, Wang J, Lorey SL. et al. Inhibition of MYC by the SMARCB1 tumor suppressor. Nat Commun. 2019; 1; 10: 2014.
20. Biegel JA, Zhou JY, Rorke LB, et al. Germ-line and acquired mutations of INI1 in atypical teratoid and rhabdoid tumours. Cancer research. 1999; 59: 74-79.
21. Imai-Sumida M, Dasgupta P, Kulkarni P, et al. Genistein represses HOTAIR/chromatin remodelling pathways to suppress kidney cancer. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology. 2020; 54: 53.
22. Zheng P, Xiong Q, Wu Y, et al. Quantitative Proteomics Analysis Reveals Novel Insights into Mechanisms of Action of Long Noncoding RNA Hox Transcript Antisense Intergenic RNA (HOTAIR) in HeLa Cells. Molecular & cellular proteomics: MCP. 2015; 14: 1447-1463.
23. Zheng P, Yin Z, Wu Y, et al. LncRNA HOTAIR promotes cell migration and invasion by regulating MKL1 via inhibition miR206 expression in HeLa cells. Cell Communication and Signaling. 2018; 16: 1-15.