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**ARTICLE**

# Immunohistochemical Localization of Transforming Growth Factor-α and Epithelial Growth Factor Receptor in Human Fetal Developing Skin, Psoriasis and Restrictive Dermopathy

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**Keratinocytes release a number of cytokines interacting with other intra- and subepidermal cells during the initiation and the perpetuation of skin inflammatory reactions. Cultured human keratinocytes overexpressing the transforming growth factor alpha (TGF-alpha) assumed a spindled morphology and displayed increased locomotion. Moreover, the receptor for TGF-alpha, the epithelial growth factor receptor (EGFR), is important for autocrine growth, promotion of cell survival, and regulation of cell migration. The expression of TGF-alpha and EGFR has not been widely studied in human developing skin and their roles in genodermatosis are not known. In this study, we investigated the expression of TGF-alpha and EGFR by immunohistochemistry in human developing skin at different gestational ages (14th week, 20th week, and 34th week), in six patients with psoriasis, and, for the first time, in an infant affected with restrictive dermopathy, a very rare lethal genodermatosis, characterized by abnormal skin growth and differentiation with thin, tightly adherent skin. TGF-alpha and EGFR were expressed in the basal layer at the 14th week and in all epidermal layers at the 20th and 34th week of gestation. In psoriasis, TGF-alpha was overexpressed in all layers of epidermis, while EGFR was expressed in the basal and first suprabasal layers. In restrictive dermopathy, we observed no expression of both TGF-alpha and EGFR at the level of the skin. The other organs showed comparable patterns to those of an age-matched infant. In conclusion, TGF-alpha and EGFR interact strictly to promote skin development during the intrauterine life. An interactive autocrine growth cycle between TGF-alpha and EGFR is present in psoriasis. A skin-localized alteration of the expression of TGF-alpha and EGFR may be at the basis of restrictive dermopathy. The delay of growth and differentiation of the skin in restrictive dermopathy may be related to the absent expression of TGF-alpha, which is probably due to a down regulation of EGFR by an abnormal autocrine mechanism.** (Pathology Oncology Research Vol 6, No 4, 250–255, 2000)

*Keywords:* TGF-α, EGFR, developing skin, psoriasis, genodermatosis

## *Introduction*

Keratinocytes release a number of cytokines interacting with other intra- and subepidermal cells during the initiation and the perpetuation of skin inflammatory reactions. Cultured human keratinocytes overexpressing the transforming growth factor alpha (TGF-alpha), a 50-amino acid polypeptide, assumed a spindled morphology and displayed increased locomotion. TGF-alpha is believed to play an important part in cell proliferation and differentiation via an autocrine mechanism showing 35% sequence homology and a nearly identical spectrum of biological activities with EGF.[4,5,8] The receptor of TGF-alpha, the epithelial growth factor receptor (EGFR), is a protein tyrosine kinase that mediates the signal transduction playing an important role for autocrine growth, promotion of cell survival, and regulation of cell migration.[4,5,8]

It is known that both TGF-alpha and EGFR are overexpressed in psoriasis,[2] but the expression of TGF-alpha and

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*Table 1.* **Clinical data of the patients with psoriasis**

| *No.* | *Type* | *Age, Sex* | *Localization* |
|---|---|---|---|
| 1 | P. vulgaris | 71 years, M | D III (hand), dorsal |
| 2 | P. vulgaris | 66 years, M | Right thorax |
| 3 | P. vulgaris | 63 years, M | Right patella |
| 4 | P. vulgaris | 76 years, M | Elbow |
| 5 | P. vulgaris | 71 years, M | Right pretibial |
| 6 | P. exudativa* | 58 years, F | Right gluteus |

*Notes:* P.: psoriasis, M: male, F: female, D: digit, * or pustular psoriasis

EGFR has not been widely studied in human developing skin and their roles in genodermatosis are not absolutely known. In this study, we investigated the immunohistochemical localization of both TGF-alpha and EGFR in human developing skin, psoriasis, and restrictive dermopathy (RD), a vary rare genodermatosis with autosomal recessive inheritance characterized by an extremely taut skin resulting in generalized joint contractures and a peculiar facial appearance with small, fixed, O-shaped mouth, low-set ears, and micrognathia.[7]

