metadata, citation and similar papers at core.ac.uk
brought to you by
provided by RERO DOC Digit

Wound-Healing Studies in Transgenic and Knockout Mice

Richard Grose[*,1,2] *and Sabine Werner*[2]

Abstract

Injury to the skin initiates a cascade of events including inflammation, new tissue formation, and tissue remodeling, that finally lead to at least partial reconstruction of the original tissue. Historically, animal models of repair have taught us much about how this repair process is orchestrated and, over recent years, the use of genetically modified mice has helped define the roles of many key molecules. Aside from conventional knockout technology, many ingenious approaches have been adopted, allowing researchers to circumvent such problems as embryonic lethality, or to affect gene function in a tissue- or temporal-specific manner. Together, these studies provide us with a growing source of information describing, to date, the in vivo function of nearly 100 proteins in the context of wound repair.

This article focuses on the studies in which genetically modified mouse models have helped elucidate the roles that many soluble mediators play during wound repair, encompassing the fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) families and also data on cytokines and chemokines. Finally, we include a table summarizing all of the currently published data in this rapidly growing field. For a regularly updated web archive of studies, we have constructed a *Compendium of Published Wound Healing Studies on Genetically Modified Mice* which is avaialble at http://icbxs.ethz.ch/members/grose/woundtransgenic/home.html.

Index Entries: Wound healing; mouse; gene targeting; growth factor; cytokine.

1. Introduction

Wound healing is a highly ordered and well-coordinated process that involves inflammation, cell proliferation, matrix deposition, and tissue remodeling ***(1)***. During the past few years, a series of candidate key players in the wound-healing scenario have been identified. These include not only a variety of different growth factors and cytokines, but also molecules that are involved in cell–cell and cell–matrix interactions, and proteins responsible for cell stability and cell migration. In most cases, the suggested function of these molecules is based on descriptive expression studies and/or functional in vitro studies. By contrast, their in vivo function in wound repair has been poorly defined.

The development of transgenic mouse technologies has already provided new insights into the function of many different genes during embryonic development. These technologies allow gain-of-function experiments (overexpression of ligands and receptors) as well as loss-of-function experiments (gene knockouts by homologous recombination in embryonic stem cells or overex–pression of dominant-negative-acting molecules). Although many unconditional knockout and transgenic animals die during embryonic development, spatial and temporal control of gene ablation and overexpression, using both inducible and cre-lox technologies, makes it possible to investigate the functions of proteins formerly precluded owing to developmental lethality. A large number of viable genetically modified mice are now available that should not only be useful in determining the role of the targeted or overexpressed genes in normal physiology, but also for different types of repair processes. Indeed, the past 5 yr have seen an exponential growth in the number of transgenic

*Author to whom all correspondence and reprint requests should be addressed:[1]London Research Institute Lab 214, Cancer Research UK, 61 Lincoln's Inn Fields, London WC2A 3PX. :[2]Institute of Cell Biology, Department of Biology, ETH Zurich, 8093 Zurich, Switzerland.E-mail: richard.grose@cancer.org.uk

Molecular Biotechnology © 2004 Humana Press Inc. All rights of any nature whatsoever reserved. 1073–6085/2004/28:2/147–166/$25.00

mice used for wound-healing experiments, and these studies have provided interesting and, in many cases, unexpected results concerning the in vivo function of growth factors, extracellular matrix molecules, proteinases, and structural proteins in wound repair. The list of transgenic wound-healing studies continues to expand, and in an attempt to retain an overview of the field, we have therefore established a regularly updated *Compendium of Genetically Modified Mouse Wound Healing Studies* available at http://icbxs.ethz.ch/members/grose/woundtransgenic/home.html.

In this chapter, we focus on the studies in which genetically modified mouse models have helped elucidate the roles that many soluble mediators play during wound repair. We begin by covering the fibroblast growth factor (FGF) family, move on to the transforming growth factor-β (TGF-β) superfamily, and then address the data from cytokine and chemokine studies. Finally, Table 1 summarizes all of the currently published data in this rapidly growing field.

2. Fibroblast Growth Factors

FGFs comprise a growing family of structurally related polypeptide growth factors, currently consisting of 22 members ***(2)***. During embryogenesis, FGFs play key roles in regulating cell proliferation, migration, and differentiation. In adult tissues, FGFs have a diverse range of effects, including mediating angiogenesis and neuroprotection, in addition to their stimulatory effects during wound repair ***(3)***. FGFs transduce their signals through four high-affinity transmembrane protein tyrosine kinases, FGF receptors 1–4 (FGFR1–4) ***(4)***. Many of the FGFs and their receptors show specific expression patterns in both developing and adult skin ***(3)***, where they are particularly involved in the regulation of hair growth ***(5)***. Being potent mitogenic and chemotactic factors, FGFs are clear candidates for contributing to the wound-healing response. This hypothesis has been corroborated by a number of studies in which local application of FGFs stimulated tissue repair ***(6)***.

