Tazarotene-induced Gene 2 (TIG2), a Novel Retinoid-Responsive Gene in Skin

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Retinoids exert their biologic effects through two families of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which belong to the superfamily of steroid/thyroid hormone nuclear receptors. By using a subtraction hybridization approach, we have identified a cDNA sequence TIG2 (Tazarotene-induced gene 2), whose expression is up-regulated by the treatment of skin raft cultures by an RAR β/γ-selective anti-psoriatic synthetic retinoid tazarotene {AGN 190168/ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)-ethynyl] nicotinate}. The retinoid-mediated up-regulation in the expression of TIG2 was confirmed by Northern blot analysis. Upon sequencing, TIG2 was found to be a cDNA whose complete sequence was not in the GenBank and EMBL data bases. The TIG2 cDNA is 830 bp long and encodes a putative protein product of 164 amino acids. TIG2 is neither expressed nor induced by tazarotene in primary keratinocyte and fibroblast cultures. Thus, TIG2 is expressed and induced by tazarotene only when keratinocytes and fibroblasts form a tissue-like 3-dimensional structure. We further demonstrate that RAR-specific retinoids increase TIG2 mRNA levels. In contrast, neither RXR-specific retinoids nor 1,25-dihydroxyvitamin D_3 increased TIG2 levels. Finally, we demonstrate that TIG2 is expressed at high levels in nonlesional psoriatic skin but at lower levels in the psoriatic lesion and that its expression is up-regulated in psoriatic lesions after topical application of tazarotene. *Key words: retinoic acid receptor/psoriasis/subtractive hybridization. J Invest Dermatol 109:91–95, 1997*

Pharmacologic and physiologic retinoid signals in skin are transduced through two families of nuclear receptors, namely, retinoic acid receptors (RARα, β, and γ) and retinoid X receptors (RXRα, β, and γ) (Chambon, 1994: Mangelsdorf *et al*, 1994; Boehm *et al*, 1995; Nagpal and Chandraratna, 1996). RARs, which belong to the superfamily of steroid/thyroid/vitamin D_3 nuclear receptors, readily heterodimerize with RXRs *in vitro* (for references see, Nagpal and Chandraratna, 1996) and function as such *in vivo* (Nagpal *et al*, 1993). RARs are ligand-dependent transcription factors that activate the expression of retinoid-responsive genes by cooperative action of their activation functions, AF-1 (ligand-independent activation function), and AF-2 (ligand-dependent activation function) (Nagpal *et al*, 1992, 1993). Retinoids have been extensively used for the treatment of various nonmalignant (psoriasis and acne), and malignant (squamous cell carcinoma, actinic keratoses, basal cell carcinoma, and Kaposi's sarcoma) skin diseases, and they hold significant potential as a therapeutic modality in various epithelial cancers (Peck and DiGiovanna, 1994; Boehm *et al*, 1995; Nagpal and Chandraratna, 1996). Despite their therapeutic efficacy in various dermatologic diseases, the molecular basis of retinoid action in skin is poorly understood. Various genes whose expression is induced in cell culture systems by retinoids have been described. Of these, cellular retinoic acid binding protein II (CRABPII) is the only marker whose expression is induced by retinoic acid (RA) in normal skin (Elder *et al*, 1993). In contrast, CRABPII expression is downregulated by RA in submerged keratinocyte cultures (Elder and Cromie, 1993), and it is also overexpressed in psoriasis (Didierjean *et al*, 1991), a hyper-proliferative and inflammatory condition of skin (Krueger and Duvic, 1994) that can be treated by retinoids (Esgleyes-Ribot *et al*, 1994; Weinstein, 1996). The increased levels of CRABPII in psoriatic plaques indicates that it may not be a useful efficacy marker of retinoid action in psoriatic lesions. In addition, several lines of evidence suggest that CRABPII functions to modulate retinoid action by sequestering RA away from the nucleus and by enhancing RA metabolism (Boylan and Gudas, 1991; Fiorella and Napoli, 1991).

To further understand the molecular mechanisms of retinoid therapeutic action in skin, we sought to identify genes whose expression is induced in a 3-dimensional skin culture system (skin rafts) by an anti-psoriatic synthetic retinoid, tazarotene (AGN 190168) (Weinstein, 1996). Since tazarotene is topically effective in the treatment of psoriasis, studying its mechanism of action would provide insight into how retinoids affect the disease. Recently, by using a subtractive hybridization approach in skin rafts, we identified a novel gene, tazarotene-induced gene 1 (TIG1), whose expression was induced in skin rafts and psoriatic lesions by tazarotene (Nagpal *et al*, 1996). With a similar strategy for identification of differentially expressed transcripts, we have now identified another novel gene, tazarotene-induced gene 2 (TIG2), whose expression is up-regulated by this anti-psoriatic retinoid in skin rafts. Interestingly, unlike TIG1, TIG2 is not expressed in

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Abbreviations: RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoic X receptor; TIG1, tazarotene induced gene 1; TIG2, tazarotene induced gene 2; VDR, vitamin D_3 receptor.

