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Function of AP-1 target genes in mesenchymal–epithelial cross-talk in skin

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Abstract

An increasing number of examples on the importance of mesenchymal–epithelial interactions in physiological (e.g. embryonic development) and pathological (tumourigenesis) processes have been described. This is best illustrated in the skin, where the well-controlled balance of keratinocyte proliferation and differentiation forms the basis for a proper histoarchitecture of the epidermis. Here, a double paracrine loop of cytokines, which are synthesised and secreted by cells of the epidermis (keratinocytes) and the underlying dermis (fibroblasts) seems to play a major role. The aim of this commentary is to review research that has investigated the role of specific subunits of transcription factor AP-1 (Jun/Fos) in this regulatory network. Using an *in vitro* skin equivalent model strong evidence was provided for a critical and specific function of c-Jun and JunB in mesenchymal–epithelial interaction in the skin by regulating the expression of interleukin-1 (IL-1)-induced keratinocyte growth factor (KGF) and GM-CSF in fibroblasts. These factors, in turn, adjust the balance between proliferation and differentiation of keratinocytes ensuring proper architecture of the epidermis. This commentary will summarise our current knowledge on the molecular mechanisms underlying AP-1-dependent mesenchymal–epithelial interactions and discuss the physiological relevance of these *in vitro* findings in skin physiology and pathology.

Keywords: AP-1; Fos; Jun; Proliferation; Differentiation; Target genes; Tumourigenesis; Wound healing; Keratinocytes; Fibroblasts

1. Structural and functional properties of the transcription factor AP-1

A tight balance of regulatory processes that lead to cell proliferation, differentiation or programmed cell death (apoptosis) determines the integrity of an organism. Under the influence of defined signal transduction pathways individual members of the AP-1 family (Jun, Fos ATF proteins) of transcription factors seems to be critically involved in the regulation of these phenotypes by addressing specific yet unknown target genes. The central role of AP-1 members is explained by their responses to numerous extracellular stimuli and by the observation that Jun, Fos and ATF proteins become active in many biological processes, such as embryonic development, tissue remodelling, tumourigenesis, inflammation and apoptosis, e.g. in response to radiation or chemical carcinogens. We must therefore expect that each AP-1 transcription factor has defined target promoters and target genes. To execute their functions in the proposed biological processes each AP-1 factor represents a homo- or heterodimer of members of the Jun, Fos and ATF proteins subfamilies. The formation of each dimer strictly depends on their relative dimerisation affinities and the relative abundance of each of the Jun, Fos and ATF proteins available in the cell at a given time. Additional diversity is generated by specific posttranslational modifications generated by signal transduction pathways to individual subunits of the transcription factors. There is strong evidence both from tissue culture experiments and knockout mice that specific members of the MAP kinase family (Erks, JNK/SAPKs, p38) selectively

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Abbreviations: AP-1, activator protein 1; IL, interleukin; KGF, keratinocyte growth factor; JNK, jun aminoterminal kinase; SAPK, stress activated protein kinase; TGF, transforming growth factor; NGF, nerve growth factor; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; TNF, tumour necrosis factor; MAPK, mitogen activated protein kinase; IKK, IκB-kinase; NFκB, nuclear factor κB; ECM, extracellular matrix; MMP, matrix metallo proteinase; TIMP, tissue inhibitor of metallo proteinases; TSK, tight skin mouse.

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address AP-1 proteins depending on the extracellular stimuli. Finally, AP-1 function is controlled by either positive or negative interactions with other cellular proteins, such as NFkB and the glucocorticoid receptor. All of these regulatory mechanisms determine the net activity of the complement of AP-1 factors and thus determine the genetic programs to be addressed (for review, see [1–3]).

Intensive investigation of AP-1 function in tissue culture cells led to the notion that different AP-1 complexes, which display subtle but important variations in DNA binding specificity, may act as tissue- and signal-specific activators of AP-1-dependent genes. This assumption is best documented by the distinct phenotypes obtained after inactivation of specific AP-1 members *in vivo* by gene targeting. Loss of c-*jun* results in embryonic lethality with impaired hepatogenesis and heart failure (for review, see [4]). Disruption of *JunB* causes also embryonic lethality due to defective establishment of a proper feto-maternal circulatory system [5]. Mice lacking individual members of the Fos family are viable and fertile but show specific defects in distinct tissues (for review, see [4]).

Analysis of the function and regulation of c-Jun and JunB is of particular interest because, based on these data as well as previous studies on cell proliferation, transformation and transactivation [6–9] both proteins have been proposed to act antagonistically in the regulation of cell proliferation, transformation and differentiation. These data suggested that either protein might regulate both overlapping and non-overlapping sets of yet unidentified target genes to control these processes. However, a complex *in vivo* model to support this concept was still missing.

