Mechanism of Regulation and Suppression of Melanoma Invasiveness by Novel Retinoic Acid Receptor-γ Target Gene Carbohydrate Sulfotransferase 10

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Abstract

Retinoic acid (RA) induces growth arrest and differentiation of S91 murine melanoma cells and serves as a valuable model for this disease. RA acts through activation of RA receptors (RAR), which are members of the nuclear receptor superfamily of ligand-inducible transcription factors. Interestingly, differentiation is mediated by RARγ, but not by RARα or RARβ, suggesting that RARγ possesses unique and uncharacterized molecular properties. To address this question, DNA microarrays in combination with RAR isoform-specific agonists were employed to identify novel RARγ target genes that may play a role in this process. Here, we identified and validated carbohydrate sulfotransferase 10 (*CHST10*) as a novel RARγ target gene in S91 cells. The RARγ-inducible CHST10 promoter was obtained, and two atypical, independently functioning RA response elements were identified in a 425 bp region. Surprisingly, this fragment is bound by RARγ, but not by RARα or RARβ, thus providing a mechanism for the observed RARγ-specific regulation. CHST10 is a sulfotransferase that forms HNK-1 glycan on neural cell adhesion proteins and glycolipids, and HNK-1 is thought to modulate cell adhesion and possibly metastasis. We show that CHST10 is also regulated by RARγ in a significant subset of human melanoma cells, and three-dimensional cell culture migration assays suggest that CHST10 functions as a suppressor of invasiveness, but not proliferation, in these cells. Induction of CHST10 by RARγ-activating retinoids may present a novel therapeutic strategy to inhibit invasiveness in a subset of melanoma patients. [Cancer Res 2009;69(12):5218–25]

Introduction

All-*trans* retinoic acid (RA) activates RA receptors (RAR), which are members of a large group of ligand-dependent transcription factors called the nuclear receptor superfamily (1, 2). RARs are a particularly interesting receptor subset because RA is not only required for mammalian embryonic development (3) but can also act as powerful differentiation-inducing agent in many tumor cell cultures *in vitro* and some (pre)malignant lesions *in vivo*, making retinoids promising anticancer drugs (4, 5).

There are three RAR isoforms, α, β, and γ, which are encoded by genes on different chromosomes (6). RARs bind with retinoid X receptor, and pending absence or presence of RAR ligand, these heterodimers are complexed with accessory proteins, which serve as corepressors or coactivators, respectively (7). RAR isoforms share high sequence homology with one another, except for the extreme NH_2- and COOH-terminal ends, where their sequences diverge considerably (6). The DNA-binding domain ensures recognition of and binding to RA response elements (RARE), which typically consist of a direct repeat spaced by 2 or 5 nucleotides, although other configurations have been reported (1, 2). Several studies have indicated that differences in activities between the three major RAR isoforms appear relatively minor (6), consistent with extensive mouse knockout experiments that also suggest considerable functional redundancy (8–10).

S91 murine melanoma cells undergo RA-dependent growth arrest and differentiation into a melanocytic cell type and serve as a valuable model for this disease (11). Interestingly, whereas growth arrest and regulation of RARβ expression in these cells is mediated by a functionally redundant mechanism, differentiation is only mediated by RARγ (11). These data suggest that RARγ possesses unique and uncharacterized molecular properties, which enable it to regulate expression of a specific set of genes in these cells. Other models support this interpretation. For instance, overexpression of RARγ causes terminal differentiation of a human embryonal carcinoma cell line (12). Gene knockout experiments in murine F9 embryonal carcinoma cells showed that ablation of *RARγ* results in loss of differentiation potential (13), with concomitant alterations in gene expression of many RA-regulated genes (14, 15), several of which appear to be primary RARγ targets (16). In addition, *RARγ*-null mice, but not *RARα*- or *RARβ*-null mice, display a myeloproliferative syndrome (17), but until now no mechanism has been presented to explain these observations. Dissection of this RARγ-specific signaling mechanism could provide important new insights into RAR-dependent control of tumor cells, which likely also apply to normally developing tissues and organs.

Here, we identified carbohydrate sulfotransferase 10 (CHST10; also called HNK-1 ST or BRGAT1) as a novel RARγ target gene that it is regulated through a mechanism in which two atypical RAREs mediate binding and activation by RARγ but not by other RAR isoforms. This novel mode of regulation also applies to a substantial proportion of human melanoma cells. CHST10 is known to form HNK-1 glycan on neural cell adhesion proteins and glycolipids (18–20). Expression of the HNK-1 epitope is tightly regulated during embryonic development, when the effects of RA are most critical, and CHST10 plays an important physiologic role in synaptic plasticity of the hippocampus (21). We present evidence that CHST10 suppresses invasiveness but not proliferation in melanoma cells. These studies suggest that induction of CHST10 by RA

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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or RARγ agonists may present a novel therapeutic approach to inhibit invasiveness in a significant subset of melanoma patients.

