Variable expression of retinoic acid receptor (RARβ) mRNA in human oral and epidermal keratinocytes; relation to keratin 19 expression and keratinization potential

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Abstract. Previous studies have revealed that the cells that form the different regions of the oral and epidermal stratified squamous epithelia represent a number of intrinsically distinct keratinocyte subtypes, each of which is developmentally programmed to preferentially express a particular pattern of keratins and type of suprabasal histology. Retinoic acid (RA) is known to modulate stratified squamous epithelial differentiation, including expression of the basal cell keratin K19 and the suprabasal keratins K1/K10 and K4/K13. We have found that all keratinocyte subtypes are similar in their steady state levels of RARα and RARγ mRNAs in culture and that these levels are only minimally affected by RA. In contrast, RARβ mRNA expression varies greatly among keratinocyte subtypes and, in eight of ten cell strains examined, directly correlated with their levels of K19 mRNA. Exposure to 10^{-6} M RA increases the levels of RARβ and K19 mRNA; conversely, complete removal of RA from the medium results in reduced levels of these messages. RA does not coordinately induce RARβ and K19 messages in nonkeratinocyte cell types: fibroblasts cultured in the presence of 10^{-6} M RA express very high levels of RARβ mRNA but do not express detectable K19, and mesothelial cells decrease their levels of RARβ and K19 mRNA in response to 10^{-6} M RA. The correlation between RARβ and K19 mRNA levels in most keratinocyte subtypes suggests a role for RARβ in specifying patterns of keratin expression and suprabasal differentiation in stratified squamous epithelia.

Introduction

The stratified squamous epithelia of the skin, oral cavity, esophagus, vagina, exocervix, and cornea differ histolog-ically and with respect to the patterns of keratins they express [11, 18, 36, 37, 40, 41]. All stratified squamous epithelia express keratins K5 and K14 in the basal layer of keratinocytes, the cell type that forms this class of epithelia [42]. In so-called “keratinizing” epithelia, such as the epidermis, the suprabasal keratinocytes express the keratin pair K1/K10 and differentiate into a superficial, flattened multilayer of pyknotic cells termed the stratum corneum. Nonkeratinizing epithelia, such as that lining the nonmasticatory surfaces of the oral cavity, lack a stratum corneum; the basal cells express K19 as well as K5/K14 and the suprabasal cells express K4/K13. The keratinizing epithelium of the oral gingiva and hard palate normally does not express K19 in the basal cells and expresses K4/K13 and K1/K10 suprabasally [6, 14, 28, 41, 43, 45, 48].

Recent studies have demonstrated that regional differences in oral epithelial suprabasal differentiation result from intrinsic, developmentally imprinted differences in gene regulation among the keratinocytes that form the keratinizing and nonkeratinizing epithelia and are not primarily determined by instructive influences of the underlying connective tissue [37, 38, 43]. We refer to these closely related but intrinsically distinct epithelial cell types as keratinocyte “subtypes” [37].

Retinoic acid (RA), a vitamin A metabolite, exerts profound effects on the growth and differentiation of many types of cells, including keratinocytes [17, 65]. High concentrations of RA suppress synthesis of the epidermal terminal differentiation keratins K1/K10 and enhance expression of K19 by epidermal and conjunctival keratinocytes in culture [12, 18, 33]. Conversely, when completely deprived of RA, keratinocytes increase their K1/K10 synthesis and reduce that of K19, adopting a more “epidermoid” pattern of differentiation [18, 33]. These changes are associated with alterations in mRNA levels and rates of gene transcription [11, 12, 21, 31, 34, 60].

The effects of RA on transcription are believed to be mediated by three structurally related nuclear proteins, the retinoic acid receptors RARα, -β, and -γ [2,
The RARs are encoded by distinct genes and belong to a larger family of DNA-binding, regulatory proteins which includes the steroid and thyroid hormone receptors [15, 23, 62]. Two RAR-related genes, RARx and RARβ [24, 39, 44], have been identified by low stringency hybridization screening with the human RARx DNA-binding domain coding sequence. However, RAR protein binds RA with only very weak affinity; thus, RA is not likely to be the natural ligand of the RARs. The RAR proteins are composed of six domains: one of these binds ligand and another binds specific DNA sequences called retinoid-responsive elements, resulting in an influence on the transcription of downstream structural genes. Retinoid-responsive elements 20–30 bp in length have been identified in several genes including laminin B1 [63], osteocalcin [53], and the RARβ gene itself [56, 58].

