Molecular Cloning and Characterization of a Novel Retinoic Acid-inducible Gene That Encodes a Putative G Protein-coupled Receptor

Yijun Cheng† and Reuben Lotan‡

From the Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

The effects of retinoids such as all-trans-retinoic acid (ATRA) on cell growth, differentiation, and apoptosis are thought to be mediated by nuclear retinoid receptors, which are involved in ligand-dependent transcriptional activation of target genes. Using differential display, we identified the cDNA of a novel gene, designated retinoic acid-inducible gene 1 (RAIG1), which was induced by ATRA in the squamous carcinoma cell line UMSCC-22B. Two RAIG1 transcripts of 2.4 and 6.8 kilobase pairs, respectively, have the same ORF that encodes a 357-amino acid polypeptide. RAIG1 mRNA is expressed at high level in fetal and adult lung tissues. Induction of RAIG1 expression by ATRA is rapid (within 2 h) and dose-dependent in the range between 1 nm to 1 μM. The constitutive RAIG1 mRNA levels, which were low in three of five head and neck and four of six lung cancer cell lines, increased after ATRA treatment in most cell lines. The deduced RAIG1 protein sequence contains seven transmembrane domains, characteristic of G protein-coupled receptors. A fusion protein of RAIG1 and the green fluorescent protein was localized in the cell surface membrane and perinuclear vesicles in transiently transfected cells. RAIG1 was mapped to chromosome 12p12.3-p13. Our results provide novel evidence for a possible interaction between retinoid and G protein signaling pathways.

Malignant transformation is often associated with abrogation of signaling pathways that are essential for maintaining normal development, differentiation, and homeostasis. One of the approaches to cancer prevention and therapy is to restore aberrant pathways to normalcy. In this regard, retinoids have been shown to affect many fundamental cellular processes including embryogenesis, differentiation, and tumorigenesis (1–3). Furthermore, these compounds have been found to exert significant preventive and therapeutic effects against cancer in human patients (4, 5). The ability of retinoids to reverse malignant transformation is best demonstrated in several successful chemoprevention trials. It has been shown that retinoids suppress oral premalignant leukoplasia lesions (6) and decrease the incidence of second primary tumors in head and neck cancer patients (7). Most of the effects of retinoids are thought to be mediated by retinoid receptors through regulation of gene expression in the cell. Retinoids interact with two classes of nuclear receptors, the retinoic acid receptors (RARs)1 and retinoid X receptors, which belong to a superfamily of ligand-inducible transcription factors that include the steroid and thyroid hormone receptors, vitamin D receptor, and a number of orphan receptors with unknown ligands (8–10). Retinoid receptors can form homodimers and heterodimers that recognize retinoic acid receptor response element (RARE) consensus sequence, usually a direct repeat (purineG/G/T/TCA) separated by 1–5 nucleotides. Upon ligand binding, nuclear receptors recognize and bind to RARE, thereby activating or repressing gene transcription (8–10). A number of cofactors have been found to interact with nuclear receptors and modulate their transcriptional activity. Co-activators are thought to serve as bridges between the receptors and components of the basic transcriptional machinery, thus mediating activation of transcription (11–13). Co-repressors may associate with ligand-free receptors and squelch their activity. Ligand-dependent activation of nuclear receptors may involve dissociation of co-repressor and recruitment of co-activator by the receptor (14–16). Various genes, which are induced directly by ATRA, were found to contain RARE in their promoter region (1, 3, 8–10). Only a few of these genes can potentially mediate retinoid signaling for modulation of cell growth (1, 3).