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2-dimensional cultures of keratinocytes and fibroblasts. The basal and retinoid-induced expression of TIG2 is observed only when keratinocytes and fibroblasts form a tissue-like structure as in skin rafts and psoriatic lesions. We also demonstrate that TIG2 is induced by RAR-specific but not significantly by RXR-specific retinoids and 1,25-dihydroxyvitamin D_3. Finally, we demonstrate that TIG2 is expressed at high levels in nonlesional psoriatic skin but only at lower levels in the psoriatic lesion and that topical application of tazarotene to psoriatic plaques results in a rapid upregulation of TIG2 expression.

MATERIALS AND METHODS

Skin Rafts and Primary Cultures of Keratinocytes and Fibroblasts Normal skin raft cultures (model ZK 1300, Advanced Tissue Sciences, San Diego, CA) are 3-dimensional models of human skin tissues that have dermal, epidermal, and cornified layers. These skin rafts were made by the manufacturer by seeding neonatal fibroblasts (pooled from 10 donors) onto a nylon mesh, which gave rise to a dermal tissue. Keratinocytes seeded on top of the dermal tissue differentiated into multilayered epidermis consisting of basal, spinous, and granular layers and stratum corneum. Primary cultures of foreskin keratinocytes and fibroblasts were purchased (Clonetics, San Diego, CA).

Subtractive Hybridization Skin raft cultures were either mock-treated or treated with the retinoid tazarotene (1 μM) on days 1 and 3 and harvested on day 4 for total RNA preparation as described previously (Nagpal *et al*, 1996). cDNA prepared from tazarotene-treated skin raft cultures was directionally cloned into *SalI–NotI* sites of pSPORT-1 (Life Technologies, Gaithersburg, MD) and single-stranded DNA (ssDNA) was prepared from 10^6 transformants as described previously (Li *et al*, 1994; Nagpal *et al*, 1996). Another directional cDNA library was constructed in *SalI–NotI* sites of pSPORT-2 (Life Technologies, Gaithersburg, MD), by using cDNA from mock-treated skin rafts, linearized with *SalI* and *in vitro*-transcribed in the presence of biotin-14-CTP as described (Li *et al*, 1994). pSPORT-1 ssDNA was blocked with a *NotI*-oligo primer 5′-GCGGCCGCCCT$_{15}$-3′ by incubation at 70°C for 20 min with 600 μM dTTP and *Taq* DNA polymerase. Poly(dA)-blocked ssDNA (600 ng) was hybridized with biotinylated RNA (80 μg) in 2× hybridization buffer (80% formamide, 100 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid), pH 7.5, 2 mM ethylenediamine tetraacetic acid, 0.2% sodium dodecyl sulfate) for 24 h at 42°C with shaking (200 rpm). The control experiment contained all the components except biotinylated RNA. After hybridization, streptavidin (25 μg) was added and the mixture was incubated at room temperature (5 min). Common ssDNA/RNA sequences were removed by phenol/chloroform extraction (five times), and unhybridized ssDNA was precipitated and electroporated into DH12S (Life Technologies, Gaithersburg, MD) cells.

Retinoids All-*trans*-retinoic acid (RA) and 1,25-dihydroxyvitamin D_3 were purchased (Sigma, St. Louis, MO, and Calbiochem, La Jolla, CA). Tazarotene {ethyl 6-[2-(4,4-dimethylthiochroman-6-yl)-ethynyl] nicotinate}, SR 11217 {4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-2-methyl-1-propenyl]benzoic acid} and SR 11237 [2-(4-carboxyphenyl)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1,3-dioxolane] were synthesized in the Department of Chemistry, Retinoid Research, Allergan.

Northern Blot Hybridization For northern blot hybridization, total RNA (10 μg) was electrophoresed on 1% agarose–1.1 M formaldehyde gels, transferred to Nytran, and probed with labeled cDNA probes at 42°C for 18 h. The most stringent wash was at 65°C in 0.1× SSPE (0.18 M NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM ethylenediamine tetraacetic acid)/1% sodium dodecyl sulfate for 30 min.

