Biochemical and Biophysical Research Communications 463 (2015) 569–574

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Long none coding RNA HOTTIP/HOXA13 act as synergistic role by decreasing cell migration and proliferation in Hirschsprung disease

Hua Xie [a, b, 1], Dongmei Zhu [a, b, 1], Cao Xu [a, b], Hairong Zhu [a, b], Pingfa Chen [a, b], Hongxing Li [a, b], Xiang Liu [c], Yankai Xia [a, b], Weibing Tang [a, b, *]

[a] Department of Pediatric Surgery, State Key Laboratory of Reproductive Medicine, Nanjing Children's Hospital Affiliated Nanjing Medical University, Nanjing 210008, China
[b] Key Laboratory of Modern Toxicology (Nanjing Medical University), Institute of Toxicology, School of Public Health, Nanjing Medical University, Ministry of Education, Nanjing 211166, China
[c] Department of Pediatric Surgery, Anhui Provincial Children's Hospital, Anhui 230000, China

ARTICLE INFO

Article history:
Received 23 April 2015
Accepted 28 May 2015
Available online 2 June 2015

Keywords:
HSCR
Long noncoding RNA
Neural cell
Pediatric disease
Hox genes

ABSTRACT

Long noncoding RNAs (lncRNAs) have been confirmed to be associated with various human diseases. However, whether they are associated with Hirschsprung disease (HSCR) progression remains unclear. In this study, we designed the experiment to explore the relationship between lncRNA HOTTIP and HOXA13, and their pathogenicity to HSCR. Quantitative real-time PCR and Western blot were performed to detect the levels of lncRNA, mRNAs, and proteins in colon tissues from 79 patients with HSCR and 79 controls. Small RNA interference transfection was used to study the function experiments in human 293T and SK-N-BE cell lines. The cell viability and activities were detected by the transwell assays, CCK8 assay, and flow cytometry, respectively. LncRNA HOTTIP and HOXA13 were significantly down-regulated in HSCR compared to the controls. Meanwhile, the declined extent of their expression levels makes sense between two main phenotype of HSCR. SiRNA-mediated knock-down of HOTTIP or HOXA13 correlated with decreased levels of each other and both reduced the cell migration and proliferation without affecting cell apoptosis or cell cycle. Our study demonstrates that aberrant reduction of HOTTIP and HOXA13, which have a bidirectional regulatory loop, may play an important role in the pathogenesis of HSCR.

1. Introduction

Hirschsprung disease (HSCR), which occurs in about 1:2000–1:5000 live births, is characterized by the deficient migration of enteric neural crest cells (ENCCs) during embryogenesis from 5 to 12 weeks [1]. The absence of enteric neurons from variable lengths of the bowel in HSCR results from a failure of neural crest-derived cells to colonize the affected gut regions [2]. Considering the outcome, patients can be classified as short segment HSCR (S-HSCR, 80% of cases) where the aganglionic segment does not extend beyond the upper sigmoid, and long segment HSCR (L-HSCR, 20% of cases) where aganglionosis extends proximal to the sigmoid. Otherwise, there are also other phenotypes such as total colonic aganglionosis (TCA) [3]. Genetic predisposition and intestinal microenvironment changes are reported to be the main contributors to the development of HSCR. Genes, which control ENCC migration, proliferation, survival, and/or differentiation, play particularly important roles in the pathogenesis of HSCR. Up to date, more than 10 genes including *RET*, *GDNF*, *GFRα1*, *NTN*, *PSPN*, *EDNRB*, *EDN3*, *ECE-1*, *SOX10*, *PHOX2B* have been identified to be associated with the pathogenesis of HSCR [4]. Our previous studies also demonstrated that some genes and miRNAs are involved in HSCR [5–9], however, whether more genes or noncoding genes, especially long noncoding RNAs, are involved in HSCR remains poorly understood.

The homeobox (HOX) genes, which consist of gene clusters (A, B, C and D), encode a family of transcriptional factors crucial to embryo patterning and cell fate [10]. During embryonic development, HOX genes expression controls the identity of body regions in accordance with the spatio-temporal colinearity rules [11]. At the cellular level, hox genes regulate cell differentiation and are involved in NCC migration [12]. Moreover, some hox genes are

* Corresponding author. Department of Pediatric Surgery, Nanjing Children's Hospital Affiliated Nanjing Medical University, Nanjing 210008, China.
E-mail address: twbcn@njmu.com (W. Tang).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.bbrc.2015.05.096
0006-291X/

known to give rise to the defect of ENCCs. It is reported that HOXA4 overexpression was related to disorganization of the ENCC proliferation, migration and differentiation. HOXB5 coordinates with other transcription factors mediates RET expression [13,14]. In addition, HOXA13, which has been reported as a marker of gut primordia posteriorization in the early ENS development in the zebrafish model and plays a crucial role in extra-embryonic vascularization and human hindgut development [15,16], also present an important role in ENCC migration.

