Regulation of keratin expression by retinoids

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Abbreviations: AP-1, activating protein-1; EBS, epidermolysis bullosa simplex; EI, epidermolytic ichthyosis; *KRT*, keratin gene; MAPK, mitogen-activated protein kinase; RA, retinoic acid; RAR, retinoic acid receptors; RXR, retinoid X receptors

Vitamin A and its natural and synthetic metabolites (retinoids) affect growth and differentiation of human skin and among the genes affected by retinoids in epidermis are keratin genes. Keratins are intermediate filament proteins that have essential functions in maintaining the structural integrity of epidermis and its appendages. Their expressions are under strict control to produce keratins that are optimally adapted to their environment. In this article, retinoid regulation of keratin expression in cultured human epidermal keratinocytes and in human skin in vivo will be reviewed. The direct and indirect mechanisms involved will be discussed and novel therapeutic strategies will be proposed for utilizing retinoids in skin disorders due to keratin mutations (e.g., epidermolysis bullosa simplex and epidermolytic ichthyosis).

Retinoid Regulation of Gene Transcription

Retinoids, i.e., natural and synthetic analogs of vitamin A, are known to affect proliferation and differentiation of a variety of cells.¹ Most biological activities exerted by retinoids are carried out through binding to specific nuclear retinoid receptors, belonging to a superfamily of receptors which includes steroid, vitamin D, thyroid, peroxisome proliferator-activated and a number of other receptors.²⁻⁴ Retinoic acid receptors (RAR α , β , γ) are ligand-dependent transcription factors which are activated by all-trans retinoic acid (RA). Another family of receptors, retinoid X receptors (RXR α , β , γ), has 9-cis RA as a natural agonist.² Usually RAR and RXR proteins form heterodimer complexes.² Both RARs and RXRs contain several functional domains of two of which are important to achieve gene transcription: the DNA-binding E-domain which mediates the binding of the receptors to the target-genes and the ligand-binding C-domain.³

In human epidermis, the predominant RAR isoforms are RAR γ but low levels of RAR α is also present.⁵ However, the protein levels of RXRs are normally higher than those of RARs with RXR α being the most abundant receptor.⁵ The most common heterodimer complex in human epidermis appears to be RAR γ /RXR α ,⁵ which are localized to the suprabasal layers whereas the

RAR α /RXR α heterodimer is dominating in the basal layer.^{6,7} The RAR/RXR heterodimers are localized to the nucleus where they bind to specific DNA regulatory sequences; retinoic acid response elements (RAREs), which are usually found in the 5' upstream region of the target genes.³ The heterodimer complexes are in their unliganded form accompanied by certain co-repressor molecules which inhibit transcription of target genes (reviewed in ref. 8). When RA binds to RARs a conformational change is induced in the receptor which dissociates the co-repressors and permits the binding of co-activators to the complex.⁸ It also gives RXR the opportunity to bind its ligand, which could further induce the RAR-mediated transcription of the retinoid-regulated gene.⁸⁻¹¹

Another mechanism by which RA and its receptors regulate the differentiation and proliferation of epidermal keratinocytes is by acting as an antagonist of activating protein-1 (AP-1).¹² AP-1 consists of combinations of fos and jun heterodimers, which are well established regulators of keratinocyte differentiation.^{13,14} The activity of AP-1 is determined by the composition of the heterodimer complexes¹⁵ and by posttranscriptional modifications of jun and fos proteins, of which phosphorylation is most important.^{16,17}

Also, reciprocal interactions between RARs and nuclear factor kappaB (NF κ B) has been shown, which can be of relevance when designing retinoids for chemo-preventive and anti-inflammatory interventions.^{18,19} NF κ B transactivation of target genes requires that heterodimers are formed between class I NF κ B proteins (p105/p50 and p100/p52) and class II proteins (RelA, RelB and c-Rel), although some homodimer combinations also are reported to activate gene transcription.²⁰ It is important to note that the antagonistic effects performed by retinoids on AP-1 and NF κ B activity do not require RAREs in the target genes.