***Subjects***

We studied skin sections from three fetuses of the 14th week, 20th and 34th week of gestation, five skin biopsies from five patients with classic psoriasis or psoriasis vulgaris, one skin biopsy from one patient with pustular psoriasis (clinical data are presented in *Table 1*) and one patient with restrictive dermopathy. This patient was a small-for-date girl weighing 1730g admitted to the neonatal intensive care unit of the University Hospital Heidelberg following a caesarian section at 34 weeks' gestation to non-consanguineous parents. On admission, the baby showed skin with erosions, generalized flexion joint contractures, a small, fixed, O-shaped mouth, downward slanting palpebral fissures and hypertelorism, pinched nose, low-set dysplastic ears, and micrognathia, reminiscent of a "porcelain doll" (*Figure 1*). Despite massive intensive care, no stable oxygen saturation could be maintained and the baby died of respiratory failure on the 6th day after birth. Chromosomal analysis revealed a numerically and structurally normal female karyotype. At autopsy, the infant had a tense and fragile skin with many erosions. Head circumference was 31.5 cm and showed wide-

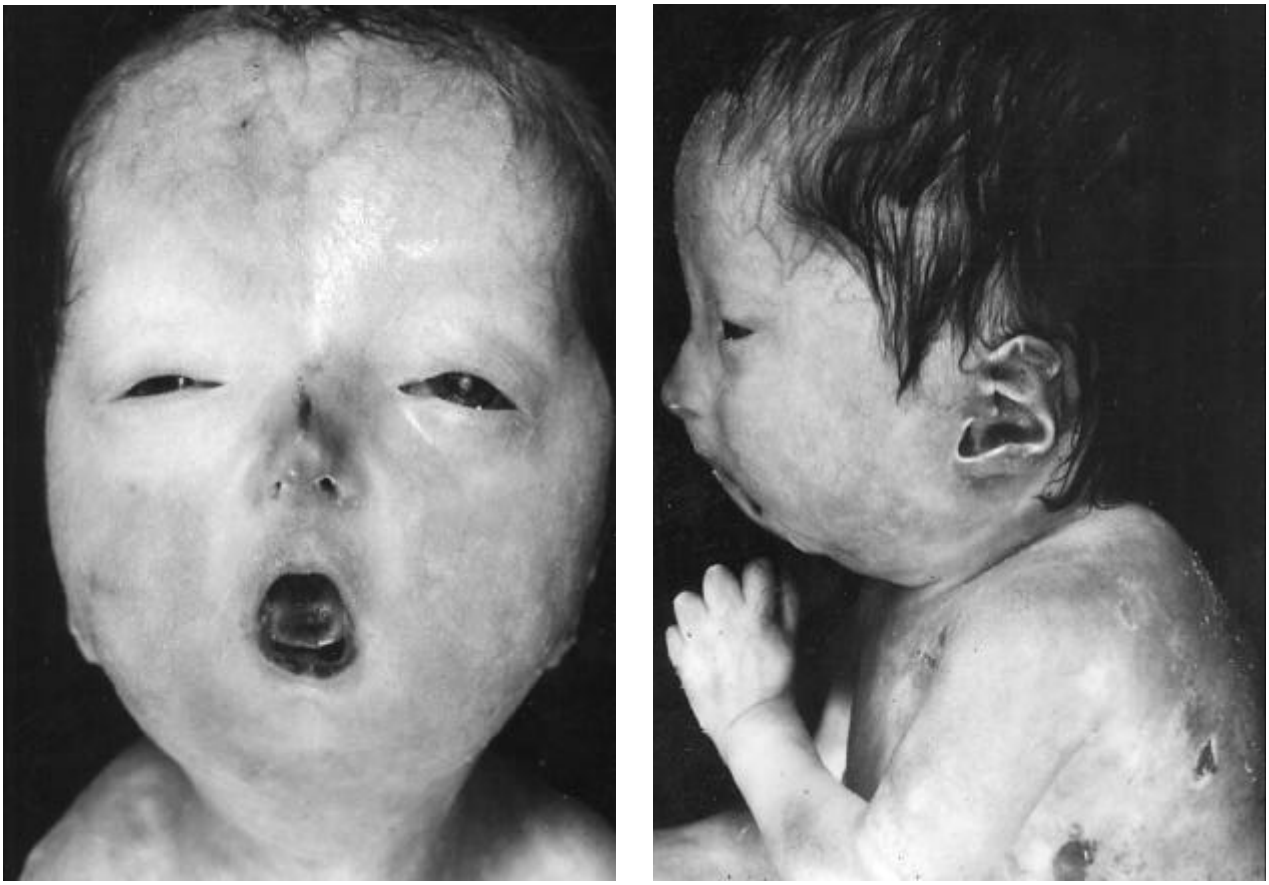

*Figure 1. Postmortem view of the infant with restrictive dermopathy showing skin erosions, a small, fixed, O-shaped mouth, downward slanting palpebral fissures and hypertelorism, pinched nose, low-set dysplastic ears, and micro- and retrogenia.*

ly patent fontanels and skull sutures. Roentgenogram of the calvaria showed bilateral hypoplasia of the parietal bone. Lungs were atelectatic (lung weight: 12 g at left, 14 g at right; normal combined lung weight at 34 weeks of gestation: 33.5 ± 16.5). There was a dilatation of the right heart with passive blood congestion of the abdominal organs. Muscle, brain and spinal cord were normal for gestational age. Skin thickness depended on the body region. On the thorax, the epidermis was 5-8 cells thick, whereas it was 7-9 cells thick