LncRNAs are defined as ncRNAs that longer than 200 nucleotides without the capacity of coding proteins [17]. Recent studies have indicated that lncRNAs may act as modular scaffolds, combining multiple nucleic acid and protein binding domains to direct the localization and/or regulate the activity of multiple effector proteins simultaneously [18]. Till data, more than 200 lncRNAs have been annotated in the 4 HOX loci [19]. The recently studied lncRNA HOTTIP gene is located in physical contiguity with HOXA13 and directly coordinate and controls the activation of several 5′ HOXA genes via interaction with the WDR5/MLL complex [20]. In hepatocellular carcinoma and osseous arthritis, HOTTIP has been reported to regulate HOXA13 expression [21,22]. However, whether it holds the true regulation in HSCR remains to be elucidated.

In this study, we design the experiments to investigate the involvement of the lncRNA HOTTIP and HOXA13 in the pathogenesis of Hirschsprung disease.

2. Materials and methods

2.1. Ethics statement and samples collection

In the study, a total of 79 HSCR samples were obtained from HSCR patients at Nanjing Children's Hospital Affiliated to Nanjing Medical University from October 2009 to May 2013 (NJMU Birth Cohort). Pathological detection of all patients was performed to make a definite diagnosis. Seventy-nine control colon tissues were obtained from isolated patients proved without HSCR or other congenital malformation. All of Children's guardians were provided informed consent for the use of the tissues before surgery. After surgical removal, the tissues were immediately frozen and stored at −80 °C. All activities in this study was approved by the Institutional Ethics Committee of Nanjing Medical University and involving subjects were done under compliance with the government policies and the Helsinki Declaration.

2.2. Clinical diagnostic criteria

Collected HSCR colon samples were classified as L-HSCR and S-HSCR by barium enema, anorectal manometry and pathological diagnosis after surgery. S-HSCR means that patient's aganglionosis segment is restricted to the rectosigmoid colon, while ones' aganglionosis segment extends proximal to the sigmoid colon are L-HSCR.

2.3. Quantitative real time polymerase chain reaction (RT-PCR)

Total RNA was obtained from tissues using TRIzol reagent as described by the manufacturer (Invitrogen Life Technologies Co, USA). Quantitative RT-PCR was performed to determine the expression levels of lncRNA HOTTIP and HOXA13. For mRNA detection, total RNAs (500 ng) were reverse transcribed using the reverse transcription kit (Takara, Tokyo, Japan) under 37 °C for 15 min and 85 °C for 30 s. RT-PCR was performed on the ABI Prism 7900HT (Applied Biosystems, USA). GAPDH was used as an endogenous control. Forward and reverse primer's sequences were shown in Supplement Table 1. PCR was performed for 5 s at 95 °C and for 30 s at 60 °C for 40 cycles.

2.4. Protein analysis

For western blot, total proteins were extracted from tissues or cultured cells using RIPA buffer containing protease inhibitors (cOmplete, ULTRA, Mini, EDTA-free, EASY pack Roche), while the membrane protein were extracted from tissues by Mem-PER Eukaryotic Membrane Protein Extraction Reagent Kit(Thermo Scientific, Rockford, USA). Protein concentrations were determined by BCA method. Equal amount of proteins (100ug) were separated on 7.5%/12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to PVDF membrane. Membrane was blocked using 5% skimmed milk and incubated with respective antibodies. Primary polyclonal antibodies were purchased from epitomics. The secondary antibodies were anti-rabbit or anti-goat HRP-linked. The blots were developed using ECL reagent (Millpore, MASS, USA). Equal amount of protein was confirmed using GAPDH antibody. All experiments were repeated at least three times.

2.5. Cell culture and SiRNA transfection

The Human 293T cell and SK-N-BE(2) cell were obtained from American Type Culture Collection (ATCC, Manassas VA, USA), which were cultured in complete growth medium: DMEM (Hyclone, UT, USA), supplemented with 10% fetal bovine serum (10%FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C, 5% CO_2. Cells, which were cultured to about 50% confluence, were transfected with 50 nM negative control and 100 nM siRNA by Lipofectamine 2000 (Invitrogen Corp, CA, USA). The sequences of siRNA, targeting either HOXA13 or HOTTIP, and off-target negative control were presented in Supplement Table 1.

2.6. Cell proliferation assays

CCK-8 assay (Beyotime, Nantong, China) was used to detect the cell proliferation after 24 h transfection. The TECAN infinite M200 Multimode microplate reader (Tecan, Mechelen, Belgium) was used to measure the absorbance at 450 nm. All experiments were performed in triplicate independently.

