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Review

Retinoic Acid-Induced Epidermal Transdifferentiation in Skin

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Abstract: Retinoids function as important regulatory signaling molecules during development, acting in cellular growth and differentiation both during embryogenesis and in the adult animal. In 1953, Fell and Mellanby first found that excess vitamin A can induce transdifferentiation of chick embryonic epidermis to a mucous epithelium (Fell, H.B.; Mellanby, E. Metaplasia produced in cultures of chick ectoderm by high vitamin A. *J. Physiol.* **1953**, *119*, 470–488). However, the molecular mechanism of this transdifferentiation process was unknown for a long time. Recent studies demonstrated that Gbx1, a divergent homeobox gene, is one of the target genes of all-*trans* retinoic acid (ATRA) for this transdifferentiation. Furthermore, it was found that ATRA can induce the epidermal transdifferentiation into a mucosal

epithelium in mammalian embryonic skin, as well as in chick embryonic skin. In the mammalian embryonic skin, the co-expression of Tgm2 and Gbx1 in the epidermis and an increase in TGF-β2 expression elicited by ATRA in the dermis are required for the mucosal transdifferentiation, which occurs through epithelial-mesenchymal interaction. Not only does retinoic acid (RA) play an important role in mucosal transdifferentiation, periderm desquamation, and barrier formation in the developing mammalian skin, but it is also involved in hair follicle downgrowth and bending by its effect on the Wnt/β-catenin pathway and on members of the Runx, Fox, and Sox transcription factor families.

Keywords: all-*trans* retinoic acid (ATRA); skin; homeobox gene; feather-bud formation; transdifferentiation; mucosal epithelium; epithelial-mesenchymal interaction

1. Introduction

Skin is composed of an epidermis, which is an epithelium derived from ectodermal tissue, and an underlying dermis, which is a connective tissue derived from mesenchyme of mesodermal origin. During the formation of skin and its appendages, e.g., feathers, scales, and hair, the epithelium, and mesenchyme are inducers and targets of each other [1]. Retinoic acids (RAs) including all-*trans*, 9-*cis*, and other derivatives, an active metabolite of retinol, regulate cell proliferation, differentiation, and morphogenesis during normal development of the skin [2–7]. When human keratinocytes are grown on their dermal equivalent (fabricated collagen lattices), physiologic concentrations (1–10 nm) of RA result in an epithelium very similar to that in normally keratinized epidermis; but a higher concentration (>0.1 μm) of RA reduces epidermal maturation and produces parakeratosis, and a deficiency of RA leads to hyperkeratosis [8]. Similar to the results obtained from keratinocyte cultures, retinol deficiency in the rat can cause squamous metaplasia and keratinization in a wide variety of nonkeratinized and secretory epithelia [9,10]; and excess retinol can induce epidermal mucous metaplasia in skin cultured from chick embryos [11]. An amount of 16.7 μm of ATRA induces glandular metaplasia in mouse embryonic upper-lip skin instead of hair vibrissa follicle [12,13]. Many of the abnormalities in pattern formation and organ formation that result from the addition of exogenous RA during embryogenesis are related in part to the ability of retinoids to change the pattern of expression of the clusters of homeobox genes in the embryo [14–17]. Many homeobox genes have been shown to change their expression in the skin as a response to RA [18–20]. It was demonstrated over a decade ago that *Gbx1* (gastrulation brain homeobox 1), a divergent homeobox gene, is one of the target genes of RA for the mucous transdifferentiation [21]. Our recent study found that 1 μm ATRA can induce mucous transdifferentiation in the mammalian embryonic skin as well as in chick embryonic skin. The molecular mechanism of this transdifferentiation was examined in the mammalian embryonic skin. Interestingly, ATRA-induced expression of Tgm2 (transglutaminase 2) and Gbx1 in the epidermis and that of TGF-β2 expression in the dermis are required for the mucosal transdifferentiation [22]. Other than the transdifferentiation, RA also plays an important role in barrier formation and hair follicle formation in skin [6,7,23–27]. This review highlights our current understanding of the role of retinoic acid in the epidermal transdifferentiation of skin and its appendages.