Keratin Expression in Normal Human Skin and Cultured Keratinocytes

Recent reports have described the tissue-specific expression of type I (*KRT* 9–20) and type II (*KRT* 1–8) keratin genes, the formation of keratin filaments and the role of other proteins in keratin filament assembly.²¹⁻²⁶ In normal human epidermis, heterodimers of keratin 5 (K5) and K14 form the cytoskeleton of undifferentiated cells in the basal layer, which is replaced in suprabasal cells by K1 and K10, accompanied by K2 in the

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Figure 1. The keratin network in epidermal keratinocytes. The basal dividing cells produce keratin (K) 5 and K14 while differentiating supra-basal cells shut off expression of these keratins and switch to expressing K1 and K10 (and later K2). In the epidermis of palms and soles, K9 is expressed in differentiated keratinocytes. The keratin filaments provide a cellular framework that reaches from the nucleus to the specialized cell-cell junctions called desmosomes and to the specialized cell-substratum junctions called hemi-desmosomes.

Table 1. A summary of reported effects of retinoic acid on keratin mRNA and protein expressions in cultured keratinocytes in vitro and human skin in vivo

Keratin (mRNA or protein)	In vitro cultured keratinocytes	In vitro Organotypic skin	In vivo human skin
<i>KRT1/K1</i>	↓ ^{36,38}	0/↓ ^{61,81}	0/↓ ^{42,47}
<i>KRT2/K2</i>	↑↓ ⁸²	↓ ^{60,61,81}	↓ ⁴⁷
<i>KRT4/K4</i>	↑↓ ⁸²	↑ ^{60,61}	↑ ^{45,47}
<i>KRT5/K5</i>	↓ ^{37,39,40}		0 ⁴²
<i>KRT6/K6</i>	↓ ^{27,37-39}	↓ ⁸¹	↑ ^{41,42,44}
<i>KRT7/K7</i>	↑ ³⁸		
<i>KRT9/K9</i>	↓ ³⁸		
<i>KRT10/K10</i>	↓ ³⁸	↓ ^{61,81,83}	0/↓ ^{42,47}
<i>KRT13/K13</i>	↑ ^{27,38}	↑ ⁶¹	↑ ^{42,45,47}
<i>KRT14/K14</i>	↓ ^{27,37,39}		0 ⁴²
<i>KRT15/K15</i>	↑ ^{27,38}		
<i>KRT16/K16</i>	↓ ^{27,38,39}		↑ ^{41,44}
<i>KRT17/K17</i>	↑ ²⁷ , ↓ ³⁷		↑ ^{41,44}
<i>KRT18/K18</i>	↓ ³⁷	↑ ⁸¹	
<i>KRT19/K19</i>	↑ ^{27,36-38,40}	↑ ^{81,84}	↑ ^{42,45}

The reported changes are indicated by ↓ (reduction), ↑ (induction), ↑↓ (an initial induction followed by a reduction), 0 (no effect) or combinations thereof.

granular layer (Fig. 1).²¹ In palms and soles, the type I keratin K9 is expressed in suprabasal layers.²¹

In cultured keratinocytes, the keratin expression profile differs from normal epidermis. Furthermore, the profile also depends on the culture conditions used. Keratinocytes grown in serum-containing medium and in the presence of feeder cells, express K5, K6, K14, K16, K17 and low levels of K13, K15 and K19,²⁷ whereas in serum-free low-calcium medium containing epidermal growth factor (EGF) they show a more restricted mRNA expression, including *KRT5*, *KRT13*, *KRT14*, *KRT6*

and *KRT17*.²⁸⁻³⁰ Upon differentiation of keratinocytes, e.g., by raising the calcium level with or without the addition of growth factors (EGF and fibroblast growth factor-10) or adding the EGF-receptor inhibitor PD153035, expression of the differentiation-related keratins K1 and K10 are induced in a subpopulation of cells.^{28,30-35}

Retinoid Regulation of Keratins in Cultured Keratinocytes and Normal Skin

Several keratins have been reported to be retinoid regulated in cultured human epidermal keratinocytes and normal human skin. As shown in Table 1, the effect of retinoids on keratins in vitro and in vivo is paradoxical. In vitro, retinoids reduces the expression of *KRT5*, *KRT14*, *KRT6*, *KRT1* and *KRT10* in cultured keratinocytes,³⁶⁻⁴⁰ whereas *KRT7*, *KRT13*, *KRT15* and *KRT19* are induced.^{27,38,40}

By contrast, a different keratin profile is observed when retinoic acid is added to reconstructed human skin in vitro or topically to human skin in vivo. Almost similar effects are generated in the two multilayered tissues including increased expression of K4, K6, K13, K16, K17 and K19 whereas no effects are observed on K5, K14, K1 and K10 proteins.⁴¹⁻⁴⁷ On the other hand *KRT1*, *KRT2*, *KRT10* and *KRT14* mRNA's, are downregulated by retinoid treatment.^{42,43,45-47} In fact, a number of these keratin genes, e.g., *KRT5*, *KRT6*, *KRT14* and *KRT17*, have been reported to carry positive or negative RAREs,⁴⁸⁻⁵⁰ whereas in *KRT2* and *KRT4* genes no RAREs have been identified so far. Thus, it is likely that these latter two retinoid-regulated keratins are among the many genes that are indirectly influenced by retinoids through mechanisms that do not require RAREs.⁵¹