2.7. Cell cycle and apoptosis analysis

Cells were transfected with HOTTIP or HOXA13 siRNA, as well as negative controls for 48 h. For the cell cycle assay, cells were fixed in 75% alcohol, and stained with propidium iodide (Sigma, MO, USA). For the apoptosis analysis, cells were washed in PBS, then mixed with Annexin V-FITC Apoptosis Detection Kit (BD Biopharmingen, NJ, USA) for 15 min in the dark. All experiments were analyzed by BD Biasciences FACS Calibur Flow Cytometry (BD Biasciences, NJ, USA). The tests were repeated for three times per experiment.

2.8. Cell transwell assays

After the transfection of HOTTIP or HOXA13 siRNA for 48 h, cells were seeded at 1×10^6 cells/ml with serum-free medium. 100 μl cell suspension was seeded to the upper chamber (Millpore, MASS, USA) in 24-well plates, while 600 μl DMEM with 10%FBS were filled in the lower chamber. Twenty four hours later, cells were stained with crystal violet staining solution (Beyotime, Nantong, China) for 15 min, then counted and photographed under 40 × magnification (five views per well). Migrated cells were counted by using Image-pro Plus 6.0 while cell numbers of normal control group was normalized to 1. All experiments were performed in triplicate independently.

Table 1
Clinical features of study subjects.

Variable	HSCR n = 79	Control n = 79	*P*
Age, (months, mean, SE)	3.99 (0.33)	3.47 (0.13)	0.26[a]
Sex (%)			
Male	61 (77.2)	55 (69.6)	0.27[b]
Female	18 (22.8)	24 (30.4)	
Weight (kg, mean, SE)	5.2 (0.08)	4.9 (0.16)	0.33[a]

[a] Student t-test.
[b] Two-sided χ^2 test.

2.9. Statistical analysis

STATA 9.2 and Graph Pad prism software were used for data analysis. Data of tissue samples are presented as box plot of the median and range of log-transformed relative expression level by Mann–Whitney U test., while the data of cell samples are presented as mean ± SEM for three times every test, that are analyzed by double-sided Student's t-test. Pearson correlation analysis were used to analyze the relationship of expression level of tissues between case and control groups. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Patients' clinical information

A total of 158 tissue samples were used in this study, including 79 HSCR and 79 control tissues. The clinical information, including age, body weight and gender, were shown in Table 1. While the result of age, the body weight had no difference statistically, the gender rate (Male/Female) of HSCR and control was 61/18 and 55/24, which matched the gender rate of this disease.

3.2. HOTTIP and HOXA13 are down-regulated in colon tissues of HSCR patients

We found that the mRNA levels of HOXA13 were significantly down-expressed in HSCR tissues as compared to the control samples, while protein levels of randomly picked six samples conformed to the results. In addition, we find that HOTTIP expression levels were also decreased in HSCR samples (Fig. 1A–C). Spearman analyses indicated that HOTTIP and HOXA13 had a high correlation score in both HSCR and control tissues (r: 0.928 and r: 0.902, respectively) (Fig. 1D, E). These results suggest a potentially interrelated role between HOTTIP and HOXA13. To explore whether the expression levels of HOTTIP and HOXA13 were associated with the clinical outcome of HSCR patients, we then examined the clinical phenotype with the help of Chi-squared test. We consider the sample which cycle time (CT) value change less than the median CT value as a HOTTIP/HOXA13 high-regulated one according to the results of quantitative RT-PCR. In these 79 HSCR samples, we screen out 4 patients diagnosed as TCA, since TCA has been reported to have a different pathogenesis with other phenotypes [23]. The result, which was shown in Table 2, revealed that HOTTIP and HOXA13 expression levels show no difference in gender, but had correlation with phenotype of L-HSCR and S-HSCR.