The mechanisms through which retinoids suppress carcinogenesis are not well understood. However, it is thought that nuclear receptors play an important role in this mechanism (1, 4, 17). In vitro studies have shown that retinoids inhibit cell proliferation and suppress squamous cell differentiation in head and neck squamous cell carcinoma (HNSCC) cell lines (18). Human oral squamous carcinoma cell UMSCC-22B is sensitive to ATRA in a clonogenic assay (19) and exhibits diminished growth as xeno-transplants in nude mice treated with RAR-selective retinoid ALRT1150 (20). To better understand molecular events in retinoid-induced cell growth inhibition and differentiation, we use the differential display tech-
null (21) to identify retinoic acid-regulated genes in UMSCC-22B cells. Here, we present the cDNA of a novel retinoic acid-responsive gene, retinoic acid-induced gene 1 (RAIG1), which encodes a putative guanine nucleotide-binding protein (G-protein)-coupled receptor. Our results suggest that retinoids may exert biological function through interaction with a yet to be defined G-protein signaling pathway.

**EXPERIMENTAL PROCEDURES**

Cell Culture and Treatment with Retinoic Acids—Human HNSCC cells lines UMSCC-22B, UMSCC-10A, UMSCC-10B, UMSCC-22A, and UMSCC-30 were kindly provided by Dr. T. Carey (University of Michigan, Ann Arbor, MI). HNSCC cell line MDA886 was obtained from Dr. P. G. Sacks (Memorial Sloan-Kettering Cancer Center, New York, NY). Human non-small cell lung cancer (NSCLC) cell line H1792 was kindly provided by Dr. Adi Gazdar (University of Texas Southwestern Medical Center, Dallas, Texas). NSCLC cell lines H157, H460, H226, Calu-1, and H596 and human cervical carcinoma cell line HeLa were purchased from the American Type Culture Collection (Rockville, MD). ATRA, 9-cis-retinoic acid (9-cis-RA) and 13-cis-retinoic acid (13-cis-RA) were obtained from Dr. Werner Bollag (Hoffmann-La Roche, Basel, Switzerland).

The cancer cells were maintained in a 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 95% air and 5% CO2. The cells were seeded at a density of 2 × 106 cells/175-cm2 dish. After 24 h, cells were treated with retinoic acids for different periods of time. Control cultures received the same amount of the solvent Me2SO as did the treated culture (0.1%).

Preparation of Total RNA and cDNA—Total RNA was extracted using the Tri-Reagent method (Molecular Research Center, Inc., Cincinnati, OH). For reverse transcription, 10 μg of RNA were treated with RQ1 RNase-free DNase (Promega, Madison, WI) for 20 min and purified with Tri-Reagent after the treatment. The first strand cDNA was synthesized from 2 μg of total RNA with Superscript Reverse Transcriptase (Life Technologies, Inc.) and oligo(dT)16-primer. The cDNA synthesized was diluted with H2O to 80 μl and served as template in differential display.

Differential Display—The Delta RNA Fingerprinting Kit from CLONTECH (Palo Alto, CA) was used to perform differential display polymerase chain reaction (DD-PCR). This kit contains nine oligo(dT) anchoring primers and 10 arbitrary primers with 90 possible combinations of upstream and downstream primers. DD-PCR was carried out in 20-μl reaction mixtures, containing 1 μl of cDNA, oligo(dT) anchoring primer and arbitrary primer (1 μM each), dNTPs (100 μM each), 1× Advantage2™ KlenTag polymerase mix (CLONTECH), and 20 μM of upstream and downstream primers. DD-PCR reaction beginning from RNA preparation. Only those bands with promising bands were re-evaluated using an independent DD-PCR reaction. Because of the fact that 22B cells. Here, we present the cDNA of a novel retinoic acid-responsive gene, retinoic acid-induced gene 1 (RAIG1), which encodes a putative guanine nucleotide-binding protein (G-protein)-coupled receptor. Our results suggest that retinoids may exert biological function through interaction with a yet to be defined G-protein signaling pathway.