Materials and Methods

Tissue culture and retinoids. Cells were obtained from the American Type Culture Collection or NIH and cultured according to standard conditions. RA was obtained from Sigma. Am580 (4-[(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)carboxamido]benzoic acid), CD2314 [2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)-4-thiophene carboxylic acid], CD666 [(*E*)-4-[1-hydroxy-1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)-2-propenyl]benzoic acid], CD2624 [4-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthylthio)benzoic acid], and CD2366 (7-[3-(1-adamantyl)-4-methoxyphenyl]-3,7-dimethyl-2,4,6-heptatrienoic acid) were provided by Galderma R&D and kept as 30 mmol/L stock solutions in DMSO at −20°C.

DNA microarray and Northern blot analysis. Cells were grown in 15 cm plates until 70% confluent and treated for 8 h with 0.1% DMSO or 1 μmol/L CD666. Total RNA was extracted by Trizol reagent (Life Technologies). RNA expression was analyzed by Genome Systems on Mouse Gem 1 Microarray. Northern blot procedures and preparation of ^{32}P-labeled complementary DNA probes were done as described previously (11).

Quantitative reverse transcription-PCR analysis. RNA (0.1 μg/μL) was used for each reaction. iQ SYBR Green kit was obtained from Bio-Rad, and reverse transcriptase was from Qiagen and used according to the manufacturer's recommendations. Primer sequences were CHST10 forward 5′-ACATGCACCACCAGTGGC and reverse 5′-CTTCCGGCATGGTTGTC and GAPDH (internal control) forward 5′-GAAGGTGAAGGTCGGAGT and reverse 5′-GAAGATGGTGATGGGATTTC-3′. PCR conditions were as follows: 48°C for 30 min and 95°C for 8.5 min followed by 40 cycles of 15 s at 95°C and 1 min at 60°C.

Cloning and characterization of the CHST10 promoter. A previously cloned 2,971-nucleotide cDNA for murine CHST10 was deposited in GenBank (AF360543). Standard PCR-based techniques were used with Platinum Taq High Fidelity (Invitrogen) to clone a 8.1 kb genomic DNA fragment based on the Celera mouse genome sequence database that at the 3′ end overlapped for 40 nucleotides with the 5′ end of the cDNA. This fragment was cloned into pGL3-basic vector (Promega) using both engineered and present *Mlu*I and *Hin*dIII sites. PCR-based techniques were used to clone promoter deletion and mutation constructs.

Transfection, luciferase, and proliferation assay. Transfection and luciferase assays were carried out essentially as described (22, 23) using LipofectAMINE Plus (Life Technologies) according to the manufacturer's protocol. Phosphorothioate-modified oligodeoxynucleotides (Life Technologies) were used that target RARγ1 mRNA around the start codon (24) and transfected as described (22). Small interfering RNA (siRNA; Ambion) were transfected at 100 nmol/L per well per 6-well plate as described (22, 23). For proliferation assays, WST-1 reagent was used according to the manufacturer's instructions (Roche).

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation assays were essentially done as described (22, 23). Briefly, S91 cells were treated with 1 μmol/L retinoid or 0.1% DMSO for 24 h and crosslinked with 1% formaldehyde. Cell pellets were lysed and sonicated and supernatants were diluted in immunoprecipitation buffer. After preclearing with 20 μg sheared salmon sperm DNA, 15 μL mouse IgG, and 50 μL of 50% protein A-agarose beads (Santa Cruz Biotechnology), immunoprecipitation was done overnight at 4°C with 20 μL IgG or anti-RAR antibodies (Santa Cruz Biotechnology). Complexes were recovered by 2 h incubation at 4°C with 50 μL protein A-agarose beads. Precipitation of chromatin complexes, reversal of formaldehyde crosslinking, and purification of DNA fragments were done as described (22, 23). Ten microliters from a 50 μL DNA extraction were used per PCR. The primers for amplifying the CD666-responsive 425 bp CHST10 fragment were forward 5′-AGATCTTAGTTTCTGGCTTTC and reverse 5′-CTATTACGGGTATAAG. PCR conditions were 2 min at 94°C and 37 cycles of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C. The *mRARβ2* promoter primers and PCR conditions have been described (22, 23).

Nuclear runoff assay. S91 cells were grown in 15 cm plates and treated for 16 h with DMSO (control) or 1 μmol/L CD666. Preparation of nuclei, reaction, and hybridization conditions were done as described (25). Nitrocellulose membranes were prepared by spotting 10 μg denatured plasmids and baking in vacuum at 80°C. After hybridization, exposure was detected by autoradiography.

Three-dimensional cell culture migration assay. Cells were grown in 96-well plates using the Three-Dimensional Collagen Cell Culture System according to the manufacturer's protocol (Chemicon) with the suspension method. Briefly, 2,000 cells were mixed in each well with 100 μL premade collagen solution, gelled at 37°C, and then covered with medium and incubated. Migration of clones was checked daily by microscopy and typically counted between 1 and 2 weeks after plating. When required, 1 μmol/L CD666 or 0.1% DMSO was mixed with the collagen solution and the covering medium.