on the scalp. An age-matched infant (fetus of the 34$^{th}$ week of gestation) showed an epidermis of 3-5 cells on the thorax. The stratum corneum was thin and intermingled with parakeratosis, while the stratum granulosum contained globular, coarse, keratohyalin granules. The dermo-epidermal junction was flat with complete lack of rete pegs. The corneal layer of the age-matched infant showed a basket-weave arrangement of the most differentiated keratinizing cells and the dermo-epidermal junction showed rete pegs. The dermis was compact with collagen bundles orientated parallel to the surface. In the dermis there were no elastic fibers, except in the vessel walls. The sweat glands and pilo-sebaceous structures were undifferentiated and very few hair shafts were identified with the exception of the scalp. The hypodermis did not show any structural abnormality. Elastic fibers were seen in the dermis of the age-matched control fetus. No differences were seen in the examination of elastic and collagen fibers in the subendocardium of the heart, in the peripheral branches of the pulmonary artery, in the capsule and septa of the spleen as well as in the portal tracts of the liver of the propositus as compared with age-matched control samples.

To investigate the interrelation of TGF-alpha with EGFR during developing skin and to determine the underlying pathogenic mechanism in RD, we studied the protein expression of TGF-alpha (anti-TGF-alpha antibody, Oncogene Research Products, Cambridge, MA, USA) and its receptor (anti-EGFR antibody, Merck, Darmstadt, Germany) by immunohistochemistry. We used the Avidin-Biotin peroxidase Complex (Vector Labs, Burlingame, CA, USA) as immunolabeling method. The ABC method was compared and confirmed with the Catalyzed Signal Amplification method (Dako CSA, Dako, Hamburg, Germany) according to.[11] Antigen unmasking procedures (750-W for 20 min in 10 mmol/L citric acid at pH 6.0) were performed. The stages of skin growth and differentiation are grouped in *Table 2.*