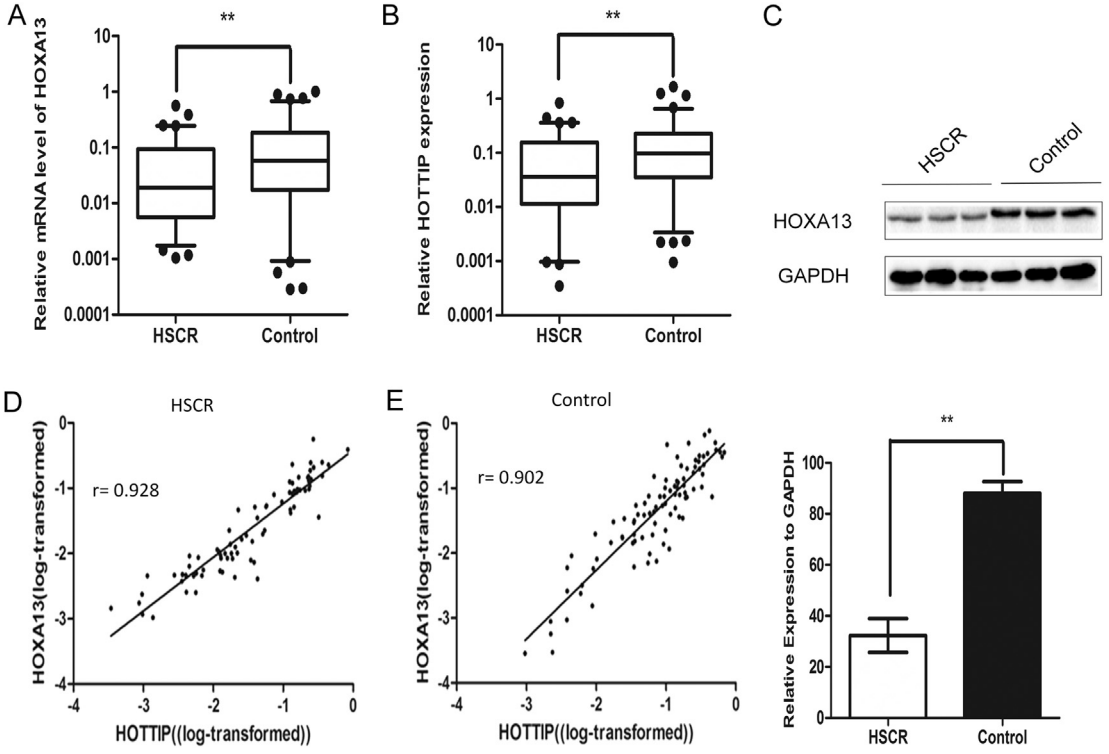

Fig. 1. LncRNA HOTTIP and HOXA13 were down-regulated is HSCR and had a high correlation both in HSCR and control tissues. A, B:The relative expression levels of HOXA13 and HOTTIP in human HSCR tissues (n = 79) and control tissues (n = 79) were evaluated by qRT-PCR. Data were presented as box plot of the median and range of log-transformed relative expression levels. The top and bottom of the box represent the seventy-fifth and twenty-fifth percentile. The whiskers indicate the 10th and 90th points. ** Significantly different compared with that of control ($P < 0.01$). C: The protein expression levels of HOXA13 in human HSCR tissues and controls (3 randomly picked representative samples from both groups are shown) (blow). Quantization of Western-blotting was done by Image J software (above). D, E: The correlations were analyzed between HOTTIP and HOXA13 in HSCR tissues and controls ($P < 0.0001$, $r = 0.928$ and $P < 0.0001$, $r = 0.902$ respectively). Data were analyzed using the Pearson correlation analysis with natural log transformed expression levels.

Table 2
Correlation of characteristics and gene expression levels.

Characteristics	HOTTIP		Chi-square[a]	P-value[a]	HOXA13		Chi-square[a]	P-value[a]
	High	Low			High	Low		
Gender								
Male	28	29	0.226	0.634	29	28	0.576	0.448
Female	10	8			11	7		
Phenotype								
L-HSCR	21	16	4.823	0.026	23	14	5.868	0.015
S-HSCR	12	26			13	25		

L-HSCR: Long-segment HSCR; S-HSCR: Short-segment HSCR.
[a] Two-sided χ^2 test.

3.3. A loop-regulation between HOTTIP and HOXA13 in 293T and SK-N-BE(2) cell lines

To explore the roles of HOTTIP and HOXA13 in HSCR, we silenced HOTTIP expression through siRNAs in 293T and SK-N-BE(2) cell lines. After 48 h of siRNA delivery, HOTTIP expression levels were decreased compared to negative control. Meanwhile, we observed the reduction of HOXA13 levels, which was consistent with its protein expression levels. In consideration of the high relevance between HOXA13 and HOTTIP in tissue samples, we knocked down HOXA13 using siRNAs to further investigate the regulation between the HOTTIP/HOXA13 gene axes in HSCR. Similarly, the result shows that HOXA13 knockdown also decreased HOTTIP mRNA and protein levels in both 293T and SK-N-BE(2) cell lines (Fig. 2A, B). Those results indicate that the interdependently regulated expression of HOTTIP and HOXA13 has a synergistic role in the pathogenicity of HSCR.

3.4. Decreased level of HOTTIP and HOXA13 both can inhibit cell migration and cell proliferation

To investigate the functional roles of HOTTIP and HOXA13, the cell migration, cell proliferation, cell apoptosis and cell cycle were analyzed after transfecting the 293T and SK-N-BE(2) cell lines with siRNA of HOTTIP and HOXA13, a decreased affection was observed in cell migration and proliferation as revealed by the transwell assay and CCK8 assay, respectively. Both the number of migrated cells and the proliferation rate were significantly lower in the cells treated with HOTTIP siRNA or HOXA13 siRNA, suggesting that reduction of HOTTIP/HOXA13 suppress cell migration and cell proliferation (Fig. 3A, B). Additionally, using flow cytometry analysis, we investigated whether HOTTIP/HOXA13 are involved in the apoptosis and cell cycle progression. However, the percentage of apoptosis cells was not statistically different from the cells transfected with HOTTIP/HOXA13 siRNA or the control. Furthermore, there were also no difference in the cell distribution in cell cycle (Supplement Fig. 1A, B).