Results

***CHST10* is a novel RARγ target gene.** To identify transcripts that are specifically induced by RARγ, DNA microarrays were used to screen for differentially expressed cDNAs between DMSO (control)-treated cells and cells treated for 8 h with RARγ agonist CD666. One transcript, which encoded CHST10, was induced 3.3-fold by CD666 on the array (data not shown). To validate these results, cells were treated with DMSO or RARα agonist Am580, RARβ agonist CD2314, CD666, or pan-RAR agonist RA. After 8 h, total RNA was isolated and gene expression levels were determined by Northern blot analysis. As shown in Fig. 1A (*a*), CHST10 is strongly induced (6.2-fold) by CD666 but only weakly, if at all, by Am580 and CD2314, suggesting that it represents a novel RARγ target gene. Indeed, induction by CD666 fully accounts for the induction levels obtained with RA. A time course analysis showed that induction of CHST10 becomes rapidly detectable within 4 h of treatment and plateaus ∼16 h (Fig. 1A, *b*), consistent with regulation of a primary target gene.

To determine whether transcriptional or posttranscriptional events are involved in CD666-dependent induction of CHST10, a runoff assay was done (Fig. 1B). CD666 induced expression of CHST10 to about a similar extent as RARβ, showing that CHST10 is transcriptionally induced. CHST10 mRNA half-life remained virtually unchanged by CD666 at ∼3.5 h (Supplementary Fig. S1A and B); thus, transcriptional activation is the critical mechanism. These results were corroborated in the following experiments. Transactivational properties of RARs are enhanced when both RAR and retinoid X receptor are liganded (26–28). To test whether enhancing effects are seen in regulation of CHST10, cells were treated for 8 h with DMSO (control), or increasing concentrations of CD666, in the absence or presence of pan-retinoid X receptor agonist CD2624. Total RNA was extracted and Northern blot analysis was done to determine gene expression levels. As shown in Fig. 1C, in the absence of CD2624, a dose-dependent gene-inducing effect of CD666 is observed reaching peak levels at 1 μmol/L. When CD2624 and CD666 are combined, increased induction of CHST10 is detected at 0.01 and 0.1 μmol/L CD666, leveling off at 1 μmol/L presumably because maximum gene expression has been achieved. Transactivation is mediated by the RARγ moiety of the heterodimer because CD2624 alone has no effect on gene expression.

