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Identification and Characterization of Human Genomic Binding Sites for Retinoic Acid Receptor/Retinoid X Receptor Heterodimers

Kenta Hosoda, Masashi Sato, Kazuyuki Yanai*

Department of Biomolecular Science, Faculty of Science, Toho University, Chiba, Japan
Email: *yanai@biomol.sci.toho-u.ac.jp

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Abstract

All-trans retinoic acid (ATRA) triggers a wide range of effects on vertebrate development by regulating cell proliferation, differentiation, and apoptosis. ATRA activates retinoic acid receptors (RARs) which heterodimerize with retinoid X receptors (RXRs). RAR/RXR heterodimers function as ATRA-dependent transcriptional regulators by binding to retinoic acid response elements (RAREs). To identify RAR/RXR heterodimer-binding sites in the human genome, we performed a modified yeast one-hybrid assays and identified 193 RAR/RXR heterodimer-binding fragments in the human genome. The putative target genes included genes involved in development process and cell differentiation. Gel mobility shift assays indicated that 160 putative RAREs could directly interact with the RAR/RXR heterodimer. Moreover, 19 functional regulatory single nucleotide polymorphisms (rSNPs) on the RAR/RXR-binding sequences were identified by analyzing the difference in the DNA-binding affinities. These results provide insights into the molecular mechanisms underlying the physiological and pathological actions of RAR/RXR heterodimers.

Keywords

All-Trans Retinoic Acid, Retinoic Acid Receptor, Retinoid X Receptor, Yeast One-Hybrid System, Polymorphism, Regulatory SNP

1. Introduction

All-trans retinoic acid (ATRA) which is a vitamin A derivative affects physiological processes ranging from

*Corresponding author.

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embryonic development to homeostasis of adult tissues and organs [1]. The actions of ATRA are mediated through the retinoic acid receptors (RARs), which is a member of the nuclear receptor super family. RARs heterodimerize with retinoid X receptors (RXRs) and function as ATRA dependent transcriptional regulators by binding to retinoic acid response elements (RAREs). Their target genes involve cell proliferation, differentiation, apoptosis, and homeostasis [2] [3]. As synthetic retinoic acids have been widely used and are known to have therapeutic effects in cancers and other various types of disease [4] [5], identification of target genes may lead to the development of more specific and effective treatments.

Methods to identify DNA-binding sites for transcription factors such as electrophoretic mobility shift assays (EMSAs), SELEX, DNase I foot printing assays, chromatin immunoprecipitation (ChIP) analysis, and reporter assays can be used to identify transcription factor binding sites, but these processes tend to be laborious and not well matched for screening large numbers of DNA elements [6]. Recently, the dramatic rising of the data for RAR target genes is generated by high-throughput technologies, such as microarrays, genome-wide ChIP analyses, and computational methods. However microarrays could not discriminate between primary and secondary target genes. Furthermore, the results of ChIP analyses actually include indirect transcription factor-DNA interactions [7]. In addition, these approaches have obstacles including high cost, reproducibility, high false positive rates, and cell-specific context. Therefore, it was important to develop alternative methods to assess regulatory regions in the genome.

Recently, we developed a modified yeast one-hybrid system which enables rapid and efficient identification of genomic targets for DNA-binding proteins [8]. Using this system, we reported here functional screening for RAR/RXR heterodimer-binding sites from human genome. As a result, we identified 193 genomic fragments including RAR/RXR heterodimer-binding motifs. At least 160 genomic fragments were confirmed as direct binding sites for the RAR/RXR heterodimer. In addition, we identified 19 functional regulatory single nucleotide polymorphisms (rSNPs) on the identified RAR/RXR-binding sequences.

2. Materials and Methods

2.1. Plasmid Constructions

Human RXR alpha and human RAR alpha was amplified by the polymerase chain reaction (PCR) from Uterus cDNA (PCR Ready-cDNA, Maxim Biotech, Inc., USA) and pituitary Cap site cDNA (Wako Pure Chemical Industries, Ltd., Japan.), respectively. These cDNA fragments were cloned into pGADT7 (CLONTECH, USA) and reamplified by PCR with primers (**Table 1**, RXR-5', RXR-3', RAR-5) to generate the restriction sites for sub-cloning. The Hind III fragment including nuclear localization signal (NLS) and GAL4 activation domain (GAL4 AD) was removed from pGADT7andthe cDNAs was inserted into the plasmid, respectively. The resulting plasmid was named pADH1_RXR and pADH1_RAR. The fragment including NLS and GAL4AD was amplified from pGADT7 by PCR and cloned. The fragment was inserted *Bgl* II sites at N-terminal end of RAR in pADH1_RAR and confirmed by sequencing. Resulting plasmid was named pADH1_NLS_GAL4AD_RAR. Foot-and-mouth disease virus (FMDV) 2A peptide sequence was amplified by annealing synthetic oligonucleotides (**Table 1**, FMDV_2A-F and FMDV_2A-R) and reamplified by PCR with primers (**Table 1**, FMDV_2A-5' and FMDV_2A-3') to generate the restriction sites. Then FMDV 2A fragment and NLS_GAL4AD_RAR was tandemly inserted at C-terminal end of RXR in pADH1_RXR. The resulting plasmid was named pADH1_RXR_2A_NLS_GAL4AD_RAR.pSUR (Gene Bank AB425277) was constructed previously and used as a reporter in the modified yeast one-hybrid system [8]. The positive control of a modified one-hybrid assay were constructed by inserting eight copies of the mouse cellular retinoic acid binding protein II (mCRABPII) RARE (**Table 1**, mCRABP2_F and mCRABP2_R) [9] at the upstream of the SPO13 promoter of pSUR. To generate the restriction sites and Kozak sequence for sub-cloning, N-terminal end side of RXR was amplified by PCR. The resulting PCR product was digested with *Hind* III and *Sma* I. C-terminal end side of RXR was obtainedby digesting with *Sma* I and *Xho*I. Both fragments were inserted into *Hind* III and *Xho*I sites pSP64 Poly (A) (Promega, USA) for *in vitro* transcription/translation. The cDNA for human RAR alpha was also inserted into the *Sal* I and the *Xba* I sites pSP64 Poly (A).

2.2. A Modified Yeast One-Hybrid Assay

The human genomic library for a modified yeast one-hybrid assay was generated as previously described [8].

Table 1. Primer sequences.