***In Situ* Hybridization** *In situ* hybridization was performed on paraffin-embedded paraformaldehyde-fixed sections by using Genius kit (Boehringer Mannheim, Indianapolis, IN). Proteinase K (2.5 μg per ml) digestion was carried on for 30 min and hybridization with digoxigenin-labeled anti-sense probe (0.4 ng per ml) was performed at 44°C. Nonspecific binding was blocked with normal sheep serum (2%), and specific binding was detected by incubation (3 h) with alkaline phosphatase-conjugated anti-digoxigenin antibody followed by a 4-h incubation with nitroblue tetrazolium substrate at 37°C. Sense probes were used in parallel as negative controls and showed no background staining.

RESULTS

Identification of a Novel Retinoid-Responsive Gene, TIG2, by Subtraction Hybridization Tazarotene (AGN 190168), an RARβ/γ-selective retinoid, is topically effective in the treatment of psoriasis (Esgleyes-Ribot *et al*, 1994; Weinstein, 1996), a skin disease characterized by keratinocyte hyper-proliferation and infiltration of immune cells into epidermis and dermis. To identify tazarotene-responsive genes in skin, we used a subtraction hybridization procedure (Li *et al*, 1994; Nagpal *et al*, 1996) to isolate differentially regulated genes from skin raft cultures. Differentially expressed genes in tazarotene-treated skin rafts, isolated after subtraction of common cDNA sequences between vehicle-treated and tazarotene-treated skin rafts, were analyzed by northern blot hybridization. The expression of one of the subtracted cDNA clones, B1, was induced after tazarotene treatment of skin rafts **(Fig 1)**. The blot was also probed with labeled Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (a housekeeping gene) cDNA to ensure that equal quantities of control and retinoid-treated RNA were used in the northern blot analysis **(Fig 1)**. Quantitation of the northern message showed a 4-fold increase in B1 expression after tazarotene treatment. Upon sequencing, B1 was found to be 830 bp in length, contained an open reading frame from 157 to 648 bp, and coded for a putative protein product of 164 amino acids **(Fig 2)**. The ATG (157–159 bp) protein initiation codon was confirmed by the presence of an in-frame stop codon (TGA) 12 bp upstream of the ATG codon **(Fig 2*A*)**. It had a canonical poly(A) signal (5′-AATAAA-3′) at bp 750 and a long poly(A) tail starting at bp 769 **(Fig 2)**. Homology searches from DNA (GenBank and EMBL) databases showed significant homology to a 176-bp mRNA sequence (GenBank accession number, L07329) isolated from retinal pigment epithelial cells **(Fig 3*A*)**. Further homology searches from protein (Swiss-Prot) database did not show identity to known protein sequences. We therefore conclude that B1 is a novel cDNA sequence. Subsequently, another cDNA clone, B13, was identified from the same library as a tazarotene-induced gene and upon sequencing was found to be identical to B1. Because we have previously reported a tazarotene-induced gene as TIG1, B1 will hereafter be referred to as tazarotene-induced gene 2, or TIG2. A hydrophobic stretch of 20 amino acids in the N-terminal region of the protein **(Fig 3*B*)** indicated that the TIG2 product might be a membrane-associated protein. TIG2 also contained putative signals for casein kinase II phosphorylation (amino acids 50–53 and 54–57), myristylation (amino acids 16–21 and 29–34), and protein kinase C phosphorylation (amino acids 76–78). These putative motifs were identified by MacDNASIS PROSITE program. The consensus sequences used for searching phosphorylation sites were (S/T)-X-X-(D/E) and (S/T)-X-(R/K), respectively, for casein kinase II and protein kinase C phosphorylation motifs (MacDNASIS).

Interestingly, TIG2 was not up-regulated by tazarotene or RA in 2-dimensional cultures of keratinocytes **(Fig 4*A*)** or fibroblasts **(Fig**

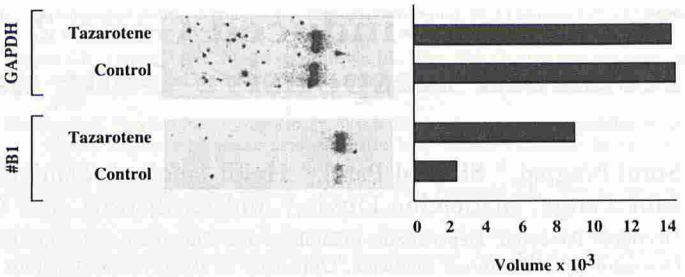

Figure 1. Identification of a tazarotene-induced cDNA clone. cDNA clone B1, obtained from subtracted cDNA library, was confirmed to be tazarotene-responsive by northern blot hybridization. Total RNA (10 μg) from mock-treated or tazarotene-treated skin raft cultures was subjected to northern blot hybridization. GAPDH and B1 sequences were detected with labeled cDNA probes. The northern blot signals, quantified in terms of total volume (an arbitrary unit) by PhosphorImager (Molecular Dynamics, Sunnyvale , CA), are also presented. The overnight exposure of the blot in the PhosphorImager screen is presented. The message level of B1 cDNA clone was induced 4-fold after tazarotene treatment of skin raft cultures.