RAR expression during development has been studied in several species. RAR mRNAs have been localized in the developing limb buds of embryonic mice [10] and in the regenerating limb and tail of the newt [47], consistent with the hypothesis that RAR plays a role in determining proximal-distal organization [57, 59]. Differentially expressed and inducible isomers of murine RARs generated by alternative splicing also have been identified [20, 29, 35, 69]. RAR mRNA has been found to be expressed at high levels in murine and human skin [13, 29, 68], prompting interest in the possibility that RAR plays a particularly important role in epidermal tissue function.

We have compared steady state mRNA levels of the three RARs under control and elevated RA culture conditions in ten normal human adult and fetal oral and epidermal keratinocyte subtypes and in two nonkeratinocyte cell types. We find that levels of RARβ, but not of RARα and RARγ, messages vary greatly among keratinocyte subtypes. RARβ mRNA levels typically are positively correlated with the levels of K19 message and inversely correlated with the tendency of that subtype to undergo an epidermoid type of differentiation (i.e., keratinization) in vivo.

**Table 1. Summary of normal human cell strains used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Keratinocyte subtype</th>
<th>Tissue of origin</th>
</tr>
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<tbody>
<tr>
<td>OKP7</td>
<td>Special oral nonkeratinizing</td>
<td>soft palate</td>
</tr>
<tr>
<td>OKP4</td>
<td>Special oral nonkeratinizing</td>
<td>soft palate</td>
</tr>
<tr>
<td>OKF4</td>
<td>Typical oral nonkeratinizing</td>
<td>floor of mouth</td>
</tr>
<tr>
<td>OKB2</td>
<td>Typical oral nonkeratinizing</td>
<td>buccal mucosa</td>
</tr>
<tr>
<td>OKHP</td>
<td>Oral keratinizing</td>
<td>hard palate</td>
</tr>
<tr>
<td>N4Ep</td>
<td>Interfollicular epidermal</td>
<td>skin</td>
</tr>
<tr>
<td>P1Ep</td>
<td>Interfollicular epidermal</td>
<td>skin</td>
</tr>
<tr>
<td>Str. N</td>
<td>Epidermal</td>
<td>skin</td>
</tr>
<tr>
<td>B40K2</td>
<td>Fetal epidermal</td>
<td>fetal skin</td>
</tr>
<tr>
<td>B40K2F</td>
<td>Fetal oral nonkeratinizing</td>
<td>fetal floor of mouth</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Strain</th>
<th>Nonkeratinocyte cell type</th>
<th>Tissue of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1F</td>
<td>Dermal fibroblast</td>
<td>skin</td>
</tr>
<tr>
<td>LP9</td>
<td>Mesothelial</td>
<td>peritoneal</td>
</tr>
</tbody>
</table>

We have compared steady state mRNA levels of the three RARs under control and elevated RA culture conditions in ten normal human adult and fetal oral and epidermal keratinocyte subtypes and in two nonkeratinocyte cell types. We find that levels of RARβ, but not of RARα and RARγ, messages vary greatly among keratinocyte subtypes. RARβ mRNA levels typically are positively correlated with the levels of K19 message and inversely correlated with the tendency of that subtype to undergo an epidermoid type of differentiation (i.e., keratinization) in vivo.

**Methods**

**Cells and culture conditions.** The derivation and properties of the normal, diploid human keratinocyte, fibroblast, and mesothelial cell strains are summarized in Table 1. OKP7 was cultured from a biopsy of normal soft palatal epithelium from a healthy adult male. The other cell strains have been described previously [9, 36, 37, 49, 67].