Primer name	Sequence (5'→3')
RXRA-5'	aaaAAGCTTACGCGTGCCGCCACCATGGACACC
RXRA-3'	tttAAGCTTTCTAGACTACTCGAGAGTCATTTGGTGCGGCGC
RARA-5'	aaaGTCGACAGATCTgccgccaccATGGCCAGCAACAGCAGC
RARA-3'	tttTCTAGACTACTCGAGCGGGGAGTGGGTGGCCGGG
FMDV_2A-F	aaaAGATCTTAAAATTGTCGCTCCTGTCAAACAAACTCTTAACTTTGATTTACT-CAAACTGGCTG
FMDV_2A-R	aaaTCTAGAGGATCCtttACTAGTTGGACCTGGATTGCTTTCTACATCCCCAGCCAGTTTGAG-TAAATCA
FMDV_2A-5'	aaaGTCGACAAAATTGTCGCTCCTGTCAA
FMDV_2A-3'	tttCTAGAGAATTCCCGCGGCTCGAGACGCGTTGGACCTGGATTGCTTTC
mCRABP2_F	GATCCGAGGCTAGAAGGGCAGAGGTCACAGCAGATCTG
mCRABP2_R	AATTCAGATCTGCTGTGACCTCTGCCCTTCTAGCCTCG
pSRU-K1-F	TCGCGTTGCATTTTTGTTCTACAAAATGAAGCAC
pSRU-K1-R	ACTTCCTTTTTGTCGGCGGCTATTTCTCAATATAC
pSRU-K2-F	TGTTCTACAAAATGAAGCACAG
pSRU-K2-R	CGGCTATTTCTCAATATACTCC
rCRBPII-F	GTCCCACTCTGCTGTCACAGGTCACAGGTCACAGGTCACAGGTCATTTT
rCRBPII-R	AAAATGACCTGTGACCTGTGACCTGTGACCTGTGACAGCAGAGTGGGAC
RARE-F	TAGGGTTCACCGAAAGTTCACTC
RARE-R	GAGTGAACTTTCGGTGAACCCTA
random_F	CGCGTTGTGTGTGTTTTATTCC
random_R	GGAATAAAACACACACAACGCG

The expression vector by placing FMDV 2A peptide sequence between RXR and RAR (pADH1_RXR_2A_NLS-GAL4_RAR) was transformed into the 5FOA-selected yeast cells. The obtained transformants were grown on synthetic complete media lacking leucine, tryptophan, and uracil but containing 25 µg/ml 6-azauracil, 10 µM LG100754 (Wako Pure Chemical Industries, Ltd., Japan.), and 1 µM TTNPB (Funakoshi, Tokyo, Japan) for 3 weeks. Human genomic fragments were recovered from the positive colonies by colony-direct PCR with primers corresponding to the vector sequences (**Table 1**, pSRU-K1-F and pSRU-K1-R). The PCR fragments were directly sequenced with the forward and reverse primers (**Table 1**, pSRU-K2-F and pSRU-K2-R).

2.3. EMSAs

The RARE of rat rat cellular retinol binding protein type II (rCRBPII) were labeled with Cy5 and used as a probe (**Table 1**, rCRBPII-F and rCRBPII-R) [10]. Synthesis of human RAR and RXR proteins was performed with the TNT SP6 High Yield System (Promega, USA). *In vitro* synthesized RAR and RXR proteins were mixed with 500 ng of Calf thymus DNA (Invitrogen, USA) and 0.25 pmol of labeled oligonucleotide at 4˚C. In competition experiments, a 25- and 200-fold molar excess of unlabeled oligonucleotide was added to the reaction mixture. Used competitor sequences were listed in **Table 2** and **Table 3**. The RARE of human RAR beta (**Table 1**, RARE-F and RARE-R) [11] and random sequences (**Table 1**, random_F and random_R) were used as positive and negative controls, respectively. The binding reaction was carried out in the EMSA binding buffer

Table 2. The validated RAR/RXR heterodimer-binding sites in the human genome.

Position	RARE on genome sequence	Motif	Symbol	Distance	EMSA competition %	
					×25	×200
chr1: 4974378-4974396	ctcAGGTCAaAGGTGAgga	DR1	AJAP1	259,282	46 ± 2.2	81.8 ± 2.7
chr1: 12160796-12160815	ttgAGGTCAagAGTTCAaga	DR2	TNFRSF8	37,372	63.8 ± 1.5	95.1 ± 3.1
chr1: 13537350-13537369	acgAGGTCAggAGTTCAaga	DR2	LRRC38	302,882	63.1 ± 1.9	96.8 ± 0.3
chr1: 25551801-25551820	ttgAGGTCAggAGTTCAaga	DR2	SYF2	7202	57.5 ± 1	88.9 ± 1.2
chr1: 28932727-28932746	tctTGAACTccTGACCTcgt	DR2	TAF12	36,867	63.1 ± 1.9	96.8 ± 0.3
chr1: 30925056-30925085	ataTGACCCccgtatcacttaTGACCTctg	DR12	MATN1	271,361	52.3 ± 1.8	92.5 ± 0.3
chr1: 42478144-42478163	ttgAGGTCAagAGTTCAaga	DR2	HIVEP3	−93,658	67.1 ± 0.3	95.2 ± 6
chr1: 45330672-45330691	ttgAGGTCAagAGTTCAaga	DR2	PTCH2	−22,066	67.1 ± 0.3	95.2 ± 6
chr1: 51547811-51547830	tctTGAACTccTGACCTcaa	DR2	C1orf185	−20,085	57.5 ± 1	88.9 ± 1.2
chr1: 77460865-77460884	tagAGGTCAggAGTTCAaga	DR2	ST6GALNAC5	127,689	69.4 ± 0.6	100 ± 0.5
chr1: 84134340-84134359	tctTGAACTccTGACCTcgt	DR2	TTLL7	330,483	63.1 ± 1.9	96.8 ± 0.3
chr1: 144530379-144530398	tggAGGTCAagAGGTCAagg	DR2	PPIAL4A	−166,143	66.5 ± 0.5	106.5 ± 0.9
chr1: 154106408-154106427	ctgAGGTCAggAGTTCAagt	DR2	NUP210L	21,174	66 ± 1.6	92.4 ± 1.1
chr1: 182413124-182413143	tctTGAACTccTGACCTcat	DR2	RGSL1	−6122	54.6 ± 2.2	93.7 ± 0.4
chr1: 194536260-194536279	tctTGAACTttTGACCTcag	DR2	NONE	NONE	65 ± 1.5	97 ± 0.8
chr1: 202259783-202259802	ctgAGGTCAggAGTTCAaga	DR2	UBE2T	51,301	62.3 ± 3.2	98.6 ± 0.3
chr1: 211421924-211421943	acaAGGTCAggAGTTCAaga	DR2	RCOR3	−10,774	53.3 ± 1.5	92.1 ± 0.5
chr1: 246944336-246944358	agaGGGTCAgagatAGGATAatg	DR5	SCCPDH	56,969	16.1 ± 0.6	56.9 ± 2.7
chr2: 20711074-20711099	ttcTGACCTctcacctcTGACCTtat	DR8	RHOB	64,252	58.2 ± 1	94.5 ± 0.4
chr2: 48263266-48263285	ttgAGGTCAaaAGTTCAaga	DR2	FBXO11	−130,344	64 ± 0.2	99.4 ± 1.1
chr2: 49177869-49177888	tctTGACCTctTGACCTttt	DR2	LHCGR	−194,999	76 ± 0.5	108 ± 1.4
chr2: 99943751-99943770	tctTGAACTccTGACCTcaa	DR2	TXNDC9	9099	57.5 ± 1	88.9 ± 1.2
chr2: 99971483-99971501	tctTGAACTcTGACCTcaa	DR1	EIF5B	17,658	64.9 ± 2.1	97.2 ± 0.9
chr2: 102169950-102169969	tctTGAACTccTGACCTcag	DR2	RFX8	−78,795	62.3 ± 3.2	98.6 ± 0.3
chr2: 114517066-114517085	acgAGGTCAagAGTTCAaga	DR2	SLC35F5	−2676	75.6 ± 0.2	109.5 ± 1.1
chr2: 130041252-130041271	ctgAGGTCAggAGTTCAaga	DR2	RAB6C	−695,973	62.3 ± 3.2	98.6 ± 0.3
chr2: 130228881-130228900	ttgAGGTCAggAGTTCAaga	DR2	RAB6C	−508,344	57.5 ± 1	88.9 ± 1.2
chr2: 176862952-176862971	acaAGGTCAagAGTTCAaga	DR2	KIAA1715	4056	65.8 ± 0.2	114.4 ± 7.8
chr2: 205912271-205912289	tttTGACCTcTCACCTctc	DR1	PARD3B	501,764	52.3 ± 0.7	87.7 ± 0.6
chr3: 3949000-3949021	caaTGAACTctggTGACCTggt	DR4	LRRN1	107,890	60.6 ± 2.4	98.3 ± 0.9
chr3: 24598921-24598946	catTGACCTtttttccaAGGTCAatt	ER8	THRB	−62,621	62.6 ± 0.6	96.5 ± 1.2
chr3: 25637996-25638015	acaTGAACCctTGACCCcaa	DR2	TOP2B	67,825	66.9 ± 0.9	97.3 ± 1.3
chr3: 75150440-75150462	cagTGACCTccactTCACCCcag	DR5	FRG2C	−563,030	63.5 ± 1	102.1 ± 1.3
chr3: 94076622-94076641	tctTGAACTccTGACCTcag	DR2	DHFRL1	−294,565	62.3 ± 3.2	98.6 ± 0.3