4B). GAPDH controls for RNAs are also shown in **Fig 4*A*** and ***B*** for keratinocytes and fibroblasts, respectively. Also, TIG2 was expressed exclusively in the epidermis of nonlesional and lesional psoriatic skin and was up-regulated in psoriatic lesions by tazarotene treatment (*vide supra*, **Fig 5**). These findings suggested two plausible reasons why TIG2 was not retinoid-inducible in keratinocyte and fibroblast cultures: (i) the retinoid might induce a fibroblast-specific factor that then up-regulates TIG2 expression in keratinocytes or (ii) TIG2 might be expressed and induced in a retinoid-dependent manner only when keratinocytes and fibroblasts form a "tissue" structure as in skin rafts and skin. To test the first possibility, keratinocytes were treated with tazarotene in the presence of fibroblast-conditioned medium. These keratinocytes also exhibited neither basal nor tazarotene-induced expression of TIG2 **(Fig 4*C*)**, therefore ruling out the possibility of a retinoid-responsive fibroblast-specific factor in the induction of TIG2. The GAPDH control for the keratinocytes grown in fibroblast-conditioned medium is also shown **(Fig 4*C*)**.

TIG2 Responds to RAR but Not to RXR or Vitamin D_3 Receptor (VDR) Agonists Tazarotene (AGN 190168) activates gene expression only through RARs and not through RXRs (Nagpal *et al*, 1995, 1996). Further, AGN 190168 is RARβ/γ-selective and exhibits only weak transactivation through RARα (Nagpal *et al*, 1995). In contrast, SR 11217 and SR 11237 are RXR-specific and do not activate gene expression through RARs (Lehmann *et al*, 1992). 1,25-Dihydroxyvitamin D_3 is a ligand for VDR and induces the expression of VDR-responsive genes through its cognate nuclear receptor. To determine the receptor specificities of TIG2 expression, skin rafts were treated with RAR-specific (Tazarotene), RXR-specific (SR11217 and SR11237), or VDR-specific (1,25-dihydroxyvitamin D_3) ligands. Only the RAR-specific ligand significantly induced TIG2 expression **(Fig 4*D*)**, thus demonstrating that the induction of TIG2 is mediated through RAR but not by RXR or VDR agonist-dependent signal transduction pathways.

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   1 CCATGATTACGCCAAGCTCTAATACGACTCACTATAGGGAAAGCTGGTACGCCTGCAGGT   60

  61 ACCGGTCCGGAATTCCCGGGTCGACCCACGCGTCCGGCGGGACGGTCAGGGGAGACCTCC  120

 121 AGGCGCAGGGAAGGACGGCCAGGGTGACACGGAAGCATGCGACGGCTGCTGATCCCTCTG  180
   1                                 M  R  R  L  L  I  P  L          8

 181 GCCCTGTGGCTGGGTGCGGTGGGCGTGGGCGTCGCCGAGCTCACGGAAGCCCAGCGCCGG  240
   8  A  L  W  L  G  A  V  G  V  G  V  A  E  L  T  E  A  Q  R  R   28

 241 GGCCTGCAGGTGGCCCTGGAGGAATTTCACAAGCACCCGCCCGTGCAGTGGGCCTTCCAG  300
  28  G  L  Q  V  A  L  E  E  F  H  K  H  P  P  V  Q  W  A  F  Q   48

 301 GAGACCAGTGTGGAGAGCGCCGTGGACACGCCCTTCCCAGCTGGAATATTTGTGAGGCTG  360
  48  E  T  S  V  E  S  A  V  D  T  P  F  P  A  G  I  F  V  R  L   68

 361 GAATTTAAGCTGCAGCAGACAAGCTGCCGGAAGAGGGACTGGAAGAAACCCGAGTGCAAA  420
  68  E  F  K  L  Q  Q  T  S  C  R  K  R  D  W  K  K  P  E  C  K   88

 421 GTCAGGCCCAATGGGAGGAAACGGAAATGCCTGGCCTGCATCAAACTGGGCTCTGAGGAC  480
  88  V  R  P  N  G  R  K  R  K  C  L  A  C  I  K  L  G  S  E  D  108