2.1. Keratinocyte Growth Factor

2.1.1. Epidermal Expression of a Dominant-Negative Keratinocyte Growth Factor Impairs Wound Reepithelialization

Keratinocyte growth factor (KGF, FGF-7) binds exclusively to a splice variant of FGF receptor 2 (FGFR2IIIb), a transmembrane protein tyrosine kinase receptor that is present only on epithelial cells ***(7)***. In previous studies, a strong upregulation of KGF expression has been observed after skin injury in mice and humans ***(8,9)***, with mouse studies revealing KGF mRNA levels more than 150-fold higher compared with the basal levels at d 1 after injury ***(8)***. KGF expression at the wound site is restricted to dermal fibroblasts and γδ T cells, and, thus, it was hypothesized to act in a paracrine manner, stimulating keratinocytes to proliferate and migrate and thereby effect wound reepithelialization. By targeting expression of dominant-negative FGFR2IIIb to the epidermis, we could block KGF receptor signaling at the wound site and clearly demonstrate a reepithelialization defect in transgenic mice. The mutant receptor lacks a functional tyrosine kinase domain ***(10,11)*** and, on ligand binding, forms nonfunctional heterodimers with full-length wild-type receptors, thereby blocking signal transduction ***(10–12)***. The truncated form of the KGF receptor is known to bind KGF; FGF-10; FGF-1; FGF-3; and, although with lower affinity, FGF-2 ***(7,13,14)***. Therefore, it should inhibit the action of all these ligands.

Although the transgenic mice generated by Werner et al. ***(15)*** appeared macroscopically normal, a histological analysis of their skin revealed epidermal atrophy, disorganization of the epidermis, abnormal hair follicle morphology, and a 60–80% reduction in the number of hair follicles. Finally, these mice revealed dermal hyper-thickening with a gradual replacement of adipose tissue by connective tissue. Histological analysis of full-thickness excisional wounds, where back skin was excised to the level including the panniculus carnosus muscle, revealed a severe delay in wound

Table 1
Wound-Healing Studies on Transgenic and Knockout Mice[a]

Gene	Strategy	Ref.
Activin A	Overexpressor - Keratin 14 promoter	***(59)***
BMP-6	Overexpressor - Keratin 10 promoter	***(62)***
BPAG1	Knockout	***(85)***
Cathepsin G	Knockout	***(86)***
CD44	Antisense knockdown - Keratin 5 promoter	***(87)***
CDK4	Knockout	***(115)***
Connexin 30/31/43	Knockouts	***(116)***
CXCR2	Knockout	***(75)***
Cyr61	Knockout	***(117)***
E2F-1	Knockout	***(118)***
EGFR	Knockout	***(88)***
FGF-1/2	Double knockout	***(119)***
FGF-2	Knockout	***(27)***
Fibrinogen	Knockout	***(89)***
Fibrinogen	Knockout	***(120)***
Plasma Fibronectin	Knockout	***(121)***
Fibronectin EDA	Knockout	***(122)***
Follistatin	Overexpressor - Keratin 14 promoter	***(123)***
Glucocorticoid receptor	Knock-in mutation	***(124)***
GM-CSF	Overexpressor - Keratin 5 promoter	***(125)***
HGF	Overexpressor - Metallothionein promoter	***(126)***
HGFL	Knockout	***(90)***
Hoxb13	Knockout	***(127)***
β2-Integrin	Knockout	***(128)***
β1-Integrin	Conditional knockout - Keratin 5 Cre	***(129)***
β5-Integrin	Knockout	***(91)***
Interleukin-6	Knockout	***(66)***
Interleukin-6	Knockout	***(130)***
Interleukin-10	Knockout	***(77)***
IP-10	Overexpressor - Keratin 5 promoter	***(72)***
c-Jun	Conditional knockout - Keratin 14 Cre	***(131)***
c-Jun	Conditional knockout - Keratin 5 Cre	***(132)***
Keratin 6a	Knockout	***(92)***
Keratin 8	Knockout	***(80)***
KGF	Knockout	***(16)***
KGFR	Dominant negative - Keratin 14 promoter	***(15)***
Krox 24/20	Knockouts	***(133)***
MCP-1 / MIP-1α	Knockout	***(134)***
MMP-1	Overexpressor - Haptoglobin promoter	***(93)***
MMP-3	Knockout	***(94)***
MMP-9	Knockout	***(135)***
c-Myc-ER	Overexpressor - Keratin 14 promoter	***(136)***
Nf-1	Knockout	***(95)***
eNOS	Knockout	***(96)***
iNOS	Knockout	***(97)***
iNOS	Knockout	***(137)***
NPY(2) receptor	Knockout	***(138)***
Nrf-2	Knockout	***(139)***

(continued)

Table 1 *(continued)*
Wound-Healing Studies on Transgenic and Knockout Mice[a]