2. Effects of RA on Morphogenesis of Chick Embryonic Skin

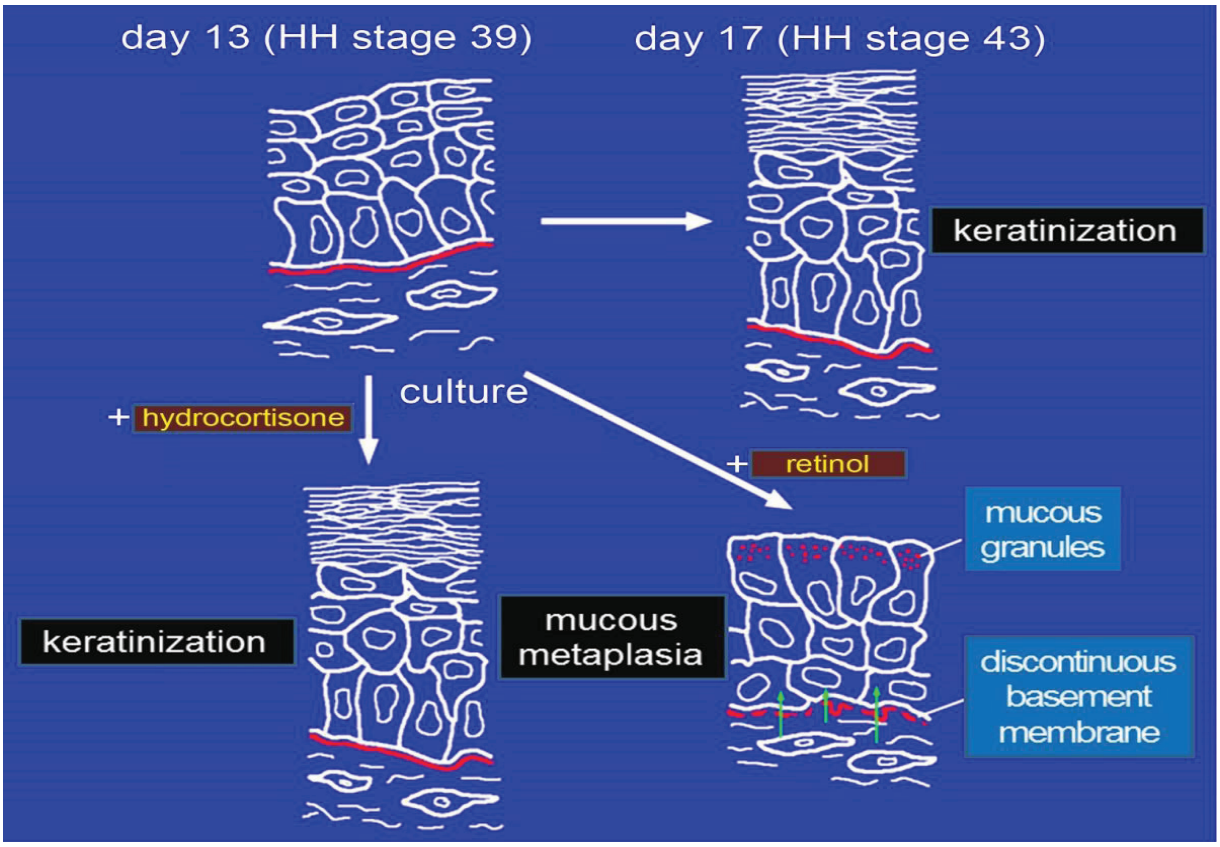
RA is known to regulate cell proliferation, differentiation, and morphogenesis during the normal development of many tissues [16,28]. Two cutaneous structures of chick, *i.e.*, scales and feathers, have been studied extensively to define the mechanism of retinoid-induced morphogenetic change [11,14,15,20,29–31]. Dhouailly *et al*. (1980) showed that the single injection of 125 μg (417 μm) of ATRA into the amniotic cavity of chick embryos at embryonic Hamburger-Hamilton (HH) stage 36 (day 10), which correspond to the beginning of scale morphogenesis, leads to the formation of feathers on chick foot scales [29]. An amount of 2.5 μm of RA also modulates axis orientation and phenotypes of skin appendages [14]. Using chick embryonic skin cultured *in vitro*, we examined the mechanism of the mucous transdifferentiation induced by RA and showed that in chick embryonic tarsometatarsal skin, this transdifferentiation to a mucous epithelium is induced by the interaction of the epidermis with the dermis, when dermal fibroblasts are pretreated with retinol (Figure 1) [21,32–35]. Furthermore, 1 μm ATRA inhibits feather bud formation and induces the transdifferentiation of the epidermis of chick embryonic dorsal skin to a mucous one [36]. In the mucous transdifferentiated epidermis, PAS-positive materials, mucous granules, and a discontinuous basement membrane are observed (Figure 1). The discrepancy between the results of Dhouailly *et al*. (1980) and ours may be ascribed to the difference in the RA concentrations used, the processing time, chicken embryonic stages, and culture conditions (*in ovo* or *in vitro*).