 481 AAAGTTCTGGGCCGGTTGGTCCACTGCCCCATAGAGACCCAAGTTCTGCGGGAGGCTGAG  540
 108  K  V  L  G  R  L  V  H  C  P  I  E  T  Q  V  L  R  E  A  E  128

 541 GAGCACCAGGAGACCCAGTGCCTCAGGGTGCAGCGGGCTGGTGAGGACCCCCACAGCTTC  600
 128  E  H  Q  E  T  Q  C  L  R  V  Q  R  A  G  E  D  P  H  S  F  148

 601 TACTTCCCTGGACAGTTCGCCTTCTCCAAGGCCCTGCCCCGCAGCTAAGCCAGCACTGAG  660
 148  Y  F  P  G  Q  F  A  F  S  K  A  L  P  R  S  *              163

 661 CTGCGTGGTGCCTCCAGGACCGCTGCGGGTGGTAACCAGTGGAAGACCCCAGCCCCCAGG  720

 721 GAGAGGAACCCGTTCTATCCCCAGCCATGATAATAAAGCTGCTCTCCCAAAAAAAAAAAA  780

 781 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA  830
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Figure 2. Nucleotide and putative protein sequence of TIG2 (B1). B1 cDNA clone was sequenced and analyzed by MacDNASIS (Hitachi, San Bruno, CA) program for open reading frames. Nucleotide and putative protein sequence of TIG2 as obtained from our subtracted cDNA library after sequencing two independent cDNA clones (B1 and B13) in both directions is shown. GenBank accession number, U77594.

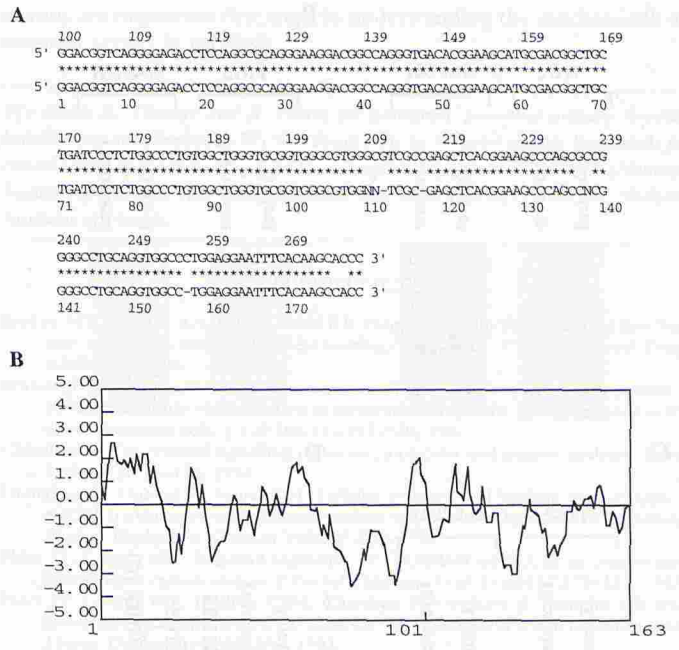

Figure 3. Nucleotide homology and hydropathic profile of TIG2. The nucleotide and putative protein sequences of TIG2 were analyzed by MacDNASIS (Hitachi, San Bruno, CA) program for nucleotide and protein homologies from GenBank, EMBL, and Swiss-Prot databases. *(A)* The homology (approximately 60%) between TIG2 and the partial cDNA clone L07329, of 176 bp from retinal pigment epithelial cells, is shown. The upper strand represents the TIG2 cDNA forward sequence and the lower strand corresponds to the L07329 cDNA sequence. *(B)* Kyte-Doolittle hydrophobicity plot of TIG2 showing hydrophobicity (*y* axis) against amino acids (*x* axis) is presented. Regions of the protein greater than +0.50 are predicted to be membrane-embedded (MacDNASIS).

Topical Application of Tazarotene (AGN 190168) Induces TIG2 Expression in Psoriatic Lesions To evaluate induction of TIG2 *in vivo* in psoriatic lesions, patients with long-standing plaque-type psoriasis were treated once daily with topical tazarotene gel (0.1%) for up to 8 wk. Skin-punch biopsies were taken before the treatment and after 3 and 14 d of tazarotene treatment. A biopsy was also taken from the nonlesional skin of the psoriatic patients before start of treatment. A typical *in situ* hybridization using digoxigenin-labeled TIG2 anti-sense RNA showed intense staining for TIG2 mRNA expression in the epidermis of the nonlesional skin biopsy of the psoriatic patient **(Fig 5*A*)**, demonstrating that TIG2 is highly expressed in uninvolved psoriatic skin. In nonlesional skin, TIG2 was expressed in basal and suprabasal layers of the epidermis, hair follicles, and endothelial cells. A reduction in TIG2 expression was observed in the psoriatic lesion compared to nonlesional skin **(Fig 5*B*)**. TIG2 was strongly induced in psoriatic lesions after 3 or 14 d of tazarotene treatment **(Fig 5*C,D*)**. Hybridization of psoriatic lesional section with sense digoxigenin-labeled TIG2 RNA probe did not show staining **(Fig 5*E*)**, thus demonstrating the specificity of TIG2 signal obtained with the anti-sense probe.