Gene	Strategy	Ref.
Osteopontin	Knockout	***(98)***
PAI-1	Knockout	***(140)***
PAI-1	Knockout	***(141)***
PAI-2 and PAI-1/2	Knockout / double knockout	***(99)***
PAR-1	Knockout	***(100)***
PDGF-B	Knockout	***(142)***
PKC-ε	Knockout	***(143)***
Placenta growth factor	Knockout	***(144)***
Plasminogen	Knockout	***(101)***
Plasminogen / Fibrinogen	Double knockout	***(89)***
PPAR α and β	Knockouts	***(145)***
PPAR β/δ	Knockout	***(146)***
PU.1	Knockout	***(147)***
P and E Selectin	Double knockout	***(102)***
Skn-1a/i	Knockout	***(103)***
SLPI	Knockout	***(104)***
Smad-3	Knockout	***(54)***
Smad-3	Knockout	***(148)***
Stat-3	Conditional knockout - Keratin 5 Cre	***(68)***
Syndecan-1	Knockout	***(149)***
Syndecan-4	Knockout	***(105)***
Tachykinin / Neurokinin-1 receptor	Knockout	***(150)***
γ/δT cell receptor	Knockout	***(151)***
Telomerase	Overexpressor - Keratin 5 promoter	***(152)***
Tenascin C	Knockout	***(106)***
TGFα	Knockout	***(153)***
TGFα	Knockout	***(154)***
TGFα	Knockout	***(155)***
TGFα/KGF	Double knockout	***(16)***
TGFβ	Knockout	***(40)***
TGFβ	Knockout	***(46)***
TGFβ	Knockout	***(156)***
TGFβ	Overexpressor - Albumin promoter	***(47)***
TGFβ	Overexpressor - Keratin 14 promoter	***(157)***
TGFβ	Overexpressor - Keratin 14 promoter	***(158)***
Type II TGFβ receptor	Dominant negative - Keratin 5 promoter	***(159)***
Thrombomodulin	Knockout	***(107)***
Thrombomodulin	Overexpressor- Keratin 14 promoter	***(108)***
Thrombospondin-1	Overexpressor- Keratin 14 promoter	***(109)***
Thrombospondin-2	Knockout	***(110)***
TNF receptor p55	Knockout	***(160)***
tPA / uPA	Double knockout	***(111)***
tPA / uPAR	Double knockout	***(112)***
Transglutaminase-1	Knockout	***(113)***
Vimentin	Knockout	***(78)***
Vitronectin	Knockout	***(114)***

[a]Regularly updated version available at http://icbxs.ethz.ch/members/grose/woundtransgenic/home.html

reepithelialization in the transgenic mice compared with control littermates ***(15)***. On d 5 after injury, the number of proliferating keratinocytes in the hyperproliferative epithelium was 80–90% reduced compared with control mice. These results demonstrated an important role for KGF receptor signaling in wound repair, although the type of KGF receptor ligand responsible for this defect was not defined.

2.1.2. KGF-Deficient Mice Show No Defect in Wound Healing

To determine further the role of KGF in development and in repair processes, Guo et al. ***(16)*** used embryonic stem cell technology to generate mice lacking KGF. The obtained knockout mice revealed no obvious defects, with the exception of the fur, which appeared matted and greasy, especially in male animals. Although KGF is widely expressed during development and in the adult animal, no histological defects could be detected in the KGF knockout mice. Most surprising, even the healing process of full-thickness incisional wounds was normal. Thus, no histological differences could be determined between normal mice and those lacking KGF, and the proliferation rate of the keratinocytes at the wound edge was not altered. These data demonstrate that incisional wounds can heal in the absence of KGF. It would, however, be interesting to study the healing process of excisional wounds in these animals, since the extent of reepithelialization is much higher in excisional than in incisional wounds.

The lack of obvious phenotypic abnormalities in the KGF knockout mice is in contrast to the results obtained with the dominant-negative KGF receptor (see this section, above). Although it might be possible that KGF is indeed not involved in these processes, this seems highly unlikely, since the pattern of KGF expression correlates very well with its postulated functions in normal and wounded skin. The most likely explanation for the discrepancies between the knockout and the dominant-negative receptor results is a redundancy in ligand signaling. Although KGF might normally be the most important KGF receptor ligand in normal and wounded skin, the lack of this gene in KGF knockout mice might be compensated for by other known KGF receptor ligands. More recent data from our laboratory and from others suggest that FGF-10 is the principal candidate for effecting this compensation since it is also expressed in the mesenchyme of normal and wounded skin ***(14,17)***. Studies using neutralizing KGF and/or FGF-10 antibodies during wound repair should help to clarify this issue further. Furthermore, the tissue-specific knockout of the KGF receptor splice variant of FGFR2 as well as double knockouts of different ligands of this receptor will shed light on the role of the KGF receptor and the various types of FGF in normal and wounded skin.

2.2. Wound Healing in Mice Deficient in TGF-α

As already described, the inhibition of KGF receptor signaling in basal keratinocytes of transgenic mice caused a severe delay in reepithelialization ***(15)***. However, the wounds in these animals finally healed (unpublished finding), demonstrating the presence of other epithelial mitogens in the wound that can at least partially compensate for the defect in KGF receptor signaling. One of these factors might be TGF-α, a strong mitogen and chemo–attractant for many different cell types, including epidermal keratinocytes ***(18)***. Furthermore, expression of TGF-α is strongly upregulated in the wound tissue on d 1 after injury ***(16)***, and addition of exogenous TGF-α to a wound has been shown to enhance epithelial wound healing ***(19)***.

To determine the role of endogenous TGF-α in wound repair, two groups generated mice lacking this growth factor ***(20,21)***. Surprisingly, these mice appeared normal with the exception of eye abnormalities and waviness of whiskers and fur. By contrast, the epidermis of these mice was indistinguishable from that of control mice. Most interestingly, no significant wound-healing abnormalities were observed in these mice, whereby two different wound-healing models (full-thickness back skin excisions and wounds generated by

tail amputation) were used. However, one group observed more variability in the rate of wound closure in TGF-α null mice ***(21)***, suggesting that the lack of this mitogen can be compensated for to a variable extent by other growth factors. Such a compensation could be achieved by growth factors that, like TGF-α, bind to the epidermal growth factor (EGF) receptor. The most likely candidates are EGF and heparin-binding EGF, which are present at high levels in wound fluid ***(22)***. This hypothesis is supported by the severe phenotypic abnormalities of mice lacking the EGF receptor ***(23,24)*** and by transgenic mice expressing a dominant-negative EGF receptor in the epidermis ***(25)***, although the wound-healing process in these animals has not been analyzed yet. By contrast, the lack of TGF-α is unlikely to be compensated for exclusively by KGF, since incisional wound healing also appeared normal in mice lacking both TGF-α and KGF ***(16)***.