Clearly, keratin gene expression is affected by retinoids in many complex ways, and it is obvious that classical RARE-activation by RAR/RXR heterodimers is not always involved.^{48,50-55} Among the indirect effects associated with the regulation of several keratin genes are interactions with AP-1 and NFκB signaling pathways (see above). Recently it was shown in mouse skin that topical application of the natural compound sulforaphane induced K16 and K17 via AP-1 activation.⁵⁶ Furthermore, using a co-transfection strategy others reported that the upstream regulatory regions of *KRT5*, *KRT6* and *KRT14* genes are *activated* by AP-1 (c-Fos and c-Jun).⁵⁷ On the other hand, certain NFκB proteins, *suppressed* the *KRT5* and *KRT14* promoters while the *KRT6* promoter was *activated* by the p65 NFκB protein.⁵⁷ These results suggest that keratin gene expression might depend on interplay between retinoid signaling with that of AP-1 and/or NFκB proteins, an interplay which is unique to each affected keratin. In the case of *KRT4*, *KRT13* or *KRT2* genes no retinoid response element has been reported yet. However, in the case *KRT2*, an anti-AP-1 mechanism was ruled out since CD2409, an anti-AP1 retinoid without affinity for RARs, did not alter the mRNA expression of this gene (Virtanen M, et al. unpublished observation).

Another possibility, as in the case of regulation of *KRT4* mRNA, is that AP-2 activation is involved. An AP-2 site in the

KRT4 gene was found that increased the activity of a reporter gene in the HaCaT cell line, although an indirect effect was not completely ruled out.⁵⁸ It is also important to keep in mind that retinoid regulation of this and other keratin mRNAs could also involve post-transcriptional changes in mRNA stability as previously noted for *KRT19*.⁴⁰

Results from a recent study also add retinoylation (acylation by RA) to the list of putative mechanisms regulating keratin expression. In this study, retinoic acid was reported to bind to a number of proteins, including K10 and K16.⁵⁹ However, the physiological importance of these direct interactions needs still to be further explored.

Keratins are Regulated by Ligands with RAR-Specificity

The pronounced upregulation of *KRT4* and *KRT13* mRNA and downregulation of *KRT2* by retinoic acid in vivo^{42,45,47} has also been verified in organotypic skin models.^{60,61} Since the effects in vivo and in reconstructed skin in vitro are almost similar, the latter model can be used to explore the role of the various isoforms of RARs in retinoid regulation of keratins. A prerequisite is that the expressions of RAR α , RAR γ and RXR α in organotypic skin are similar to human skin which was recently shown.⁶¹ This suggests that RAR α and RAR γ , but not RAR β mediate the effects of retinoids on keratin expression.⁶¹

Over the last 20 years a number of synthetic retinoids that are highly selective for a single RAR-isoform or two or more isoforms have been developed. In a recent study, it was found that the most potent compounds affecting keratin gene expression were two RAR α agonists and a pan-RAR agonist.⁶¹ One of the two RAR α agonists, Am580/CD336, has previously been shown to downregulate VEGF and NOS-2 with similar efficiency as a pan-RAR agonist.^{62,63} However, the role of RAR α agonists as potent modulators of gene transcription in keratinocytes is obviously not a general phenomenon, since Am580/CD336 was less effective than retinoic acid in increasing the expression of other retinoid-regulated genes in cultured keratinocytes.⁶⁴

Keratin Mutations in Epidermolysis Bullosa Simplex (EBS) and Epidermolytic Ichthyosis (EI)

Almost two decades ago it was disclosed by several groups that EBS and EI are caused by mutations in *KRT5* or *KRT14* and *KRT1* or *KRT10* genes, respectively.^{65,66} The disease-causing mutations are point and dinucleotide mutations and deletions.^{67,68} The mutations are usually located in the conserved domains of the α -helix (1A and 2B),²¹ but can also be found in the non-helical linker regions.⁶⁹ These dominant-negative mutations cause destabilization of the cytoskeleton since the mutant keratin forms a defective heterodimer with its keratin partner. This may cause cells to become less resistant to mechanical trauma and other types of stress, and result in epidermolysis in the basal or suprabasal parts of epidermis in EBS and EI patients, respectively.^{21,70}