For the purposes of comparative RNA analysis all cells were cultured in a "consensus medium" [25] consisting of Dulbecco's modified Eagle (DME)/F12 medium (3:1 v/v) plus 5% fetal bovine serum, 0.4 mg/ml hydrocortisone, 10 ng/ml epidermal growth factor, and 5 μg/ml insulin. This medium permits nearly optimal growth of high density cultures of all the keratinocyte and nonkeratinocyte cell types studied here. Keratinocytes were co-cultivated with lethally irradiated 3T3 fibroblast feeder cells [51]. Stock cultures of keratinocytes were maintained in complete keratinocyte medium [1], which is the above formulation plus 10^-5 M cholaera toxin and 24 μg/ml adenosine. Stock cultures of human fibroblasts were cultured in DME plus 10% bovine serum and mesothelial cells in M199/MCDDB105 (1:1 v/v) plus 7% bovine serum, 0.4 μg/ml hydrocortisone, and 10 ng/ml epidermal growth factor [7].

Some cultures received all-trans RA (Sigma, St. Louis, Mo. USA) for the final two days of their culture before extraction. RA was dissolved in 95% ethanol at a concentration of 1 mM and diluted 1:1000 to a final concentration of 10^-5 M into the medium of cultures that had reached about 80% confluence. Control cultures received the 0.1% ethanol vehicle alone. The RA concentration in control medium containing 5% normal serum was estimated to be 10^-9 M, based on earlier calculations [8, 18]. In one experiment, retinoids were extracted from the serum prior to its use as a medium supplement by the acetone:alcohol liquid extraction method of Rothblat et al. [18, 52].

**RNA isolation.** Confluent keratinocyte cultures had displaced most of the 3T3 feeder cells before the time of extraction. Any remaining 3T3 cells were selectively removed by a brief incubation and rinse with an 0.02% EDTA solution. The cultured keratinocytes were then rinsed three times with isotonic phosphate buffered saline (pH 7.2) and lysed by the addition of a 7 M guanidine hydrochloride solution [64]. After shearing the DNA by passing the lysate ten times through an 18 gauge needle, protein and RNA were precipitated with 95% ethanol at -20°C. Protein was removed by extracting twice with phenol/chloroform and RNA was precipitated by the addition of 0.3 M NaCl and 95% ethanol. The RNA precipitate was washed with 70% ethanol and dissolved in 10 mM TRIS-HCl/1 mM EDTA (pH 7.4). The concentration and purity of RNA samples were determined by assessing spectrophotometric absorbance at 260 and 280 nm. The integrity of the RNA was determined by UV fluorescence examination of ethidium bromide-stained 18S and 28S rRNA in electrophoretic agarose gels.

**Agarose gel electrophoresis and Northern blot analysis.** Prior to their loading on gels, RNA samples were heated to 65°C for 5 min in a buffer consisting of a 7:2:1 (v:v:v) mixture of formamide, 10× MOPS (=0.2 M morpholinopropanesulfonic acid, 50 mM sodium acetate, 10 mM EDTA), and 37% formaldehyde. Thirty μg of total RNA was loaded per lane onto denaturing 1% agarose gels, containing 2.2 M formaldehyde. The samples were electrophoretically separated at 120 V using a 1× MOPS running buffer and the RNA was then blot-transferred to a nitrocellulose membrane. Blots were UV-cross-linked using a Stratalinker Model 1800 (Stratagene, La Jolla, Calif., USA), wetted briefly in 4× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), and prehybridized in a buffer consisting of 50% formamide, 5× SSC, 50 mM NaPO4, NaH2PO4 (pH 7.4), 5 mM EDTA, 0.02% Ficoll, and 0.02% polyvinylpyrrolidone, 100 μg/ml calf thymus DNA, and 100 μg/ml bovine serum albumin at 42°C for 18 h. CDNA hybridization probes
were radiolabeled with \( ^{32}P \) dCTP (specific activity 3000 Ci/mmole, Dupont NEN) using the random primer method [16].