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chr3: 118151024-118151046	caaTGTCCTaaacaTGACTGaga	DR5	IGSF11	602,641	2.6 ± 3	21.3 ± 0.6
chr3: 121266626-121266645	tctCGAACTccTGACCTcaa	DR2	POLQ	−1783	50.2 ± 0.9	86.9 ± 1
chr3: 145586120-145586139	tctTGAACTccTGACCTcaa	DR2	PLOD2	293,152	57.5 ± 1	88.9 ± 1.2
chr3: 149024261-149024280	ttgAGGTCAggAGTTCAaga	DR2	CP	−84,439	57.5 ± 1	88.9 ± 1.2
chr3: 151488824-151488850	aaaAGGTCAactggtaaaAGGTCAagg	DR9	AADACL2	37,133	64 ± 1.3	101.1 ± 1.4
chr3: 186241436-186241456	ctgTGACCCcagTGACCCcaa	DR3	CRYGS	20,721	70.3 ± 2.4	106.6 ± 0.5
chr4: 49258702-49258721	tctTGAACTccTGACCTcaa	DR2	CWH43	270,447	57.5 ± 1	88.9 ± 1.2
chr4: 74427504-74427523	tctTGAACTccTGACCTcag	DR2	RASSF6	58,826	62.3 ± 3.2	98.6 ± 0.3
chr4: 103004436-103004455	ttgAGGTCAggAGTTCAgga	DR2	SLC39A8	262,209	54.4 ± 1.9	98 ± 4.2
chr4: 112083722-112083741	ccaTGACCCaaACACCTtcc	DR2	PITX2	−520,453	−1.5 ± 0.6	27.8 ± 1.4
chr4: 169802530-169802550	caaAGGTCAtggATGTCAgtg	DR3	CBR4	128,928	42.2 ± 1.7	87.1 ± 1.2
chr5: 16950445-16950464	ttgAGGTCAggAGTTCAaaa	DR2	MYO10	−14,070	66.3 ± 0.3	99.6 ± 1.5
chr5: 21398739-21398756	attAGGTCATGACCTttt	IR0	GUSBP1	−60,841	70 ± 0.8	105.8 ± 1.3
chr5: 23515290-23515309	tcaTGAACTccTGACCTcaa	DR2	PRDM9	7576	66.8 ± 0.9	100.7 ± 0.6
chr5: 69214462-69214481	tctTGAACTttTGACCTcaa	DR2	SERF1B	−106,600	64 ± 0.2	99.4 ± 1.1
chr5: 72858058-72858077	tctTGAACTccTGACCTcaa	DR2	ANKRA2	3443	57.5 ± 1	88.9 ± 1.2
chr5: 73742058-73742077	ttgAGGTCAggAGTTCAaca	DR2	ENC1	195,181	61.8 ± 0.4	106.3 ± 6.5
chr5: 80685562-80685581	tctTGAACTtcTGACCTcag	DR2	ACOT12	4416	51 ± 2.7	88.8 ± 3.2
chr5: 138039418-138039437	ttgAGGTCAggAGTTCAaga	DR2	CTNNA1	−49,679	57.5 ± 1	88.9 ± 1.2
chr5: 143214556-143214575	tctTGAACTccTGACCTagt	DR2	YIPF5	335,712	52.8 ± 1.1	91.9 ± 0.2
chr5: 145898007-145898026	ccgAGGTCAggAGTTCAaga	DR2	GPR151	−2341	63.2 ± 0.6	97.1 ± 0.7
chr5: 166602931-166602950	tctTGAACTccTGACCTcaa	DR2	ODZ2	−108,902	57.5 ± 1	88.9 ± 1.2
chr5: 180015655-180015674	cggTGTCCCcaTGACCTtgt	DR2	SCGB3A1	2822	26.8 ± 0.9	80.1 ± 3.3
chr6: 22196558-22196577	ctgAGGTCAggAGTTCAaga	DR2	PRL	106,514	62.3 ± 3.2	98.6 ± 0.3
chr6: 31915297-31915315	aggTGACCTtTGACCTgta	DR1	SKIV2L	−11,275	72.6 ± 1.4	103.2 ± 0.3
chr6: 38272104-38272123	acgAGGTCAagAGGTCAaga	DR2	BTBD9	335,810	75.8 ± 3.3	104.5 ± 0.7
chr6: 42310079-42310097	ctgGGGTCAaAGGTCAcca	DR1	TRERF1	109,695	72.1 ± 2.9	104.2 ± 0.3
chr6: 42437740-42437759	ggtGGATCAcaAGGTCAgga	DR2	TRERF1	−17,967	21.9 ± 2.3	66.5 ± 2.2
chr6: 93902272-93902291	tctTGAACTccTGACCTcaa	DR2	EPHA7	227,018	57.5 ± 1	88.9 ± 1.2
chr6: 107377360-107377379	atgAGGTCAagAGGTCAaga	DR2	C6orf203	27,963	73 ± 0.6	99.9 ± 2.1
chr6: 127695151-127695170	tctTGAACTccTGACCTcat	DR2	ECHDC1	−31,609	54.6 ± 2.2	93.7 ± 0.4
chr6: 137349337-137349356	ctgAGGTCAggAGTTCAaga	DR2	IL20RA	16951	62.3 ± 3.2	98.6 ± 0.3
chr7: 2263677-2263699	cggAGACCCtacaaTGACCCctc	DR5	MAD1L1	8895	52.3 ± 1.7	100.7 ± 0.8
chr7: 18015916-18015935	tctTGAACTccTGACCTcat	DR2	SNX13	−35,795	54.6 ± 2.2	93.7 ± 0.4