Retinoid Therapy for Keratinization Disorders of the Skin

The use of retinoids in keratinization disorders depends on the severity of skin manifestations and the patients' general health, sex, age, etc.⁷¹ Mild forms of ichthyosis can be treated with topical retinoids such as RA (tretinoin) and tazarotene whereas severe cases respond better to oral therapy with synthetic retinoids (acitretin or isotretinoin).⁷¹ Despite being in use for a long time the exact mechanism of action in these disorders is not completely understood. The general hypothesis is that retinoids normalize keratinocyte differentiation. Other possible mechanisms of retinoids could include downregulation of desmosomal proteins, an anti-proliferative effect or regulation of lipid synthesis, growth factors and cytokines.⁷²⁻⁷⁴

In EI, an alternative hypothesis is that retinoids act through the regulation of suprabasal keratins. This is supported by the fact that EI-patients with *KRT10* mutations respond to retinoid treatment, whereas patients with *KRT1* mutations do not respond of unknown reasons.⁷⁵ One explanation might be that patients with *KRT10*-mutations are not as sensitive to the negative effect of retinoid-induced downregulation of both type II keratins (K1 and K2) in the suprabasal compartment as patients with *KRT1*-mutations⁷⁵ or that the mechanism involve the recently reported interaction between retinoids and K10.⁵⁹ However, it would be tempting to suggest that RAR α ligands would be suitable as intervention for epidermolytic keratinopathies due to either dominant negative K2 mutations (i.e., Ichthyosis bullosa of Siemens; IBS) or K1/10 mutations (epidermolytic ichthyosis) where upregulation of K4/13 might compensate for the retinoid-induced reduction in K1/K10. In the case of IBS, pharmacological silencing of the mutated *KRT2* gene by retinoids or Retinoic acid metabolism blocking agents (RAMBAs⁷⁶) should hypothetically be the perfect treatment and IBS-patients actually respond well to low-dosage of synthetic retinoids.⁷⁸ However, in healthy control skin it has been reported that retinoids or RAMBAs reduces the *KRT2* mRNA levels, but less effect was observed at the protein level, thus suggesting involvement of other mechanisms than solely downregulation of *KRT2*.^{47,77}

It is well established that a common adverse effect during retinoid treatment is skin irritation. Interestingly, the retinoid-induced skin irritation has been shown to be a receptor-mediated process involving activation of RAR β and RAR γ , but not RAR α ,^{79,80} which suggests that RAR α agonists could be a better therapeutic option than RAR β/γ agonists from a clinical perspective with respect to both efficacy and tolerance.

In conclusion, the regulation of keratins by retinoids in human keratinocytes is complex and putatively a RAR α -mediated event. An improved knowledge of keratin regulation may be of considerable significance under both physiologic and pathological conditions as well as during retinoid therapy of disorders of keratinization.