2. Experimental systems to study the role of AP-1 in skin

A very attractive *in vivo* system to study the function of Jun proteins in proliferation and differentiation is the skin. The formation and maintenance of the mature epidermis rely on a tightly balanced process of keratinocyte proliferation and terminal differentiation. The distinct expression pattern of AP-1 subunits in multiple, well defined keratinocyte layers of the epidermis as well as the large number of AP-1 target genes in epidermal cells indicate that AP-1 plays a pivotal role in normal and pathological skin physiology. Moreover, the regulatory function of AP-1 in wound healing, photoaging and tumourigenesis is demonstrated by numerous studies in tissue culture cells and mouse models (for review, see [10] and references therein). These data strongly suggest that AP-1 transactivation of target genes contributes to normal development of the epidermis as well as protective and pathological responses in individual cells of the skin.

Regulation of keratinocyte proliferation and differentiation during skin development and skin regeneration is controlled both by cell-autonomous functions as well as by interactions with the adjacent connective tissue [11–13]. So far their functional significance of the dermal–epidermal interplay to regulate tissue repair and homeostasis is only poorly understood. Moreover, the underlying molecular mechanisms in cells of the connective tissue, which regulate these interactions (predominantly dermal fibroblasts) are not conclusively resolved.

There is accumulating evidence that the fibroblast–keratinocyte interplay to control epidermal tissue homeostasis is regulated by a cytokine network between both cell types [11,14,15]. These studies are mostly based on various tissue engineered skin equivalent model in which mesenchymal support of keratinocyte proliferation and differentiation is based on matrix components and diffusible factors produced by fibroblasts (Fig. 1). This includes a simple two-dimensional feeder-layer coculture of keratinocytes and postmitotic 3T3 cells [16]. In the more advanced three-dimensional organotypic cultures keratinocyte proliferation and differentiation is maintained under the control of cocultured matrix-embedded dermal fibroblasts from human or mouse to form a three-dimensionally organised epithelium (Fig. 1, [17–19]). Keratinocytes form a typical epidermal tissue architecture expressing essentially all characteristic differentiation markers including a basement membrane [20]. Importantly, this *in vitro* system mimics many aspects of the re-epithelialisation process during wound closure, e.g. the relation of proliferation and integrin expression pattern and development of the stratum corneum barrier [21]. Therefore, organotypic cocultures are the most advanced method to obtain three-dimensionally organised epithelia, and are considered a true tissue engineered skin equivalent model.

3. A cytokine network regulating mesenchymal–epithelial interaction

The experimental conditions used in organotypic cultures, in which fibroblasts were embedded in a collagen matrix showed that fibroblast-mediated control of keratinocyte proliferation and differentiation does not depend on cell–cell contact but rather on soluble paracrine-acting factors. Several growth factors and interleukins have been detected in normal and wounded skin, such as IL-1, IL-6, IL-8, GM-CSF, TGF-α and TGF-β, NGF, PDGF as well as members of the FGF family (for review, see [12]). Using the *in vitro* tissue model a critical function of at least some of these factors could be documented. In a double paracrine fashion keratinocytes release interleukin-1 (IL-1) resulting in the induction of growth factors such as keratinocyte growth factor (KGF) in dermal cells, which in turn stimulate keratinocyte proliferation [22]. These *in vitro* findings are likely to be of physiological relevance since the dynamic and reciprocal modulation of cytokine and growth factor expression in keratinocytes and fibroblasts involving IL-1 and KGF share many aspects of the initial phase of