2.3. Mice Lacking FGF-2 Show Delayed Reepithelialization

In contrast to the restricted pattern characteristic of many of the FGFs, FGF-2 is expressed in many different tissues and cell types. It exerts a plethora of effects, both in vivo and in vitro, including mitogenic, chemotactic, angiogenic, and developmental activities. Thus, it is reported to act as a survival factor in many models of tissue repair, ranging from neural injury models to corneal and skin wounds ***(26)***. Surprisingly, considering the myriad of potential functions, mice lacking FGF-2 appeared superficially indistinguishable from wild-type littermates. However, when these mice were challenged by full-thickness excisional wounding, they showed delayed healing ***(27)***. In addition to a retardation in the rate of reepithelialization, mice null for FGF-2 show reduced collagen deposition at the wound site and also have thicker scabs. Expression of FGF-2 is known to be enhanced following injury ***(8,28)***, and topical application of FGF-2 has been reported to accelerate both dermal and epidermal repair ***(29–31)***. In addition, neutralizing antibodies to FGF-2 were shown to inhibit granulation tissue formation in sponges implanted into rats ***(32)***. Taken together, these findings suggest a specific role for FGF-2 during wound healing that, despite the apparent redundancy of FGF signaling, cannot be covered for by other FGF family members.

3. TGF-β Superfamily Members and Downstream Signaling Molecules

The TGF-β superfamily encompasses a diverse range of proteins, many of which play important roles during development and differentiation. Mammalian members include TGF-β1-3, bone morphogenetic proteins (BMPs), Mullerian inhibiting substance, inhibins, and activins ***(33)***. Their biological effects are mediated by heteromeric receptor complexes, which signal via activation of intracellular Smad signaling pathways ***(34)***. TGF-β is one of the most studied molecules in the wound-healing scenario. This growth and differentiation factor is found in large amounts in platelets and is also produced by several cell types that are present in a wound, including activated macrophages, fibroblasts, and keratinocytes ***(35)***. Three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) are present in mammals and have both distinct and overlapping functions. In vitro, these molecules have been shown to be mitogenic for fibroblasts, but they inhibit proliferation of most other cells. Furthermore, TGF-βs modulate differentiation processes and are very potent stimulators of the expression of extracellular matrix (ECM) proteins and integrins ***(33)***. Therefore, they have the properties expected of wound cytokines. Indeed, a series of studies has demonstrated a beneficial effect of exogenous TGF-β for wound repair ***(35)***. Furthermore, endogenous TGF-β is likely to play an important role in wound healing, since all three types of mammalian TGF-β are expressed during repair, with each isoform having a characteristic distribution in the wound tissue ***(36,37)***. TGF-β induction is modulated in a complicated manner by systemic glucocorticoid treatment of wild-type mice, suggesting that aberrant expression of TGF-β1, TGF-β2, and TGF-β3 is associated with the wound-healing defect seen in these mice ***(37)***. Additionally, TGF-β is particularly important for the scarring response; it has been shown that TGF-β1 and TGF-β2 induce cu-

taneous scarring, whereas TGF-β3 seems to inhibit this effect *(38,39)*.

3.1. TGF-β1-Deficient Mice Show Severely Impaired Late-Stage Wound Repair

To clarify further the role of the TGF-β1 isoform in wound repair, Brown et al. *(40)* wounded transgenic mice deficient in TGF-β1 owing to a targeted disruption of the TGF-β1 gene *(41,42)*. These mice exhibit no obvious developmental abnormalities and appear phenotypically normal. However, at approx 3 wk of age, they develop a severe wasting syndrome, which is accompanied by a pronounced multifocal inflammatory response and tissue necrosis, resulting in multisystem organ failure and death *(41,42)*. To overcome this problem, the animals were wounded at d 10 after birth.

Full-thickness excisional wounds were created on the backs of TGF-β1 null mice and control mice and covered with a nonabsorbent dressing. The percentage of wound closure was determined at different time points after injury. Mice were sacrificed at d 10 after wounding and the wounds were analyzed histologically. Surprisingly, early wound healing proceeded almost normally in the TGF-β1-deficient mice, suggesting that other TGF-β isoforms or even different growth factors can compensate for the lack of TGF-β1 *(40)*. Alternatively, maternal rescue in utero by transplacental transfer of TGF-β1 and postnatally by transmission in the milk *(43)* might explain the lack of abnormalities in early wounds. However, the lack of TGF-β1 ultimately caused a severe inflammatory response in the wound, as well as in many other tissues and organs, which is likely to be responsible for the wound-healing abnormalities seen at later stages. Thus, histological analysis of the wounds at d 10 after injury revealed a thinner, less vascular granulation tissue in the knockout mice, which was dominated by a marked inflammatory cell infiltrate. Furthermore, decreased reepithelialization and decreased collagen deposition were observed in mutant animals when compared with control mice *(40)*. These defects in wound repair are likely to be a secondary effect of the severe wasting syndrome observed in these mice. Malnutrition and weight loss have been associated with impaired wound healing *(44,45)*, and the weight loss that accompanies the inflammatory response is likely to exert an adverse effect on repair.