4. Discussion

HSCR, which is characterized by an absence of enteric neurons in the distal gut, is a congenital digestive tract in newborns [24]. The absence of enteric neurons from variable lengths of the bowel in HSCR results from a failure of neural crest-derived cells to colonize the affected gut regions [25]. HSCR are regarded as a

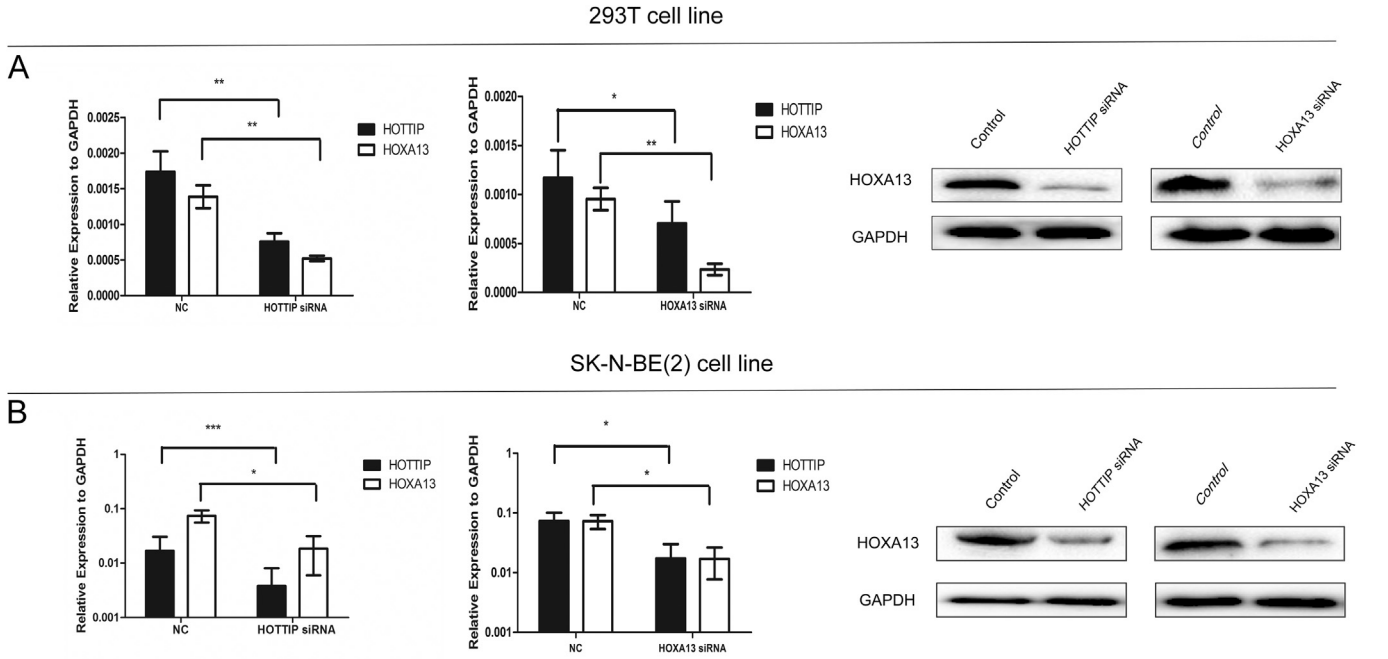

Fig. 2. A loop regulation between HOTTIP and HOXA13 in 293T and SK-N-BE(2) cell lines. A, B: Cells were transfected with HOTTIP or HOXA13 siRNA for 48 h in 293T and SK-N-BE(2) cell line respectively. qRT-PCR was performed to evaluate the mRNA level of HOXA13 and HOTTIP (left two). HOXA13 protein expression levels were analyzed by western-blotting (right one) (*,**,*** indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively; NC means negetive control.).