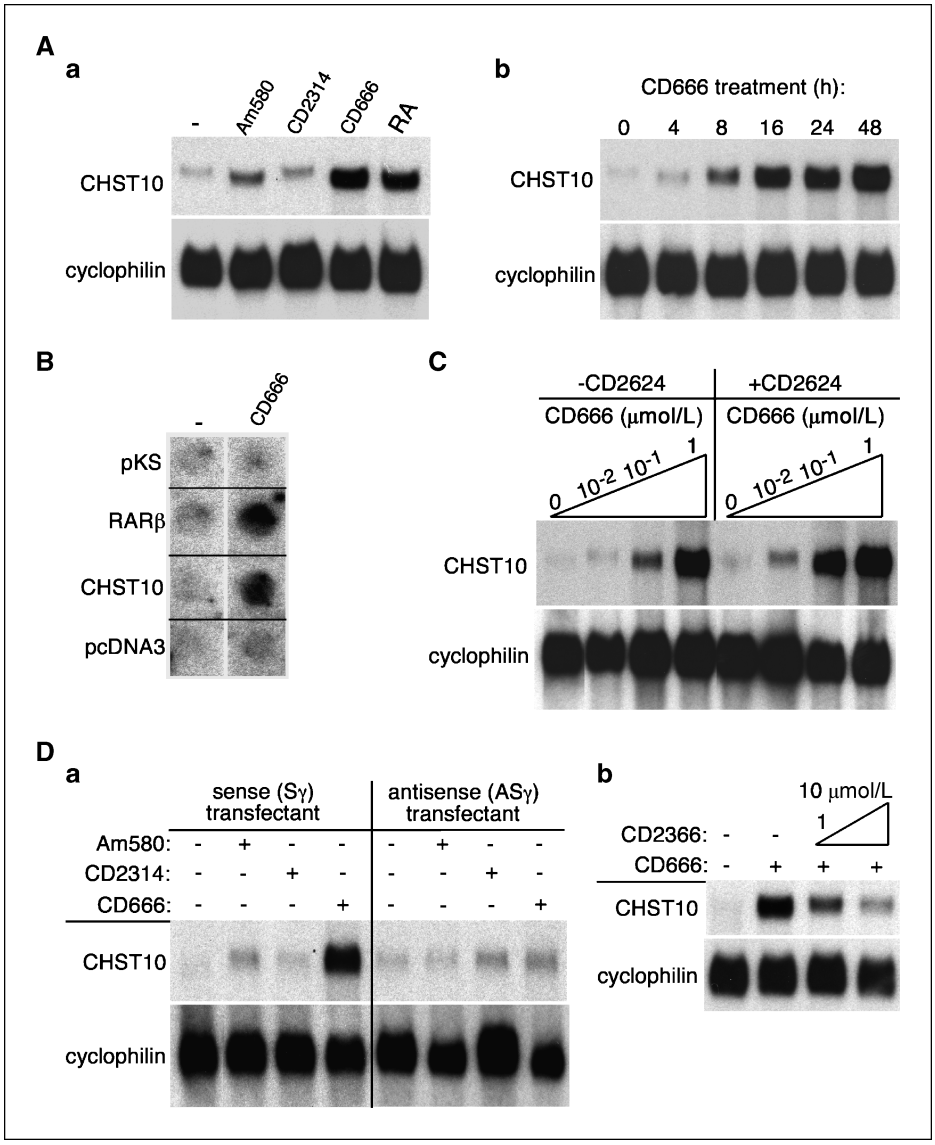

Figure 1. *CHST10* is a novel RARγ target gene in S91 cells. *A,* Northern blot analysis (15 μg RNA/lane) of cells treated for 8 h with the indicated agonists (1 μmol/L) showing that CHST10 is induced to the same extent by CD666 and RA but not or only weakly by Am580 and CD2314 (*a*). Induction by CD666 occurs within 4 h and reaches maximum levels after 16 h (*b*). Cyclophilin serves as loading control. *B,* nuclear runoff assay showing that 1 μmol/L CD666 (16 h treatment) induces transcription of CHST10. RARβ serves as positive controls; plasmids pKS and pcDNA3 serve as negative controls. *C,* Northern blot analysis showing enhanced induction of CHST10 when cells are cotreated (8 h) with indicated doses (μmol/L) of CD666 and 0.1 μmol/L retinoid X receptor agonist CD2624. Cyclophilin serves as loading control. *D,* Northern blot analysis showing that transfection of cells with 5 μmol/L antisense RARγ (ASγ) but not control sense (Sγ) oligodeoxynucleotides 24 h before retinoid treatment (1 μmol/L, 8 h) blocks CD666-dependent induction of CHST10 (*a*). *b,* Northern blot analysis of cells showing dose-dependent repression of 1 μmol/L CD666-mediated induction of CHST10 by CD2366. Cyclophilin serves as loading control.

If this interpretation is correct, then repression of RARγ expression should result in loss of CD666-dependent induction of CHST10. To test this hypothesis, cells were pretreated with sense (Sγ) or antisense (ASγ) RARγ oligodeoxynucleotides that effectively repress RARγ protein expression (24). Next, cells were treated for an additional 8 h with DMSO or CD666, and gene expression was determined by Northern blot analysis. Figure 1D (*a*) shows that CHST10 is again strongly induced by CD666 in Sγ-treated cells, but this effect is virtually absent in ASγ-treated cells. This supports our evidence that *CHST10* is a RARγ target gene, but an alternative mechanism must still be addressed. Unliganded RARγ has been found to act as a repressor under certain conditions (24, 29–31), which could potentially explain the lack of CHST10 induction by Am580 and CD2314. However, these agonists are still incapable of inducing CHST10 expression in RARγ-depleted cells, arguing against a repressor function for RARγ (Fig. 1D, *a*). Finally, to complement the oligodeoxynucleotide experiments, we used RARγ antagonist CD2366 (29) and studied its effects on CD666-dependent regulation of CHST10. As shown in Fig. 1D (*b*), CD2366 blocked CD666-mediated induction in a dose-dependent fashion. These combined results show that retinoid X receptor/RARγ heterodimers specifically regulate expression of CHST10 in S91 cells.

Cloning and characterization of a CD666-inducible CHST10 promoter region. To identify the RARγ-specific mechanism of regulation of CHST10, a 8.1 kb genomic fragment was obtained and cloned into a promoter-less luciferase reporter plasmid. After

transfection into S91 cells, CD666 responsiveness was assessed by determining ratio of luciferase activity in the presence and absence of CD666. As shown in Fig. 2A, the 8.1 kb fragment shows a strong 5-fold induction by CD666 and thus likely represents the major RARγ-regulated region. Subsequent deletion analysis showed that a 425 bp fragment contained all essential functionality. Further analysis of this fragment by smaller deletions (Fig. 2B) showed that induction by CD666 decreases by ~2-fold when sequences between −425 and −394 are deleted, suggesting that this region contains RARE1. Remaining induction is prevented after deletion of region −275 to −267, suggesting that this sequence contains RARE2. Thus, there are two moderately strong independent RAREs that together confer complete responsiveness to CD666. The downstream transcriptional start site has not been established but appears to lack a typical TATA box (data not shown).

Typical RAREs consist of a direct repeat of (A/G)G(T/G)TCA spaced by two (DR2) or five (DR5) nucleotides, but as shown in Fig. 2C, only RARE1 to some extent resembles a DR0 configuration (5′-ACATCA/ATATCA-3′) on the reverse strand. To analyze RARE1, two guanines were mutated to thymines to destroy this RARE1 if used as predicted from canonical RAREs. As shown in Fig. 2B, mutant promoter M-406 lost half of its responsiveness to CD666, confirming that RARE1 is critically involved in RARγ regulation. RARE2 does not resemble a direct repeat, and mutation of the two guanines in RARE2 did not inhibit CD666-dependent induction (data not shown). However, when RARE2 was replaced by an *Eco*RI restriction site in mutant promoter M-275, responsiveness to CD666 was lost. This result was corroborated in double-mutant promoter M-406/M-275, which has no functional RAREs; here, as expected, CD666-dependent induction was completely inhibited. Thus, the CHST10 promoter contains an atypical pair of independently functioning RAREs that together confer strong regulation by RARγ.

