Estrogen Receptor β Promotes Renal Cell Carcinoma Progression via Regulating LncRNA HOTAIR-miR-138/200c/204/217 Associated CeRNA Network

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Abstract

Recent studies indicated that the estrogen receptor beta (ERβ) could affect the progression of prostate and bladder tumors, however, its roles in the renal cell carcinoma (RCC), remain to be elucidated. Here we provide clinical evidence that ERβ expression is correlated in a negative manner with the overall survival/disease-free survival in RCC patients. Mechanism dissection revealed that targeting ERβ via silencing with ERβ-shRNA and stimulating the transactivation of ERβ with 17β-estradiol or environmental endocrine disrupting chemicals, all resulted in altering the long non-coding RNA (lncRNA) HOTAIR expression. The ERβ-modulated HOTAIR is able to function via antagonizing several microRNAs, including miR-138, miR-200c, miR-204, or miR-217 to impact various oncogenes, including ADAM9, CCND2, EZH2, VEGFA, VIM, ZEB1, and ZEB2, to promote RCC proliferation and invasion. Together, the identification of the ERβ-HOTAIR axis may provide us new biomarkers and/or therapeutic targets to better suppress RCC progression in the future.

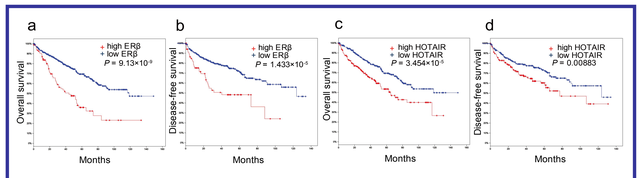

Figure 1. High expression levels of ERβ mRNA and lncRNA HOTAIR were associated with poor prognosis in RCC patients. (a-d) Survival curve based on TCGA database showed RCC patients with high levels of ERβ had significantly shorter (a) Overall survival (OS) and (b) disease-free survival (DFS), and those with high levels of HOTAIR also had significantly shorter (c) OS and (d) DFS.

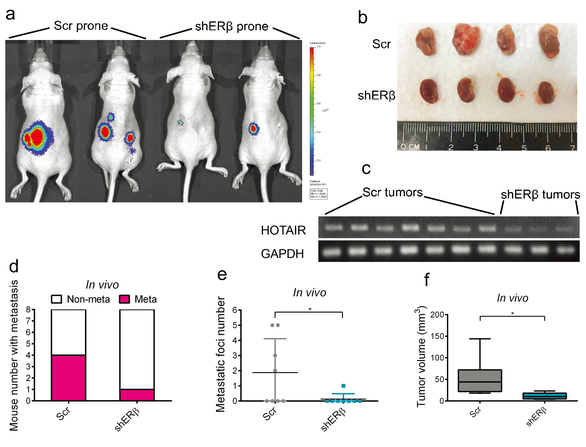

Figure 2. In vivo experiment showed targeting ERβ strongly suppressed RCC growth/invasion. (a) Representative IVIS images of nude mice after implanting 786-O/Scr and 786-O/shERβ into nude mice for 8 weeks. (b) Representative orthotopic tumor xenografts from both groups. (c) Agarose gel of PCR products using HOTAIR/GAPDH primer and xenografted RCC RNA samples. (d) Number comparison of mice with Metastasis (Meta) vs. no Metastasis (Non-meta) between mice xenografted with Scr and shERβ RCC tumors. (e) Metastastic foci number per mouse comparison between 786-O/Scr and 786-O/shERβ xenografted mice. (f) Tumor size comparison between 786-O/scr and 786-O/shERβ RCC xenografts. For e and f , data are mean ± SD, * P< 0.05.