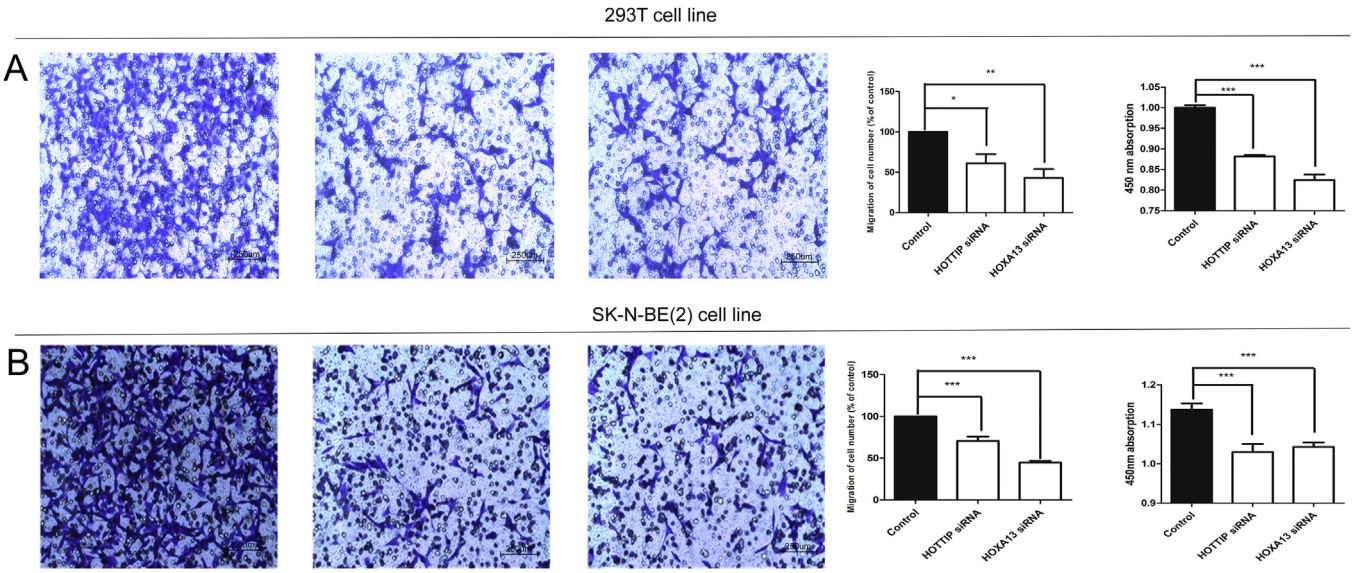

Fig. 3. Cytobiology change after treating cell lines with HOTTIP or HOXA13 siRNA. A, B: Transwell and cck-8 assay was performed as described in Materials and methods. The representative images of invasive cells at the bottom of the membrane stained with crystal violet were visualized as shown (left). The quantifications of cell migration were presented as percentage migrated cell numbers. * indicates significant difference compared with control group ($P < 0.05$). Absorbance at 450 nm was presented with Mean ± SE. * indicates significant difference compared with control group $P < 0.05$ (A, B, right). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

multigenic disorder and a lot of genes, as well as none coding genes, have been reported to be involved in the pathogenesis of HSCR. Except the susceptibility gene RET and other classical genes, hox genes, as a cluster belonging to one of 13 paralog groups (Hox1–Hox13), are found in all animal species and have conserved roles in body patterning [26,27]and involved in ENCC migration.

In HOX genes, HOXA4 transgenic mice develops congenital megacolon [28], while HOXB5 are correlated with the migration and differentiation of NCCs, suggesting a regulatory role of HOXB5 in the development of NCCs [29]. What is more, perturbation of HOXB5 signaling in NCC from the entire neural tube causes apoptosis and neurocristopathies in mice [30]. Besides, HOXA9 and HOXA13 are considered as early and organized pattern of ENS development in the zebrafish model [16]. Hoxa13 expression has also been described in the chick model and is strongly expressed in the endoderm of the hindgut and cloaca through early development of the gut and its overexpression failed to produce a tail or gut phenotype [31]. Thus, we desire to investigate whether or not HOXA13 are involved in HSCR. We found that both mRNA and protein expression levels of HOXA13 were markedly down regulated in HSCR, which indicates that HOXA13 may participate in the pathogenesis of HSCR.

Recently, lncRNAs have emerged as an important player in the biology of numerous diseases, especially in tumor and stem cell differentiation [32–34]. There also have been some lncRNAs reported in the pathogenesis of the central neural system and neuron degeneration diseases [35]. The recently studied lncRNA HOTTIP located in physical contiguity to HOXA13. HOTTIP RNA directly interacts with WDR5 and targets WDR5/MLL complexes, which drives histone H3 lysine 4 trimethylation and regulate gene transcription including HOXA13 [20]. Our next results found that lncRNA HOTTIP was also marked down-regulated and the expression levels of these two genes have a high internal correlation and both can predict HSCR clinical phenotypes independently.

In order to detect how HOTTIP and HOXA13 are participated in the process of HSCR, we conducted experiments in vitro. When we transfected HOTTIP siRNA with human 293T and SK-N-BE(2) cell lines, we found the knockdown of HOXA13 expression. In addition, HOTTIP expression levels were also decreased, when siRNA of HOXA13 was transfected in human 293T and SK-N-BE(2) cell lines. As reported, it seems that a loop regulation may also exists in our cell lines and may be a critical mechanism to maintain HOXA13 expression. Indeed, we admit that the current results can't explain how HOXA13 regulate HOTTIP expression, which needs further experiments in our next study. Of note, knocking down either HOTTIP or HOXA13 results in reduced cell proliferation and migration. Our results highlight that lncRNA, which is used to be considered as transcript noise and are confirmed to participate in the occurrence and development of cancers mostly, could also have vital roles in congenital diseases, just like HSCR.