### *Results*

The examination of the skin of a fetus at the 14$^{th}$ week of gestation showed an epidermal layer composed of basal cells, three-layered stratum intermedium composed of glycogenated intermediate cells, and a superficial periderm composed of cell undergoing keratinization and desquamation (the exfoliated peridermal cells forming part of the vernix caseosa). The dermal-epidermal junction underlying the dermis was flat. Some mesenchymal condensations of crowded basal cells at the dermal-epidermal junction were also seen. At the 20$^{th}$ week of gestation a stratified epithelium (stratum spinosum) occurred with major differentiation of the cutaneous appendages. At the 20$^{th}$ week of gestation some peridermal zones were adjacent to corneal layer zones. At the

*Table 2.* **Steps of the Embryogenesis of the Skin and its Appendages**

The skin is composed of two layers, the epidermis and the dermis, that are derived from two diferent germ layers: ectoderm and mesoderm.

| *Age of gestation* | *Stage* | |
|---|---|---|
| 4 wks | Ectoderm and mesoderm | Monolayer of *ectodermal* cells underlying the *mesoderm*. |
| 7 wks | Periderm | *Periderm*, *basal layer*, *mesenchyma* with colonization by neural crest-derived dendritic cells (melanocytes) between 40 and 50 days.. |
| 11 wks | Epidermal stratification | *Periderm*, *intermediate layer*, *basal layer*, *epidermal / dermal ridges*, *developing collagenous and elastic fibers in the dermis.* |
| 21 wks | Periderm shedding | Complete shedding of *periderm* and formation of the *corneal layer*. |
| | Skin appendages | Development of *hair follicle* at 10 wks by mesenchymal condensations beneath budding groups of crowded basal cells.<br>Development of the *sebaceous glands* at 14-15 wks and *apocrine glands* at 20-24 wks from the outer sheath of hair follicles.<br>Development of the *eccrine sweat glands* at 20 wks as epidermal dowgrowths into the underlying mesenchyma. |

*Notes:* wks: weeks. Table according to Urmacher CD. "Cutaneous Tissue" In: Histology for Pathologists. Sternberg SS (ed). Lippincott- Raven Publishers Philadelphia – New York 2$^{nd}$ edition 25-45, 1997 and to Moore KL, Persaud TVN. "The Integumentary System" In: The Developing Human. W.B. Saunders Company, 1998.