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References

- Fisher GJ, Voorhees JJ. Molecular mechanisms of retinoid actions in skin. *FASEB J* 1996; 10:1002-13.
- Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J* 1996; 10:940-54.
- Giguere V. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. *Endocr Rev* 1994; 15:61-79.
- Giguere V. Orphan nuclear receptors: from gene to function. *Endocr Rev* 1999; 20:689-725.
- Fisher GJ, Talwar HS, Xiao JH, Datta SC, Reddy AP, Gaub MP, et al. Immunological identification and functional quantitation of retinoic acid and retinoid X receptor proteins in human skin. *J Biol Chem* 1994; 269:20629-35.
- Karlsson T, Rollman O, Vahlquist A, Törmä H. Immunofluorescence localization of nuclear retinoid receptors in psoriasis versus normal human skin. *Acta Derm Venereol* 2004; 84:363-9.
- Reichrath J, Mittmann M, Kamradt J, Muller SM. Expression of retinoid-X receptors (-alpha,-beta,-gamma) and retinoic acid receptors (-alpha,-beta,-gamma) in normal human skin: an immunohistochemical evaluation. *Histochem J* 1997; 29:127-33.
- Lefebvre P, Martin PJ, Flajollet S, Dedieu S, Billaut X, Lefebvre B. Transcriptional activities of retinoic acid receptors. *Vitam Horm* 2005; 70:199-264.
- Minucci S, Horn V, Bhattacharyya N, Russanova V, Ogryzko VV, Gabriele L, et al. A histone deacetylase inhibitor potentiates retinoid receptor action in embryonal carcinoma cells. *Proc Natl Acad Sci USA* 1997; 94:11295-300.
- Forman BM, Umesono K, Chen J, Evans RM. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell* 1995; 81:541-50.
- Westin S, Kurokawa R, Nolte RT, Wisely GB, McInerney EM, Rose DW, et al. Interactions controlling the assembly of nuclear-receptor heterodimers and co-activators. *Nature* 1998; 395:199-202.
- Schule R, Rangarajan P, Yang N, Klierer S, Ransone LJ, Bolado J, Verma IM, Evans RM. Retinoic acid is a negative regulator of AP-1-responsive genes. *Proc Natl Acad Sci USA* 1991; 88:6092-6.
- Briata P, D'Anna F, Franzini AT, Gherzi R. AP-1 activity during normal human keratinocyte differentiation: evidence for a cytosolic modulator of AP-1/DNA binding. *Exp Cell Res* 1993; 204:136-46.
- Angel P, Szabowski A, Schorpp-Kistner M. Function and regulation of AP-1 subunits in skin physiology and pathology. *Oncogene* 2001; 20:2413-23.
- Cook SJ, Aziz N, McMahon M. The repertoire of fos and jun proteins expressed during the G₁ phase of the cell cycle is determined by the duration of mitogen-activated protein kinase activation. *Mol Cell Biol* 1999; 19:330-41.
- Bannister AJ, Oehler T, Wilhelm D, Angel P, Kouzarides T. Stimulation of c-Jun activity by CBP: c-Jun residues Ser63/73 are required for CBP induced stimulation in vivo and CBP binding in vitro. *Oncogene* 1995; 11:2509-14.
- Deng T, Karin M. c-Fos transcriptional activity stimulated by H-Ras-activated protein kinase distinct from JNK and ERK. *Nature* 1994; 371:171-5.
- Andela VB, Rosier RN. The proteasome inhibitor MG132 attenuates retinoic acid receptor trans-activation and enhances trans-repression of nuclear factor kappaB. Potential relevance to chemo-preventive interventions with retinoids. *Mol Cancer* 2004; 3:8.
- Rockel JS, Kudirka JC, Guzi AJ, Bernier SM. Regulation of Sox9 activity by crosstalk with nuclear factor kappaB and retinoic acid receptors. *Arthritis Res Ther* 2008; 10:3.
- Li Q, Verma IM. NFkappaB regulation in the immune system. *Nat Rev Immunol* 2002; 2:725-34.
- Lane EB, McLean WH. Keratins and skin disorders. *J Pathol* 2004; 204:355-66.
- Rugg EL, Leigh IM. The keratins and their disorders. *Am J Med Genet C Semin Med Genet* 2004; 131:4-11.