**RESULTS**

Isolation and Characterization of RAIG1 cDNA—Differential display was carried out using RNA isolated from untreated and ATRA (1 μM for 4 and 24 h)-treated UMSCC-22B cells to identify novel retinoic acid-responsive genes. One differentially expressed cDNA fragment, DD1, identified two mRNA species about 2.4 and 6.8 kbp that were up-regulated in UMSCC-22B cells treated with 1 μM ATRA (Fig. 1A). Two other natural retinoids, 9-cis-RA and 13-cis-RA, also increased the level of these two transcripts (Fig. 1B). However, whereas 9-cis-RA was as effective as ATRA, 13-cis-RA appeared to be less potent. A comparison of DD1 sequence with those deposited in the National Center for Biotechnology Information (NCBI) through Internet services.

Cloning RAIG1 cDNA and Sequencing—Two cDNA libraries were screened to isolate RAIG1 cDNA: a ZAP cDNA library custom-made by Stratagene from HNSCC cell line ScCYY1 treated with ATRA and a λgt10 cDNA library made from normal human lung tissue (CLONTECH). cDNA sequencing was carried out at the Institutional DNA Core Facility (Department of Molecular Genetics, M. Anderson Cancer Center) using the fluorescence-labeled automatic sequencing approach. The sequence data were analyzed with the Seq-Analyses program. Sequence homology to published data base was analyzed with BLAST program at the National Center for Biotechnology Information (NCBI) through Internet services.

Southern blotting analysis was carried out as described (22) using a 1.1-kbp EcoRI cDNA probe of RAIG1.

In Vitro Transcription-Translation of RAIG1 Protein—RAIG1 protein was translated in vitro with the TNT T7 Coupled Reticulocyte Lysate system (Promega). Template DNA was obtained by subcloning ORF fragment of RAIG1 cDNA into expression vector pcDNA3 (Invitrogen). The in vitro transcription-translation was carried out with 1 μg of plasmid DNA in 50 μl of reaction mixture supplemented with 40 μCi of [35S]cysteine (ICN) and various amounts of canine pancreatic microsomal membranes (Promega) for 90 min at 30 °C. The microsomal membrane fraction was isolated by high speed centrifugation (100,000 × g for 60 min). The products were separated by 10% SDS-polyacrylamide gel electrophoresis, and dried gels were analyzed by autoradiography.

Subcellular Localization of RAIG1 Protein—A fusion protein with green fluorescent protein (GFP) attached to the carboxyl terminus of RAIG1 protein was made to detect the subcellular localization of RAIG1 protein. To prepare the RAIG1-GFP expression vector, the ORF fragment of the RAIG1 was amplified by PCR using appropriate primers containing Xhol and BgII sites at the 5′- and 3′-ends, respectively, and inserted into Xhol/BgII sites of pEGFP-N1 vector (CLONTECH). Modification was made to place a perfect Kozak sequence 5′-CCACC-3′ (23) immediately before the first codon of RAIG1’s ORF. Two micrograms of RAIG1-GFP plasmid were transfected into 5 × 104 H1792 cells or HeLa cells in chamber slides with LipofectAMINE™ (Life Technologies, Inc., Inc.). Two to three days after transfection, cells were examined under a fluorescence microscope or by confocal laser scanning microscopy.