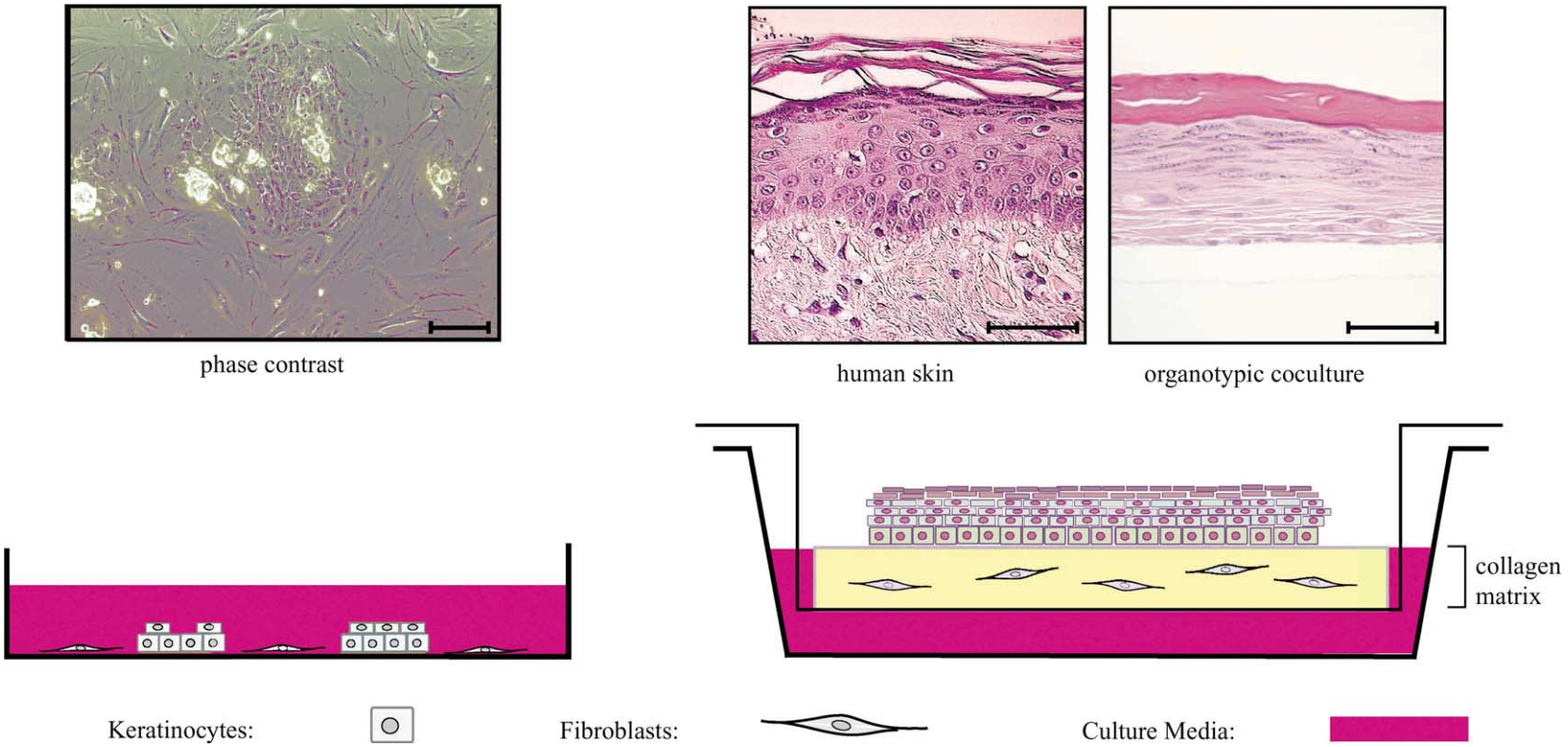

Fig. 1. Experimental systems allowing keratinocyte proliferation and differentiation *in vitro*. *Left panel*: Two-dimensional feeder-layer cocultures. Keratinocytes grow on a feeder of growth-arrested (by X-ray) fibroblasts. Under these conditions, keratinocytes proliferate and form keratinocyte islands with low efficiency of terminal differentiation. On top a typical keratinocyte island is shown by phase contrast microscopy. Bar: 100 μm. *Right panel*: Three-dimensional organotypic cocultures. Human epidermal keratinocytes grow air exposed on a matrix of collagen type I containing homologous human primary fibroblasts or heterologous wild-type and genetically modified mouse fibroblasts. Under these conditions, keratinocytes form a typical epidermal tissue architecture expressing characteristic differentiation markers. A haematoxylin/eosin stained cross section of such cultures is shown on the top as compared to a cross section from a biopsy of human skin. Bar: 100 μm (for details, see [25,26]).

re-epithelialisation and epidermis regeneration after wound healing [14].

4. Function of AP-1 subunits in cytokine-mediated fibroblast–keratinocyte cross-talk

Despite a large body of data on the expression pattern of AP-1 members and potential AP-1 target genes in the epidermis and in keratinocyte cell lines, the phenotypes of mice lacking c-Fos, FosB or JunD in the epidermis did not provide evidence for a critical role of these proteins in normal skin development (for review, see [4]). Moreover, specific deletion of c-Jun in cells of the *stratum basale* did not yield an obvious skin phenotype,[1] suggesting that these AP-1 subunits may not be required for cell-autonomous functions in keratinocytes, or that other members of the protein family may functionally compensate their loss.

In contrast, different lines of evidence pointed to an involvement of AP-1 in cytokine-mediated mesenchymal–epithelial interactions by altering gene expression in fibroblasts. First, certain factors involved in wound healing, such as EGF, FGF, members of the TGF-β, TNF-α and interleukin protein families stimulate AP-1 activity through the activation of the MAP kinases JNK and p38, which, in turn hyperphosphorylate and thereby activate c-Jun and ATF-2, the main mediators of c-*jun* expression in response to cytokines and genotoxic agents (for review, see [23]). Upregulated gene expression resulting in enhanced levels of Jun and Fos proteins trigger alterations in the expression of AP-1 target genes, for example matrix metalloproteinases [24]. Moreover, a number of cytokines produced in fibroblasts have been suggested to be potential AP-1 target genes (for review, see [10]).

To identify the specific contribution of AP-1-dependent cytokine expression in fibroblasts regulating keratinocyte proliferation and differentiation fibroblasts from c-*jun* or *junB*-deficient mice were employed in two-dimensional feeder-layer cocultures [25] and three-dimensional organotypic cultures [26] containing primary human keratinocytes. Since cytokine-mediated cross-talk involves a variety of cell types in the epidermis and in the mesenchyme the use of cells from different species allowed precise definition of the cell serving as the source of the cytokine of interest.