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chr7: 28744762-28744781	acgAGGTCAggAGTTCAaga	DR2	TRIL	253,257	63.1 ± 1.9	96.8 ± 0.3
chr7: 41717021-41717040	ttgAGGTCAggAGTTCAaga	DR2	INHBA	25,675	57.5 ± 1	88.9 ± 1.2
chr7: 56952727-56952746	tcaTGAACTccTGACCTcaa	DR2	ZNF479	254,834	66.8 ± 0.9	100.7 ± 0.6
chr7: 67465473-67465492	ctgAGGTCAagAGTTCAaga	DR2	NONE	NONE	65.7 ± 1.3	98.9 ± 0.9
chr7: 88072665-88072684	gcaTGACCTccTGACCTctg	DR2	STEAP4	−136,447	67.9 ± 1.6	100.2 ± 0.3
chr7: 92803857-92803876	ttaAGGTCAggAGTTCAaga	DR2	HEPACAM2	51,915	63.3 ± 1.5	91.4 ± 1.9
chr7: 152384707-152384726	acaAGGTCAggAGTTCAaga	DR2	XRCC2	−11,467	53.3 ± 1.5	92.1 ± 0.5
chr8: 53147041-53147060	tctTGAACTccTGACCTcat	DR2	ST18	175,388	54.6 ± 2.2	93.7 ± 0.4
chr8: 56752302-56752321	ttgAGGTCAtaAGTTCAaga	DR2	LYN	−40,074	68.1 ± 5.6	102.8 ± 0.8
chr8: 104877729-104877748	tctTGAACTccTGACCTcag	DR2	RIMS2	364,763	62.3 ± 3.2	98.6 ± 0.3
chr8: 104984015-104984034	tctTGAACTccTGACCTcaa	DR2	TM7SF4	−368,029	57.5 ± 1	88.9 ± 1.2
chr8: 141528665-141528684	tctTGAACTccTGACCTcaa	DR2	CHRAC1	7278	57.5 ± 1	88.9 ± 1.2
chr9: 10621119-10621138	tctTGAACTccTGACCTcgt	DR2	PTPRD	−8406	63.1 ± 1.9	96.8 ± 0.3
chr9: 25217250-25217269	ctgAGGTCAagAGTTCAaga	DR2	LOC100506422	−849,413	65.7 ± 1.3	98.9 ± 0.9
chr9: 83921356-83921375	ttgAGGTCAagAGTTCAaga	DR2	TLE1	382,230	67.1 ± 0.3	95.2 ± 6
chr9: 92908690-92908709	tctTGAACTccTGACCTcag	DR2	DIRAS2	496,408	62.3 ± 3.2	98.6 ± 0.3
chr9: 128762284-128762303	ttgAGGTCAggAGTTCAaga	DR2	PBX3	252,677	57.5 ± 1	88.9 ± 1.2
chr10: 101670478-101670496	gatAGGTCAaAGGGCAcag	DR1	DNMBP	99,189	51.3 ± 1.5	93.6 ± 1.8
chr10: 120477590-120477612	tggTGACCCttcttTGACCTtag	DR5	PRLHR	−122,441	67.5 ± 0.5	103 ± 0.7
chr10: 122751598-122751617	atgAGGTCAggAGTTCAaga	DR2	WDR11	140,921	54.6 ± 2.2	93.7 ± 0.4
chr10: 127772676-127772694	ttgTGAACTtTGACCTctg	DR1	FANK1	187,577	67.3 ± 1	104.5 ± 1.2
chr11: 9987853-9987872	tctTGAACTccTGACCTcaa	DR2	SWAP70	302,235	62.3 ± 3.2	98.6 ± 0.3
chr11: 10151333-10151352	ttgAGGCCAcgAGTTCAaga	DR2	SBF2	164,411	37.8 ± 2.1	78.1 ± 0.6
chr11: 73263784-73263803	ttgAGGTCAgaAGTTCAaga	DR2	PLEKHB1	−94,800	58.6 ± 1.3	94.4 ± 0.8
chr11: 84351765-84351784	atgAGGTCAggAGTTCAaga	DR2	DLG2	986,539	54.6 ± 2.2	93.7 ± 0.4
chr11: 102121325-102121344	tccTGACCTcaTGATCTgcc	DR2	BIRC3	−66,846	20.9 ± 1.1	62.9 ± 1.8
chr11: 113168930-113168949	ctgAGGTCAggAGTTCAaga	DR2	TTC12	−16,311	62.3 ± 3.2	98.6 ± 0.3
chr11: 133310404-133310423	ctgAGGTCAagAGTTCAaga	DR2	SPATA19	404,978	65.7 ± 1.3	98.9 ± 0.9
chr12: 1757331-1757350	tggAGGTCAggAGTTCAaga	DR2	WNT5B	18,929	60.7 ± 1.4	93.7 ± 1.2
chr12: 5137518-5137541	actTGACCCcactgtTGACCTctc	DR6	KCNA5	−15,555	71.2 ± 1.1	105.4 ± 0.4
chr12: 13637881-13637900	tctTGAACTccTGACCTcaa	DR2	EMP1	288,289	57.5 ± 1	88.9 ± 1.2
chr12: 53367382-53367401	aagAGGTCAggAGTTCAaga	DR2	KRT18	24,549	59.3 ± 1.6	91.8 ± 0.7
chr12: 111147348-111147367	agaAGGCCAtgGGGTCAaat	DR2	HVCN1	−20,412	56.6 ± 0.7	88.3 ± 0.5
chr12: 122916626-122916645	ctgAGGTCAggAGTTCAaga	DR2	CLIP1	−9520	62.3 ± 3.2	98.6 ± 0.3
chr13: 64476525-64476544	ctgAGGTCGggAGTTCAaga	DR2	NONE	NONE	66.9 ± 2	103 ± 0.8
chr13: 77978240-77978259	actTGAACTccTGACCTcaa	DR2	MYCBP2	−77,073	62.3 ± 0.4	99.4 ± 1.6