The high expression of TIG2 message in uninvolved relative to lesional skin suggested that it may play a role in maintaining the normal physiology of skin and that of other tissues. Thus, various normal adult tissues were analyzed for TIG2 expression. Apart from skin, TIG2 was also expressed in pancreas, liver, spleen, prostate, ovary, small intestine, and colon but not in heart, brain, placenta, lung, skeletal muscle, kidney, thymus, testis, or peripheral blood

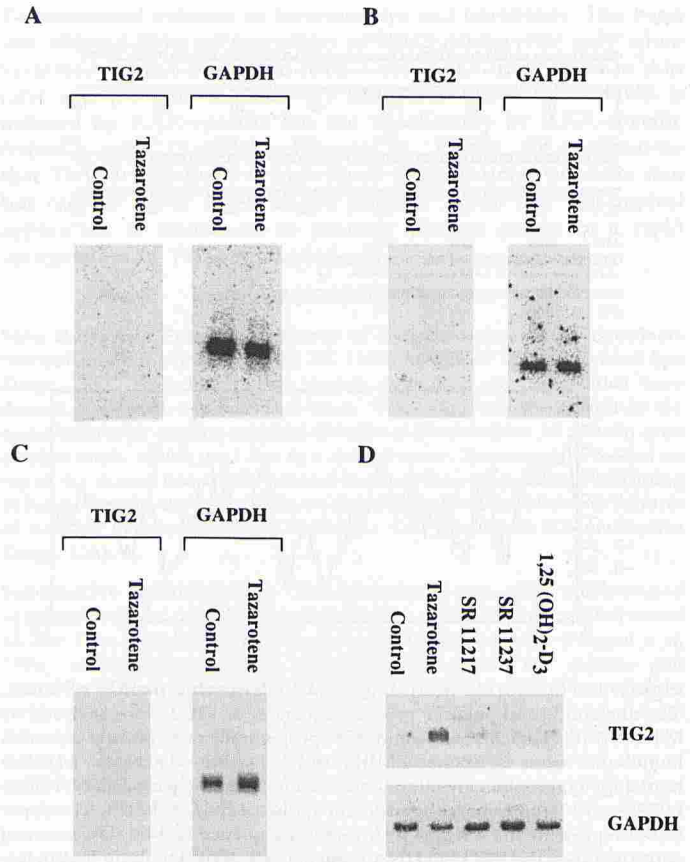

Figure 4. TIG2 is retinoid-inducible in a 3-dimensional culture system but not in 2-dimensional cultures of keratinocytes and fibroblasts. Total RNA (10 μg) from mock-treated (Control) or tazarotene-treated keratinocytes *(A)*, fibroblasts *(B)*, and keratinocytes *(C)* cultured in fibroblast-conditioned medium were subjected to northern blot hybridization. TIG2 and GAPDH sequences were detected with ^{32}P-labeled cDNA probes prepared by random priming. The overnight PhosphorImager exposures of the blots are presented. *(D)* TIG2 expression is induced in an RAR-ligand–dependent manner. Total RNA (10 μg) from mock-treated (Control), tazarotene-, SR11217-, SR11237-, or 1,25-dihydroxyvitamin D_3-treated skin raft cultures was subjected to northern blot hybridization. TIG2 and GAPDH sequences were detected with labeled cDNA probes.

leukocytes **(Fig 6)**, demonstrating the tissue-specific expression of TIG2 *in vivo*. GAPDH control of the multiple tissue northern blot is also shown.

Chromosomal Localization of TIG2 BLAST search of TIG2 cDNA sequence against human sequence tag site genomic clones database identified a clone (HSRSTS 367; GenBank accession number, Y07966) that displayed 100% nucleic acid homology over a stretch of 68 bp. The location of this STS clone fragment in the human genome maps the TIG2 gene to p13.3 region of chromosome 17. Therefore, TIG2 is located at 17p13.3 position. Interestingly, this region is associated with pancreatic tumorigenesis (for references, see GenBank accession number Y07966).