In summary, Brown et al. *(40)* demonstrated that the lack of TGF-β1 can be compensated in the early stage of wound repair. However, the severe inflammation seen in the mice ultimately caused a severe wound-healing defect.

3.2. Immunodeficient Mice Lacking TGF-β1 Show Retarded Healing

To try to dissect the TGF-β1-dependent wound-healing defects from the effects of severe inflammation, Crowe et al. *(46)* crossed TGF-β1 null mice onto the immunodeficient Scid$^{-/-}$ background *(46)*. Scid$^{-/-}$ mice lack T- and B-cells and therefore do not have the machinery to mount the large inflammatory response seen in non-immunocompromised mice lacking TGF-β1 *(40)*. In contrast to what was predicted, the absence of inflammation in TGF-β1$^{-/-}$ Scid$^{-/-}$ mice resulted in a major delay in all the primary phases of repair by around a week compared to TGF-β1$^{+/+}$ Scid$^{-/-}$ controls. This delay was not singly owing to either the lack of TGF-β1 or the lack of lymphocytes, but to the combination of the two. This suggests that TGF-β1 and lymphocytes may affect compensatory pathways during repair. Alternatively, the delay may be a side effect of the absence of TGF-β1 in wounds leading to delayed expression of the other two TGF-β isoforms, TGF-β2 and TGF-β3. Although unable to distinguish between which of these hypotheses may be true, Crowe et al.'s *(46)* study presents an elegant method for bypassing a knockout phenotype that would otherwise mask a defect in wound repair.

3.3. Mice Overexpressing TGF-β1 Show Severely Impaired Late-Stage Wound Repair

In contrast to the knockout approaches described above, Shah et al. *(47)* investigated the effect of excess levels of TGF-β1 on wound repair. Their hypothesis was that elevated levels of circulating TGF-β1 would accelerate healing but

also enhance scarring. Mice with elevated plasma levels of active TGF-β1 were generated by cloning a modified porcine TGF-β1 construct, generating constitutively active TGF-β1, downstream of the mouse albumin promoter region. Using a dorsal incisional wounding model, complemented by ventral subcutaneous implantation of polyvinyl alcohol (PVA) sponges, Shah et al. ***(47)*** were able to study both normal cutaneous wound repair and cellular infiltration as a model of granulation tissue formation.

Surprisingly, they found that while the PVA sponges yielded the expected results, with increased cellularity, granulation tissue formation, and collagen deposition in transgenic animals, local TGF-β1 levels were lower in the incisional wounds of transgenic mice than in their control littermates. As such, the data show that increased circulating levels of TGF-β1 do not necessarily lead to increased levels of TGF-β1 at the wound site. Concomitant with the decreased TGF-β1 level in transgenic wounds, an increase in levels of TGF-β3 and type II TGF-β-receptor at the wound site were observed, and this resulted in an improved neodermal architecture in the healed transgenic wounds.

3.4. Smad3 Null Mice Show Accelerated Cutaneous Wound Healing With Increased Rate of Reepithelialization and Reduced Inflammation

TGF-βs and activin, both of which regulate key cellular functions during cutaneous wound repair, are known to require the nuclear transcriptional activators Smad2 and Smad3 for their intracellular signaling functions ***(48–50)***. Smad2 and Smad3 proteins are recruited to ligand-bound TGF-β and activin receptor complexes, where they are phosphorylated by the type I receptor. The phosphorylated Smads 2 and 3 undergo a conformational change, which allows them to bind to cytoplasmic Smad4, after which they are able to translocate to the nucleus and activate their downstream targets ***(51)***.

In contrast to Smad2 null mice, which die during embryogenesis ***(52)***, mice lacking functional Smad3 survive into adulthood ***(53)***. Following full-thickness incisional wounding, Smad3 null mice show a marked augmentation in repair. This accelerated healing was shown to be characterized by an increased rate of reepithelialization and a reduced local inflammatory infiltrate ***(54)***. In addition to neutrophils and monocytes being almost absent in the Smad3 knockout wounds, there was a dramatic decrease in granulation tissue formation, resulting in an overall decrease in wound area. Wounds of Smad3 knockout mice were found to have significantly lower levels of TGF-β expression, likely owing to the decreased monocyte concentration, since these cells form a major supply line delivering TGF-β to the early wound.

To determine whether the lack of TGF-β was a cause of rather than an effect of the lack of inflammatory response, exogenous TGF-β1 was applied to the wounds of control and Smad3 null mice. While this treatment resulted in an augmented neutrophil infiltration into the wounds of control mice, it failed to rescue the inflammatory response in Smad3 null animals, indicating that Smad3 signaling may underpin TGF-β1-mediated inflammatory cell chemotaxis. Contrastingly, exogenous TGF-β1 did rescue the granulation tissue phenotype, resulting in a stimulation of matrix production in the wounds of Smad3 null mice, though the fibroblast numbers were not increased. Thus, TGF-β1-dependent matrix deposition seems to function in a Smad3-independent fashion in these mice, in agreement with previous studies suggesting a c-Jun-dependent pathway ***(55)***.

