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Retinoic acid-induced HOXA5 expression is co-regulated by HuR and miR-130a

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ABSTRACT

Retinoic acid (RA) has been used as a chemopreventive agent for breast cancer. It has been shown that HOXA5 is a critical mediator of RA-induced cell growth inhibition. However, the molecular mechanisms underlying RA-induced HOXA5 expression remain largely unknown. Here we report that in addition to transcriptional regulation, post-transcriptional regulation also contributes to RA-induced HOXA5 expression. miR-130a, a c-Myc responsive miRNA, represses HOXA5 cellular levels under unstressed condition. Upon RA treatment, c-Myc is quickly degraded via the proteasome-dependent pathway. This in turn decreases miR-130a levels and de-represses the translation of HOXA5. We also show that the de-repression of HOXA5 translation is dependent on the RNA-binding protein Human antigen R (HuR), which binds to 3′UTR of HOXA5 mRNA and increases its stability in response to RA treatment. Collectively, these results demonstrate that HuR and miR-130a dynamically regulate HOXA5 gene expression via modulating HOXA5 mRNA turnover and translation, respectively, thereby contributing to RA-induced growth inhibition.

1. Introduction

Unlike prokaryotes, whose gene expression is mainly regulated at the transcription level, eukaryotes regulate gene expression not only through the transcriptional modification, but also via the post-transcriptional mechanisms. It has been well recognized that RNA-binding proteins (RBPs) and microRNAs (miRNAs) are two major factors involved in the post-transcriptional regulation of gene expression. miRNAs are 20- to 22-nt-long regulatory RNAs expressed in both plants and metazoan animals [1]. It has been estimated that approximately 30% of all human genes are regulated by miRNAs [2]. In principle, miRNAs base-pair with their target mRNAs, promote mRNA degradation and/or inhibit mRNA translation, thereby regulating gene expression post-transcriptionally. Over the past decade, the function of miRNAs has been intensely investigated. It has been shown that miRNAs play critical roles in the regulation of a variety of cellular functions as well as many disease processes [3,4]. Similar to miRNAs, RBPs are solidly established regulators of mRNA stability and translation in response to environmental changes in metazoans. By binding to the specific RNA sequences in the target mRNAs, RBPs can modulate mRNA turnover and translation [5]. Given the critical roles of RBPs in the post-transcriptional regulation of gene expression, a great deal of work has been done with RBPs [6–8]. It is not surprising that RBPs exert profound effects on cellular adhesion and invasion during cancer progression. It is well accepted that both miRNAs and RBPs have the opportunity to regulate the common target mRNA, yet existence of the dynamic regulation of mRNA orchestrated by miRNAs and RBPs remains to be determined.

Retinoic acid (RA), the natural or synthetic derivative of vitamin A, participates in the regulation of a vast spectrum of biological processes such as cell growth, differentiation, apoptosis, and morphogenesis [9,10]. RA has been increasingly used for the treatment of a variety of cancers, underscoring the important role of RA in the anti-cancer therapy. Because of this, understanding the molecular mechanisms by which RA induces cytotoxicity of tumor cells has attracted increasing attentions recently. HOXA5 has been well recognized as a critical mediator of RA-induced growth inhibition. It has been shown that HOXA5 is strongly up-regulated following RA treatment [11]. Here, we set out to investigate the molecular mechanisms underlying the RA-induced HOXA5 expression and the subsequent cell death. Our findings reveal that both miR-130a and HuR are involved in the up-regulation of HOXA5 in response to RA treatment. Also, miR-130a- and HuR-mediated HOXA5 regulation is functionally important for RA-induced cell death. Our data highlight the importance of post-transcriptional modulation in the

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regulation of RA-induced HOXA5 expression, and also implicate miR-130a and HuR as potential therapeutic targets for cancer treatment.

2. Materials and methods

2.1. Reagents and antibodies

The following antibodies were used in this study: anti-HOXA5 (Sigma), anti-β-Actin, anti-RARβ and anti-HuR (Santa Cruz), anti-c-Myc and anti-Ago2 (Cell Signaling Technology), mouse IgG1 (M075-3, MBL), and rabbit IgG (Sigma). MG132 was purchased from Calbiochem (La Jolla, CA, USA). 9-Cis-RA, 13-cis-RA, all-trans-RA, α-amanitin and cycloheximide were obtained from Sigma-Aldrich Corporation.

2.2. Oligonucleotides

Mimics and inhibitors of miR-130a were synthesized by Genepharma Company (Shanghai, China). Transfection of miR-130a mimics and inhibitors using lipofectamine 2000 (Invitrogen) was performed according to the manufacturer's instruction. The sequences of the oligonucleotides used in this study are as listed below:

Primers used in qRT-PCR/RT-PCR assays
HOXA5 Fw: 5′AGATCTACCCCTGGATGCGC3′
Rev: 5′CCTTCTCCAGCTCCAGGGTC3′
β-Actin Fw: 5′GACCTGACTGACTACCTCATGAAGAT3′
Rev: 5′ GTCACACTTCATGATGGAGTTGAAGG3′
U6(RT): 5′CGCTTCACGAATTTGCGTGTCAT3′
U6 Fw: 5′GCTTCGGCAGCACATATACTAAAAT3′
U6 Rev: 5′CGCTTCACGAATTTGCGTGTCAT3′
miR-130a (RT): 5′GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACATGCCC3′
miR-130a Fw: 5′GTCAGTGCAATGTTAAAAGGGCAT3′
miR-130a Rev: 5′CAGTGCGTGTCGTGGAGT3′
pri-miR-130a Fw: 5′GGTGGTCTCTGTGCTGGGGGTCAGG3′
pri-miR-130a Rev: 5′ ATGCTGAGGAGGCAGCCAGCGCTGGGTAG3′
c-Myc Fw: 5′ CAGCTGCTTAGACGCTGGATT3′
c-Myc Rev: 5′ GTAGAAATACGGCTGCACCGA3′
GAPDH Fw: 5′ CCTGTTCGACAGTCAGCCG3′
GAPDH Rev: 5′ CGACCAAATCCGTTGACTCC3′.
Synthetic Renilla luciferase gene (hRluc)
hRluc Fw: 5′AGACAAGATCAAGGCCATCGTCCA3′
hRluc Rev: 5′TTTCTCGCCCTCTTCGCTCTTGAT3′.
Synthetic Firefly luciferase gene (hluc)
hluc Fw: CTGCTGAACAGCATGGGCATTTCT
hluc Rev: ATGTGTACATGCTCTGGAAGCCCT
miR-130a mimic sequence: sense 5′CAGUGCAAUGUUAAAAGGGCAU3′ and antisense 5′GCCCUUUUAACAUUGCACUGUU3′
miR-130a inhibitor sequence: 5′AUGCCCUUUUAACAUUGCACUG3′.
Primers used in ChIP assays
miR-130a c-Myc responsive element:
Fw: 5′TCTGTGCTGGGGGTCAGGGGGTTG3′
Rev: 5′CCTCCTCACTCCTTCTCCCAGTCCC3′.
Oligonucleotide sequence of shRNAs:
siAgo2-1: CGGCAAGAAGAGATTAGCAAA
siAgo2-2: CGTCCGTGAATTTGGAATCAT
shc-Myc-1: CAGTTGAAACACAAACTTGAA
shc-Myc-2: CCTGAGACAGATCAGCAACAA
shHuR: GAGGCAATTACCAGTTTCA
shHOXA5: GCCATTATAGCGCCTGTATAA, CCGCAGAAGGAGGATTGAAAT
shRARβ: CTGGGTAAATACACCACGAAT,GCCACCTCTCATTCAAGAAAT.

2.3. Plasmid construction

HuR and HOXA5 cDNAs were amplified by RT-PCR from total RNA purified from HeLa cells. The generated DNA fragments were cloned into p3XFLAG-myc-CMVTM-24 expression vector (Sigma). DNA fragments containing wild-type or mutant c-Myc responsive elements of miR-130a promoter were amplified by PCR, digested using XhoI and HindIII, and then ligated into a linearized pGL3-Basic vector (Promega). The HOXA5 mRNA 3′UTR and the deletion mutants were generated and ligated into psiCHECK-2 vector (Promega). All PCR products were verified by DNA sequencing.

2.4. Real-time RT-PCR and RT-PCR

Total RNA was isolated using TRIzol (Invitrogen). 1 μg of RNA was used to synthesize cDNA using PrimeScript™ RT reagent kit (Takara) according to the manufacturer's instruction. Real-time PCR was performed using SYBR Green real-time PCR analysis (Takara). PCR results, recorded as threshold cycle numbers (Ct), were normalized against an internal control (β-actin). RT-PCR was carried out by using One-Step RNA PCR kit (AMV) (Takara, Tokyo, Japan) according to the supplier's protocol. For miR-130a and U6 RNA level examination, reverse transcription reaction was carried out with the indicated RT-primer (miR-130a-RT, U6-RT) before real-time PCR analysis.

2.5. Luciferase assay

To investigate whether miR-130a is transcriptionally regulated by c-Myc, MCF7 cells expressing either control shRNA or c-Myc shRNA were transfected with the pGL3-based construct containing miR-130a promoter plus Renilla luciferase reporter plasmid. Twenty-four hours later, the reporter activity was measured by using a luciferase assay kit (Promega) and plotted after normalizing with respect to Renilla luciferase activity (mean ± SD).

To determine the effect of HuR on HOXA5 mRNA stability, MCF7 cells expressing either control shRNA or HuR shRNA were transfected with the psiCHECK-2 based construct containing full length or the indicated fragment of HOXA5 3′UTR. Twenty-four hours later, the reporter activity was measured by a luciferase assay kit and plotted after normalizing with respect to Firefly luciferase activity. The data were shown as mean ± SD of three independent experiments.

2.6. RNA interference

To generate lentiviruses expressing RARβ, HuR, c-Myc, HOXA5, or control shRNA, HEK293T cells grown on a 6-cm dish were transfected with 2 μg of RARβ shRNA, HuR shRNA, c-Myc shRNA, HOXA5 shRNA (cloned in PLKO.1) or control vector, 2 μg of pREV, 2 μg of pGag/Pol/PRE, and 1 μg of pVSVG. 24 h after transfection, cells were cultured with DMEM medium containing 20% FBS for another 24 h. The culture medium containing lentivirus particles was cleaned by centrifugation to get rid of the cell debris at 12,000 ×g for 5 min, and used for the target cell infection.

2.7. ChIP

The ChIP assay was performed as previously described [12].

2.8. IP–RT-PCR

Immunoprecipitation–Reverse transcription polymerase chain reaction (IP–RT-PCR) was performed as described previously [13]. Briefly,