3. Effects of RA on the Expression of Homeobox Genes in Chick Embryonic Skin

Many of the abnormalities in pattern formation and organ formation that result from the addition of exogenous RA during embryogenesis are related in part to the ability of retinoids to change the pattern of expression of the clusters of homeobox (*Hox*) genes in the embryo [14,17]. *Hox* genes are master control genes that specify the body plan and regulate the development and morphogenesis of higher organisms [37]. Apart from these classic homeobox genes, there are other groups of homeobox genes (divergent homeobox genes), which are located outside the *Hox* loci and also play an important role in regulating growth and differentiation during the development of many organs. Using a degenerate RT-PCR (reverse transcriptase-polymerase chain reaction)-based screening method, we previously isolated divergent homeobox genes *Gbx1* [21], *Hex* (currently *Hhex*) [38–40], and *HB9* (currently *Mnx1*) [41,42] from chick embryonic tarsometatarsal scale skin in addition to *Hox* genes. Furthermore, among the many homeobox genes isolated, *Gbx1* shows a strong increase in expression in the epidermis of tarsometatarsal scale skin during the course of retinol-induced epidermal transdifferentiation to mucosal epithelium; and its expression is induced by the interaction of the epidermis with dermal fibroblasts pretreated with retinol [21].

In the feather bud, homeobox genes other than *Gbx1* are reported to be expressed, such as *Gbx2* [43]; *Msx-1* and *Msx-2* [19], *HB9* [42]; *Hox b-4*, *Hox a-7*, and *Hox c-8* [44]; *Dlx 2, 3, 5* [45]; and *Hex* [39,40]. *Gbx2* is a homeobox gene closely related to *Gbx1* and is required for proper segregation of early regional identities anterior and posterior to the mid-hindbrain boundary in the case of vertebrates [46].

Figure 1. Schematic diagram summarizing the effect of RA on epidermal differentiation of tarsometatarsal chick embryonic skin. Excess retinol can induce epidermal mucous metaplasia (transdifferentiation) in cultured skin obtained from chick embryos [11]. Epidermal mucous metaplasia of 13-day-old chick embryonic tarsometatarsal skin (HH stage 39) can be induced by culturing the skin with excess retinol for only 8 h and then without retinol for six days [34], after which the mucous granules and a discontinuous basement membrane are observed. Retinol primarily affects the dermal cells [32,35], which then transform the epidermal cells into mucus-secreting cells [33,34], suggesting the importance of epithelial–mesenchymal interaction in retinol-induced epidermal mucous metaplasia.



Studies on *Gbx1* have concentrated on the brain or neurons. In mice, during development *Gbx1* is expressed in the central nervous system [47,48]; and combined expression of *Lhx7* and *Gbx1* plays a role in the development of the cholinergic system of the basal forebrain [46]. In zebrafish, *Gbx1* and *Otx2* are expressed in the neuroectoderm [48]. It was recently shown for the first time that the *Gbx1* gene is also expressed in the feather bud of chick embryonic dorsal skin [36]. Treatment of organ cultures of chick embryonic dorsal skin with 1 μm ATRA for 24 h induces transdifferentiation of the epidermis to mucosal epithelium with a concomitant increase in *Gbx1* mRNA expression in the epidermis.

Gbx1 is expressed mainly in the epithelium of the feather bud [36]. In contrast, *Gbx2* is expressed in the mesenchyme of it [43]. The gene expression pattern of *Gbx1* in the epidermis of feather buds is almost the same as that of the *HB9* homeobox gene, as we reported previously [42]. Other than homeobox genes, the genes encoding morphogenetic proteins such as fibroblast growth factors (FGFs) [49], bone morphogenetic proteins (BMPs) [50], sonic hedgehog (Shh) [51–53], Wnt 7a [54],

Notch, Delta, Serrate [55], and β-catenin [56] are involved in the early stage of feather development [20]. Especially, FGF-, BMP-, Shh, and Wnt-signaling pathways play important roles in the morphogenesis of feather [20,49,50,53,54].