Overall, the data suggest that Smad3 signaling plays an inhibitory role during wound repair, since its abrogation leads to enhanced reepithelialization and contraction of wound areas, at least in an incisional wound-healing scenario. As with the KGF knockout mice, it would be interesting to see how efficiently Smad3 null mice manage to repair full-thickness excisional wounds, where the granulation tissue formation is thought to play a more important role.

3.5. Overexpression of Activin A in Basal Keratinocytes Stimulates Wound Repair

Activin A, a TGF-β superfamily member, is a homodimeric protein comprising two activin βA

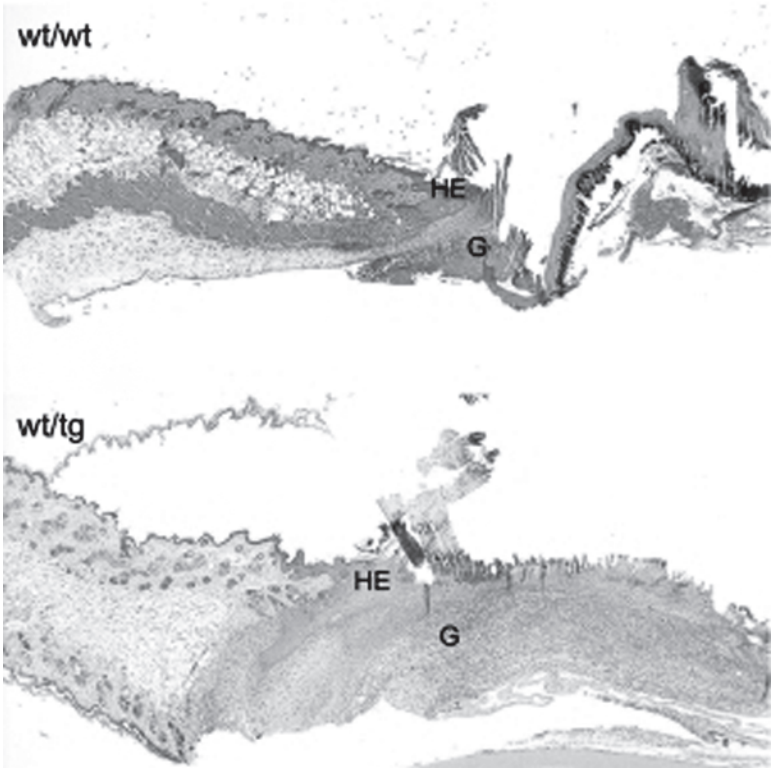

Fig. 1. Wound-healing phenotype of activin-overexpressing mice. Full-thickness excisional wounds were made on the back of 3-mo-old female transgenic mice (wt/tg) and female control littermates (wt/wt). Mice were killed on d 5 after injury. Sections (6 mm) from the middle of the wound were stained with hematoxylin and eosin. G, granulation tissue; HE, hyperproliferative epithelium. Note the larger area of granulation tissue in the transgenic mice. Magnification: ×25. (Reprinted from **ref. *59***. Copyright 1999 Oxford University Press.)

monomers connected by disulfide linkage. That it might play a role in the skin was first suggested by knockout mouse studies, of activin βA ***(56)*** and of the activin antagonist follistatin ***(57)***, which both showed clear phenotypes in hair follicle development. Further studies from our laboratory demonstrated activin βA to be strongly induced following wounding ***(58)***. Therefore, mice overexpressing the human activin βA chain in the epidermis, under the control of the keratin 14 promoter, were generated to further investigate its role in tissue repair ***(59)***.

A study by Munz et al. ***(59)*** found that unwounded mice overexpressing activin displayed epidermal hyperthickening and dermal fibrosis. Following full-thickness excisional wounding, the mice also showed enhanced granulation tissue formation and more rapid wound reepithelialization **(Fig. 1)**. This augmentation of the granulation tissue was accompanied by an earlier increase in expression of the ECM molecules fibronectin and tenascin-C, although collagen expression remained unaffected. Thus, in addition to revealing novel activities of activin in keratinocyte differentiation and dermal fibrosis, this study implicates activin as a stimulatory factor during wound repair.

3.6. Epidermal Overexpression of BMP-6 Inhibits Wound Reepithelialization

BMP-6 is strongly expressed in the developing murine epidermis, with mRNA levels falling after 6 d postpartum to a low level in adult skin ***(60)***. As such, it is closely associated with the most active phases of skin proliferation. To address further the role of BMP-6 in the skin, Blessing et al. **(61)** engineered transgenic mouse lines overex–

pressing BMP-6 in the suprabasal layers of the epidermis, using the keratin 10 promoter. Different lines with varied patterns of transgene expression showed completely opposite skin phenotypes. Strong and uniform expression of the BMP-6 transgene inhibited cell proliferation but had little effect on differentiation, whereas weak and patchy expression evoked strong hyperproliferation and parakeratosis in adult epidermis and severe perturbations of the usual pattern of differentiation, resulting in a psoriasis-like phenotype.

Since BMP-6 was found to be upregulated in human skin ulcers, where it may be involved in the inhibition of reepithelialization, the same laboratory decided to investigate wound repair in these mice strongly overexpressing BMP-6 ***(62)***. Although all the major phases of tissue repair could be observed in transgenic mice, they showed significant delays in eschar detachment, reepithelialization, and granulation tissue maturation. Thus, BMP-6 may well be a causal factor in the failure of repair seen in chronic wounds.