- Arin MJ, Mueller FB. Keratins and their associated skin disorders. *Eur J Dermatol* 2007; 17:123-9.
- Pekny M, Lane EB. Intermediate filaments and stress. *Exp Cell Res* 2007; 313:2244-54.
- Arin MJ. The molecular basis of human keratin disorders. *Hum Genet* 2009; 125:355-73.
- Kölsch A, Windoffer R, Leube RE. Actin-dependent dynamics of keratin filament precursors. *Cell Motil Cytoskeleton* 2009; 66:976-85.
- Korge B, Stadler R, Mischke D. Effect of retinoids on hyperproliferation-associated keratins K6 and K16 in cultured human keratinocytes: a quantitative analysis. *J Invest Dermatol* 1990; 95:450-5.
- Brysk MM, Arany I, Brysk H, Chen SH, Calhoun KH, Tying SK. Gene expression of markers associated with proliferation and differentiation in human keratinocytes cultured from epidermis and from buccal mucosa. *Arch Oral Biol* 1995; 40:855-62.
- Leigh IM, Navsaria H, Purkis PE, McKay IA, Bowden PE, Riddle PN. Keratins (K16 and K17) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro. *Br J Dermatol* 1995; 133:501-11.
- Curto EV, Lambert GW, Davis RL, Wilborn TW, Dooley TP. Biomarkers of human skin cells identified using DermArray DNA arrays and new bioinformatics methods. *Biochem Biophys Res Commun* 2002; 291:1052-64.
- Marchese C, Rubin J, Ron D, Faggioni A, Torrisi MR, Messina A, et al. Human keratinocyte growth factor activity on proliferation and differentiation of human keratinocytes: differentiation response distinguishes KGF from EGF family. *J Cell Physiol* 1990; 144:326-32.
- Poumay Y, Pittelkow MR. Cell density and culture factors regulate keratinocyte commitment to differentiation and expression of suprabasal K1/K10 keratins. *J Invest Dermatol* 1995; 104:271-6.
- Pol A, Bergers M, van Ruissen F, Pfundt R, Schalkwijk J. A simple technique for high-throughput screening of drugs that modulate normal and psoriasis-like differentiation in cultured human keratinocytes. *Skin Pharmacol Appl Skin Physiol* 2002; 15:252-61.
- Sugiura K, Muro Y, Futamura K, Matsumoto K, Hashimoto N, Nishizawa Y, et al. The unfolded protein response is activated in differentiating epidermal keratinocytes. *J Invest Dermatol* 2009; 129:2126-35.
- Chamcheu JC, Pihl-Lundin I, Mouyobo CE, Gester T, Virtanen M, Moustakas A, et al. Immortalized keratinocytes derived from epidermolytic ichthyosis patients reproduce the disease phenotype: a useful in vitro model for testing new treatments. *Br J Dermatol* 2011; 164:263-72.
- Fuchs E, Green H. Regulation of terminal differentiation of cultured human keratinocytes by vitamin A. *Cell* 1981; 25:617-25.
- Gilfix BM, Eckert RL. Coordinate control by vitamin A of keratin gene expression in human keratinocytes. *J Biol Chem* 1985; 260:14026-9.
- Lee DD, Stojadinovic O, Krzyzanowska A, Vouthounis C, Blumenberg M, Tomic-Canic M. Retinoid-responsive transcriptional changes in epidermal keratinocytes. *J Cell Physiol* 2009; 220:427-39.
- Stellmach V, Leask A, Fuchs E. Retinoid-mediated transcriptional regulation of keratin genes in human epidermal and squamous cell carcinoma cells. *Proc Natl Acad Sci USA* 1991; 88:4582-6.
- Crowe DL. Retinoic acid mediates post-transcriptional regulation of keratin 19 mRNA levels. *J Cell Sci* 1993; 106:183-8.
- Eichner R, Kahn M, Capetola RJ, Gendimenico GJ, Mezick JA. Effects of topical retinoids on cytoskeletal proteins: implications for retinoid effects on epidermal differentiation. *J Invest Dermatol* 1992; 98:154-61.