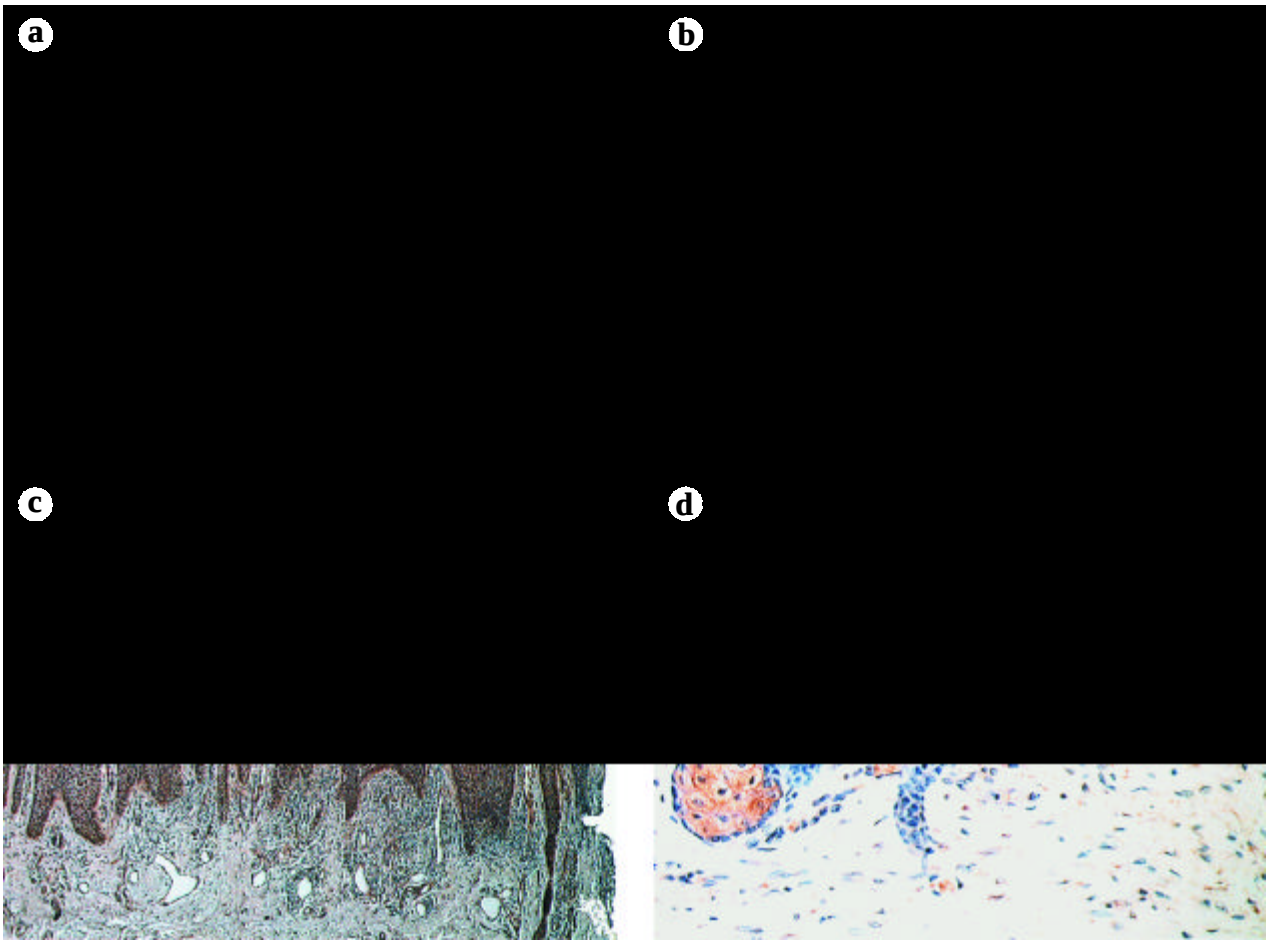


*Figure 2 a. Skin of a fetus of the 14th week of gestation showing a weak immunostaining for TGF-a localized at the basal layer (stratum germinativum) of the epidermis. Little membranous staining is also seen in the other layers (intermediate layer) of the epidermis (anti-TGF-a immunostain, ×200 original magnification). b. Skin of a fetus of the 34th week of gestation showing strong immunostaining for TGF-a throughout the epidermis (anti-TGF-a immunostain, ×200 original magnification). c. Skin of a patient with psoriasis (case no. 6) showing prominent acanthosis with elongation of the rete pegs and strong overexpression of TGF-a. Superficially, the formation of conspicuous Munro microabscesses with transmigration of polymorphonuclear leukocytes through the reactive epidermis (anti-TGF-a immunostain, × 50 original magnification). d. Skin of the infant with restrictive dermopathy showing no expression of TGF-a with the exception of some adnexal appendages (anti-TGF-a immunostain, × 200 original magnification).*

34th week of gestation the stratum corneum was evident thoroughly.

TGF-alpha showed a vertical progressive increase of the expression in the skin of fetuses of different ages of gestation. At 14 weeks the immunostaining was weak and localized at the basal cells and at the junctions of the cells of the intermediate layer (*Figure 2a*). A progressive expression in the cytoplasm of the cells of the intermediate layer was seen at 20 weeks and at 34 weeks of gestation (*Figure 2b*). In all six biopsy specimens with psoriasis, we found parakeratosis, prominent acanthosis and basal cell hyperproliferation. In the pustular psoriasis (psoriasis exudativa) there were Munro microabscesses. In both psoriasis vulgaris and psoriasis exsudativa, we detected a strong overexpression of TGF-alpha (*Figure 2c*). In RD no expression of TGF-alpha could be identified with the exception of very few adnexal appendages (*Figure 2d*).