Chromosome Assignment of RAIG1—The following two pairs of primers from each of the radiation hybrid clones (Research Genetics, Inc., Huntsville, AL) was analyzed by PCR with RAIG1A and RAIG1B primers. The PCR conditions were 35 cycles at 94 °C for 30 s, 64 °C for 30 s, and 72 °C for 45 s for both primers. The PCR products from RAIG1A and RAIG1B primers were 246 and 156 bp, respectively. The results were submitted to the Radiation Hybrid Server of Stanford Human Genome Center2 to calculate linkage of RAIG1 to reference markers. YAC clones 929e11, 829h11, 787g9, 738b11, 954g10, 763g6, and 964e10 were selected from a YAC contig mapped with RAIG1-linked reference markers and purchased from Research Genetics. RAIG1 was assigned to specific YAC clones by PCR-screening YAC DNA with RAIG1A and RAIG1B primers as described (25).
FIG. 1. RAIG1, a novel retinoic acid induced gene. **A**, differentially expressed cDNA fragment DD1 obtained by differential display identifies two mRNA species induced by 1 μM ATRA in UM-SCC-22B cells. Cells were treated with Me2SO alone or with 1 μM ATRA for 4 or 24 h, and total RNA was isolated and analyzed by Northern blotting using DD1 fragment as a probe. **B**, effects of natural retinoic acids on RAIG1 expression. UM-SCC-22B cells were treated with 1 μM ATRA, 9-cis-RA and 13-cis-RA for 24 h, and total RNA was isolated and analyzed by Northern blotting using RAIG1 cDNA probe. **C**, structure of RAIG1 cDNAs; RAIGIL has an additional 4.3-kbp sequence extending from the 3’-end of RAIG1S. **D**, nucleotide and deduced amino acid sequence of RAIG1S. Kozak sequence adjacent to initiation codon is indicated by underlining and boldface type. The poly(A) signal AATAAA is underlined.
GenBank™ data base revealed no homology to any existing gene. The gene represented by DD1 was named RAIG1. We decide to clone the full-length cDNA of RAIG1. To this end, the DD1 fragment was used as a starting probe to screen cDNA libraries by plaque hybridization. After three rounds of screening two cDNA libraries, 23 clones were identified and sequenced. Sequence data of two expressed sequence tag clones from the I.M.A.G.E. consortium (clone ID 266633 and 840956) were also used in assembling the cDNA sequence. Two cDNA sequences, 2302 and 6610 bp long, were assembled and named RAIG1S and RAIG1L, respectively. RAIG1S cDNA includes 99 bp of 5'-end untranslated region, 1074 bp of coding sequence, and 1129 bp of 3'-end untranslated region. The 2.3-kbp sequence at the 5'-end of RAIG1L cDNA is identical to RAIG1S, and RAIG1L has an additional 4.3-kbp sequence in its 3'-end (Fig. 1C). Both transcripts have the same ORF that encodes a protein of 357 amino acid residues with a calculated molecular mass of 40,256 daltons. (Fig. 1D). The region adjacent to the initiation codon contains a favorable Kozak sequence (23). Both RAIG1S and RAIG1L mRNA contain polyadenylation signals AAUAAA located 34 and 19 bp upstream of cleavage and polyadenylation sites, respectively. It is likely that the selection of alternative polyadenylation sites during RNA processing leads to the production of two species of RAIG1 mRNA. The polyadenylation signal at nucleotides 2249–2254 was recognized to generate mature RAIG1S mRNA, whereas the use of a downstream site at nucleotides 6571–6776 results in RAIG1L mRNA with unusually long 3'-end untranslated sequence (5440 bp). The lengths of RAIG1S and RAIG1L cDNA are close to the apparent size determined by electrophoretic migration of two mRNA species detected by Northern blotting, suggesting that they are likely to be near full length.

A 1.1-kbp cDNA probe of RAIG1 was hybridized to blots of human genomic DNA digested with different restriction enzymes and genomic DNA from different species digested with EcoRI. Southern hybridization results suggested that the 1.1-kbp cDNA fragment forms part of a single copy gene (Fig. 2A). Human RAIG1 cDNA probe only hybridized to human and monkey genomic DNAs by Southern blotting analysis (Fig. 2B), suggesting that RAIG1 may not be a well conserved gene during evolution.

The RAIG1 Protein Is a Member of the G Protein-coupled Receptor Superfamily—Hydropathy analysis based on the method of Kyte and Doolittle (26) revealed the presence of seven transmembrane helices in RAIG1 protein (Fig. 3A), which represent a signature motif of the superfamily of G-protein-coupled receptors (GPCRs) (27). By searching the SWISS-PROT data base, several sequences with a low degree of similarity to RAIG1 protein were found, including the Droshaila bride of sevenless (boss) gene, a membrane-associated ligand for sevenless which has seven transmembrane domains (28), and metabotropic glutamate receptor (mGluR) type 2 and type 3, which are G protein-coupled receptors (29). RAIG1 protein exhibits 28% identity with boss and 25% with human mGluR2 and mGluR3 in transmembrane domains, whereas other portions of the sequences share little similarity.