- Rosenthal DS, Griffiths CE, Yuspa SH, Roop DR, Voorhees JJ. Acute or chronic topical retinoic acid treatment of human skin in vivo alters the expression of epidermal transglutaminase, loricrin, involucrin, filaggrin and keratins 6 and 13 but not keratins 1, 10 and 14. *J Invest Dermatol* 1992; 98:343-50.
- Brown LJ, Geesin JC, Rothnagel JA, Roop DR, Gordon JS. Retinoic acid suppression of loricrin expression in reconstituted human skin cultured at the liquid-air interface. *J Invest Dermatol* 1994; 102:886-90.
- Eichner R, Gendimenico GJ, Kahn M, Mallon JP, Capetola RJ, Mezick JA. Effects of long-term retinoic acid treatment on epidermal differentiation in vivo: specific modifications in the programme of terminal differentiation. *Br J Dermatol* 1996; 135:687-95.
- Steijlen PM, Happle R, van Muijen GN, van de Kerkhof PC. Topical treatment with 13-cis-retinoic acid improves Darier's disease and induces the expression of a unique keratin pattern. *Dermatologica* 1991; 182:178-83.
- Steijlen PM, Reifenschweiler DO, Ramaekers FC, van Muijen GN, Happle R, Link M, et al. Topical treatment of ichthyoses and Darier's disease with 13-cis-retinoic acid. A clinical and immunohistochemical study. *Arch Dermatol Res* 1993; 285:221-6.
- Virtanen M, Torma H, Vahlquist A. Keratin 4 upregulation by retinoic acid in vivo: a sensitive marker for retinoid bioactivity in human epidermis. *J Invest Dermatol* 2000; 114:487-93.
- Tomic-Canic M, Sunjevaric I, Freedberg IM, Blumenberg M. Identification of the retinoic acid and thyroid hormone receptor-responsive element in the human K14 keratin gene. *J Invest Dermatol* 1992; 99:842-7.
- Navarro JM, Casarotres J, Jorcano JL. Elements controlling the expression and induction of the skin hyperproliferation-associated keratin K6. *J Biol Chem* 1995; 270:21362-7.
- Radoja N, Diaz DV, Minars TJ, Freedberg IM, Blumenberg M, Tomic-Canic M. Specific organization of the negative response elements for retinoic acid and thyroid hormone receptors in keratin gene family. *J Invest Dermatol* 1997; 109:566-72.
- Balmer JE, Blomhoff R. Gene expression regulation by retinoic acid. *J Lipid Res* 2002; 43:1773-808.
- Lu B, Rothnagel JA, Longley MA, Tsai SY, Roop DR. Differentiation-specific expression of human keratin 1 is mediated by a composite AP-1/steroid hormone element. *J Biol Chem* 1994; 269:7443-9.
- Eckert RL, Welter JF. Transcription factor regulation of epidermal keratinocyte gene expression. *Mol Biol Rep* 1996; 23:59-70.
- Tomic-Canic M, Freedberg IM, Blumenberg M. Codominant regulation of keratin gene expression by cell surface receptors and nuclear receptors. *Exp Cell Res* 1996; 224:96-102.
- Jho SH, Vouthounis C, Lee B, Stojadinovic O, Im MJ, Brem H, et al. The book of opposites: the role of the nuclear receptor co-regulators in the suppression of epidermal genes by retinoic acid and thyroid hormone receptors. *J Invest Dermatol* 2005; 124:1034-43.
- Kerns M, DePianto D, Yamamoto M, Coulombe PA. Differential modulation of keratin expression by sulforaphane occurs via Nrf2-dependent and -independent pathways in skin epithelia. *Mol Biol Cell* 2010; 21:4068-75.
- Ma S, Rao L, Freedberg IM, Blumenberg M. Transcriptional control of K5, K6, K14 and K17 keratin genes by AP-1 and NFkappaB family members. *Gene Expr* 1997; 6:361-70.
- Wanner R, Zhang J, Dorbic T, Mischke D, Henz BM, Wittig B, Rosenbach T. The promoter of the HaCaT keratinocyte differentiation-related gene keratin 4 contains a functional AP-2 binding site. *Arch Dermatol Res* 1997; 289:705-8.
- Takahashi N, Fujii Y. Cytokeratins 16 and 10 bind to retinoic acid covalently in skin tissue of mice. *Br J Dermatol* 2010; 162:974-9.