For analysis of RAR and keratin mRNA and 18S rRNA, blots were hybridized with solutions containing \( 5 \times 10^6 \), \( 4 \times 10^6 \), and \( 3 \times 10^6 \) cpm/ml of radioactive probe, respectively. Blots were hybridized for 18 h at 42°C followed by three washes in 2× SSC/0.1% sodium dodecyl sulfate (SDS) for 45 min at 22°C and one wash with 0.2× SSC/0.1% SDS at 60°C for 30 min. Radioactive bands were quantitated using a Betascope Model 603 blot analyzer (Betalog, Waltham, Mass., USA), which measures radioactivity directly from the hybridized blots. Average background counts on each hybridized blot were subtracted from the counts detected within each radiographic band. The levels of RAR and K19 mRNA detected in each sample were then normalized to the level of 18S rRNA in the same sample. Hybridized blots were then exposed to Kodak Xomat AR film at −70°C for two days to one month using an intensifying screen, and then developed and photographed.

**Hybridization probes.** The structures of plasmids bearing the human RAR cDNA sequences, kindly provided by Dr. Pierre Chambon, Strasbourg, France, have been described previously [26]. Plasmid pK19-1, containing 3' untranslated sequences of the human K19 gene [12] and pK5, containing a partial coding sequence of human K5, were obtained from Dr. Howard Green, Harvard Medical School. The human 18S rRNA plasmid [3] was obtained from Dr. Geoffrey Cooper, Dana-Farber Cancer Institute. In most cases, the hybridization probe sequences were cut from their respective plasmids. The RAR\( \alpha \) probe was an 0.6 kb \( PstI \) restriction fragment, the RAR\( \beta \) probe an 0.6 kb \( EcoRI \) restriction fragment, the RAR\( \gamma \) probe a 1.2 kb \( AaII/EcoRI \) restriction fragment, the 18S probe a 1 kb \( BamHI/EcoRI \) restriction fragment, and the K19 probe an 0.27 kb \( PstI \) restriction fragment. The entire K5 plasmid was used as a hybridization probe for K5 message.

**Results**

**Expression of RAR\( \alpha \), -\( \beta \), and -\( \gamma \) mRNA by human keratinocyte subtypes**

Keratinocyte cell strains cultured from adult (OKF4) and 20 week fetal (B4OKF2) floor-of-mouth tissue, adult soft palate (OKP7), and adult (P1EP) and 20 week fetal (B4EP2) epidermis were examined by Northern blot analysis to determine their relative levels of the three RAR mRNAs. As shown in Fig. 1, all of the strains expressed similar levels of RAR\( \alpha \) mRNA and similar levels of RAR\( \gamma \) mRNA when cultured either in the consensus medium (containing approximately \( 10^{-9} \) M RA, described in Methods) or in the presence of \( 10^{-6} \) M RA. In contrast, the oral and epidermal keratinocyte strains displayed a great variability in their levels of RAR\( \beta \) message (Fig. 2). Although RAR\( \beta \) message was not detectable in the epidermal keratinocyte strains P1EP and B4EP2 after three days exposure to film (Fig. 2a), blots exposed to film for 30 days revealed extremely low, but detectable, levels of RAR\( \beta \) message in epidermal cells (Fig. 2c) which were near the detection limit of the Betascope (Fig. 2b). The adult and fetal floor of mouth and soft palatal keratinocyte strains OKF4, OKP7, and B4OKF2 expressed higher and easily detectable RAR\( \beta \) mRNA levels; B4OKF2 expressed RAR\( \beta \) message levels >50-fold higher than the epidermal keratinocytes. Addition of \( 10^{-6} \) M RA to the medium elicited an increase of RAR\( \beta \) message in all keratinocyte subtypes (Fig. 2b), except for the fetal oral strain B4OKF2; these cells had exhibited the highest baseline level of RAR\( \beta \) message and were not further induced by \( 10^{-6} \) M RA.