4. Cytokines and Chemokines

Cytokines are small, secreted proteins of up to 20 kDa that affect the behavior of immune cells as well as other cells. They include the interleukins, lymphokines, and several related signaling molecules, such as tumor necrosis factor-α (TNF-α), TNF-β, and interferons. Chemokines are a subset of cytokines that stimulate chemotaxis and extravasation of immune cells via binding to G-protein-coupled receptors on the surface of target cells. It has long been thought that the proinflammatory cytokines, including interleukin-1α (IL-1α) and IL-1β, IL-6, and TNFα, play an important role in wound repair. They likely influence a series of crucial biological effects at the wound site, including stimulation of keratinocyte and fibroblast proliferation, synthesis and breakdown of ECM proteins, fibroblast chemotaxis, and regulation of the immune response.

4.1. IL-6 Knockout Mice Show Severe Deficits in Cutaneous Repair

IL-6 expression is rapidly upregulated following wounding, being produced mainly by keratinocytes, but also by macrophages, Langerhans cells, and fibroblasts ***(63)***. A mitogen for keratinocytes ***(64)***, its overexpression is associated with several skin pathologies, including psoriasis ***(65)***. Using a full-thickness punch biopsy wounding model on IL-6 knockout mice, Gallucci et al. ***(66)*** showed that IL-6 is essential for reepithelialization, inflammation, and granulation tissue formation.

Excisional wounds to IL-6 null mice took up to three times longer to heal than those of wild-type controls and were characterized by a dramatic delay in reepithelialization and granulation tissue formation. This impaired phenotype was completely rescued by administration of recombinant murine IL-6 protein 1 h prior to wounding. Thus, it appears that IL-6 is crucial for kick-starting the wound response, both via its mitogenic effects on wound edge keratinocytes and via its chemoattractive effect on neutrophils, the first inflammatory cells to reach the clot.

4.2. Stat-3-Mediated Transduction of IL-6 Signaling Is Essential for Tissue Repair

STATs (signal transducers and activators of transcription) are cytoplasmic molecules that transduce signals from a variety of growth factors, cytokines, and hormones. Once activated by tyrosine phosphorylation, they dimerize and translocate to the nucleus, where they bind to specific DNA elements and thus activate target gene expression ***(67)***. Stat-3 is activated by IL-6 signaling and is thus a likely candidate for a role in wound repair. Since Stat-3 null mice die during embryogenesis ***(68)***, Sano et al. ***(69)*** used a Cre-lox approach to knock out Stat-3 in keratinocytes. Consistent with a low level of IL-6 expression in normal skin, they saw no effect on skin morphogenesis. However, following full-thickness excisional wounding, the healing process was severely compromised, with dramatically reduced reepithelialization **(Fig. 2)**, showing clear similarities to the reepithelialization phenotype of the IL-6 knockout mice. The overall effect on repair was less dramatic than in the IL-6 null mice, since the cell types involved in both the inflammatory response and granulation tissue formation were unaffected by the tissue-specific approach.

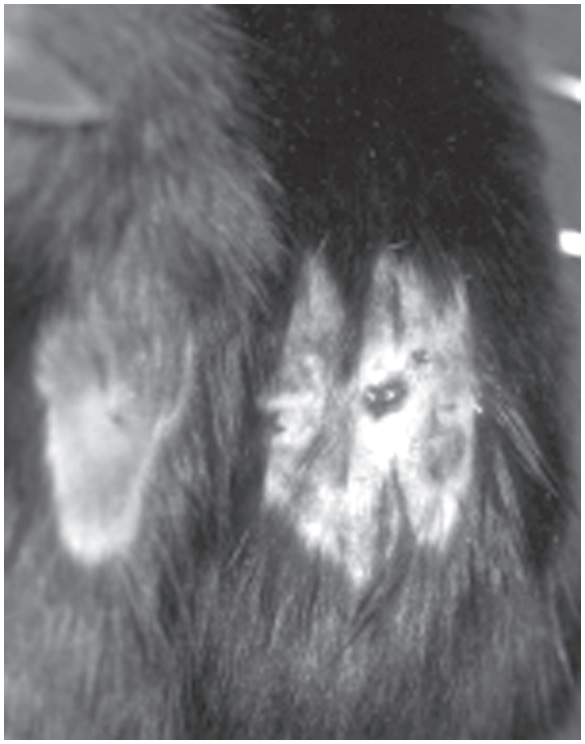

Fig. 2. Retardation of skin wound healing in Stat3-disrupted mice. A comparison of skin wound healing in a Stat3-disrupted mouse (–/–, *right*) and a control littermate (+/+, *left*) is shown. The photograph was taken on d 8 after wounding. (Figure courtesy of Prof. Junji Takeda. Reprinted from **ref. *69*** by permission. Copyright 1999 Oxford University Press.)

4.3. Epidermal Overexpression of IP-10 Delays Wound Repair

Interferon-γ-inducible protein-10 (IP-10) is a chemokine that is detected at high levels in several chronic inflammatory conditions, including psoriasis. It is a member of the CXC family of chemokines and acts primarily in the recruitment of neutrophils and lymphocytes ***(70)***. It is also one of a group of several chemokines that are upregulated following wounding, with an expression pattern that correlates well with recruitment of inflammatory cells to the wound site ***(71)***. To determine whether IP-10 could modulate an in vivo inflammatory response, Luster et al. ***(72)*** engineered mice that constitutively express IP-10 in keratinocytes. These mice showed no obvious abnormalities, until they were subjected to full-thickness excisional wounding. Following injury, IP-10-overexpressing mice showed a more intense inflammatory phase, delayed reepithelialization, and a prolonged, disorganized granulation phase with impaired angiogenesis compared with control littermates. These data suggest that IP-10 is able to inhibit wound repair by disrupting the normal development of the granulation tissue.