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chr14: 70604077-70604096	tctTGAACTccTGACCTcgt	DR2	SLC8A3	51,700	63.1 ± 1.9	96.8 ± 0.3
chr14: 74027852-74027871	ctgAGGTCAaaAGTTCAaga	DR2	HEATR4	−2211	65 ± 1.5	97 ± 0.8
chr14: 85281297-85281316	tctTGAACTctTGACCTttt	DR2	FLRT2	−715,181	68.9 ± 3.1	101.4 ± 1.8
chr14: 98241445-98241464	ctgAGGTCAggAGTTCAaga	DR2	VRK1	977,771	62.3 ± 3.2	98.6 ± 0.3
chr15: 25002465-25002484	ttgAGGTCAggAGTTCAaga	DR2	C15orf2	81,934	57.5 ± 1	88.9 ± 1.2
chr15: 45889090-45889109	tctTGAACTtcTGACCTcag	DR2	PLDN	9683	51 ± 2.7	88.8 ± 3.2
chr15: 63680120-63680140	tggAGGACAgtgTGACCTgga	IR3	CA12	−6055	5.5 ± 1.9	35 ± 1.7
chr15: 74836867-74836889	tgaAGGCCAgagagGGGTCAtgg	DR5	ARID3B	3330	39.3 ± 1.2	84.5 ± 0.5
chr16: 2926654-2926673	ctgAGGTCAagAGTTCAaga	DR2	FLYWCH2	−6532	65.7 ± 1.3	98.9 ± 0.9
chr16: 8744664-8744683	ctgAGGTCAggAGTTCAaga	DR2	ABAT	−23,770	62.3 ± 3.2	98.6 ± 0.3
chr16: 21194228-21194247	ttgGGGTCAggAGTTCAaga	DR2	DNAH3	−23,476	69.3 ± 1.2	97.1 ± 1
chr16: 24396387-24396406	tctTGAACTctTGACCTcag	DR2	CACNG3	129,523	65.7 ± 1.3	98.9 ± 0.9
chr16: 28365690-28365709	tctCCAACTcgTGACCTctt	DR2	NPIPL1	2275	67.1 ± 0.3	95.2 ± 6
chr16: 56152959-56152978	tctTGAACTccTGACCTcaa	DR2	GNAO1	−72,282	57.5 ± 1	88.9 ± 1.2
chr16: 57488795-57488816	gaaAGGTCAgtcgAGGTCAaat	DR4	CIAPIN1	−7437	75.5 ± 1.7	104.1 ± 1.8
chr16: 65859695-65859714	aagAGGTGAagAGGTCAaag	DR2	CDH5	−540,820	67 ± 0.7	102.5 ± 0.2
chr16: 79019910-79019932	ggaGGGGCAtcccaGGGTCAgaa	DR5	MAF	614,701	23.1 ± 1.1	62.8 ± 0.8
chr16: 79567620-79567653	cagTGACCTctccgccacctcccag TGACCTctg	DR16	MAF	66,985	64.5 ± 2.2	97.6 ± 1.4
chr17: 35224339-35224358	ctgAGGTCAggAGTTCAaga	DR2	LHX1	−70,423	62.3 ± 3.2	98.6 ± 0.3
chr17: 40470361-40470380	tctTGAACTccTGACCTcag	DR2	STAT5A	30,806	62.3 ± 3.2	98.6 ± 0.3
chr17: 43654747-43654766	ttgAGGTCAgaAGTTCAaga	DR2	PLEKHM1	−86,611	58.6 ± 1.3	94.4 ± 0.8
chr17: 55839651-55839679	cgaTGACCTtacaaaggctcTCACCTaaa	DR11	MSI2	505,734	27.3 ± 1.7	70.4 ± 1.5
chr17: 60825539-60825558	acgAGGTCAggAGTTCAagc	DR2	10-Mar	60,156	58.1 ± 1.6	92.2 ± 2.7
chr17: 64280560-64280579	ctgGGGTCAggAGTTCAaga	DR2	PRKCA	−18,356	65.1 ± 2.3	96.3 ± 0.8
chr17: 65785502-65785521	tctTGAACTccTGACCTcaa	DR2	BPTF	−36,268	57.5 ± 1	88.9 ± 1.2
chr17: 65810594-65810613	ctgAGGTCAggAGTTCAaga	DR2	BPTF	−11,176	62.3 ± 3.2	98.6 ± 0.3
chr17: 72730765-72730784	ctaAGGTCAggAGTTCAaga	DR2	RAB37	−2581	53.2 ± 1.7	95.3 ± 0.7
chr17: 73619194-73619213	ctgAGGTAGgaGTTGCAgac	DR2	C17orf109	−10,310	62.3 ± 3.2	98.6 ± 0.3
chr18: 8161336-8161361	ctgAGTTCAgatcctaaAGGTCAtag	DR8	RAB12	−448,094	35.1 ± 0.7	78 ± 2.5
chr18: 12322082-12322101	ttgAGGTCAggAGTTCAaga	DR2	TUBB6	13,835	57.5 ± 1	88.9 ± 1.2
chr18: 20703035-20703054	ttgAGGTCAggAGTTCAagg	DR2	CABLES1	−12,682	58.8 ± 0.8	96.2 ± 0.5
chr18: 55414467-55414486	ctaAGGTCAggAGTTCAaga	DR2	ATP8B1	55,850	53.2 ± 1.7	95.3 ± 0.7
chr19: 14416278-14416297	tctTGAACTctTGACCTtag	DR2	CD97	−75,668	65.9 ± 1.6	106.7 ± 1.4
chr19: 21290261-21290280	tctTGAACTccTGACCTcag	DR2	ZNF714	25,318	62.3 ± 3.2	98.6 ± 0.3