The polypeptide products from in vitro transcription and translation of RAIG1 cDNA include a major band migrating with an apparent molecular mass of 32 kDa in SDS-polyacrylamide gel electrophoresis (Fig. 3B, lane b). The discrepancy between in vitro translated product and the predicted molecular mass of 40 kDa is probably due to migration behavior of the RAIG1 protein molecules. We further examined the possible post-translational modification of RAIG1 protein by adding microsomal membranes into in vitro translation reaction (Fig. 3B, lanes c–f). With increasing amounts of microsomal membranes, more [35S]cysteine-labeled peptides shifted from 32 to

![Image](Fig. 2. RAIG1 structure. Southern blotting analyses were performed on human genomic DNA digested with different restriction enzymes and genomic DNA from different species digested with EcoRI (B).)

![Image](Fig. 3. Analysis of RAIG1 protein. A, hydropathy analysis of deduced RAIG1 amino acid sequence. B, in vitro translation of RAIG1 protein. Lane a, control with plasmid pcDNA3; lanes b–f, in vitro translation with plasmid pcDNA3-RAIG1 supplemented with increasing amount of canine pancreas microsomal membranes. C, membrane association of RAIG1 protein. In vitro translation without (lane a) or with 2 μl of microsomal membranes (lane b); supernatant fraction of the sample shown in lane b (lane c); membrane fraction of the sample shown in lane b (lane d).)
35 kDa. The increase in molecular weight is likely due to glycosylation of RAIG1 protein. By separating membrane pellet from supernatant, we found that all of the glycosylated peptides and most of the unprocessed peptides were membrane-associated (Fig. 3C, lane d). A potential N-linked glycosylation site (Asn-X-Ser/Thr) is located at residue 158 of RAIG1 peptide, at an extracellular loop between transmembrane domains IV and V.

**Induction of RAIG1 Expression by Retinoic Acid**—The induction of RAIG1 expression in UMSCC-22B cells was apparent as early as 2 h after treatment with 1 μM ATRA. After 4 h of ATRA treatment, the increase in RAIG1S mRNA leveled off. The induction lasted for at least 72 h (Fig. 4, A and B). At their peak, the levels of induced RAIG1 mRNAs were about 7- and 9-fold higher than control. The level of RAIG1L mRNA increased at a somewhat slower initial rate. The induction of RAIG1 expression by ATRA exhibited a dose-dependent pattern (Fig. 4, C and D). The RAIG1S transcript was increased after 24 h of treatment with 0.01 μM ATRA, whereas the induction for RAIG1L was apparent after treatment with 0.1 μM ATRA.

**Expression Pattern of RAIG1 mRNA in Normal Tissues**—Northern blotting analysis of poly(A)⁺-RNA derived from normal human fetal and adult tissues using RAIG1 cDNA as a probe revealed that both normal fetal lung and adult lung were the tissues with the highest RAIG1 mRNA level (Fig. 5). RAIG1 transcripts were also constitutively expressed in fetal kidney and adult placenta, kidney, prostate, testis, ovary, small intestine, and colon at low to moderate levels. RAIG1 mRNAs were not detectable in fetal heart, brain, and liver and adult heart, brain, liver, skeletal muscle, pancreas, spleen, thymus, and peripheral leukocytes. In normal tissues, the ratio of the two mRNA species (2.4:6.8 kbp) was in the range of 1:1 to 2:1.