Fig. 1. RAR\( \alpha \) and RAR\( \gamma \) mRNA levels in cultured oral and epidermal keratinocytes. Thirty \( \mu \)g of total RNA was isolated from cells cultured under standard conditions (−) or for the final two days of culture in the presence of \( 10^{-6} \) M RA (+). a Autoradiograms of blots hybridized with \( ^{32}P \)-labeled cDNA probes. b Quantitation using the Betascope of blots shown in a. The RAR level of each sample is normalized to the level of 18S rRNA in that sample. Indicated values are arbitrary units; one unit represents the uninduced level expressed by strain OKP7.
Comparative expression of K19 and K5 mRNA by keratinocyte subtypes

The levels of K19 message varied greatly among the oral and epidermal keratinocyte subtypes examined, as expected from previous studies of relative K19 protein synthesis by these subtypes in culture [37]. As shown in Fig. 2a, the epidermal strains had very low levels of K19 mRNA, whereas the oral nonkeratinizing strains had levels three to >30 times higher. K5 message was expressed at high levels by all keratinocyte subtypes and was reduced slightly, and essentially to the same extent, in all subtypes in response to $10^{-6}$ M RA (Fig. 2c), confirming earlier reports [12].

A relation between RARβ and K19 mRNA levels

The above results were also observed for another series of five oral and epidermal strains examined in the same way (Fig. 3 and Table 2): RARα and -γ mRNA levels did not vary substantially among the subtypes or in response to RA, whereas RARβ message varied and, in eight of the ten strains examined, was highest in subtypes expressing the highest level of K19 mRNA. The oral “typical nonkeratinizing” subtypes, strains OKF4 and OKB2 differed from the others in that they expressed higher levels of K19 message relative to RARβ message than the other strains. Based on previous studies of RA induction of K19 mRNA in keratinocytes [12, 18, 54], we expected to observe a significant induction in all the keratinocyte subtypes. The soft palatal strain OKP7, the...
Table 2. RARβ and K19 mRNA expression in keratinocyte subtypes

<table>
<thead>
<tr>
<th>Strain</th>
<th>Subtype</th>
<th>RARβ mRNA</th>
<th>K19 mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C</td>
<td>+RA</td>
</tr>
<tr>
<td>P1Ep</td>
<td>epidermal</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>B4Ep2</td>
<td>fetal epidermal</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>OKF4</td>
<td>oral nonker.</td>
<td>58</td>
<td>100</td>
</tr>
<tr>
<td>OKP7</td>
<td>oral special nonker.</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td>B4OKF2</td>
<td>fetal oral nonker.</td>
<td>700</td>
<td>500</td>
</tr>
<tr>
<td>N4Ep</td>
<td>epidermal</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Str.N</td>
<td>foreskin epidermal</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>OKHP</td>
<td>oral keratinizing</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>OKB2</td>
<td>oral nonker.</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>OKP4</td>
<td>oral special nonker.</td>
<td>100</td>
<td>160</td>
</tr>
</tbody>
</table>

Numbers shown were taken from Figs. 2b and 3. Note that values less than 15 cannot be compared reliably to one another because they did not greatly exceed the level of background radiation detected by the Betascope.

fetal epidermal strain B4Ep2, the newborn foreskin strain N, and the hard palatal strain OKHP showed RA induction of K19 mRNA. However, keratinocyte subtypes that expressed the highest (OKF4 and B4OKF2) or lowest (P1Ep and N4Ep) levels of K19 mRNA under control conditions were not substantially induced further by increased RA. The relation between control and 10⁻⁶ M RA-induced levels of RARβ and K19 mRNAs for the ten keratinocyte strains studied is summarized in Table 2.

Interestingly, the fetal epidermal strain B4Ep2 expressed levels of K19 protein [37] and message (Fig. 2) indistinguishable from that of the adult epidermal keratinocyte strains P1Ep and N4Ep when cultured under control conditions, but was induced by 10⁻⁶ M RA to express much higher levels of K19 mRNA than were adult epidermal cells. RARβ mRNA expression by B4Ep2, however, was only slightly increased above baseline by RA addition; this was similar to the behavior of P1Ep.
Fig. 4. RAR and keratin mRNA levels in keratinocytes cultured with delipidized serum. Autoradiogram of a Northern blot prepared with RNA from OKP7 soft palate keratinocytes cultured in medium containing normal serum or delipidized serum with and without 10^{-6} M RA. Hybridization intensity of sample in each lane can be compared with the 18S and 28S rRNA bands stained with ethidium bromide in the bottom panel in order to normalize for slight differences in sample loading.

Levels of RARβ mRNA expressed by keratinocytes are partly dependent upon serum RA.