Lack of c-*jun* in fibroblasts results in decreased keratinocyte proliferation and differentiation and is associated with a loss of IL-1-induced KGF and GM-CSF expression. In contrast, JunB suppresses expression of both genes thus identifying KGF and GM-CSF as the first endogenous target genes of opposite regulatory activity of c-Jun and JunB. Growth-inhibited thin epithelia in c-$jun^{-/-}$ fibroblasts containing cultures could be partially rescued by KGF to a proliferative but less differentiating epithelium, whereas addition of both KGF and GM-CSF completely restored the wild-type phenotype. On the other hand, blocking antibodies to GM-CSF and IL-1 reduced the phenotype obtained by $junB^{-/-}$ fibroblasts. As schematically illustrated in Fig. 2, these data suggest that the relative abundance and activation state of a given AP-1 subunit in a non-cell autonomous, trans-regulatory fashion directs the adjustment of the appropriate balance between keratinocyte proliferation and differentiation to allow proper re-epithelialisation and regeneration of the epidermis, e.g. during wound healing [26].

In wounded skin both fibroblasts [27] and keratinocytes [28] produce GM-CSF. Since fibroblasts do not express the GM-CSF receptor [29,30], fibroblast-derived GM-CSF will act only on keratinocytes. In the *in vitro* organotypic cocultures the amount of keratinocyte-derived human GM-CSF was very similar, regardless of the genotype of the fibroblasts.[2] However, these levels were obviously not sufficient to induce keratinocyte proliferation and differentiation in cocultures containing c-$jun^{-/-}$ fibroblasts but rely on GM-CSF produced by (mouse) fibroblasts. This is supported by the strong decrease of keratinocyte differentiation in the presence of neutralising antibodies, which recognise mouse, but not human GM-CSF [26].

What are the Il-1-induced signalling pathways regulating KGF and GM-CSF in fibroblasts? IL-1 and inflammation-associated cytokines, such as TNF-α, in addition to activation of AP-1, also stimulate the activity of transcription factor NFκB. These effects are mediated through activation of the MAP kinases JNK and p38, and IκB kinases (IKKs), respectively. While the components of the signalling pathways initiated at the activated receptor have been investigated in great detail including cross-talk between Jun and NFκB [31] and pathways upstream of JNK, p38 and IKK [32,33] (for review, see [34–36]) nothing is known about the critical signalling pathways initiated by IL-1 in fibroblasts to mediate keratinocyte growth and differentiation. Importantly, the signalling pathway involving JNK activation is not affected by the lack of c-Jun or JunB, because JNK is equally expressed and activated by IL-1 in fibroblasts regardless of the genotype (wt, c-$jun^{-/-}$, $junB^{-/-}$, A. Szabowski and P. Angel, unpublished). In light of the synergistic activity of AP-1 and NFkB to regulate expression of AP-1 target genes including collagenase [31,37] it is still possible that in addition to the JNK/AP-1 pathway, activation of NFkB is also required for KGF and GM-CSF expression. Employing fibroblasts derived from mice, which lack specific members of the JNK and IKK protein families (for review, see [34,38]) may be the most straightforward approach to identify the contribution of either one of these pathways in the regulation of KGF and GM-CSF expression.

[1] E.F. Wagner, personal communication.

[2] A. Szabowski, N. Maas-Szabowski, N.E. Fusening, P. Angel, unpublished results.