DISCUSSION

In this manuscript, we describe the identification of TIG2, a new retinoid-responsive gene. TIG2 was isolated by a subtraction hybridization approach aimed at identifying genes regulated by tazarotene, an RARβ/γ-selective anti-psoriatic synthetic retinoid. TIG2 is induced in a retinoid-dependent manner in skin rafts and in the epidermis of psoriatic lesions **(Fig 1, 5)**. In contrast, it was neither expressed nor induced by retinoids in 2-dimensional cultures of keratinocytes and fibroblasts **(Fig 4)**. Thus, TIG2 is a unique retinoid-responsive gene in that it is retinoid-inducible only when keratinocytes and fibroblasts form a 3-dimensional tissue structure as observed in skin rafts **(Fig 1)** and psoriatic lesions **(Fig 5)**. We also demonstrate that TIG2 expression is reduced in psoriatic lesions in comparison to nonlesional skin **(Fig 5*A,B*)**, suggesting that its expression may play a role in maintaining the normal phenotype of the skin. TIG2 expression was significantly induced *in vivo* in psoriatic lesions after 3 d or 2 wk of topical application of tazarotene **(Fig 5*C,D*)**. The early induction of TIG2 expression in the epidermis of the psoriatic lesion after tazarotene treatment, along with the observation that TIG2 was induced in skin rafts in a retinoid-dependent manner, suggest that the TIG2 induction is a result of direct effect of tazarotene on keratinocyte gene expression rather than an overall tazarotene effect on the lesional therapeutic status. Whether TIG2 is a marker of retinoid efficacy in psoriasis can be determined only by elucidation of its biologic functions.

TIG2 was induced only by RAR and not by RXR or VDR-specific agonists **(Fig 6)**, although human keratinocytes contain

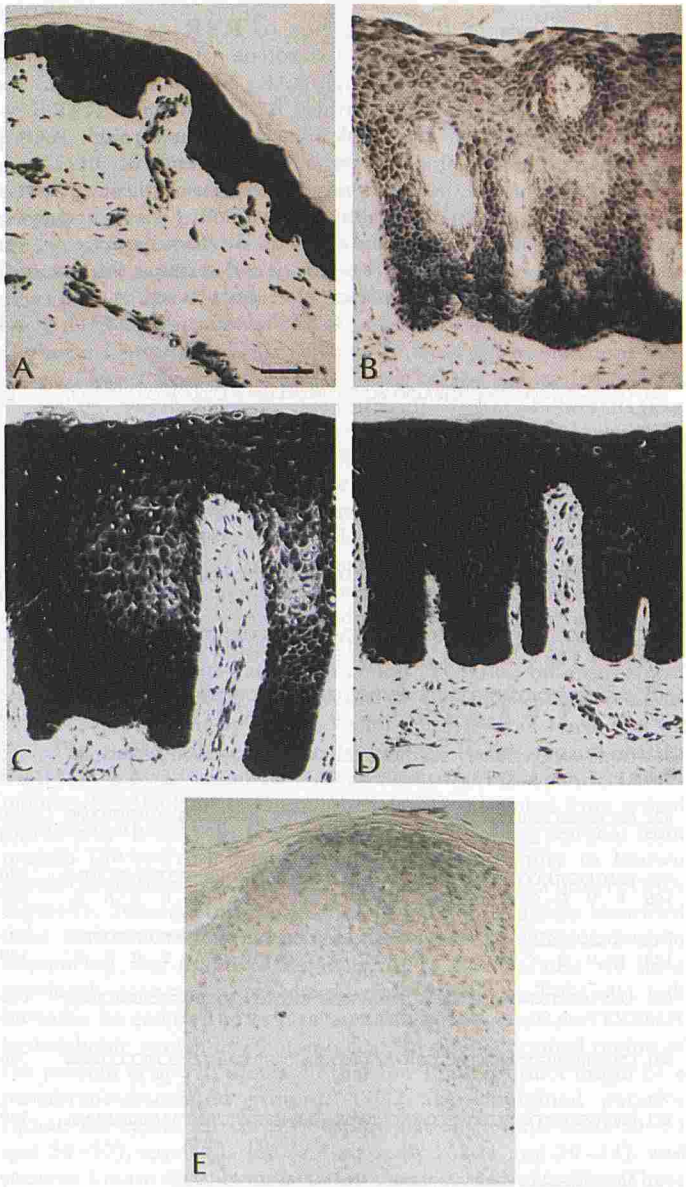

Figure 5. Retinoid-dependent induction of TIG2 in psoriatic lesions. *In situ* hybridization using digoxigenin labeled anti-sense TIG2 RNA probes was performed in nonlesional psoriatic skin *(A)*, lesional psoriatic skin *(B)*, or lesional psoriatic skin after a 3-d *(C)* or 14-d *(D)* treatment with topical tazarotene gel (0.1%). *In situ* control with retinoid-treated psoriatic skin probed with digoxigenin-labeled sense TIG2 probe was also performed *(E)*. *Scale bar*, 100 μm.