We showed that treatment of organ cultures of chick embryonic dorsal skin with 1 μm ATRA at HH stage 31 or 33 inhibits feather-bud formation and induces transdifferentiation of the epidermis to a non-keratinized stratified mucous epithelium concomitant with an increase in Gbx1 expression as microvilli develop on the upper surface of the epithelium [36]. In addition, a well-developed Golgi apparatus and PAS-positive materials are observed in the treated epidermis. These results are consistent with our previous reports stating that epidermal mucous transdifferentiation is induced by RA in chick embryonic tarsometatarsal skin at HH stage 39 along with an increase in Gbx1 expression and that typical goblet cells are induced in it by retinol [21,32,34].

Modulation of the axis orientation of feather buds by treatment with 1 μm ATRA throughout the culture period results in a higher frequency of small feather buds and many buds showing random orientations [14]. However, when 1 μm ATRA was used for treatment for only one day, along with 10 nm hydrocortisone which induces the differentiation (keratinization) of epidermis [57], and the skin was cultured for an additional four days without these chemicals, a different result was obtained. ATRA did not modulate the axis orientation, but rather caused the transdifferentiation of the epidermis to a mucous epithelium [36]. Thus RA has important roles in transdifferentiation, as well as in modulation of pattern formation.

4. Effects of Gbx1 on Chick Skin

As ATRA induces *Gbx1* mRNA expression in the epidermis, we also examined whether Gbx1 alone (without ATRA) could induce epidermal transdifferentiation. Feather buds were elongated by transient transfection of the dorsal epidermis with *Gbx1* cDNA, indicating stimulation of epidermal cell proliferation by Gbx1; however, no transdifferentiation was induced [36]. These results suggest that Gbx1 alone does not induce mucous transdifferentiation and that other signals playing a key role cooperatively with Gbx1 are involved in the epidermal to mucous transdifferentiation.

5. ATRA Induces Transdifferentiation of Rat Embryonic Epidermis to Mucosal Epithelium with Up-Regulation of Esophageal Markers MUC4 and Keratin 4

To determine the molecular mechanism of the transdifferentiation induced by ATRA, we established a culture system using rat embryonic skin in which ATRA induces epidermal transdifferentiation that is accompanied by the expression of markers of esophageal epithelium. We examined what genes are up-regulated and down-regulated in the 16E rat embryonic skin by ATRA. Representative genes up-regulated after 24 h ATRA treatment are shown in Table 1. The remainder of the up-regulated genes is shown in Supplementary Table 1. The down-regulated genes are shown in Supplementary Table 2. Bioinformatic pathway analysis of the microarray data identifies the up-regulated and down-regulated genes involved in retinol signaling pathway. *Stra6* (*stimulated by retinoic acid gene 6*), *Cyp2b3*, *d1* and *Cyp26a1*, *b1* (*cytochrome P450*, *family 2 and 26*), *Lrat* (*lecithin-retinol acyltransferase*), and *Ugt2b* (*UDP glycosyltransferase 2 family*, *polypeptide B*) are increased in expression at the mRNA level (Table 1 and Supplementary Table 1), while *Adh7* (*alcohol dehydrogenase 7*), *Cyp1a1*, *Cyp2c12*,

Cyp3a23/3a1, and *Ugt1a1(UDP glucuronosyltransferase 1 family*, *polypeptide A1)* are decreased (Supplementary Table 2). Other than these up-regulated genes, not only *Tgm2* but also *Mucin 4* (*MUC4*) and *keratin 4* (*K4*), which are markers of esophageal cells, are increased in expression at the mRNA level [22]. K4 and MUC4 are expressed in non-keratinized squamous stratified epithelium and in respiratory and digestive epithelium, respectively [58,59]. Cytokeratin, an intermediate filament observed mainly in epithelial cells, is an essential cytoskeleton component involved in the maintenance of cell morphology. The cytoskeleton of epithelia is formed by 20 subtypes of cytokeratins whose expression depends primarily on the epithelial cell type and degree of differentiation [60]. As keratin filaments, e.g., tonofibrils, K1 and K10 are not at all observed throughout the epidermis; but both K4 and MUC4 proteins are expressed in RA-induced transdifferenti ated epithelial cells, but not in control epidermal cells [22]. According to the results by electron microscopic and immunofluorescent analyses on tonofibrils, we consider that all the cells in the epidermis are induced by ATRA to undergo transdifferentiation. This transdifferentiation of whole epidermal tissue to mucosal tissue occurs without any gene transfection and requires only a one-day treatment with ATRA. Thus, this is an exciting model for induction of tissue organization, regeneration, and transdifferentiation because the generation of these transdifferentiated cells is fast and efficient.