**Induction of RAIG1 mRNA Expression by ATRA in Head and Neck and Lung Cancer Cells**—The levels of RAIG1 mRNA in different cancer cells varied greatly from nondetectable to high (Fig. 6). The panel of lung cancer cells included different histological types: squamous carcinoma (H157, H226, and Calu-1), adenocarcinoma (H1792 and H596), and large cell carcinoma (H460), whereas all head and neck cancers cell lines were squamous carcinoma. There was no apparent relation between the type of cancer cells and RAIG1 expression level. RAIG1 mRNA levels increased by ATRA treatment (1 μM for 24 h) in all HNSCC cell lines and in four of six NSCLC cell lines, most...
of which had a low level of constitutive RAIG1 mRNA. In
contrast, the constitutive expression of RAIG1 in lung cancer
cell lines H226 and Calu-1 was high and not affected by ATRA
treatment.

Subcellular Localization of RAIG1 Gene Product—To detect
the subcellular localization of RAIG1 protein, green fluorescent
protein was attached to the carboxyl terminus of RAIG1 pep-
tide, and the fusion product was transiently transfected and
expressed in H1792 and HeLa cells. The location of a similar
fusion protein of the cholecystokinin receptor type A (a G pro-
tein-coupled receptor) and GFP has been shown to represent
accurately the normal receptor localization and trafficking (30).
The RAIG1-GFP fusion protein was localized in the plasma
membrane and intracellular vesicles (Fig. 7, A and B). The
membrane distribution appeared to be even; however, a few
microclusters of higher expression were observed. Confocal la-
er scanning microscope images confirmed the presence of the
fusion protein in the plasma membrane and perinuclear vesi-
cles (Fig. 7, C and D), which are most likely the endoplasmic reticulum and Golgi apparatus involved in processing of
RAIG1-GFP synthesis. The above observations support the
prediction that RAIG1 protein is a cell membrane receptor.

Chromosome Localization of RAIG1—PCR-screening of radia-
tion hybrids (Stanford G3 RH panel) with RAIG1 STS primers
RAIG1A and RAIG1B resulted in a group of 12 framework
markers, which exhibit significant linkage to RAIG1 (Table I)
(31). All of these framework markers were located on chromo-
some 12. Six RAIG1-linked markers (D12S864, D12S98,
D12S364, D12S308, D12S358 and D12S89) were found in a
high resolution YAC contig map of chromosome 12, all located
between gene loci PRB3 and Ly-GDI (D12S1114) in a single
YAC contig (32). From this YAC contig, several YACs sur-
rrounding the RAIG1 linked markers were tested for the pres-
ence of the RAIG1 (Fig. 8A). RAIG1 was mapped to YAC
929e11, 738b11, 954g10, and 763g6. YAC 929e11 was previous-
ly assigned to chromosome 12p12.3-p13 by fluorescent in
situ hybridization (32). Because D12S847 was developed from
an insert at the end of YAC 929e11, the gene locus for RAIG1
was placed between D12S358 and D12S847 on chromosome 12
(Fig. 8B).

DISCUSSION

In this study, we cloned and characterized the cDNA of a
novel retinoic acid-responsive gene, RAIG1, from UMSSC-22B
cells. A unique feature of RAIG1 is the presence of two mRNA
species, which presumably results from alternative utilization
of different polyadenylation signals. There are four potential
poly(A) signals in the primary transcript unit of RAIG1, of
which only the first and the last are used in 3' end RNA
polyadenylation/cleavage reaction. In many occasions, RNA
processing involves a selection among multiple polyadenylation
sites (33). Polyadenylation signals can be strong or weak rela-
tive to one another, depending on their surrounding sequence
context (34). The relative stable ratios between the two RAIG1
mRNA species in different cell and tissue types suggested that
the competition between the upstream and downstream poly-
adenylation sites is well balanced regardless of the cell type
and the level of transcription. Because both RAIG1 transcripts encode the same protein, it would be interesting to analyze the effects of 3'-end untranslated region on translation and stability of RAIG1 mRNA.