EGFR expression was seen at 14 weeks with a not uniform membranous staining at basal and intermediate layers. At 20 weeks EGFR staining was particularly accentuated at the basal layer. At 34 weeks strong EGFR expression was seen throughout the epidermis (*Figure 3a*). In psoriasis, EGFR was expressed at the basal and deepest layers of the stratum spinosum (*Figure 3b*). In RD no expression of EGFR was seen *(Figure 3c).* The other organs of the patient with RD showed similar immunolabeling patterns as the age-matched control fetus. No staining was obtained when phosphate-buffered saline or nonimmune serum was used instead of the primary antibodies.

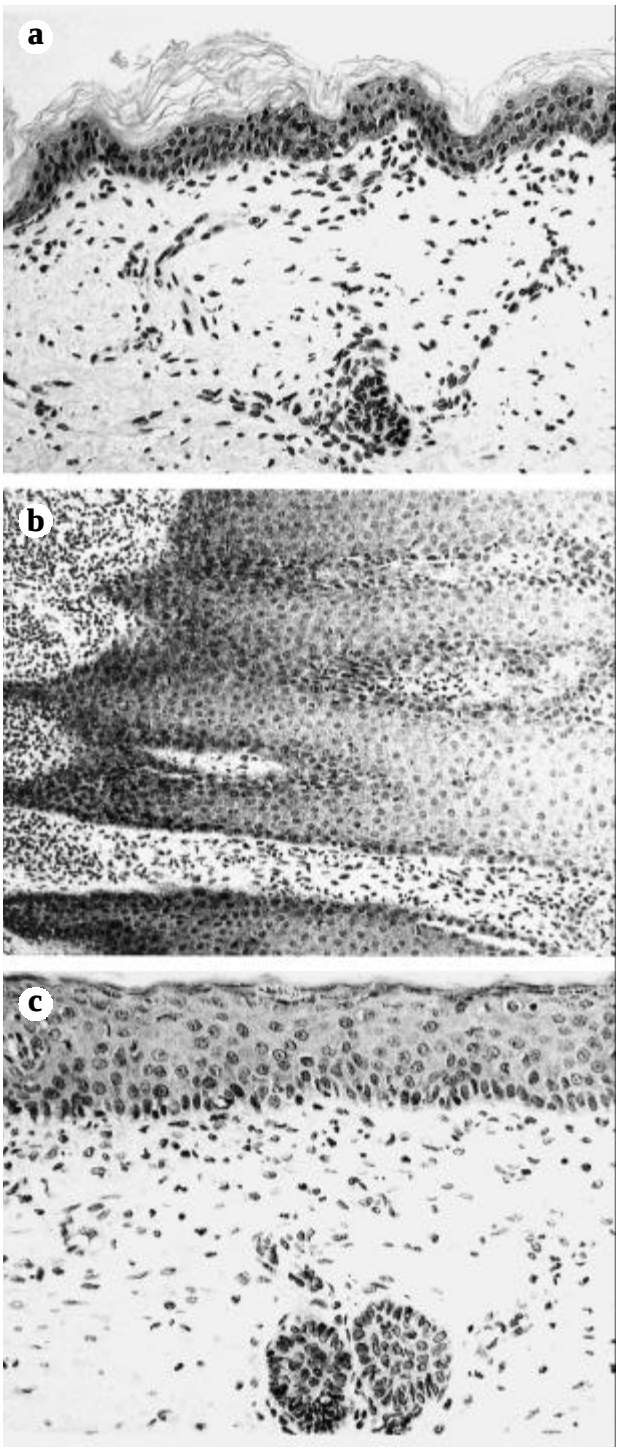


*Figure 3. a. Skin of a fetus of the 34th week of gestation showing EGFR expression throughout the epidermis (anti-EGFR immunostain, × 200 original magnification). b. Skin of a patient with psoriasis (case no. 6) showing EGFR expression at the basal layer and the deepest layers of the stratum spinosum (anti-EGFR immunostain, × 125 original magnification). c. Skin of the infant with restrictive dermopathy showing no expression of EGFR (anti-EGFR immunostain, × 200 original magnification).*