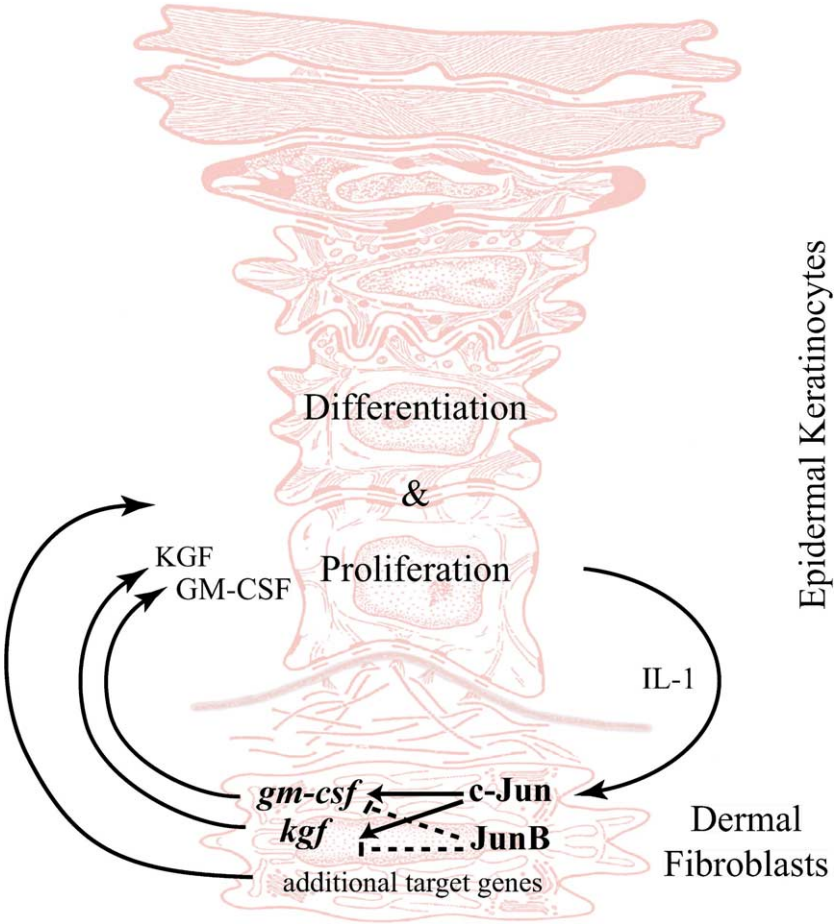

Fig. 2. Schematic illustration of the double-paracrine regulation of keratinocyte proliferation and differentiation. Keratinocytes synthesise and secrete IL-1α and -1β (e.g. after wounding) regulating the activity of components of transcription factor AP-1 (c-Jun, JunB) in fibroblasts. In turn, expression of KGF, GM-CSF and additional, yet unknown target genes gets modified. These factors are released by fibroblasts and stimulate keratinocyte proliferation and differentiation in a paracrine fashion. In addition to Jun/AP-1 regulated genes, other yet unknown fibroblast-derived cytokines, whose expression are regulated in an AP-1-independent manner also contribute to this regulatory mechanism.

5. Other factors controlling skin homeostasis

In addition to autocrine and paracrine cytokine and growth factor action, there is convincing evidence that cell–cell and cell matrix interactions are important regulators of keratinocyte proliferation, migration and differentiation. Moreover, to allow tissue remodelling, e.g. during wound healing the composition of the extracellular matrix has to be altered. This can be achieved by transcriptional regulation of cellular genes encoding ECM components as well as by proteolytic enzymes (particularly matrix metalloproteinases) and their inhibitors that control the ECM turnover on the post-translational level (for review, see [24,39]). Work from many laboratories has provided different lines of evidence for a critical role for AP-1 in the regulation of ECM components. For example, an AP-1 binding site mediates expression of human alpha2 (I) collagen (COL1A2) gene by TGF-β and TNF-α [40]. Expression of syndecan-1, a cell surface heparan sulphate proteoglycan that binds growth factors, is activated during cutaneous wound healing through a so-called AP-1-driven FGF-inducible response element (FiRE). Members of the MAP kinase family including ERKs and p38 seem to mediate selective upregulation of FiRE activity in migrating keratinocytes at the edge of the wound [41]. However, the exact composition of AP-1 complexes binding to this element has not yet been identified.

AP-1 plays a dominant role in the transcriptional activation of the majority of matrix-metalloproteinases (MMPs) that are required for cell migration, ECM degradation and tissue reorganisation during the wound healing process. Basal transcription as well as activation of MMP promoters in response to phorbol esters, cytokines, growth factors, cell matrix interactions and altered cell–cell contacts require the specific interaction of AP-1 and ETS proteins (for review, see [42]). In general, MMPs are not constitutively expressed in skin but are induced temporarily in response to the above mentioned exogenous signals to trigger the proteolytic remodelling of the ECM in physiological and pathological situations, such as tissue morphogenesis, tissue repair, dermal photoaging and tumour cell invasion (for review, see [43]). In acute murine excisional

skin wounds interstitial collagenase (MMP-1, MMP-13), MMP-9, MMP-3, MMP-10 and their physiological inhibitor TIMP-1 are strongly induced showing a unique spatial and temporal transcription pattern [44]. Tissue culture as well as animal studies have provided evidence for the importance of the AP-1 target genes MMP-1 and MMP-3 during wound healing in keratinocyte migration (MMP-1, [45]) and wound contraction, respectively (MMP-3, [46]).