RARγ, but not RARα or RARβ, occupies the CHST10 promoter *in vivo*. One possible mechanism that can account for the observed RARγ-specific regulation is a differential ability of the RAR isoforms to bind to the RAREs in the CHST10 promoter. To test this hypothesis, chromatin immunoprecipitation assays were employed. Cells were cultured for 24 h in the presence or absence of CD666, and crosslinked chromatin was immunoprecipitated from the lysates with antibodies against all three RAR isoforms or IgG control. As shown in Fig. 3, RARγ, but not RARα or RARβ, occupies the

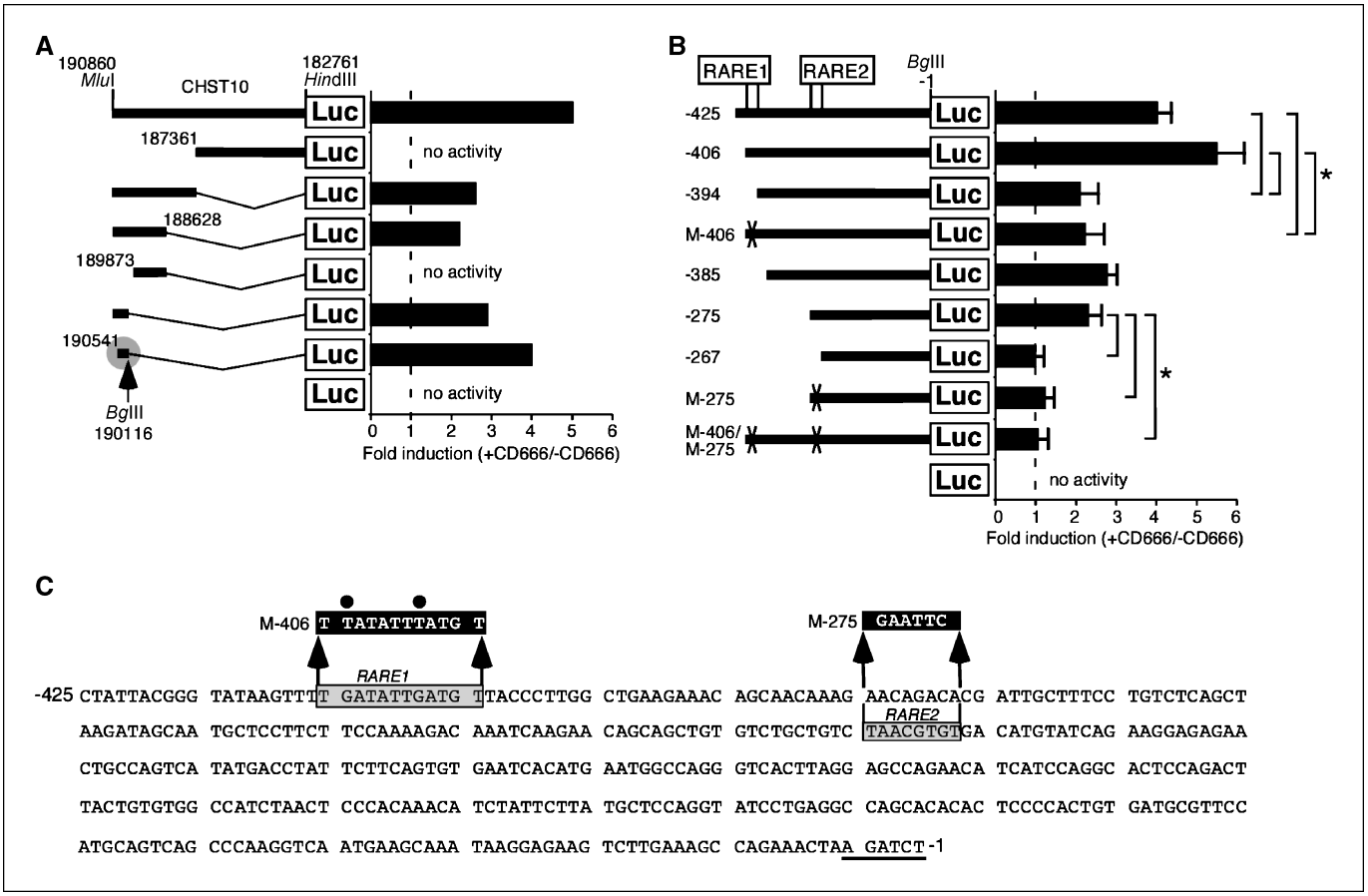

Figure 2. CHST10 promoter analysis showing presence of two atypical RAREs that confer CD666 responsiveness. *A,* promoter analysis showing CD666 responsiveness of an 8.1 kb *CHST10* chromosomal fragment (coordinates correspond to the Celera mouse genome sequence database) and deletion mutants cloned into pGL3-basic vector using indicated restriction sites. Twenty-four hours after transfection, fold induction (+CD666/-CD666) of normalized luciferase activity was calculated. A 425 bp fragment (*gray*) still retained full CD666-responsive promoter activity. *B,* deletion analysis of the 425 bp fragment showing location of RARE1 between −394 and −406 and RARE2 between −275 and −267. RARE1 and RARE2 are mutated in promoter constructs M-406 and M-275, respectively, and both are mutated in M-406/M-275. *, $0.0001 < P < 0.02$ (Student's *t* test analysis). *C,* sequence of the functional 425 bp promoter fragment. *Shaded boxes,* locations of RARE1 and RARE2; *arrows,* sequences of mutated RARE1 in M-406 (two guanines mutated to thymines; *black dots*) and RARE2 in M-275 (sequence replaced by *Eco*RI restriction site). *Bgl*II cloning site is underlined.