Table 1. List of representative genes up-regulated in 16E rat embryonic dorsal skin after 24 h incubation with 1 μm retinoic acid (RA) (Fold change).

Fold	Gene Symbol	Gene Title	Public ID
22.07	Stra6	stimulated by retinoic acid gene 6	BI284420
17.40	LOC363060	similar to RIKEN cDNA 1600029D21	AI599133
11.37	Lrat	lecithin-retinol acyltransferase	NM_022280
9.18	A2m	alpha-2-macroglobulin	NM_012488
8.61	Amy1a	amylase, alpha 1A (salivary)	AB057450
8.43	Crisp1	cysteine-rich secretory protein 1	NM_022859
8.04	Gpr85	G protein-coupled receptor 85	AF203907
7.94	Cldn7	claudin 7	AJ011811
7.41	Dhrs3	dehydrogenase/reductase (SDR family) member 3	BI276935
7.12	Nupl1	nucleoporin like 1	AF000901
7.10	Dusp14	dual specificity phosphatase 14	AI236997
6.83	Il6r	interleukin 6 receptor	NM_017020
6.66	Ces1d	carboxylesterase 1D	L46791
6.57	Tmprss2	transmembrane protease, serine 2	AI412136
6.35	LOC685158	similar to CG8138-PA	AI639305
6.24	Igfbp5	insulin-like growth factor binding protein 5	BE113270
5.62	Zfp667	zinc finger protein 667	BF402458
5.51	Cyp26b1	cytochrome P450, family 26, subfamily b, polypeptide 1	BE105541
5.37	Tgm2	transglutaminase 2, C polypeptide	BI275994
5.27	Amy2	amylase 2, pancreatic	NM_031502
5.17	Trdn	triadin	AF220558
5.14	Sorl1	sortilin-related receptor, LDLR class A repeats-containing	AI177589
5.11	Cyp26a1	cytochrome P450, family 26, subfamily a, polypeptide 1	NM_130408
4.95	Akap5	A kinase (PRKA) anchor protein 5	NM_133515

Table 1. *Cont.*

Fold	Gene Symbol	Gene Title	Public ID
4.69	Mcpt10	mast cell protease 10	X68657
4.59	Klf2	Kruppel-like factor 2 (lung)	BF288243
4.50	Ush1c	Usher syndrome 1C homolog (human)	BI291932
4.50	Cfi	complement factor I	NM_024157
4.48	Tpc1808	tropic 1808	NM_022625
4.46	Car5b	carbonic anhydrase 5b, mitochondrial	AI411132
4.45	Scgb1d2	secretoglobin, family 1D, member 2	BI285057
4.37	Srpx2	sushi-repeat-containing protein, X-linked 2	AA818334
4.37	Synpr	synaptoporin	BG666364
4.36	Slc6a1	solute carrier family 6 (neurotransmitter transporter, GABA), member 1	NM_024371
4.34	St6gal1	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1	M83143
4.32	Oprk1	opioid receptor, kappa 1	L22536
4.15	Mmp11	matrix metallopeptidase 11	NM_012980
4.11	Dcx	doublecortin	NM_053379
4.08	Kalrn	kalirin, RhoGEF kinase	AI639313
4.05	Wfdc1	WAP four-disulfide core domain 1	BI279661
4.04	Scaper	S-phase cyclin A-associated protein in the ER	BF405311
4.03	Igfbp6	insulin-like growth factor binding protein 6	NM_013104
3.95	Prelp	proline/arginine-rich end leucine-rich repeat protein	AI011747
3.90	Aoc3	amine oxidase, copper containing 3 (vascular adhesion protein 1)	AI070137
3.88	Tmem176a	transmembrane protein 176A	BM388911
3.83	Egr4	early growth response 4	NM_019137
3.75	Naaa	N-acylethanolamine acid amidase	AI412627
3.74	RGD1562533	similar to mKIAA0774 protein	BI299098
3.74	Slc19a1	solute carrier family 19 (folate transporter), member 1	NM_017299
3.69	Rnf207	ring finger protein 207	BF408540
3.68	Il17d	Interleukin 17D	AI407169
3.67	Pkia	Protein kinase (cAMP-dependent, catalytic) inhibitor alpha	AA996685
3.66	Klk8	kallikrein related-peptidase 8	BI282567
3.62	Map7d1	MAP7 domain containing 1	AW253217
3.62	Mapk11	mitogen-activated protein kinase 11	BF414412
3.61	Muc4	mucin 4, cell surface associated	BM391100