6. The role of mesenchymal–epithelial interaction in skin diseases

Previous work from different laboratories on skin diseases has suggested dysregulation of expression of genes controlling mesenchymal–epithelial interaction. For example, an aberrant or disturbed balance between cytokines may be implicated in pathological diseases in human, such as lichen planus, psoriasis or dermatofibrosis [47]. Histologically cutaneous lichen planus is characterised by epidermal hypergranulosis (a pronounced *stratum granulosum* with high amounts of keratohyalin granula), irregular acanthosis, hyperkeratosis, dermal fibrosis and a dense infiltrate [48]. By contrast, absence of granular cells, hyperproliferation and parakeratosis is observed in psoriatic epidermis [47]. *In vitro*, normal keratinocytes develop psoriatic-skin like epidermal phenotypes when grown on fibroblasts from psoriatic lesions [49], providing evidence that the epidermal alterations in psoriasis and dermatofibroma are caused by changes in the underlying dermal fibroblasts. In dermatofibroma a significant hyperplasia of the epidermis can be observed overlying the benign fibroblastic tumour nodule. Apparently, the fibrotic cells stimulate the epidermis in a fashion similar to that of embryonic mesenchyme [47]. A loss of a negatively acting AP-1 complex in fibroblasts was proposed to be involved in the phenotype of the tight skin (TSK) mouse, representing a model of fibrosis [50]. While the identification of the specific subunits of this complex remains to be determined, it is possible that it's loss of activity is linked to the enhanced binding of TGF-β to the mutated Fibrillin-1 protein representing a characteristic feature of cells of TSK mice [51]. Moreover, aberrant expression of the AP-1 target gene collagenase in fibroblasts derived from patients with cutis laxa was observed [52]. Finally, enhanced mRNA and protein levels of c-Jun and c-Fos were found in keloids and hypertrophic scars representing a model of altered wound healing characterised by hyperproliferation of dermal fibroblasts and accelerated production of components of the extracellular matrix [53]. In light of these highly complex and as yet ill-defined phenotypes there is a clear need for genetically defined mouse mutants lacking c-Jun or JunB in dermal fibroblasts, which may mimic at least some of the phenotypes described above and to identify critical target genes.

7. Outlook

The availability of appropriate mouse models is complicated by the fact that at present no fibroblast-specific promoter has been described, which would be required to specifically inactivate genes in dermal fibroblasts by conditional gene targeting. Therefore, the organotypic coculture system containing c-*jun*$^{-/-}$ fibroblasts will still be the system of choice to study functional complementation of deficiencies in order to identify additional cytokines and, most importantly, to define their specific activity to promote keratinocyte proliferation, differentiation, or both.

Fibroblast-derived KGF and GM-CSF, whose functions in skin homeostasis has already been confirmed in mice (for review, see [54]) represent the first members of a potentially growing list of critical AP-1-regulated cytokines, which are produced by the various cell types during the different phases of the wound healing process. Although the existence of cellular genes, whose expression is antagonistically regulated by c-Jun and JunB, has been suggested for a decade [6,7], KGF and GM-CSF are the first examples of this class of AP-1 target genes and represent ideal tools to define the underlying mechanism of transcriptional regulation. Moreover, KGF and GM-CSF alone in the absence of fibroblasts were not sufficient to allow keratinocytes to proliferate and to form a proper epithelium (A. Szabowski and P. Angel, unpublished). Therefore, at least one additional soluble factor whose expression is regulated in a Jun-independent manner is essential for mesenchymal–epidermal cross-talk (Fig. 2). This class of genes may include members of the TGF-β family, their receptors [55] and downstream targets of the activin A pathway [56].

Based on the phenotype of organotypic cultures containing *junB*$^{-/-}$ fibroblasts novel drug targets, such as low molecular weight inhibitors may be identified, which will allow neutralisation of the phenotype of accelerated keratinocyte differentiation. Therefore, data obtained in this genetically defined *in vitro* skin equivalent model may serve as the basis for the dissection of disease mechanisms and the design of novel and more specific strategies of therapeutic intervention of skin diseases including psoriasis, dermatofibrosis and deficiencies in wound healing.

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References

[1] Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta 1991;1072:129–57.

[2] Karin M, Liu Z, Zandi E. AP-1 function and regulation. Curr Opin Cell Biol 1997;9:240–6.

[3] Wagner EF. AP-1. Oncogene 2001;20:2334–497.

[4] Jochum W, Passegue E, Wagner EF. AP-1 in mouse development and tumourigenesis. Oncogene 2001;20:2401–12.

[5] Schorpp-Kistner M, Wang ZQ, Angel P, Wagner EF. JunB is essential for mammalian placentation. EMBO J 1999;18:934–48.

[6] Chiu R, Angel P, Karin M. Jun-B differs in its biological properties from, and is a negative regulator of, c-Jun. Cell 1989;59:979–86.

[7] Schütte J, Viallet J, Nau M, Segal S, Fedorko J, Minna J. jun-B inhibits and c-fos stimulates the transforming and trans-activating activities of c-jun. Cell 1989;59:987–97.

[8] Passegue E, Wagner EF. JunB suppresses cell proliferation by transcriptional activation of p16(INK4a) expression. EMBO J 2000;19:2969–79.

[9] Passegue E, Jochum W, Schorpp-Kistner M, Mohle-Steinlein U, Wagner EF. Chronic myeloid leukemia with increased granulocyte progenitors in mice lacking JunB expression in the myeloid lineage. Cell 2001;104:21–32.

[10] Angel P, Szabowski A, Schorpp-Kistner M. Function and regulation of AP-1 subunits in skin physiology and pathology. Oncogene 2001;20: 2413–23.

[11] Luger TA, Schwarz T. Evidence for an epidermal cytokine network. J Invest Dermatol 1990;95:100S–4S.