Continued

chr19: 33629174-33629193	tctTGAACTccTGACCTcag	DR2	LRP3	−56,415	62.3 ± 3.2	98.6 ± 0.3
chr19: 36456308-36456326	cggAGGTCAgAGTTCGaga	DR1	LRFN3	28,295	45.1 ± 2.8	83.3 ± 1
chr19: 37396556-37396575	tctTGAACTccTGACCCcag	DR2	ZNF829	10,357	65.1 ± 2.3	96.3 ± 0.8
chr19: 45539547-45539566	tctTGAACTtcTGACCTcag	DR2	CLASRP	−2741	51 ± 2.7	88.8 ± 3.2
chr20: 16197032-16197054	gagTCACCTggtcgTGACCTttg	DR5	KIF16B	357,036	66.3 ± 1.2	102.6 ± 0.3
chr20: 24050829-24050848	tctTGAACTccTGACCTcgt	DR2	GGTLC1	−81,423	63.1 ± 1.9	96.8 ± 0.3
chr20: 29578657-29578676	tctTGAACTccTGACCTcag	DR2	DEFB115	−266,800	62.3 ± 3.2	98.6 ± 0.3
chr20: 32549760-32549779	ttgAGGTCAggAGTTCAaga	DR2	RALY	−31,688	57.5 ± 1	88.9 ± 1.2
chr20: 35935942-35935961	ttgAGGTCAggAGTTCAaga	DR2	MANBAL	17,901	57.5 ± 1	88.9 ± 1.2
chr20: 49728437-49728456	tctCGAACTccTGACCTcaa	DR2	KCNG1	−88,772	57.5 ± 1	88.9 ± 1.2
chr21: 11186672-11186691	acaAGGTCAagAGATCAaga	DR2	BAGE	−87,745	57.1 ± 1.8	96.4 ± 0.5
chr21: 32961765-32961784	ctgAGGTCAggAGTTCAaca	DR2	TIAM1	−30,485	64.7 ± 0.7	101.6 ± 2
chr21: 33535792-33535813	ggcAGGTCAacccAGGCCAagg	DR4	MIS18A	115,573	16.1 ± 2.7	49.5 ± 6.3
chr21: 37807490-37807509	ttgAGGTCAggAGTTCAaga	DR2	CLDN14	31,225	57.5 ± 1	88.9 ± 1.2
chr22: 31732573-31732592	ctgAGGTCAggAGTTCAaga	DR2	PATZ1	9666	62.3 ± 3.2	98.6 ± 0.3
chr22: 33943562-33943581	atgAGGTCAagAGTTCAaga	DR2	LARGE	372,844	66.7 ± 1.5	106.1 ± 0.2
chr22: 35512814-35512833	tctTGAACTtcTGAACTcct	DR2	ISX	50,694	57.6 ± 3.4	91.4 ± 0.9
chr22: 37370457-37370476	tctTGATCTccTGACCTcgt	DR2	TST	45,024	42.5 ± 1.7	90.7 ± 0.3
chr22: 45947191-45947210	ctgTGGACTtgTGACCTctc	DR2	FBLN1	48,482	51.5 ± 1.8	90.3 ± 0.2
chr22: 48865816-48865835	tctTGAACTctTGACCTcaa	DR2	FAM19A5	−19,462	67.1 ± 0.3	95.2 ± 6
chrUn_gl000225:106816-106836	acaTAACCTacgTGACCTgtg	DR3	NONE	NONE	35.8 ± 1.5	84.1 ± 0.3
chrY: 13899010-13899029	tctTGAACTccTGACCTcag	DR2	USP9Y	−914,140	62.3 ± 3.2	98.6 ± 0.3

containing 12 mM HEPES (pH 7.9), 60 mM KCl, 4 mM $MgCl_2$, 1 mM EDTA, 12% glycerol, and 0.5% Nonidet P-40. The reaction mixtures were directly loaded onto a 4% nondenaturing polyacrylamide gels made in 0.5xTBE. After electrophoresis was performed at 4˚C, the gels were analyzed by using a bio-imaging analyzer (FLA-7000 FUJIFILM).

2.4. Bioinformatics

To map the obtained sequences on the human genomic assembly (GRCh37), these sequences were analyzed using NCBI's BLAST and were searched for AGGTCA motifs. For the stringency of the search, we allowed up to 2-bp mismatches. For each predicted RAR/RXR binding site, the nearest gene and the distance from the center of the binding site to the transcriptional start site of the gene within 1000 kb was identified with GREAT (http://bejerano.stanford.edu/great/public/html/).

3. Results

3.1. Identification of Human Genomic Binding Sites for the RAR/RXR Heterodimer

To identify RAR/RXR heterodimer—but not RXR homodimer-binding sites by a modified yeast one-hybrid system, the simultaneous expression of two transcription factors in yeast was required. Internal ribosome entry site (IRES) has been widely used for this purpose, but it has a major limitation. Namely, translation efficiency of a gene placed after IRES is much lower than that of a gene located before IRES [12]. The limitation can be over

Table 3. Identified functional rSNPs in RAR/RXR binding sites.

dbSNP ID	Gene (positon)	Sequence	EMSA (×25) Competition %	p-Value
rs191541	HIVEP3	ttgAGGTCAagAGTTCAaga	80.7 ± 2.0	9.00E−05
	(−93658)	ttgAGGTCAagACTTCAaga	21.4 ± 3.1	
rs3781025	FANK1	cagAGGTCAaAGTTCAcaa	77.9 ± 1.6	1.10E−05
	(+187577)	cagAGATCAaAGTTCAcaa	33.1 ± 0.6	
rs6907105	EPHA7	ttgAGGTCAggAGTTCAaga	69.2 ± 1.0	2.50E−04
	(+227018)	ttgAAGTCAggAGTTCAaga	43.3 ± 1.8	
rs12164613	CWH43	ttgAGGTCAggAGTTCAaga	63.5 ± 0.9	5.70E−05
	(+270447)	ttgAGGCCAggAGTTCAaga	38.2 ± 1.1	
rs36129646	unplaced	cacAGGTCAcgtAGGTTAtgt	34.4 ± 1.9	1.70E−03
		cacAGGTCAcgtACGTTAtgt	11.7 ± 2.3	
rs36129646	unplaced	cacAGGTCAcgtAGGTTAtgt	34.4 ± 1.9	2.40E−02
		cacAGGTCAcgtATGTTAtgt	18.3 ± 4.1	
rs72422438	GNAO1	ttgAGGTCAggAGTTCAaga	69.2 ± 1.0	3.70E−05
	(−72282)	ttgAGGTCAggAGTCAAgac	41.5 ± 1.0	
rs112102213	TOP2B	ttgGGGTCAagGGTTCAtgt	74.4 ± 0.5	5.60E−04
	(+67825)	ttgGGGCCAagGGTTCAtgt	43.7 ± 3.0	
rs113359665	SKIV2L	tacAGGTCAaAGGTCAcct	73.4 ± 1.6	4.80E−04
	(−11275)	tacAGGTCAaAGGTCTcct	45.7 ± 2.1	
rs114889780	RALY	ttgAGGTCAggAGTTCAaga	69.2 ± 1.0	1.20E−05
	(−31688)	ttgAGGTCAggATTTCAaga	27.0 ± 1.2	
rs138010912	INHBA	ttgAGGTCAggAGTTCAaga	69.2 ± 1.0	1.20E−02
	(+25675)	ttgATGTCAggAGTTCAaga	42.2 ± 6.1	
rs139841157	SCGB3A1	acaAGGTCAtgGGGACAccg	36.7 ± 2.6	2.80E−04
	(+2822)	acaAGATCAtgGGGACAccg	0.3 ± 1.7	
rs145976536	SCGB3A1	acaAGGTCAtgGGGACAccg	36.7 ± 2.6	3.80E−03
	(+2822)	acaAGGTCTtgGGGACAccg	9.2 ± 3.8	
rs140387758	RAB12	ctgAGTTCAgatcctaaAGGTCAtag	47.1 ± 5.9	7.40E−03
	(−448094)	ctgAGTTCAgatcctaaAGGTTAtag	14.1 ± 2.8	
rs147157947	RAB12	ctgAGTTCAgatcctaaAGGTCAtag	47.1 ± 5.9	4.40E−02
	(−448094)	ctgAGTTCTgatcctaaAGGTCAtag	27.4 ± 3.3	
rs148188266	TM7SF4	ttgAGGTCAggAGTTCAaga	63.5 ± 0.9	1.40E−03
	(−368029)	ttgAGGTTAggAGTTCAaga	45.2 ± 2.1	
rs149916363	TTC12	ctgAGGTCAggAGTTCAaga	68.7 ± 0.3	5.10E−04
	(−16311)	ctgAGATCAggAGTTCAaga	38.9 ± 2.9	
rs191800593	CDH5	aagAGGTGAagAGGTCAaag	63.5 ± 1.7	2.10E−02
	(−540820)	aagTGGTGAagAGGTCAaag	47.9 ± 3.9	
rs192782031	MANBAL	ttgAGGTCAggAGTTCAaga	69.2 ± 1.0	1.90E−05
	(+17901)	ttgAGGTCAggAGTTAAaga	41.8 ± 0.6	