6. ATRA Increases Expression of *Tgm2* and *Gbx1* mRNA and Protein in Rat Embryonic Epidermis

We focused on three genes, *i.e.*, *Tgm2*, *Gbx1,* and *TGF-β*, because we [21] and others [61,62] showed these genes are induced by RA. At first we examined the expression of two of them, *Tgm2* and *Gbx1*, in rat embryonic skin. The expression of both *Tgm2* and *Gbx1* mRNAs is increased in the epidermis of the skin cultured for one day with ATRA and then for four days without RA [22]. The expression of both Tgm2 and Gbx1 proteins is also increased throughout the epidermis cultured for one day in the presence of ATRA and then for an additional four days in its absence. Tgm2 protein is

detected in the cytoplasm, nuclei, and the area of cell adhesion in the intermediate and upper layers of the epidermis, reflecting the multifunctional nature of this protein [63–66]; whereas the expression of Gbx1 protein is seen mainly in the nuclei of the epidermal skin cells. Interestingly, *in situ* transamidase activity of Tgm2 is not detected in RA-treated Tgm2 protein [22], suggesting that the enzymatic activity might be dispensable and that other potential functions of Tgm2 [63] (discussed below) might be involved in transdifferentiation. In fact, RA-induced transdifferentiation is not inhibited by a transamidase inhibitor, ZM449829 [67], in the range of 100–300 nm (unpublished data).

To examine the function of *Tgm2* and *Gbx1* in the epidermis, we transfected the epidermis with these genes by electroporation. Epidermal keratinization and expression of K10, which is specifically expressed in the epidermis, are inhibited. In addition, rounded cells in the upper layer of the epidermis are observed in the skin by overexpression of both *Tgm2* and *Gbx1* [22]. However, epidermal keratinization is observed in the skin by overexpression of either *Tgm2* or *Gbx1* [22]. However, neither K4 nor MUC4 expression is observed in the skin overexpressing both *Tgm2* and *Gbx1* genes [22]. Thus these findings indicated that co-expression of *Tgm2* and *Gbx1* in the epidermis is required for ATRA-induced mucous transdifferentiation but that *Tgm2* or *Gbx1* alone cannot induce esophagus-like mucosal transdifferentiation. RA is a consistent inducer of *Tgm2* expression in various cells and tissues [68–70]. In mammalian epidermal cells, 3 μM RA induces tissue transglutaminase (Tgm2) [71]. Tgm2 knock-out mice do not have any defects in their keratinocyte differentiation program [72]. Tgm2 is the most diverse and ubiquitous enzyme of the transglutaminase family [73] and has been implicated in diverse processes such as inflammation, wound healing, apoptosis, neurodegenerative disorders, and cancer [63,74,75]. The most characteristic enzyme function of the transglutaminase family is calcium-dependent transamidation activity, resulting in the formation of ε-(g-glutamyl) lysine cross-links between proteins and, thus, polymerization. Tgm1, Tgm3, and Tgm5 of the transglutaminase family are found in mammalian keratinocytes and play an important role in the formation of the stratum corneum in the skin by the introduction of cross-links between proteins [76–78]. In addition to its transamidation activity, Tgm2 functions as a signal-transducing GTP-binding protein [64] and as a protein-disulfide isomerase that regulates adenylate cyclase [66].