Previous reports have demonstrated that the levels of K19 message and protein synthesized by keratinocytes in culture are dependent upon the levels of RA in the culture medium, and that K19 expression is substantially reduced during growth under RA-free conditions [18, 31, 54]. In other cell types, it has been reported that RARβ gene expression itself is inducible by RA [26, 58]. In order to determine whether the basal RARβ mRNA levels in cultured keratinocytes are dependent upon the low levels of RA present in the serum component of the medium, we analyzed RNA isolated from the soft palatal strain OKP7 cultured in the presence of delipidized serum. As shown in Fig. 4, RARβ and K19 messages were both reduced when cells were grown in delipidized serum. The level of RARβ mRNA was increased again when RA was added back to the medium, consistent with the interpretation that RA deprevation was responsible for reduction in RARβ mRNA expression during growth in delipidized serum medium. K19 mRNA levels were not restored by readdition of RA. It should be noted that the solvent-extraction proce-

Fig. 5. RARβ, K19, and K5 mRNA levels in human fibroblasts and mesothelial cells. a Autoradiograms of Northern blots of human fibroblast strain P1F and mesothelial strain LP9 compared with soft palate keratinocyte strain OKP7, hybridized with 32P-labelled probes. b Hybridized blots shown in a quantitated and normalized as described in the legend of Fig. 1.
dure used to treat the serum also removes serum constituents other than retinol and retinoic acid, including, perhaps, other factors which may also affect the level of RARβ and K19 message expressed by keratinocytes. K5 mRNA levels were not noticeably altered by growth in delipidized serum compared with control conditions. These results suggest that the RARβ and K19 mRNA levels in keratinocytes cultured in the absence of added exogenous RA are at least partly dependent upon low levels of RA in the serum.

Expression of RARβ and K19 mRNA by nonkeratinocyte cell types

Normal fibroblasts (P1F) and normal mesothelial cells (LP9) were compared to soft palatal keratinocytes (OKP7) with respect to RARβ, K19, and K5 mRNA expression (Fig. 5). Fibroblasts expressed a low level of RARβ message under control conditions but the level increased 30-fold when cells were grown in 10⁻⁶ M RA, the highest induction in all the cell types we examined. As expected, fibroblasts expressed no detectable K19 or K5 mRNA. Mesothelial cells expressed detectable RARβ and K19 mRNA under control conditions and the levels of both were reduced about threefold when cells were cultured in 10⁻⁶ M RA. As expected (see [7, 30, 32, 67]), mesothelial cells expressed no detectable K5 message when cultured in either condition. Thus, nonkeratinocyte cell types show RA-responsive RARβ gene expression, but the RA response is different from that exhibited by keratinocytes either in its direction or by absence of an association with K19 mRNA expression.

Discussion

A recent report by one of us [37] demonstrated that regional variability in the suprabasal differentiation patterns in the epithelia lining the oral cavity is based on an intrinsically determined, permanent specialization of the regional keratinocyte stem cells, and that the oral keratinocyte subtypes are different from epidermal keratinocytes. We have now identified different levels of RARβ mRNA expression by these keratinocyte subtypes, positively correlated with their level of K19 mRNA expression and their propensity toward a non-keratinized type of differentiation in vivo. We have also observed a coordinate induction of RARβ and K19 mRNA levels in most keratinocyte subtypes in response to elevated concentrations of RA. Inasmuch as K19 expression in culture is related to the degree of keratinization in vivo, these results suggest that RARβ may play a special role in selecting alternate patterns of keratin expression and suprabasal differentiation in stratified squamous epithelium. In contrast, we have found that the various keratinocyte subtypes differ only slightly in their expression ofRARx or RARy. These RARs are more likely to play a role in regulating functions common to all keratinocytes, such as K5/K14 or K6/K16 expression, or cornified envelope formation. These hypotheses remain to be tested, of course.