60. Pavez Lorie E, Chamcheu JC, Vahlquist A, Törmä H. Both all-trans retinoic acid and cytochrome P450 (CYP26) inhibitors affect the expression of vitamin A metabolizing enzymes and retinoid biomarkers in organotypic epidermis. *Arch Dermatol Res* 2009; 301:475-85.
61. Virtanen M, Sirsjö A, Vahlquist A, Törmä H. Keratins 2 and 4/13 in reconstituted human skin are reciprocally regulated by retinoids binding to nuclear receptor RARalpha. *Exp Dermatol* 2010; 19:674-81.
62. Diaz BV, Lenoir MC, Ladoux A, Frelin C, Demarchez M, Michel S. Regulation of vascular endothelial growth factor expression in human keratinocytes by retinoids. *J Biol Chem* 2000; 275:642-50.
63. Sirsjö A, Gidlöf AC, Olsson A, Törmä H, Ares M, Kleinert H, et al. Retinoic acid inhibits nitric oxide synthase-2 expression through the retinoic acid receptor-alpha. *Biochem Biophys Res Commun* 2000; 270:846-51.
64. Yoshimura K, Uchida G, Okazaki M, Kitano Y, Harii K. Differential expression of heparin-binding EGF-like growth factor (HB-EGF) mRNA in normal human keratinocytes induced by a variety of natural and synthetic retinoids. *Exp Dermatol* 2003; 12:28-34.
65. Ishida-Yamamoto A, McGrath JA, Chapman SJ, Leigh IM, Lane EB, Eady RA. Epidermolysis bullosa simplex (Dowling-Meara type) is a genetic disease characterized by an abnormal keratin-filament network involving keratins K5 and K14. *J Invest Dermatol* 1991; 97:959-68.
66. Ishida-Yamamoto A, McGrath JA, Judge MR, Leigh IM, Lane EB, Eady RA. Selective involvement of keratins K1 and K10 in the cytoskeletal abnormality of epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma). *J Invest Dermatol* 1992; 99:19-26.
67. Joh GY, Traupe H, Metze D, Nashan D, Huber M, Hohl D, et al. A novel dinucleotide mutation in keratin 10 in the annular epidermolytic ichthyosis variant of bullous congenital ichthyosiform erythroderma. *J Invest Dermatol* 1997; 108:357-61.
68. McLean WH, Morley SM, Higgins C, Bowden PE, White M, Leigh IM, Lane EB. Novel and recurrent mutations in keratin 10 causing bullous congenital ichthyosiform erythroderma. *Exp Dermatol* 1999; 8:120-3.
69. Kremer H, Lavrijsen AP, McLean WH, Lane EB, Melchers D, Ruiter DJ, et al. An atypical form of bullous congenital ichthyosiform erythroderma is caused by a mutation in the L12 linker region of keratin 1. *J Invest Dermatol* 1998; 111:1224-6.
70. Ross R, DiGiovanna JJ, Capaldi L, Argenyi Z, Fleckman P, Robinson-Bostom L. Histopathologic characterization of epidermolytic hyperkeratosis: a systematic review of histology from the National Registry for Ichthyosis and Related Skin Disorders. *J Am Acad Dermatol* 2008; 59:86-90.
71. Vahlquist A, Gänemo A, Virtanen M. Congenital ichthyosis: an overview of current and emerging therapies. *Acta Derm Venereol* 2008; 88:4-14.
72. Gatto H, Richard MH, Viac J, Charveron M, Schmitt D. Effects of retinoic acid on interleukin-1 alpha and -1beta expression by normal human keratinocytes cultured in defined medium. *Skin Pharmacol* 1993; 6:10-9.
73. Humphries JD, Parry EJ, Watson RE, Garrod DR, Griffiths CE. All-trans retinoic acid compromises desmosome expression in human epidermis. *Br J Dermatol* 1998; 139:577-84.
74. Xiao JH, Feng X, Di W, Peng ZH, Li LA, Chambon P, Voorhees JJ. Identification of heparin-binding EGF-like growth factor as a target in intercellular regulation of epidermal basal cell growth by suprabasal retinoic acid receptors. *EMBO J* 1999; 18:1539-48.
75. Virtanen M, Gedde-Dahl T Jr, Mork NJ, Leigh I, Bowden PE, Vahlquist A. Phenotypic/genotypic correlations in patients with epidermolytic hyperkeratosis and the effects of retinoid therapy on keratin expression. *Acta Derm Venereol* 2001; 81:163-70.
76. Verfaillie CJ, Borgers M, van Steensel MA. Retinoic acid metabolism blocking agents (RAMBAs): a new paradigm in the treatment of hyperkeratotic disorders. *J Dtsch Dermatol Ges* 2008; 6:355-64.
77. Pavez Lorie E, Cools M, Borgers M, Wouters L, Shroot B, Hagforsen E, et al. Topical treatment with CYP26 inhibitor talarozole (R115866) dose dependently alters the expression of retinoid-regulated genes in normal human epidermis. *Br J Dermatol* 2009; 160:26-36.
78. Steijlen PM, van Dooren-Greebe RJ, Happle R, Van de Kerkhof PC. Ichthyosis bullosa of Siemens responds well to low-dosage oral retinoids. *Br J Dermatol* 1991; 125:469-71.
79. Chen S, Ostrowski J, Whiting G, Roalsvig T, Hammer L, Currier SJ, et al. Retinoic acid receptor gamma mediates topical retinoid efficacy and irritation in animal models. *J Invest Dermatol* 1995; 104:779-83.
80. Standeven AM, Teng M, Chandraratna RA. Lack of involvement of retinoic acid receptor alpha in retinoid-induced skin irritation in hairless mice. *Toxicol Lett* 1997; 92:231-40.
81. Bernard FX, Pedretti N, Rosdy M, Deguercy A. Comparison of gene expression profiles in human keratinocyte mono-layer cultures, reconstituted epidermis and normal human skin; transcriptional effects of retinoid treatments in reconstituted human epidermis. *Exp Dermatol* 2002; 11:59-74.
82. Lee DD, Zavadi J, Tomic-Canic M, Blumenberg M. Comprehensive transcriptional profiling of human epidermis, reconstituted epidermal equivalents and cultured keratinocytes using DNA microarray chips. *Methods Mol Biol* 2010; 585:193-223.
83. Kopan R, Traska G, Fuchs E. Retinoids as important regulators of terminal differentiation: examining keratin expression in individual epidermal cells at various stages of keratinization. *J Cell Biol* 1987; 105:427-40.
84. Asselineau D, Darmon M. Retinoic acid provokes metaplasia of epithelium formed in vitro by adult human epidermal keratinocytes. *Differentiation* 1995; 58:297-306.