[12] Schröder JM. Cytokine networks in the skin. J Invest Dermatol 1995;105:20S–4S.

[13] Werner S, Peters KG, Longaker MT, Fuller-Pace F, Banda MJ, Williams LT. Large induction of keratinocyte growth factor expression in the dermis during wound healing. Proc Natl Acad Sci USA 1992;89:6896–900.

[14] Werner S, Smola H, Liao X, Longaker MT, Krieg T, Hofschneider PH, Williams LT. The function of KGF in morphogenesis of epithelium and re-epithelialisation of wounds. Science 1994;266:819–22.

[15] Maas-Szabowski N, Shimotoyodome A, Fusenig NE. Keratinocyte growth regulation in fibroblast cocultures via a double paracrine mechanism. J Cell Sci 1999;112:1843–53.

[16] Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinising colonies from single cells. Cell 1975;6:331–43.

[17] Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T. Living tissue formed *in vitro* and accepted as skin-equivalent tissue of full thickness. Science 1981;211:1052–4.

[18] Mackenzie IC, Fusenig NE. Regeneration of organised epithelial structure. J Invest Dermatol 1983;81:189S–94S.

[19] Asselineau D, Prunieras M. Reconstruction of 'simplified' skin: control of fabrication. Br J Dermatol 1984;111:219–22.

[20] Smola H, Stark HJ, Thiekotter G, Mirancea N, Krieg T, Fusenig NE. Dynamics of basement membrane formation by keratinocyte–fibroblast interactions in organotypic skin culture. Exp Cell Res 1998; 239:399–410.

[21] Garlick JA, Taichman LB. Fate of human keratinocytes during re-epithelialisation in an organotypic culture model. Lab Invest 1994;70:916–24.

[22] Maas-Szabowski N, Stark HJ, Fusenig NE. Keratinocyte growth regulation in defined organotypic cultures through IL-1-induced keratinocyte growth factor expression in resting fibroblasts. J Invest Dermatol 2000;114:1075–84.

[23] Davis RJ. Signal transduction by the JNK group of MAP kinases. Cell 2000;103(2):239–52.

[24] Borden P, Heller RA. Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit Rev Eukaryot Gene Expr 1997;7:159–78.

[25] Maas-Szabowski N, Szabowski A, Stark HJ, Andrecht S, Kolbus A, Schorpp-Kistner M, Angel P, Fusenig NE. Organotypic cocultures with genetically modified mouse fibroblasts as a tool to dissect molecular mechanisms regulating keratinocyte growth and differentiation. J Invest Dermatol 2001;116:816–20.

[26] Szabowski A, Maas-Szabowski N, Andrecht S, Kolbus A, Schorpp-Kistner M, Fusenig NE, Angel P. c-Jun and JunB antagonistically control cytokine-regulated mesenchymal–epidermal interaction in skin. Cell 2000;103:745–55.

[27] Fibbe WE, Van Damme J, Billiau A, Duinkerken N, Lurvink E, Ralph P, Altrock BW, Kaushansky K, Willemze R, Falkenburg JH. Human fibroblasts produce granulocyte-CSF, macrophage-CSF, and granulocyte–macrophage–CSF following stimulation by interleukin-1 and poly(rI).poly(rC). Blood 1988;72:860–6.

[28] Kupper TS, Lee F, Birchall N, Clark S, Dower S. Interleukin 1 binds to specific receptors on human keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. A potential autocrine role for interleukin 1 in epidermis. J Clin Invest 1988;82: 1787–92.

[29] Braunstein S, Kaplan G, Gottlieb AB, Schwartz M, Walsh G, Abalos RM, Fajardo TT, Guido LS, Krueger JG. GM-CSF activates regenerative epidermal growth and stimulates keratinocyte proliferation in human skin *in vivo*. J Invest Dermatol 1994;103:601–4.

[30] Eder M, Griffin JD, Ernst TJ. The human granulocyte–macrophage colony-stimulating factor receptor is capable of initiating signal transduction in NIH3T3 cells. EMBO J 1993;12:1647–56.

[31] Stein B, Baldwin Jr AS, Ballard DW, Greene WC, Angel P, Herrlich P. Cross-coupling of the NF-kappa B p65 and Fos/Jun transcription factors produces potentiated biological function. EMBO J 1993;12:3879–91.

[32] Baud V, Liu ZG, Bennett B, Suzuki N, Xia Y, Karin M. Signaling by proinflammatory cytokines: oligomerisation of TRAF2 and TRAF6 is sufficient for JNK and IKK activation and target gene induction via an amino-terminal effector domain. Genes Dev 1999;13:1297–308.

[33] Wang C, Fu M, D'Amico M, Albanese C, Zhou JN, Brownlee M, Lisanti MP, Chatterjee VK, Lazar MA, Pestell RG. Inhibition of cellular proliferation through IkappaB kinase-independent and peroxisome proliferator-activated receptor gamma-dependent repression of cyclin D1. Mol Cell Biol 2001;21:3057–70.