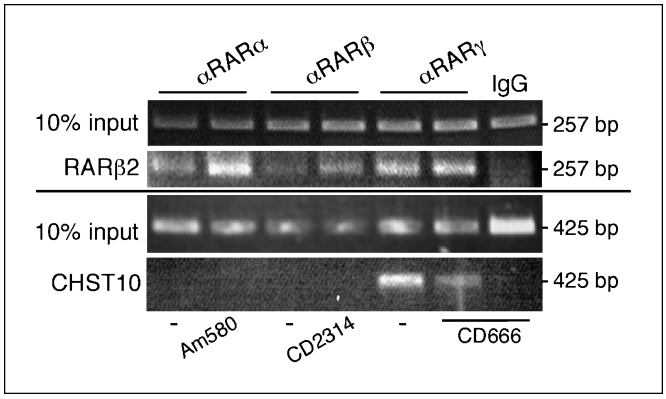

Figure 3. Chromatin immunoprecipitation assay showing that RARγ, but not RARα or RARβ, occupies the *CHST10* promoter *in vivo* in a largely ligand-independent manner. Cells were treated for 24 h with DMSO (control) or 1 μmol/L of indicated ligands. Chromatin was immunoprecipitated by indicated antibodies or IgG control and the 425 bp fragment containing RARE1 and RARE2 was detected by PCR analysis. A similar assay using the redundantly regulated *RARβ2* promoter fragment containing the βRARE shows binding by all RAR isoforms.

CHST10 promoter in a largely CD666-independent manner, although binding was somewhat diminished in the presence of ligand, which was not seen during shorter treatment (Supplementary Fig. S2). In contrast, all RAR isoforms occupy the redundantly regulated *RARβ2* promoter in a ligand-independent manner, consistent with our earlier studies (11, 22). These results provide a mechanism for the observed RARγ-specific regulation of CHST10 and suggest that the promoter architecture of *CHST10* differs dramatically from that of *RARβ2*. Indeed, histone deacetylase inhibitors strongly repress CD666-dependent gene expression of CHST10 (Supplementary Fig. S3), whereas this treatment increases RA-dependent RARβ2 expression (32).

***CHST10* is also a RARγ target gene in a subset of human melanoma cells.** Next, we tested CHST10 expression in response to CD666 in a panel of human melanoma cells by quantitative reverse transcription-PCR analysis. As shown in Fig. 4, 4 of 9 cells (UACC257, UACC62, MMAC, and Malme-3M) showed 2.5- to 6.4-fold induction of CHST10 by CD666 relative to DMSO control-treated cells, whereas one cell line, 501mel, showed repression. Treatment of the four inducible cell lines with Am580 or CD2314 gave no or much less up-regulation of CHST10, suggesting that this gene is also a RARγ-specific target in a subset of human melanoma cells. It remains to be established which factors determine CD666 responsiveness in these cells, but it is not correlated with basal RARγ mRNA levels (data not shown).

CHST10 suppresses melanoma invasiveness. Some reports suggested that the HNK-1 epitope functions in cell-cell and cell-substrate adhesion, which may potentially affect invasive traits of melanoma cells (33–36). To address this question, we manipulated CHST10 levels in human melanoma cells by RNA interference and studied migration in three-dimensional cultures as a model for invasiveness. Repression of basal CHST10 expression in 501mel and UACC257 cells by siRNA transfection resulted in dramatically increased migration compared with control siRNA-transfected cells (Fig. 5A). Treatment of CD666-responsive Malme-3M, UACC62, and UACC257 cells with CD666 significantly inhibited migration, but not proliferation, compared with DMSO control-treated cells (Fig. 5B), suggesting that induction of CHST10 caused this effect. To test this interpretation, UACC62 cells were transfected with siRNA, and 24 h later, cells were treated with DMSO or CD666. As shown in Fig. 5C, repression of basal CHST10 expression in DMSO control-treated cells greatly increased migration, whereas treatment with CD666 again decreased migration of cells relative to control siRNA-transfected cells. Repression of CD666-dependent induction of CHST10 in these cells by siRNA resulted in increased migration, suggesting that the inhibitory effects of CD666 on cell migration were mediated by RARγ-dependent induction of CHST10. Thus, CHST10 functions as a novel invasiveness-suppressing molecule in melanoma.