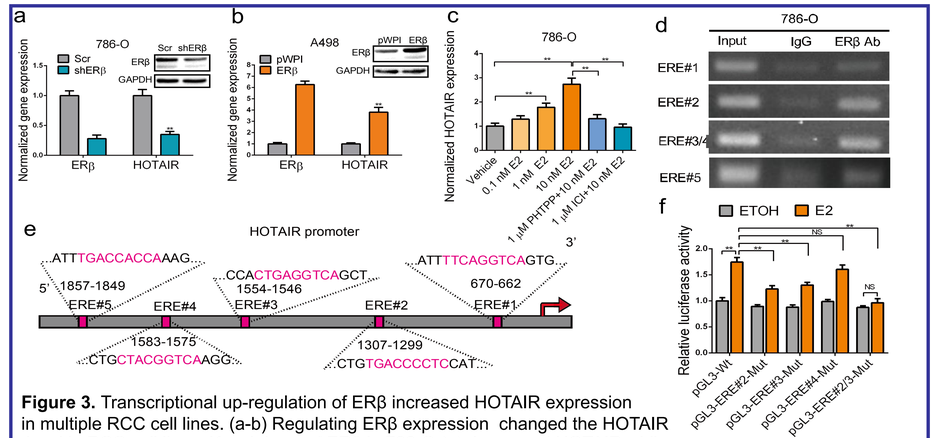

Figure 3. Transcriptional up-regulation of ERβ increased HOTAIR expression in multiple RCC cell lines. (a-b) Regulating ERβ expression changed the HOTAIR level in RCC cell lines. Knockdown of ERβ in 786-O (a) decreased HOTAIR while overexpression of ERβ in A498 (b) increased HOTAIR.(c) HOTAIR expression level after treating with different doses of E2 treatment with/without anti-estrogens in 786-O. (d) DNA agarose gel electrophoresis of PCR products from ChIP precipitated DNA and different ERE primers. (e) Five putative EREs were predicted within the 2 Kb region of HOTAIR promoter. (f) Luciferase assay using wild-type (Wt) or mutant (Mut) pGL3 constructs, with or without 10 nM E2 treatment. ** P< 0.01. Scale bar: 10 μm.

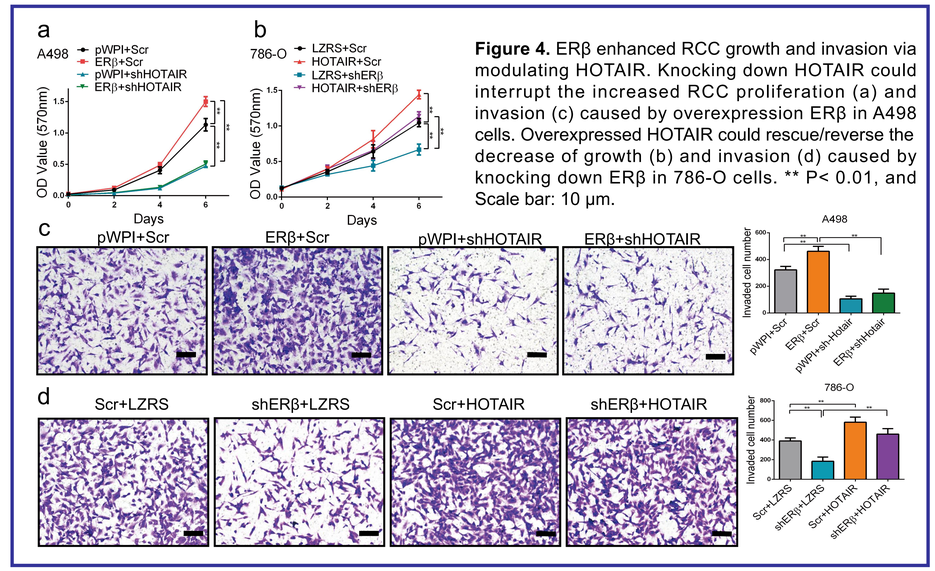

Figure 4. ERβ enhanced RCC growth and invasion via modulating HOTAIR. Knocking down HOTAIR could interrupt the increased RCC proliferation (a) and invasion (c) caused by overexpression ERβ in A498 cells. Overexpressed HOTAIR could rescue/reverse the decrease of growth (b) and invasion (d) caused by knocking down ERβ in 786-O cells. ** P< 0.01, and Scale bar: 10 μm.

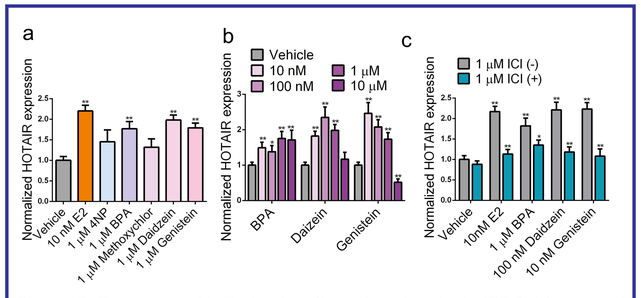

Figure 5. Treatment with Endocrine disrupting chemicals (EDCs) increased HOTAIR in RCC cell. (a) The qPCR of HOTAIR levels in 786-O cells treated with 1 μM 4-nonylphenol (4NP), bisphenol A (BPA), methoxychlor, daidzain, and genistein, or with 10 nM E2 for 24 h. (b) BPA, daidzein, genistein exhibited differential dose-effect on HOTAIR expression. (c) The qPCR assay showed that the anti-estrogen ICI182780 treatment could suppress 10 nM E2 or optimal-concentrations of EDCs (1 μM for BPA, 100 nM for daidzein and 10 nM for genistein) induced HOTAIR expression. Data are mean ± SD, ** P< 0.01