In conclusion, we find that down-regulation of HOTTIP and HOXA13 was associated with early migration and proliferation in HSCR tissue and cell lines, which may corporately contributes to the development of HSCR.

Author contributions

Conceived and designed the experiments: YX, WT, HX, DMZ; Performed the experiments: HX, DMZ, CX, HRZ, PFC, HXL, LX; Analyzed the data: CX, HRZ, PFC; Wrote the paper: HX, DMZ. All authors discussed the results and commented on the manuscript.

Acknowledgments

This study was supported by Natural Science Foundation of China (NSFC 81370473), Natural Science Foundation of China (NSFC 81400574), Natural Science Foundation of Jiangsu Province of China (BK20131388), and Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.05.096.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.096.

References

[1] T.A. Heanue, V. Pachnis, Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies, Nat. Rev. Neurosci. 8 (2007) 466–479.
[2] M.D. Gershon, Developmental determinants of the independence and complexity of the enteric nervous system, Trends Neurosci. 33 (2010) 446–456.
[3] E. Ruiz Aja, L. Vega Hernandez, N. Martinez Ezquerra, E. De Diego Garcia, A. Perez Marrodan, P.L. Alvarez-Buhilla, Genetic, population and phenotypic characteristics of patients with Hirschsprung disease, Cir. Pediatr. 25 (2012) 135–139.
[4] N.E. Butler Tjaden, P.A. Trainor, The developmental etiology and pathogenesis of Hirschsprung disease, Transl. Res. 162 (2013) 1–15.
[5] W. Tang, J. Tang, J. Qin, Q. Geng, Z. Zhou, B. Li, J. Zhang, H. Chen, Y. Xia, X. Wang, Involvement of down-regulated E2F3 in Hirschsprung's disease, J. Pediatr. Surg. 48 (2013) 813–817.
[6] H. Li, J. Tang, H. Lei, P. Cai, H. Zhu, B. Li, X. Xu, Y. Xia, W. Tang, Decreased MiR-200a/141 suppress cell migration and proliferation by targeting PTEN in Hirschsprung's disease, Cell. Physiol. Biochem. 34 (2014) 543–553.
[7] H. Lei, J. Tang, H. Li, H. Zhang, C. Lu, H. Chen, W. Li, Y. Xia, W. Tang, MiR-195 affects cell migration and cell proliferation by down-regulating DIEXF in Hirschsprung's disease, BMC Gastroenterol. 14 (2014) 123.
[8] W. Tang, J. Qin, J. Tang, H. Zhang, Z. Zhou, B. Li, Q. Geng, W. Wu, Y. Xia, X. Xu, Aberrant reduction of MiR-141 increased CD47/CUL3 in Hirschsprung's disease, Cell. Physiol. Biochem. 32 (2013) 1655–1667.
[9] Z. Zhou, J. Qin, J. Tang, B. Li, Q. Geng, W. Jiang, W. Wu, V. Rehan, W. Tang, X. Xu, Y. Xia, Down-regulation of MeCP2 in Hirschsprung's disease, J. Pediatr. Surg. 48 (2013) 2099–2105.
[10] P. Philippidou, J.S. Dasen, Hox genes: choreographers in neural development, architects of circuit organization, Neuron 80 (2013) 12–34.
[11] R. Krumlauf, Hox genes in vertebrate development, Cell 78 (1994) 191–201.
[12] M. Vieux-Rochas, B. Mascrez, R. Krumlauf, D. Duboule, Combined function of HoxA and HoxB clusters in neural crest cells, Dev. Biol. 382 (2013) 293–301.
[13] T.C. Carter, D.M. Kay, M.L. Browne, A. Liu, P.A. Romitti, D. Kuehn, M.R. Conley, M. Caggana, C.M. Druschel, L.C. Brody, J.L. Mills, Hirschsprung's disease and variants in genes that regulate enteric neural crest cell proliferation, migration and differentiation, J. Hum. Genet. 57 (2012) 485–493.
[14] V.M. Tennyson, M.D. Gershon, P.R. Wade, D.A. Crotty, D.J. Wolgemuth, Fetal development of the enteric nervous system of transgenic mice that overexpress the Hoxa-4 gene, Dev. Dyn. 211 (1998) 269–291.
[15] M.M. Garcia-Barcelo, X. Miao, V.C. Lui, M.T. So, E.S. Ngan, T.Y. Leon, D.K. Lau, T.T. Liu, X. Lao, W. Guo, W.T. Holden, J. Moore, P.K. Tam, Correlation between genetic variations in Hox clusters and Hirschsprung's disease, Ann. Hum. Genet. 71 (2007) 526–536.