***Discussion***

Free fetal movements are necessary for a normal intrauterine development of the fetus. In fetuses with reduced movements a complex of severe consequences occurs that have been termed fetal akinesia deformation sequence.[7] To this group belongs RD, which has to be distinguished from other forms, such as the Pena-Shokeir syndrome (I-II) and the Neu-Laxova syndrome. In contrast to RD, these forms do not present tautness of the skin, but the reduction of the body movements is due to a primary defect of the central nervous system (hypoxic brain damage, microcephaly, lack of anterior horn cells in the spinal cord).

A few hypotheses about the pathogenesis of RD have been proposed.[6] The clinical and histological features were variably interpreted either as the expression of an ectodermal dysplasia[14] or as a more specific morphogenetic alteration of the epidermis and dermis.[3] Holbrook et al[3] found an abnormal differentiation of keratins with arrested development of hair follicles and eccrine sweat glands and suggested an underlying disorder of skin differentiation, involving dermal-epidermal interactions. Impaired skin maturation was also demonstrated by the weak or no labeling of cytokeratins with antibodies. Witt et al. found a decreased amount of high molecular weight keratins in the skin of a brother and a sister with RD.[14] Dean et al[1] proposed that RD may be a disorder of skin differentiation due to an impairment of the cell adhesion, but Sillevis Smitt et al[13] showed no abnormal alpha1beta1 and alpha2beta1 integrin expression in fibroblast cultures.

We found an abnormal growth of the skin due to the absence of the immunolabeling of TGF-alpha and EGFR throughout the epidermis, a pattern similar to that of very early skin differentiation stages. No extra-cutaneous tissue showed a similar pattern indicating that this disease is very probably localized only to the skin. TGF-alpha is a close relative of EGF and like EGF, exerts its effects on cells through binding to the EGFR. EGFR is present at early stages of human developing skin.[9] We found EGFR expression as early as at 14 weeks of gestation, but a strong expression was found after the periderm shedding. In psoriasis, TGF-alpha was overexpressed in all layers of epidermis, while EGFR was expressed in the basal and first suprabasal layers.

Imbalance between amplification of TGF-alpha expression and an inadequate mechanism of growth inhibition may be relevant in the pathogenesis of psoriasis. Receptor tyrosine kinases form a large and important class of cell-surface receptors whose ligands are soluble or membrane-bound peptide or protein hormones. Post-translational changes of the EGFR in the skin of patients with RD may be able to down-regulate this receptor and (to produce) an inhibition of the growth and differentiation of the skin

through an altered autocrine signaling for TGF-alpha. Organogenesis and histogenesis are regulated at least in part through interactions between mesenchymal and endodermal tissues. Such interactions may be mediated through expression of genes for soluble signaling molecules, extracellular matrix proteins, and their receptors. Abnormal expression of such genes may result in abnormal organo- and histogenesis.[12]

TGF-alpha transgenic mice have been created by gene targeting in embryonic stem cell.[10] Sandgren et al. created a TGF-alpha transgenic line MT-TGF- -mini 1745-1758 (Tg (Mt-I, Tgf-alpha) Bril4c) by injecting a transgene consisting of the metallothionein-I enhancer/promoter (MT), a TGF-alpha cDNA/genomic fusion sequence carrying introns 4 and 5, and a human growth hormone noncoding sequence containing the poly(A) adenylation site into fertilized mouse eggs. The MT promoter is inducible with $Zn^{2+}$ and has been proven to be transcriptionally active in many adult tissues.[10] These authors showed a hyperplasia of adult liver, pancreas, stomach, small intestine, coecum, and colon after induction with $Zn^{2+}$ However, the possibility that other genes may be overexpressed as a result of the engineered deletion represents one limitation of the use of null mutation (knockout) strains. Probably growth factors inhibitors present in the amniotic fluid of infants with RD may play a role in inhibiting the well-orchestrated development of skin.

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