The deduced RAIG1 amino acid sequence contains a characteristic secondary structure of seven transmembrane α-helical domains. The presence of this characteristic motif and the plasma membrane localization of the RAIG1-GFP chimeric protein support the conclusion that RAIG1 is a member of GPCRs superfamily. GPCRs represent an increasingly large and functionally diverse superfamily of receptors that mediate their intracellular actions by signaling pathways involving G proteins and downstream secondary messengers (27, 35, 36). Receptors of this class respond to a variety of extracellular signals including light, lipid-derived messengers, neurotransmitters, and peptide hormones. Because the seven transmembrane domains are common to all members of GPCRs, most GPCRs bear sequence similarity with one another, primarily in the transmembrane regions (37). Among known members of GPCRs, RAIG1 protein shares a low sequence homology (25%) in the transmembrane domains only with mGluR2 and 3, which indicates that RAIG1 protein may be a novel GPCR rather than a member of the mGluR subfamily. Because GPCRs are targets for many types of therapeutics, studies on orphan receptors have become a seminal point for drug discovery using reverse molecular pharmacology strategy (38). Therefore, it will be of interest to elucidate the function of RAIG1 protein and determine whether it too is a potential target for therapeutics.

The lack of a sequence homology between RAIG1 protein and known GPCRs makes it difficult to predict the specific ligand for RAIG1 protein and the identity of its coupled G protein and secondary messengers. However, RAIG1's expression pattern provides some hints about its physiological functions. In normal tissues, RAIG1 mRNA was detected in various epithelial tissues and not in mesoderm-derived tissues. The expression of RAIG1 in epithelial tissues, notably the earodigestive tract, indicates its possible function in modulating differentiation (2, 39, 40). Such actions are carried out by regulating the expression of downstream effectors, and RAIG1 could be one such target gene.

RAIG1 mRNA level increased 3-fold within 2 h of ATRA (1 μM) treatment and almost reached a plateau by 4 h of treatment. The rapid response to ATRA suggests that RAIG1 is a primary target gene. Indeed, preliminary analysis of the upstream regulatory sequence of RAIG1 demonstrated that it confers transcriptional activation by ATRA in a promoter-luciferase construct (41). 9-cis-RA, a ligand for both RARs and retinoid X receptors, can up-regulate RAIG1 expression, whereas another natural retinoid acid, 13-cis-RA shows little effect. 13-cis-RA does not bind to retinoic acid receptors and is thought to act through its metabolites including ATRA (3). Therefore, treatment with 13-cis-RA may not result in sufficiently high levels of biological active retinoids to stimulate RAIG1 expression.

Several previous reports linked effects of ATRA on cell differentiation to G-protein signaling pathways. ATRA induces F9 mouse teratocarcinoma cells to differentiate into primitive endoderm-like cells and decreases the steady-state level of the Gαi2 subunit (42). The reduction of the Gαi2 protein by antisense RNA has ATRA-like effects on F9 cell differentiation. Overexpression of constitutively active mutant Goi2 blocks the ATRA-induced F9 cell differentiation (43). These results indicate that suppression of Gαi2 expression is required for ATRA-induced F9 differentiation. In contrast, ATRA induces P19 mouse embryonic carcinoma cells to differentiate to endoderm by up-regulating expression of Gα12 and Gα13, which in turn activate c-Jun amino-terminal kinase (44). In ATRA-induced human TC teratocarcinoma cells differentiation, protein kinase

---

**Table I**

Mapping of RAIG1 gene locus using radiation hybrids

| Linked markers | lod score |
|---------------|-----------|
| D12S1580      | 12.32     |
| D12S1581      | 12.32     |
| D12S1864      | 12.32     |
| D12S684       | 12.29     |
| D12S1804      | 11.46     |
| D12S1697      | 8.59      |
| D12S98        | 8.59      |
| D12S364       | 8.52      |
| D12S308       | 8.50      |
| D12S358       | 7.87      |
| D12S89        | 7.81      |
| D12S1898      | 7.18      |