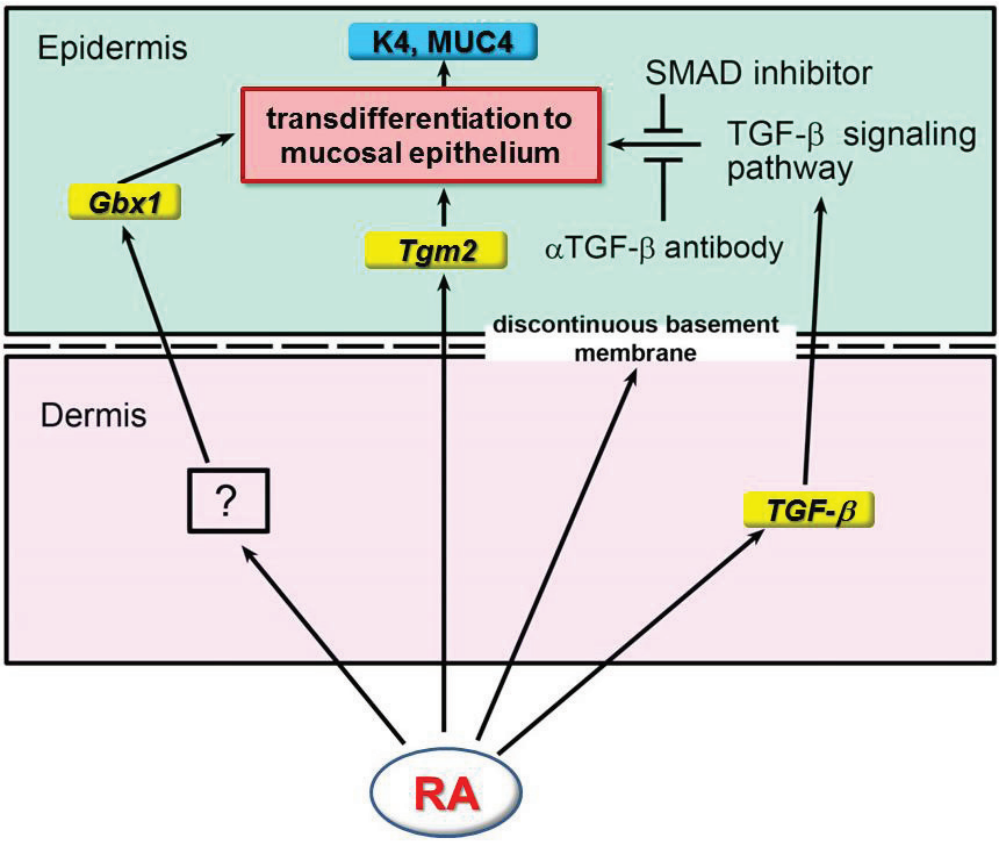
Hence, Gbx1 may be a key regulator of epithelial differentiation in addition to its known role in brain development. We showed immunohistochemically that the expression of Gbx1 protein starts to increase in the nuclei of all cells of the epidermis after one day in culture. The expression of *Gbx1* mRNA in the epidermis is up-regulated not only after 8 h of treatment with RA, but also by the physical interaction of untreated skin with the RA-pretreated dermal fibroblasts in which these fibroblasts actively migrate into the dermis, suggesting that *Gbx1* expression would appear to be regulated by some unidentified factor in the dermis (Figure 2) [21,22].

7. Involvement of TGF-β Signaling Pathway in ATRA-Induced Epidermal Transdifferentiation to Mucous Epithelium in Rat Embryonic Skin

RA added to cultures of human scalp hair follicles increases the expression of TGF-β in the dermal sheath, dermal papilla, and hair follicle [62]. In addition to Tgm2 and Gbx1, ATRA also increases the expression of both *TGF-β* mRNA and protein in the dermis of rat embryonic skin, but does so only slightly in the epidermis [22]. As ATRA increases the expression of TGF-β in the dermis, this finding

suggests that the interaction between epidermis and dermis might have an important role in epidermal transdifferentiation to a mucous epithelium. Furthermore, SB431542, a specific inhibitor of ser/thr kinase of the TGF-β type II receptor [79], and pan-TGF-β1, -β2, -β3 mAb, a neutralizing antibody, partially suppress the expression of K4 and MUC4, indicating that the TGF-β signaling pathway is involved in ATRA-induced epidermal transdifferentiation to a mucous epithelium (Figure 2).

Figure 2. Schematic diagram summarizing the effect of RA on epidermal mucosal transdifferentiation. RA up-regulates the expression of *Tgm2* and *TGF-β* in the epidermis and dermis, respectively, after 8–24 h of treatment, which is followed by the appearance of a discontinuous basement membrane. On the other hand, *Gbx1* expression is up-regulated in the epidermis after 8 h by an unknown factor, which is induced in the dermis by RA [21]. The TGF-β signaling pathway activated by *TGF-β* and acting together with *Gbx1* induces epidermal transdifferentiation to an esophagus-like mucosal epithelium [22] (reproduced with permission from *Int. J. Dev. Biol.* **2011**, *55*, 933–943).



