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Coordinated regulation of transcription and alternative splicing by the thyroid hormone receptor and its associating coregulators

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ABSTRACT

Emerging evidence has indicated that the transcription and processing of precursor mRNA (pre-mRNA) are functionally coupled to modulate gene expression. In collaboration with coregulators, several steroid hormone receptors have previously been shown to directly affect alternative pre-mRNA splicing coupled to hormone-induced gene transcription; however, the roles of the thyroid hormone receptor (TR) and its coregulators in alternative splicing coordinated with transcription remain unknown. In the present study, we constructed a luciferase reporter and CD44 alternative splicing (AS) minigene driven by a minimal promoter carrying 2 copies of the palindromic thyroid hormone-response element. We then examined whether TR could modulate pre-mRNA processing coupled to triiodothyronine (T3)-induced gene transcription using luciferase reporter and splicing minigene assays in HeLa cells. In the presence of cotransfected TRβ1, T3 increased luciferase activities along with the inclusion of the CD44 variable exons 4 and 5 in a dose- and time-dependent manner. In contrast, cotransfected TRβ1 did not affect the exon-inclusion of the CD44 minigene driven by the cytomegalovirus promoter. T3-induced two-exon inclusion was significantly increased by the cotransfection of the TR-associated protein, 150-kDa, a subunit of the TRAP/Mediator complex that has recently been shown to function as a splicing factor. In contrast, T3-induced two-exon inclusion was significantly decreased by cotransfection of the polypyrimidine tract-binding protein-associated splicing factor, which was previously shown to function as a corepressor of TR. These results demonstrated that liganded TR in cooperation with its associating cofactors could modulate alternative pre-mRNA splicing coupled to gene transcription.

1. Introduction

Gene transcription is orchestrated by the coordinated efforts of ATP-dependent chromatin remodeling, histone modification, transcription initiation, elongation, and termination, and RNA processing [1,2]. Although each of these biochemical reactions is accomplished by diverse protein complexes, communication between transcription factors and RNA splicing factors indicates co-transcriptional RNA splicing, which is performed by one general gene expression machine [1,2]. Alternative pre-mRNA splicing is regulated temporally and spatially and is a major source of protein diversity for higher eukaryotes. More than 90% of genes in humans have been estimated to generate multiple protein isoforms derived from alternative splicing (AS) [3]. Aberrant pre-mRNA splicing caused by mutations in consensus splice regulatory sequences and functional mutations in splicing factors have been reported to play pathogenic roles in various human disorders including endocrine and metabolic diseases, cancers, hematological malignancies, and neurodegenerative diseases [4–6]. Therefore, elucidating the mechanisms underlying pre-mRNA processing in more detail is important for understanding the pathogenesis of human diseases caused by deranged RNA splicing and the development of new treatment strategies.

Nuclear hormone receptors (NRs) are transcription factors that bind hormone response elements located in the responsible genomic regions of target genes and regulate gene transcription in ligand-dependent and -independent manners [7,8]. Previous findings confirmed that NRs dynamically interacted with diverse classes of transcriptional coregulators (TCRs) to regulate target

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gene transcription [9–11]. These TCRs possess intrinsic and associated enzymatic activities and modulate the recruitment of RNA polymerase II (pol II) to the transcription start site mainly by the ATP-dependent remodeling of chromatin structures and epigenetic modification of histone tails [9–11]. Emerging evidence has indicated that several NRs including the peroxisome proliferator-activated receptor (PPAR)γ, estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) may coordinate hormone-induced gene transcription with pre-mRNA processing in collaboration with specific TCRs and/or splicing factors [12–16]. Triiodothyronine (T3) activates the thyroid hormone receptor (TR), which binds to the thyroid hormone-response element (TRE) in both the absence and presence of a ligand [17], and has previously been shown to modulate the AS of beta-amyloid and TRα genes expressed in cultured cells [18,19]. The TR-associated protein, 150-kDa (TRAP150) (also known as TR-associated protein3, THRAP3), was originally isolated as a subunit of the TRAP/Mediator complex, which can be recruited to liganded TR and facilitate the recruitment of pol II to initiate transcription [20], and has recently been shown to play a role in pre-mRNA splicing [21–23]. In addition, the polypyrimidine tract-binding protein (PTB)-associated splicing factor (PSF) (also known as splicing factor proline/glutamine-rich, SFPQ), initially isolated as a protein that interacted with PTB [24] and was recently shown to associate with the DBIRD complex, which integrates AS and pol II transcript elongation [25], has previously been reported to function as a transcriptional corepressor of NRs including TR [26–28]. Taken together, these findings suggest that TR could regulate not only gene transcription, but also pre-mRNA processing in coordination with TRAP150 and/or PSF. However, the co-transcriptional regulation of AS by TR has not yet been examined.

In the present study, we constructed a luciferase reporter vector and CD44 AS minigene driven by the identical minimal promoter carrying the palindromic TRE and examined whether liganded TR could modulate the alternative splicing of CD44 variable exons coupled with gene transcription in collaboration with TRAP150 and/or PSF.