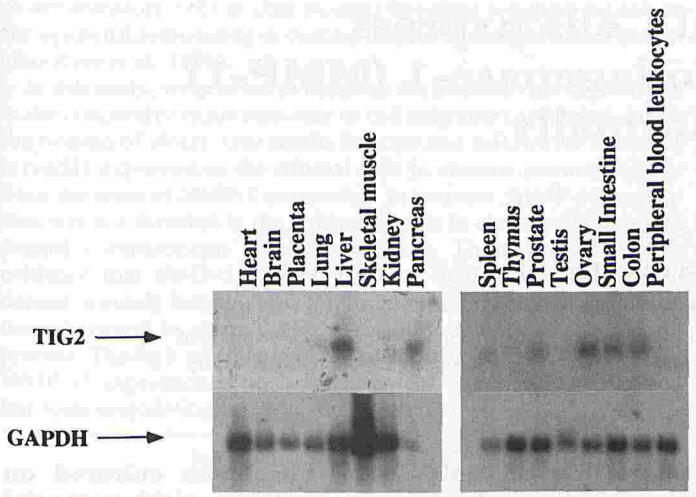

Figure 6. TIG2 is expressed in various adult normal human tissues. TIG2 expression was analyzed in multiple tissue northern blots (CLONTECH, Palo Alto, CA) containing poly(A)$^+$ RNA from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes.

these receptors in addition to RARs (Feldman *et al*, 1980; Epstein and Bonifas, 1982; Fisher *et al*, 1994). Similarly, TIG1 was also responsive to RAR agonists and not to RXR or VDR ligands (Nagpal *et al*, 1996; unpublished observations). It is also surprising that inspite of its relative abundance in skin, RXRs are not able to transduce the signals of RXR-specific ligands in skin and other systems reported (Gendimenico *et al*, 1994; Sheikh *et al*, 1994; Nagpal *et al*, 1996). RARs function *in vivo* as RAR–RXR heterodimers (Nagpal *et al*, 1993), and it appears that for TIG2 induction, only the occupation of the RAR ligand binding site of the heterodimer is required and that ligand binding to the RXR site is ineffective. Alternatively, the RXR ligand-binding site is not available for ligand binding as reported for RAR–RXR heterodimers on a DR-5 DNA recognition motif (Kurokawa *et al*, 1994). Further, the inactivity of RXR ligands in TIG1 and TIG2 induction (Nagpal *et al*, 1996; Fig. 6) suggests that the RXR homodimer pathway (Zhang *et al*, 1992) is silent in skin.

CRABPII is known to be an inducible marker of retinoid action in normal skin (Elder *et al*, 1993), but it is expressed at a high level in psoriatic skin and cultured keratinocytes (Didierjean *et al*, 1991; Elder and Cromie, 1993). Further, its expression is inhibited by RA in cultured keratinocytes. These results suggest that in hyperproliferative or abnormal differentiation states (as observed in psoriasis and cultured keratinocytes), another mechanism of CRABPII regulation is dominant over its regulation by retinoids. Therefore, our previously discovered retinoid responsive gene TIG1 (Nagpal *et al*, 1996) and another novel gene TIG2 are the only genes known that are retinoid-inducible in psoriatic lesional skin.

At present we can only speculate about the function of TIG2 and its role in RAR-mediated biology. The presence of an N-terminal hydrophobic sequence **(Fig 3*A*)** suggests that TIG2 is a membrane-associated protein. Therefore, TIG2 may play a role in cell–cell or cell–extracellular matrix interaction. The putative protein sequence of TIG2 did not contain calcium binding or hyaluronic acid binding motifs. The high level of TIG2 expression in uninvolved skin relative to lesional psoriatic skin suggests that TIG2 may play a role in the maintenance of the normal physiology of the skin. The presence of TIG2 in various normal tissues including the pancreas **(Fig 6)** and the association of its genetic locus (17p13.3) with pancreatic tumorigenesis (see GenBank accession number Y07966) suggests that it may play a role in the normal physiology of other organs as well. The actual physiologic functions of TIG2 and the relevance of its up-regulation to the therapeutic activity of tazarotene in psoriasis should be addressed. The identification of retinoid-responsive molecular markers, such as TIG1 and TIG2, in psoriatic lesions are important first steps in understanding the mechanisms of retinoid action in psoriasis.

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