Discussion

Gene knockout experiments showed that none of the three RAR isoforms are critically required for embryonic development (11–13). They can all fully or partially restore differentiation potential of HL-60R myeloid leukemia cells (37) and *RARγ*$^{-/-}$ F9 embryonal carcinoma cells, respectively (15), suggesting that they are essentially functionally redundant. However, there is compelling evidence for RAR isoform-specific mechanisms of gene regulation. For instance, RARα is likely responsible for RA-induced

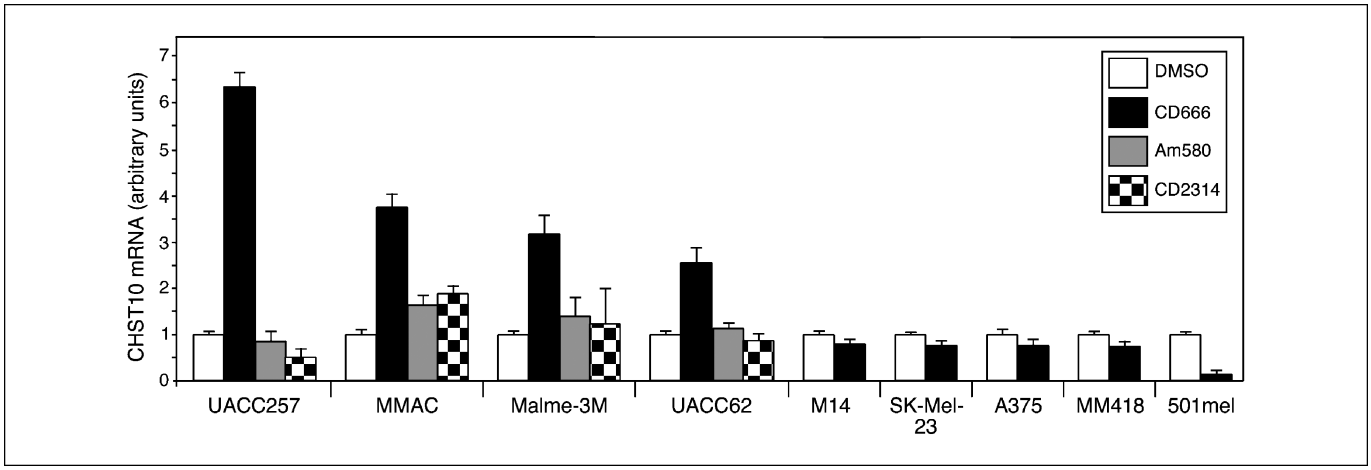

Figure 4. RARγ-dependent regulation of CHST10 in human melanoma cells. Quantitative reverse transcription-PCR analysis of DMSO or CD666 (1 μmol/L)-treated cells showing that CHST10 expression is induced in 4 of 9 melanoma cell lines. This effect does not occur with Am580 or CD2314. Expression was normalized for internal GAPDH expression control in each reaction. RNA levels in DMSO controls are arbitrarily set at 1.