* Only markers with lod score > 6 were considered to have significant linkage with RAIG1.
A-associated Gαs and Go2 expression level increased (45). Exposure to ATRA induced HL-60 human leukemia cells to differentiate into granulocyte-like cells with increased expression of receptors for the chemotactants interleukin-8, C5a (46), and leukotriene B4 (47). Those receptors are members of the GPCR superfamily and are required for normal leukocyte function in inflammatory response and host defense against infection. The ability of ATRA to modulate the expression of G proteins and GPCRs suggests that at least some aspects of ATRA-induced cell differentiation are mediated by G protein signaling pathways. Therefore, modulation of RAIG1 expression could contribute to the effects of retinoids on epithelial cell differentiation.

RAIG1 expression in HNSCC and NSCLC cells varies from high abundance to undetectable levels. Because of the high level of RAIG1 mRNA in normal lung tissue, it is notable that RAIG1 was expressed at a low level in three of five HNSCC and four of six NSCLC cell lines tested. This finding raises the hypothesis that the decrease in RAIG1 expression is associated with the malignant transformation of some airway epithelial tissues. The observation that ATRA treatment can increase RAIG1 expression in cancer cells with low constitutive expression level indicates that retinoids can restore aberrant target gene expression in cancer cells and suggests that this may play a role in suppression of carcinogenesis. Further, RAIG1 induction may serve as a marker for ATRA-induced epithelial cell differentiation.

Acknowledgment—We thank Dafna Lotan for excellent technical assistance.

REFERENCES
1. Love, J. M., and Gudas, L. J. (1994) Curr. Opin. Cell Biol. 6, 825–831
2. Morriss-Kay, G. M., and Sokolova, N. (1996) FASEB J. 10, 961–968
3. Sporn, M. B., Roberts, A. B., and Goodman, D. S. (1994) The Retinoids: Biology, Chemistry and Medicine, 2nd Ed., Raven Press, New York, NY
4. Lotan, R. (1996) FASEB J. 10, 1031–1039
5. Hong, W. K., and Sporn, M. B. (1995) Science 268, 1073–1077
6. Hong, W. K., Endicot, J., Itri, L. M., Doos, W., Batsakis, J. G., Bell, R., Fofonoff, S., Byers, R., Atkinson, E. N., Vaughan, C., Toth, B. B., Kramer, A., Dimery, I. W., Skipper, P., and Strong, S. (1986) Nature 323, 2547–2561
7. Chambon, P. (1996) DNA Cell Biol. 15, 841–850
8. Stadel, J. M., Wilson, S., and Bergsma, D. J. (1997) Trends Pharmacol. Sci. 18, 430–437
9. Edwards-Gilbert, G., Veraldi, K. L., and Milcarek, C. (1997) Nucleic Acids Res. 25, 2547–2561
10. Baldino, J. M. (1996) Curr. Opin. Cell Biol. 6, 180–190
11. Probst, W. C., Snyder, L. A., Schuster, D. I., Brosius, J., and Sealfon, S. C. (1992) DNA Cell Biol. 11, 1–20
12. Jadhav, B. V., Nadkar, M., Weissenbach, J., Paleri, D., Le Palier, D., Rigault, S., Chumakov, I., Cohen, D., Miller, P., Ward, D., and Kucherlapati, R. (1995) Nature 377, (suppl.) 321–323
Molecular Cloning and Characterization of a Novel Retinoic Acid-inducible Gene That Encodes a Putative G Protein-coupled Receptor
Yijun Cheng and Reuben Lotan

J. Biol. Chem. 1998, 273:35008-35015.
doi: 10.1074/jbc.273.52.35008

Access the most updated version of this article at http://www.jbc.org/content/273/52/35008

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 13 of which can be accessed free at http://www.jbc.org/content/273/52/35008.full.html#ref-list-1