2. Materials and methods

2.1. Cell cultures

HeLa cells were were split 24 h before transfection and cultured in DMEM containing resin–charcoal double stripped 10% fetal bovine serum during the T3 treatment as previously described [29,30].

2.2. Plasmids

The expression vectors of human TRβ1 (pKCR$_2$-hTRβ1), TRAP150 (pSV-SPORT-hTRAP150), and PSF (pCS3+MT-hPSF) were described previously [29–31]. The pGL4.23[*luc2*/minP] vector containing firefly luciferase (*Luc2*) cDNA under the control of a minimal promoter containing a TATAA box was obtained from Promega Corporation (Madison, MI). The DNA fragment containing two copies of palindromic (PAL) TRE (AGGTCATGACCT) was amplified by PCR using a primer pair as described [32] and a 2 × PAL-thymidine kinase luciferase vector [32] as a template. The PCR amplified fragment was ligated into the pGEM-T Easy vector (Promega Corporation) to yield pGEM-T Easy-PAL, and the *Eco*RI digested fragment was then ligated into the *Eco*RI site of pGEM11Zf (Promega Corporation) to yield pGEM11Zf-PAL. The DNA fragment obtained by the *Sac*I digestion of pGEM11Zf-PAL was finally ligated into the *Sac*I site of pGL4.23[*luc2*/minP] (pGL4.23 PAL-Luc). The cytomegalovirus (CMV) promoter-driven CD44 minigene (CMV-CD44) was described previously [13]. The CMV-CD44 minigene contains a genomic DNA fragment including variable exons 4 (v4) and 5 (v5) of the CD44 gene along with their surrounding introns in the intron between exon 1 and exon 2 of the human β globin gene (CD44 minigene cassette) [33]. *Luc2* cDNA in pGL4.23 PAL-Luc or pGL4.23[*luc2*/minP] was excised by *Nco*I and *Xba*I digestion and replaced by the PCR-amplified CD44 minigene cassette to obtain pGL4.23 PAL-CD44 or pGL4.23 minP-CD44, respectively. The proper construction of pGL4.23 PAL-Luc, pGL4.23 PAL-CD44, and pGL4.23 minP-CD44 was verified by nucleotide sequencing.

2.3. Lipofection, RNA isolation, and RT-PCR

Plasmids were transfected into HeLa cells in 60-mm culture dishes using Lipofectamine 2000 reagent (Invitrogen, Life Technology Corporation, Tokyo, Japan). The total amounts of the transfected plasmids were adjusted using empty expression vectors. After incubation with T3 (Sigma Aldrich Japan, Osaka, Japan), total RNA was isolated using Isogen (Nippon Gene Co., Ltd., Tokyo, Japan). AS minigene assays were performed according a previously described protocol [13] with modifications. Briefly, 5 μg of total RNA was treated with RQ1 DNase (Promega Corporation) at 37 °C for 30 min followed by the inactivation of DNase with the addition of the Stop buffer (Promega Corporation) at 65 °C for 10 min. DNase-treated RNA was denatured for 5 min at 65 °C and annealed with an antisense primer complementary to the sequence in exon 2 of the human β globin gene (HBB-AS: 5′-CCATAACAGCATCAGGAGTG-3′). First strand cDNA was synthesized using Superscript III reverse transcriptase (Invitrogen) at 50 °C for 30 min according to the manufacturer's protocol. One microliter of the cDNA sample was subjected to PCR in a total volume of 50 μl using AmpliTaq DNA polymerase (Applied Biosystems by Roche Molecular Systems, Inc., Branchburg, NJ) and a PCR primer pair; sense 5′-ACGTGGATGAAGTTGGTGGT-3′, which was complementary to exon 1 of HBB (HBB-S), and HBB-AS. The PCR conditions used were denaturing at 94 °C for 30 s, annealing at 60 °C for 45 s, and extension at 72 °C for 1 min for 35 cycles. The PCR products were subjected to electrophoresis in agarose gels containing ethidium bromide, and band intensities were quantitated using an image analyzer. The proper amplification of AS products was verified by nucleotide sequencing.

2.4. Luciferase assay

HeLa cells were split into 6-well plates and pGL4.23PAL-Luc along with TR expression vectors or an empty expression vector were transfected using a calcium phosphate precipitation method. The luciferase assay was performed as described [29,30].

2.5. Statistical analysis

Statistical analysis was performed using ANOVA, followed by Turkey's multiple comparison tests. Significance was set at $p < 0.05$.

3. Results

To examine whether TR could modulate transcription-coupled alternative pre-mRNA splicing, we constructed a luciferase reporter vector and CD44 AS minigene driven by the identical minimal promoter carrying PAL-TRE (Fig. 1). Three alternatively spliced RNA products could theoretically be generated at different levels from this CD44 minigene cassette that included two exons (v4 and v5), one exon (v4 or v5), or no exon in the context of the