[34] Karin M, Delhase M. JNK or IKK, AP-1 or NF-kappaB, which are the targets for MEK kinase 1 action? Proc Natl Acad Sci USA 1998;95:9067–9.

[35] Israel A. The IKK complex: an integrator of all signals that activate NF-kappaB? Trends Cell Biol 2000;10:129–33.

[36] Wajant H, Scheurich P. Tumour necrosis factor receptor-associated factor (TRAF) 2 and its role in TNF signaling. Int J Biochem Cell Biol 2001;33:19–32.

[37] Mengshol JA, Vincenti MP, Coon CI, Barchowsky A, Brinckerhoff CE. Interleukin-1 induction of collagenase 3 (matrix metalloproteinase 13) gene expression in chondrocytes requires p38, c-Jun N-terminal kinase, and nuclear factor kappaB: differential regulation of collagenase 1 and collagenase 3. Arthritis Rheum 2000;43: 801–11.

[38] Karin M. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. Oncogene 1999;18:6867–74.

[39] Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev 2000;14:2123–33.

[40] Chung KY, Agarwal A, Uitto J, Mauviel A. An AP-1 binding sequence is essential for egulation of the human alpha2(I) collagen (COL1A2) promoter activity by transforming growth factor-beta. J Biol Chem 1996;271:3272–8.

[41] Jaakkola P, Maatta A, Jalkanen M. The activation and composition of FiRE (an FGF-inducible response element) differ in a cell type- and growth factor-specific manner. Oncogene 1998;17:1279–86.
[42] Benbow U, Brinckerhoff CE. The AP-1 site and MMP gene regulation: what is all the fuss about? Matrix Biol 1997;15:519–26.
[43] Kähäri VM, Saarialho-Kere U. Matrix metalloproteinases in skin. Exp Dermatol 1997;6:199–213.
[44] Madlener M, Parks WC, Werner S. Matrix metalloproteinases (MMPs) and their physiological inhibitors (TIMPs) are differentially expressed during excisional skin wound repair. Exp Cell Res 1998;242:201–10.
[45] Lund LR, Romer J, Bugge TH, Nielsen BS, Frandsen TL, Degen JL, Stephens RW, Dano K. Functional overlap between two classes of matrix-degrading proteases in wound healing. EMBO J 1999;18:4645–56.
[46] Bullard KM, Lund L, Mudgett JS, Mellin TN, Hunt TK, Murphy B, Ronan J, Werb Z, Banda MJ. Impaired wound contraction in stromelysin-1-deficient mice. Ann Surg 1999;230:260–5.
[47] Lever WF. Histopathology of the skin, 6th ed. London: Pitman Medical Publishing, 1984.
[48] Stefanidou MP, Ioannidou DJ, Panayiotides JG, Tosca AD. Low molecular weight heparin: a novel alternative therapeutic approach for lichen planus. Br J Dermatol 1999;141:1040–5.
[49] Saiag P, Coulomb B, Lebreton C, Bell E and Dubertret L. Psoriatic fibroblasts induce hyperproliferation of normal keratinocytes in a skin equivalent model *in vitro*. Science 1985;230:669–72.
[50] Philips N, Bashey RI, Jimenez SA. Increased alpha 1(I) procollagen gene expression in tight skin (TSK) mice myocardial fibroblasts is due to a reduced interaction of a negative regulatory sequence with AP-1 transcription factor. J Biol Chem 1995;270:9313–21.
[51] Saito S, Nishimura H, Brumeanu TD, Casares S, Stan AC, Honjo T, Bona CA. Characterisation of mutated protein encoded by partially duplicated fibrillin-1 gene in tight skin (TSK) mice. Mol Immunol 1999;36:169–76.
[52] Hatamochi A, Arakawa M, Mori K, Mori Y, Ueki H, Yoshioka H. Increased expression of type VI collagen genes in cutis laxa fibroblasts. J Dermatol Sci 1996;11:97–103.
[53] Teofoli P, Barduagni S, Ribuffo M, Campanella A, De Pita O, Puddu P. Expression of Bcl-2, p53, c-jun and c-fos protooncogenes in keloids and hypertrophic scars. J Dermatol Sci 1999;22:31–7.
[54] Werner S, Smola H. Paracrine regulation of keratinocyte proliferation and differentiation. Trends Cell Biol 2001;11:143–6.
[55] Frank S, Madlener M, Werner S. Transforming growth factors beta1, beta2, and beta3 and their receptors are differentially regulated during normal and impaired wound healing. J Biol Chem 1996;271: 10188–93.
[56] Munz B, Smola H, Engelhardt F, Bleuel K, Brauchle M, Lein I, Evans LW, Huylebroeck D, Balling R, Werner S. Overexpression of activin A in the skin of transgenic mice reveals new activities of activin in epidermal morphogenesis, dermal fibrosis and wound repair. EMBO J 1999;18:5205–15.