come by a 2A peptide, a "self-cleaving" small peptide, which was identified in the FMDV [13]. As 2A-me-diated cleavage is a universal phenomenon in eukaryotic cells [14] [15], we constructed a single expression vector by placing FMDV 2A segment between RXR and RAR. To minimize the effect of RXR homodimer on the reporter activity, RXR is expressed as the native protein, although RAR is expressed as a fusion to the GAL4 AD (**Figure 1**). To evaluate the function of RAR/RXR heterodimer, yeast cells were transformed with these effectors and the indicated reporters (**Figure 2**). The transformants were grown on synthetic complete media includ-

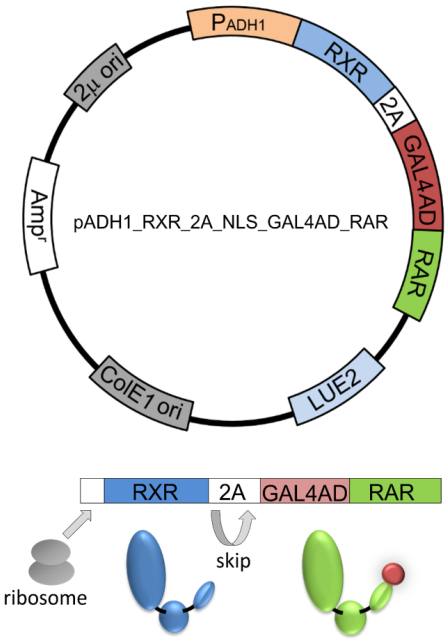

Figure 1. Schematic of bicistronic protein expression from a single expression vector. A bicistronic expression vector contains FMDV 2A peptide sequence (2A) between RXR cDNA and the GAL4 activation domain-RAR fusioncDNA under the control of the ADH1 promoter. The plasmid also contains Col E1 and 2 micro origins for the autonomous replication in *E. coli* and yeast respectively, ampicillin resistance, and a *LEU*2 selectable marker.Schematic of RXR and RAR expression via 2A-mediated translational skip mechanism is presented.

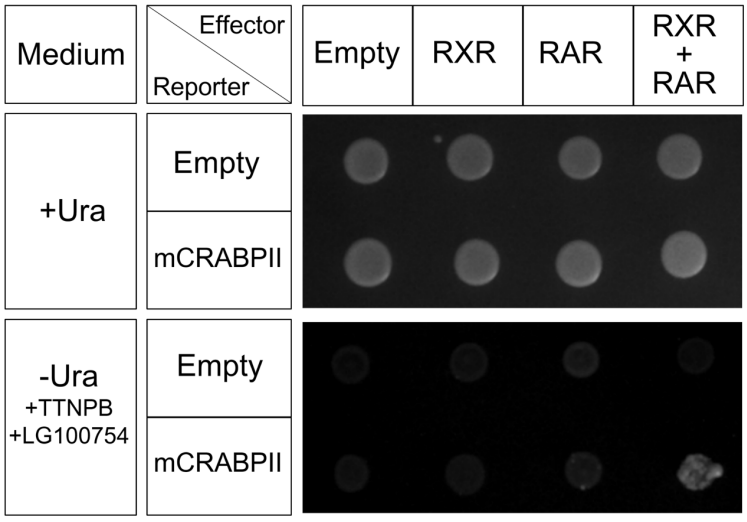

Figure 2. A modified yeast one-hybrid system. Yeast cells transformed with the indicated plasmids. The transformants were grown on synthetic complete media including uracil (*Ura*) or lacking uracil but containing the RAR-specific ligand (*TNPB*) and the antagonist of RXR homodimers (*LG*100754). The plates were photographed after 2 days (+Ura) or 5 days (−Ura + TNPB + LG100754) growth at 30˚C.

ing RAR-specific ligand TTNPB [16] and antagonist of RXR homodimers LG100754 [17] [18]. The transformants expressing either RAR or RXR alone could not grow, whereas the transformants expressing both RAR and RXR could grow in a RARE-dependent manner. These results indicated that RAR/RXR heterodimer could activate the reporter gene via the RARE.

The human genomic library after 5FOA selection [8] was transformed with the human RAR/RXR expression vector (**Figure 1**). More than 1×10^7 of the library was selected for uracil prototroph. Human genomic fragments were recovered from the validated colonies by colony-direct PCR and sequenced. Two hundred and eleven unique sequences were obtained from 364 clones.

3.2. Experimental Validation of the RAR/RXR-Binding Sites

RAREs are typically composed of two directrepeats of a core motif, RGKTCA [19]. The classical RARE is a 5 bp-spaced direct repeat (DR5). Furthermore, the heterodimers also bind to direct repeats separated by 1 bp (DR1) or 2 bp (DR2) [20]. To analyze the obtained genomic sequences, AGGTCA motifs were computationally searched. For the stringency of the search, we allowed up to 2-bp mismatches. As an initial test, we examined direct interaction *in vitro* synthesized RAR/RXR and the known RARE (rCRBPII RARE) (**Figure 3**). Incubation of the the cy5-labeled rCRBPII oligonucleotides with the combination of RAR and RXR retarded complexes, but no shifted band was observed with either receptor alone (**Figure 3**, lanes 2-4). The complexes represented a sequence-specific interaction between the rCRBPII probe and the RAR/RXR heterodimer, since the formation of this complex was specifically reduced with molar excess of unlabeled competitors (**Figure 3**, lanes 5-7). Moreover, the addition of anti-RXR or anti-RAR antibody created a slower-migrating complex (**Figure 3(a)**, lanes 7 and 8). No super shifted bands were observed with anti-HNF4 antibodies (**Figure 3**, lane 9). These results indicated that the sequence-specific binding complex contained both RAR and RXR, presumably as a heterodimer.