The relationship between RA and RARβ levels and the expression of genes associated with keratinocyte differentiation (such as K19) is not the same in all cell types. For example, dermal fibroblasts express very high levels of RARβ message in response to high RA but do not express K19 (Fig. 5; ref. [15]). Keratin gene regulation clearly involves other cell type-specific transcriptional regulation factors which must be present at the correct levels in cells in order to permit RARβ-mediated regulation. In this regard, Glass et al. [22] have detected multiple, cell type-specific nuclear proteins that appear to alter the affinity of RARx for several RA-response sequences in DNA.

Even within the keratinocyte family, the level of RARβ expression does not appear to be sufficient to account completely for the level of basal cell K19 expression or the differences in the type of suprabasal differentiation expressed in vivo by all of the keratinocyte subtypes we have examined. The oral non-keratinizing cell strains that were from regions other than the soft palate expressed lower levels of RARβ mRNA than expected for the levels of K19 mRNA they expressed. Interestingly, this subtype may exhibit the widest reversible range of suprabasal differentiation in vivo, from the normal non-keratinized state to keratinized in response to local irritation (see ref. [36]). Additionally, as we have described here and elsewhere [25], the oral keratinizing subtype (i.e., hard palate and gingiva) expresses RARβ mRNA at levels nearly as low as that of epidermal keratinocytes. Nevertheless, this oral keratinocyte subtype frequently displays a “parakeratinized” form of suprabasal differentiation different from that of the epidermis, in vivo, and in culture is induced by 10⁻⁶ M RA to express much higher levels of K19 than are epidermal keratinocytes. Keratinocyte subtypes that express similar low levels of RARβ mRNA may respond differently to RA because they express different RARβ isoforms or different coregulators of RAR-mediated gene transcription. Alternatively, their metabolism of retinoic acids may differ such that the intracellular retinoids bound to the RARs in epidermal cells is significantly different quantitatively or qualitatively from that in oral keratinizing cells. If the latter were true, two keratinocyte subtypes would be able to regulate their gene expression differently even in the presence of the same extracellular RA concentration and the same intracellular level of RA receptors. It will be necessary to determine the levels of RAR protein to confirm this hypothesis.

Our experiments have also identified a specific difference in retinoid regulation of keratin gene expression between adult and 20-week fetal epidermal keratinocytes. One of us had recently reported that, despite the basal cell K19 protein expression by 20-week gestational age fetal epidermis in vivo, when fetal epidermal cells are placed in culture under control conditions they resemble adult epidermal cells in their lack of K19 protein expression [37]. That observation would be consistent with the conclusion that, by the time that the fetus has essentially completed its tissue and organ formation, the
epidermal keratinocytes are intrinsically “adult”, even though the fetal epidermis appears histologically immature. However, our finding here that fetal epidermal keratinocytes respond to RA by a much larger induction of K19 mRNA clearly indicates that at 20 weeks gestation the cells are intrinsically different from those of the adult. Interestingly, the fetal keratinocytes stably retain this difference during serial culture.

Epithelia formed by other subtypes of the keratinocyte family such as the cornea and conjunctiva, as well as epithelia formed by nonkeratinocyte epithelial cell types such as the tracheobronchial epithelium and urothelium, are also subject to RA regulation of their differentiation. All convert to a keratinized stratified squamous epithelium under conditions of severe dietary vitamin A deprivation [5, 18, 27, 61]. Determination of the RAR expression patterns of these epithelia would be an initial approach to understanding the mechanisms regulating their differentiation programs.

In a separate publication [25], we report that RARβ mRNA expression and its correlation with K19 expression are frequently lost in squamous cell carcinomas (SCCs) derived from the oral epithelium and epidermis. These aberrations may contribute to the abnormal control of growth and terminal differentiation exhibited by SCC cells [49, 50, 66].

RARβ expression generally correlates with K19 mRNA expression (and, by extension the nonkeratinized, stratified squamous differentiation pathway) in keratinocytes, but it is not associated with K19 expression in normal fibroblasts (this paper) or in malignant keratinocytes [25]. This discovery suggests a promising approach to identifying coregulators involved in retinoid-mediated transcriptional regulation. Proteins necessary for RARβ-binding to its target sequences and for RA-regulated transcription might be identified by comparing extracts of the cells we have examined in this paper. Such experiments could clarify the role of retinoids and their receptors in modulating differentiation-related gene expression in epithelial cells.

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