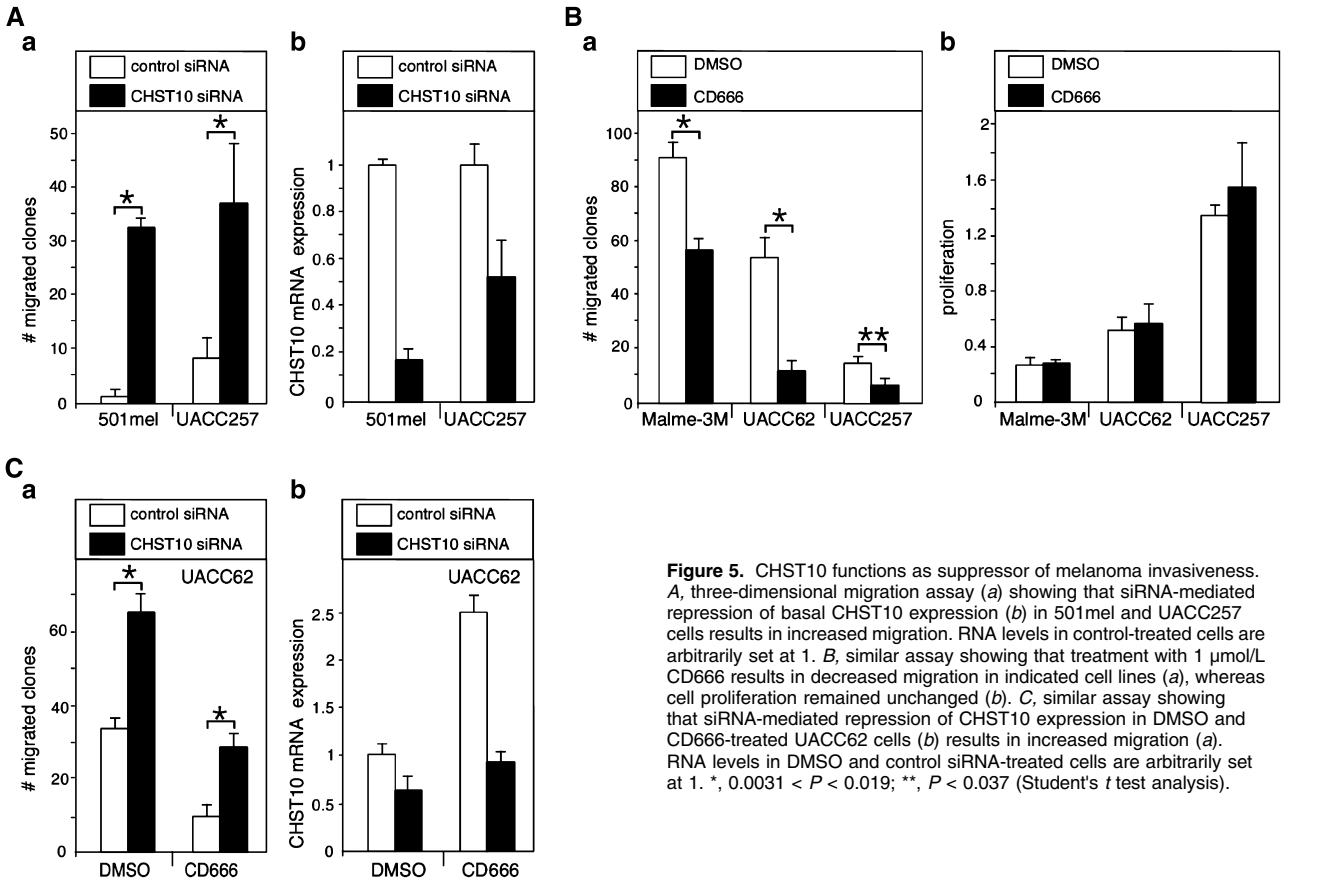

Figure 5. CHST10 functions as suppressor of melanoma invasiveness. *A,* three-dimensional migration assay (*a*) showing that siRNA-mediated repression of basal CHST10 expression (*b*) in 501mel and UACC257 cells results in increased migration. RNA levels in control-treated cells are arbitrarily set at 1. *B,* similar assay showing that treatment with 1 μmol/L CD666 results in decreased migration in indicated cell lines (*a*), whereas cell proliferation remained unchanged (*b*). *C,* similar assay showing that siRNA-mediated repression of CHST10 expression in DMSO and CD666-treated UACC62 cells (*b*) results in increased migration (*a*). RNA levels in DMSO and control siRNA-treated cells are arbitrarily set at 1. *, $0.0031 < P < 0.019$; **, $P < 0.037$ (Student's *t* test analysis).

differentiation (38) and restoration of CD38 expression in response to RA treatment in HL-60R cells (39) and this isoform also appears to be responsible for RA-dependent induction of transglutaminase II and transforming growth factor-β2 and insulin-like growth factor binding protein-3 in tracheobronchial epithelial cells (40, 41). RARα may also regulate expression of specific genes and proliferation in several other cell types (42–44), whereas RARβ may perform this function in breast cancer cells (45). Furthermore, overexpression of RARγ, but not RARα or RARβ, induces constitutive mesenchymal differentiation of a human embryonal carcinoma cell line (12). Ablation of *RARγ*, but not *RARα*, results in loss of differentiation potential of F9 cells in response to RA (13), whereas only *RARγ*-null mice display a myeloproliferative syndrome (17). RARγ also induces differentiation of S91 melanoma cells (11) and neuroblastoma cells (46). Unfortunately, few primary genes have been linked to regulation by RARγ, and those are exclusive to F9 embryonal carcinoma cells (16). Here, we identified and validated a new RARγ target gene in melanoma cells and present evidence for a novel mechanism whereby the nucleosomal context of the *CHST10* promoter selectively allows RARγ to bind (directly or indirectly) and activate transcription through two atypical RAREs despite the extremely high degree of conservation of the DNA-binding domain between RAR isoforms (>94%). This surprising result illustrates the complex nature of genomic DNA-nuclear receptor-coregulator interactions.

CHST10 is known to play an important role in hippocampal plasticity (21), but this is the first report linking its expression to melanoma invasiveness. CHST10, together with two glucuronyltransferases (GlcAT-P and GlcAT-S), directs the biosynthesis of HNK-1 glycan on neural cell adhesion proteins such as NCAM and glycolipids (47). Whereas little else is known about CHST10 other than its enzymatic activities, HNK-1 has been more studied. Expression of the HNK-1 epitope is highly regulated during fetal development and is found on migrating neural crest cells, cerebellum, and Schwann cells in motor neurons (20). HNK-1 has been implicated in a diverse array of biological activities such as cell migration, recognition, and particularly adhesion (33, 35, 47, 48), but unfortunately, the effects of HNK-1 on tumor cell properties still remain unclear. Confounding issues are that different anti-HNK-1 antibodies can recognize different HNK-1 epitopes on different proteins (18), and whereas some epitopes are important for cell adhesion, others are not (35). This issue may at least be partially responsible for differences in reported HNK-1 expression in primary and metastatic tumors and cell lines (33, 35, 36, 49). For instance, HNK-1 was detected in 42% of metastases to the skin but 0% of metastases to lymph nodes (49). Another study detected HNK-1 in 27% of distant organ metastases of uveal melanoma, but only 1 of 15 liver tumors was HNK-1 positive, its main site of metastasis (33). We failed to detect any HNK-1 expression in all primary and metastatic melanomas (Supplementary Fig. S4). More systematic approaches are needed to understand the relationship

between CHST10 levels and HNK-1 expression and their role in melanoma progression.

Retinoids are well-established anticancer agents that can induce a host of biochemical effects in tumor cells, such as growth arrest, differentiation, and apoptosis (4, 5, 11, 41). Unfortunately, however, for various reasons, retinoids as well as many other compounds have shown little efficacy against melanoma (50). Our studies suggest an alternative use of retinoids possibly as an adjuvant therapy that aims to induce CHST10 in the tumor cells as a novel strategy to inhibit melanoma invasiveness in a subset of patients that are genetically competent to respond to these RARγ-activating drugs. The feasibility of this strategy will have to await further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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