As a next step, we examined whether the 193 predicted RARE should interact with RAR/RXR heterodimer. At least 160 predicted RAREs in the obtained genomic sequences could directly interact with RAR/RXR heterodimer (**Table 2**). These RAR/RXR-binding sites were located around or in the genes with various functions,

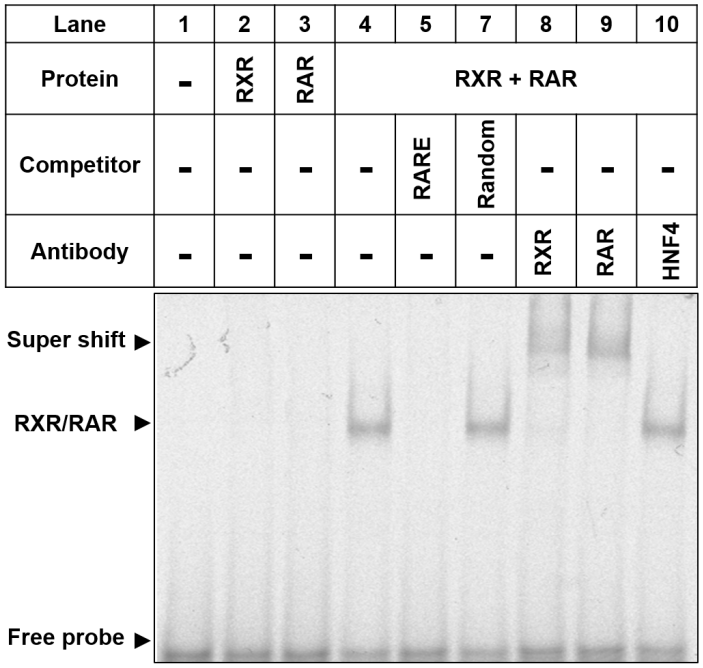

Figure 3. Binding of RAR/RXR heterodimer to RARE by mobility gel shift assays. Cy5-labeled double-stranded rCRBPII RARE was incubated with *in vitro* transcribed/translated human RAR alpha (RAR) and human RXR alpha (RXR). In a competition assay, 100-fold molar excess of the unlabeled oligonucleotides (RARE or Random) were added to the reaction mixture. In a supershift experiment, the indicated antibodies were incubated in the reaction mixture. Binding reactions were resolved by electrophoresis on a 4% acrylamide gel in 0.5 × TBE.

such as cytoskeleton (TUBB6, KIF16B, KRT18 and CTNNA1), extracellular matrix (LARGE), signal transduction (CREB5 and STAT3), transcription (TAF12, POLQ and TRERF1), translation (EIF2B3 and EIF5B) and development (INHBA, ISX, KIAA1715, LHX1, and PBX3). Remarkably, several genes previously known to be regulated by ATRA including FBLN1 [21] and RAR beta [11] [22] were also included. Although the RARE of human RAR betagene was already reported, the exon 9 was a novel binding site. Furthermore, we confirmed that RAR/RXR could directly interact with non-canonical motifs, such as inverted repeats (IRs) and everted repeats (ERs) (**Table 2**).

3.3. Regulatory SNPs in RAR/RXR-Binding Sites

Functional rSNPs in transcription factor-binding sites may predictably lead to differences in gene expression and associate with disease susceptibility. Then, we identify 23SNPs on each RAR/RXR-binding sequence by using the NCBI's SNP database and examined the effect on the RAR/RXR-binding affinity. As a result, 19 functional rSNPs were identified by analyzing the difference in the DNA-binding affinities (**Table 3**).

4. Discussion

We identified 211 of the human genomic fragments containing putative RAREs using the modified yeast one-hybrid. Theses sequences contain canonical RAREs (DR1, DR2, and DR5) but also contain half-site arrangements. At least 160 of putative RAREs in the obtained genomic fragments could directly interact with RAR/RXR heterodimer. Interestingly, some putative RAREs differed from canonical RAREs also directly bound to RAR/RXR heterodimer. Furthermore, 19 functional rSNPs on the RAR/RXR-binding sites were identified by analyzing the difference in DNA-binding affinity.

Most of researches historically focused on promoter regions to find transcription factor-binding sites, but 90% of the identified RAR/RXR-binding sites located in over 10 kb from the transcription start site of genes (**Table 2**). In this study, we could not identify all previously reported canonical RAR/RXR binding sites [11] [23]-[25], although a modified yeast one-hybrid assay is one of the effective methods to identify transcription factor-binding sites as a genome-wide scale. There are some explanations for missed RAR/RXR-DNA interactions. First, the quality of a library will affect the efficiency of identification of protein-DNA interactions. Second, some RAREs may exist adjacent to sequences recognized by yeast transcription factors and may be discarded during the negative selection by 5FOA [8].

RAR/RXR heterodimers contribute to transactivation through widely spaced (up to 150 bp) DR elements [26]. A response element compose of palindromic arrangement of consensus motifs with no spacer nucleotide between the two half-sites (IR0) is known to be bound and activated by RAR/RXR heterodimers [27] [28]. In this study, we confirmed that several types of RAREs including IR0 and DR elements spaced by more than 5 bp directly interact with RAR/RXR heterodimerby EMSA (**Table 2**). These results are consistent with above previous researches.

Genome-scale sequencing has led to the discovery of millions of human SNPs [29]. There are several examples of rSNPs associated with disease susceptibility [30]-[32]. In this study, we identified 19 functionalr SNPs including the 50 kb downstream region of EPHA7 gene. A recent study revealed a strong correlation between expression of EPHA7 and glioblastoma multiforme patient survival [33]. ATRA induces cell differentiation and causes inhibition of cell proliferation in a variety of cancer cell lines including glioblastoma cell lines [34]-[37]. ATRA enhances cytotoxicity of paclitaxel in glioblastoma xenografts and can be therapeutically useful against glioblastoma [38]-[40]. Our results suggest that the direct interaction RAR/RXR with the sequence around EPHA7 gene may possibly affect early stage of neural differentiation and the therapy of glioblastoma. Further studies will be necessary to clarify the relationship between the EPHA7 polymorphism (rs6907105) and the effects of ATRA therapy.

Recently, genome-wide ChIP analyses of RAR/RXR-binding sites were reported using several human cancer cell lines, but such studies are insufficient to get a complete overview of the target genes under its control [41] [42]. As mentioned above, ChIP techniques potentially include indirect transcription factor-DNA interactions [7]. In contrast, our strategy for identification of RAR/RXR sites in human genome took a fundamentally different approach based on the direct interaction between the RAR/RXR heterodimer and human genomic sequences using a yeast genetic selection. Our finding will provide insights into the molecular mechanisms underlying the physiological and pathological actions of RAR/RXR heterodimer.

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