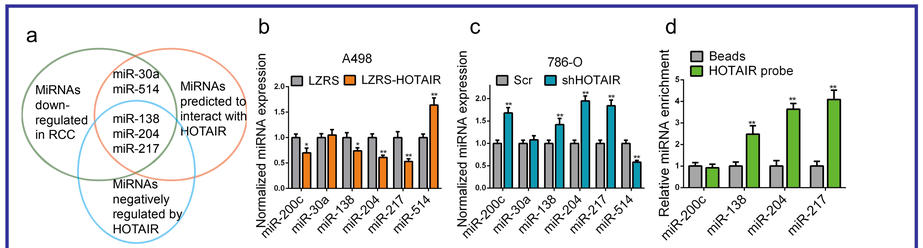

Figure 6. ERβ-HOTAIR interacts with and regulates multiple tumor suppressor miRNAs in RCC cells. (a) Diagram showing the approach of narrowing down miRNA candidates that may interact with HOTAIR in RCC. (b) qPCR assay found oeHOTAIR in A498 decreased miR-200c/138/204/ 217. (c) qPCR assay found shHOTAIR in 786-O cells increased miR-200c/138/204/217. (d) MiR-138/204/217, but not miR-200c, were enriched in biotinylated HOTAIR anti-sense oligo pull-down product. Data are mean ± SD, ** P< 0.01.

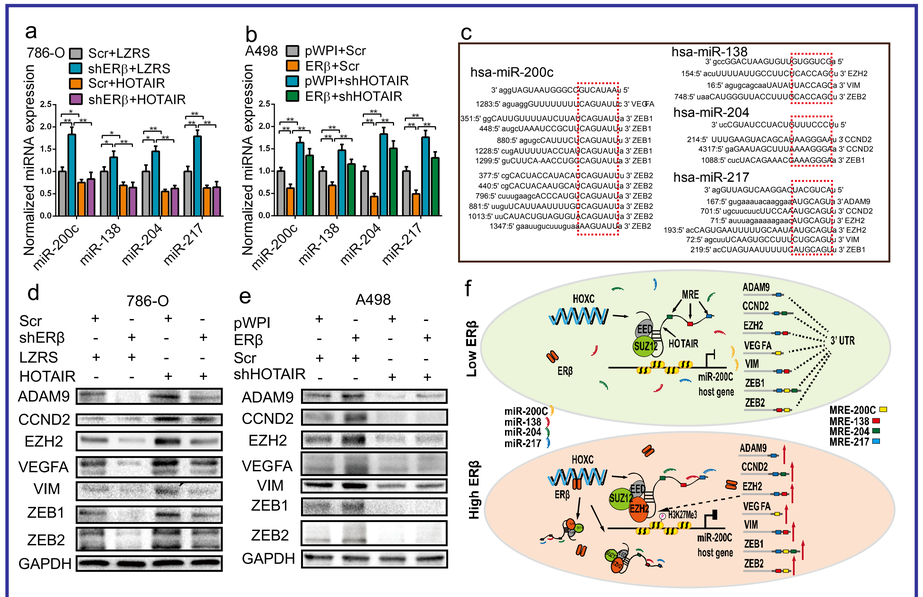

Figure 7. ERβ/HOTAIR-modulated ceRNA system regulates multiple oncogenes in RCC cells. (a) The oeHOTAIR interrupted the ERβ knockdown-mediated increase of miR-200c/138/204/217. (b) The shHOTAIR reversed the ERβ-mediated decrease of miRNAs. (c) Sequences of the four tumor suppressor miRNAs with respective confirmed/putative miRNA targeting site(s) in the 3' UTR sequences. (d,e) Western blot demonstrated that HOTAIR could rescue those oncogene protein levels change induced by ERβ in 786-O cells and A498 cells. (f) Diagram illustrating the entire ERβ/HOTAIR mediated oncogene signaling network in RCC. Data are mean ± SD, ** P< 0.01.

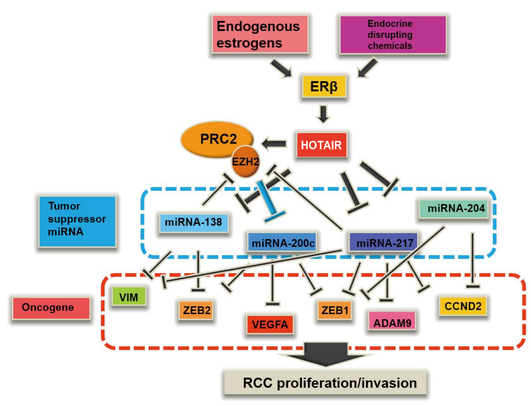

Figure 8. Detailed mechanistic diagram illustrates the impact of ERβ in RCC. ERβ, activated by endogenous estrogens or EDCs, promotes RCC progression though transcriptionally up-regulating lncRNA HOTAIR and shifting the entire associated ceRNA network.

Summary

1. **High expression levels of ERβ mRNA and lncRNA HOTAIR were associated with poor prognosis in renal cell carcinoma patients.**
2. **HOTAIR regulates miR-138/-204/-217 by directly antagonizing and miR-200c through enhancing the epigenetic modulation effect of PRC2.**
3. **ERβ-HOTAIR-miRNAs may function via post-transcriptional regulation of a complicated ceRNA network involving multiple crucial oncogenes to influence the RCC progression.**
4. **EDCs may increase HOTAIR when in their physiological reachable concentrations, and adequate protections from environmental exposure is warranted for either early or late stage RCC patients.**