8. Development of Skin in Retinoid Signaling Deficient Mice

RA is commonly used in the treatment of skin diseases such as acne and psoriasis, as well as in chemotherapy for leukemia. A frequent adverse effect of these therapies is RA-induced hair loss [62,80]. The mechanisms to explain how excess RA arrests hair follicle growth have been explored [27,62]. ATRA induces a catagen-like stage in human hair follicles, presumably via up-regulation of TGF-β2 in the dermal papilla, resulting in hair loss [62]. In a mouse model which knocked out *cytochrome P450*, *family 26*, *subfamily b*, *polypeptide 1* (*Cyp26b1*), which encodes an RA-degrading enzyme, the *in vivo*

administration of RA to pregnant mice or its addition to skin cultures demonstrated that appropriate RA levels are important for periderm desquamation, embryonic skin differentiation, and barrier formation in the developing mammalian skin [26]. Furthermore knock-out mouse lacking Cyp26b1 in their skin exhibit major hair follicle development defects [27]. By acting on the Wnt/β-catenin pathway and on members of the Runx, Fox, and Sox transcription factor families, RA modulates pathways and factors implicated in hair follicle downgrowth and bending [27]. In the human and mouse cicatricial alopecia and alopecia areata, retinoid metabolism is altered and genes involved in RA synthesis increases, while RA degradation genes are decreased [81,82]. Appropriate RA metabolism is needed for the normal formation of the hair follicle [6,27,81,82].

Retinoic acid receptors (RARα, β, γ) and retinoic X receptors (RXRα, β, γ) are expressed in the skin and play an important role in the development of epidermis [2,3,16,25,83–86]. In epidermis of skin, RARα, RARγ, RXRα, RXRβ, and RXRγ are highly expressed, while expression of RARβ is weak or absent [84]. In dermis of skin, RARβ, RXRα, RXRβ, and RXRγ are weakly expressed, while RARα and RARγ are moderately expressed [84]. In the mouse lacking RXRα selectively in adult keratinocytes, hair follicle degeneration, marked hair loss, and hyperplastic interfollicular epidermis are observed [3,85]. It was shown that RXRα has key roles in hair development and in epidermal keratinocyte proliferation and differentiation [3,84]. The knock-out of the RARα, RARβ, or RARγ genes does not prevent RA-induced mouse upper-lip skin glandular metaplasia, but RARγ knock-out dramatically decreases its ratio [87]. Both RARα and RARγ can initiate a glandular metaplasia, whereas RARβ cannot give rise to any metaplasia, but the following progression of metaplasia requires RARβ, indicating that these receptors have both redundant and unique functions [87].

Neutral lipid synthesis enzyme acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) functions as the major acyl-CoA:retinol acyltransferase (ARAT) in murine skin. When dietary retinol is abundant, DGAT1 deficiency results in elevated levels of RA in skin and cyclical hair loss [88]. Deletion of DGAT1 specifically in the epidermis causes alopecia. DGAT1 functions as an ARAT in the skin, where it acts to maintain retinoid homeostasis and modulate RA signaling [88].

9. Concluding Remarks

RA plays important roles in skin development by affecting signaling pathways and the expression of many genes including homeobox genes. RA induces epidermal transdifferentiation in chick and rodent embryonic skin. We identified *Gbx-1*, *Tgm2* and *TGF-β* as the key target genes regulated by ATRA during transdifferentiation. Thus far we don't have any information on which isoforms of RARs and RXRs are directly involved in the ATRA-induced transdifferentiation, and to what extent are 9-*cis* retinoic acid and non-genomic signals, *i.e.*, posttranslational modification, important to the transdifferentiation. These issues remain to be explored. An embryonic skin culture system where RA induces epidermal transdifferentiation provides a useful model in which RA can direct cell reprogramming to another cell. As the generation of these transdifferentiated cells is fast, efficient, and devoid of tumorigenic pluripotent stem cells, these cells can provide a novel system for regenerative medicine.

Further study is required to elucidate the role of RA in the skin and skin appendage development by identifying additional developmental signaling pathways and transcriptional regulatory proteins regulated by RA.

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Conflicts of Interest

The authors declare no conflict of interest.

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