[16] R. Doodnath, M. Wride, P. Puri, The spatio-temporal patterning of Hoxa9 and Hoxa13 in the developing zebrafish enteric nervous system, Pediatr. Surg. Int. 28 (2012) 115–121.
[17] A. Fatica, I. Bozzoni, Long non-coding RNAs: new players in cell differentiation and development, Nat. Rev. Genet. 15 (2014) 7–21.
[18] J.T. Lee, Epigenetic regulation by long noncoding RNAs, Science 338 (2012) 1435–1439.
[19] H.W. Brock, J.W. Hodgson, S. Petruk, A. Mazo, Regulatory noncoding RNAs at Hox loci, Biochem. Cell. Biol. 87 (2009) 27–34.
[20] K.C. Wang, Y.W. Yang, B. Liu, A. Sanyal, R. Corces-Zimmerman, Y. Chen, B.R. Lajoie, A. Protacio, R.A. Flynn, R.A. Gupta, J. Wysocka, M. Lei, J. Dekker, J.A. Helms, H.Y. Chang, A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression, Nature 472 (2011) 120–124.
[21] L. Quagliata, M.S. Matter, S. Piscuoglio, L. Arabi, C. Ruiz, A. Procino, M. Kovac, F. Moretti, Z. Makowska, T. Boldanova, J.B. Andersen, M. Hammerle, L. Tornillo, M.H. Heim, S. Diederichs, C. Cillo, L.M. Terracciano, Long noncoding RNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients, Hepatology 59 (2014) 911–923.
[22] D. Kim, J. Song, J. Han, Y. Kim, C.H. Chun, E.J. Jin, Two non-coding RNAs, MicroRNA-101 and HOTTIP contribute cartilage integrity by epigenetic and homeotic regulation of integrin-alpha1, Cell. Signal 25 (2013) 2878–2887.
[23] (!!! INVALID CITATION !!!).
[24] S.J. McKeown, L. Stamp, M.M. Hao, H.M. Young, Hirschsprung disease: a developmental disorder of the enteric nervous system, Wiley Interdiscip. Rev. Dev. Biol. 2 (2013) 113–129.
[25] F.R. Demehri, I.F. Halaweish, A.G. Coran, D.H. Teitelbaum, Hirschsprung-associated enterocolitis: pathogenesis, treatment and prevention, Pediatr. Surg. Int. 29 (2013) 873–881.
[26] M. Maconochie, S. Nonchev, A. Morrison, R. Krumlauf, Paralogous Hox genes: function and regulation, Annu Rev. Genet. 30 (1996) 529–556.
[27] R. Keynes, R. Krumlauf, Hox genes and regionalization of the nervous system, Annu Rev. Neurosci. 17 (1994) 109–132.
[28] V.M. Tennyson, M.D. Gershon, D.L. Sherman, R.R. Behringer, R. Raz, D.A. Crotty, D.J. Wolgemuth, Structural abnormalities associated with congenital megacolon in transgenic mice that overexpress the Hoxa-4 gene, Dev. Dyn. 198 (1993) 28–53.
[29] M. Fu, V.C. Lui, M.H. Sham, A.N. Cheung, P.K. Tam, HOXB5 expression is spatially and temporarily regulated in human embryonic gut during neural crest cell colonization and differentiation of enteric neuroblasts, Dev. Dyn. 228 (2003) 1–10.
[30] M.K. Kam, M.C. Cheung, J.J. Zhu, W.W. Cheng, E.W. Sat, P.K. Tam, V.C. Lui, Perturbation of Hoxb5 signaling in vagal and trunk neural crest cells causes apoptosis and neurocristopathies in mice, Cell. Death Differ. 21 (2014) 278–289.
[31] D.J. Roberts, R.L. Johnson, A.C. Burke, C.E. Nelson, B.A. Morgan, C. Tabin, Sonic hedgehog is an endodermal signal inducing Bmp-4 and Hox genes during induction and regionalization of the chick hindgut, Development 121 (1995) 3163–3174.
[32] B.C. Ellis, P.L. Molloy, L.D. Graham, CRNDE: a long non-coding RNA involved in CanceR, Neurobiology, and DEvelopment, Front. Genet. 3 (2012) 270.
[33] N. Lin, K.Y. Chang, Z. Li, K. Gates, Z.A. Rana, J. Dang, D. Zhang, T. Han, C.S. Yang, T.J. Cunningham, S.R. Head, G. Duester, P.D. Dong, T.M. Rana, An evolutionarily conserved long noncoding RNA TUNA controls pluripotency and neural lineage commitment, Mol. Cell. 53 (2014) 1005–1019.
[34] S.Y. Ng, G.K. Bogu, B.S. Soh, L.W. Stanton, The long noncoding RNA RMST interacts with SOX2 to regulate neurogenesis, Mol. Cell. 51 (2013) 349–359.
[35] P. Wu, X. Zuo, H. Deng, X. Liu, L. Liu, A. Ji, Roles of long noncoding RNAs in brain development, functional diversification and neurodegenerative diseases, Brain Res. Bull. 97 (2013) 69–80.