4.4. CXCR2 Null Mice Show Multiple Defects in Wound Healing

IP-10 exerts its biological effects via binding to the CXCR3 chemokine receptor. This receptor is reported to antagonize the signal transduction pathway downstream of another chemokine receptor, CXCR2 ***(72)***. CXCR2 receptors are expressed on keratinocytes, neovascularizing endothelial cells, and neutrophils and bind the chemokines MIP-2 and KC, both of which are upregulated in mouse wounds ***(73)***. To determine their role in wound repair, Devalaraja et al. ***(74)*** made full-thickness excisional punch biopsy wounds in mice lacking CXCR2. Following wounding, these mice exhibited defective neutrophil recruitment, delayed monocyte recruitment, and decreased secretion of the proinflammatory cytokine IL-1β. Histologically, they also showed delayed reepithelialization and decreased neovascularization ***(75)***.

4.5. IL-10 Causes Scarring in a Model of Fetal Wound Repair

Fetal wound healing is characterized by rapid reepithelialization, minimal inflammation, and scar-free repair ***(76)***. Fetal wounds also show diminished expression of the proinflammatory cytokines IL-6 and IL-8, a phenomenon that was hypothesized to be owing to their negative regulation by the anti-inflammatory cytokine IL-10. To test this hypothesis, Liechty et al. ***(77)*** wounded embryonic skin from IL-10 null mice that had been grafted onto strain-matched adult mice. Wounds of control embryonic skin grafts showed little inflammation and normal restoration of der-

mal architecture. However, wounded IL-10 null grafts showed significantly higher inflammatory cell infiltration and collagen deposition more akin to the scarring associated with adult repair. This study suggests that IL-10 plays an important role in regulating the expression of proinflammatory cytokines at the fetal wound site, and thus modulates downstream matrix deposition that leads to scar-free repair.

4.6. Embryo Studies Address Roles for Intermediate Filaments in Wound Repair

In a further study into the mechanisms behind the perfect healing that occurs in the embryo, Eckes et al. ***(78)*** wounded midgestational mouse embryos lacking the intermediate filament protein vimentin. Embryonic day 11.5 mouse embryos are capable of healing an excisional hind leg amputation wound within 24 h ***(79)***. Another study, using the same model, had revealed that one of the major intermediate filaments in early embryonic skin, keratin 8, was not essential for normal embryonic repair ***(80)***. Using DiI-labeling of wound margin mesenchymal cells, Eckes et al. ***(78)*** showed that, while reepithelialization proceeded normally, vimentin null embryos failed to contract the mesenchyme of their wound bed **(Fig. 3)**. Thus, vimentin is essential for the generation of the tractional forces that drive mesenchymal contraction in the embryonic wound. This defect in repair was also seen in adult wounds to vimentin null mice, again in a defect limited to the connective tissue, which displayed delayed granulation tissue formation and contraction.

5. Conclusion

Studies of wound healing using genetically modified mice have already revealed crucial roles for several genes in the repair process. However, some of the normal functions of the genes targeted might not be revealed owing to redundancy or compensation. This hypothesis is supported by the lack of obvious wound-healing abnormalities in various knockout mice, such as mice deficient in KGF or TGF-α ***(16)***. Although it cannot be excluded that these proteins are indeed not important for wound repair, their strong induction in healing skin wounds supports their functional significance. In the case of TGF-α and KGF, other growth factors, which bind to the same receptor, might compensate for the lack of these mitogens. Wound-healing studies using animals deficient in two or more homologous molecules, as well as studies with dominant-negative-acting molecules that can inhibit the function of several members of a protein family, will be very useful in answering these areas of question.

At the other extreme, secondary effects, which are owing to systemic defects caused by the transgene, or to transgene-mediated defects in nonwounded skin, might obscure the normal function of a gene in wound repair. Thus, it has long been known that the wound-healing process is significantly impaired by systemic abnormalities such as malnutrition, weight loss, impaired oxygenation, and ageing ***(44,45)***. This was also the case in the TGF-β1 knockout mice, which developed a severe wasting syndrome at approx 3 wk of age, accompanied by a severe inflammatory response in various tissues and organs, including the wound. These abnormalities are likely to be responsible for the impaired wound healing seen in these mice, making it impossible to study the local effects of the lack of TGF-β1 on wound repair in this model. One approach to circumvent this problem, as mentioned under **Subheading 3.2.**, was to cross the mice onto an immunodeficient background. However, these problems might also be solved by the generation of mice that have a tissue-specific knockout or tissue-specific overexpression of a transgene ***(81)***. Ideally, inducible systems that allow the induction of a transgene or the deletion of an endogenous gene in a time- and tissue-specific manner should be used. Such systems thus allow the study of the role of a particular gene under specific conditions such as during wound repair.

The first successful results with inducible systems in the skin have recently been published. Two have adopted an estrogen receptor-based approach, where Cre recombinase was fused in frame with the tamoxifen-responsive hormone-binding domain of the estrogen receptor. This fusion protein was expressed under the control of the Keratin 5 promoter ***(82)*** or Keratin 14 pro-

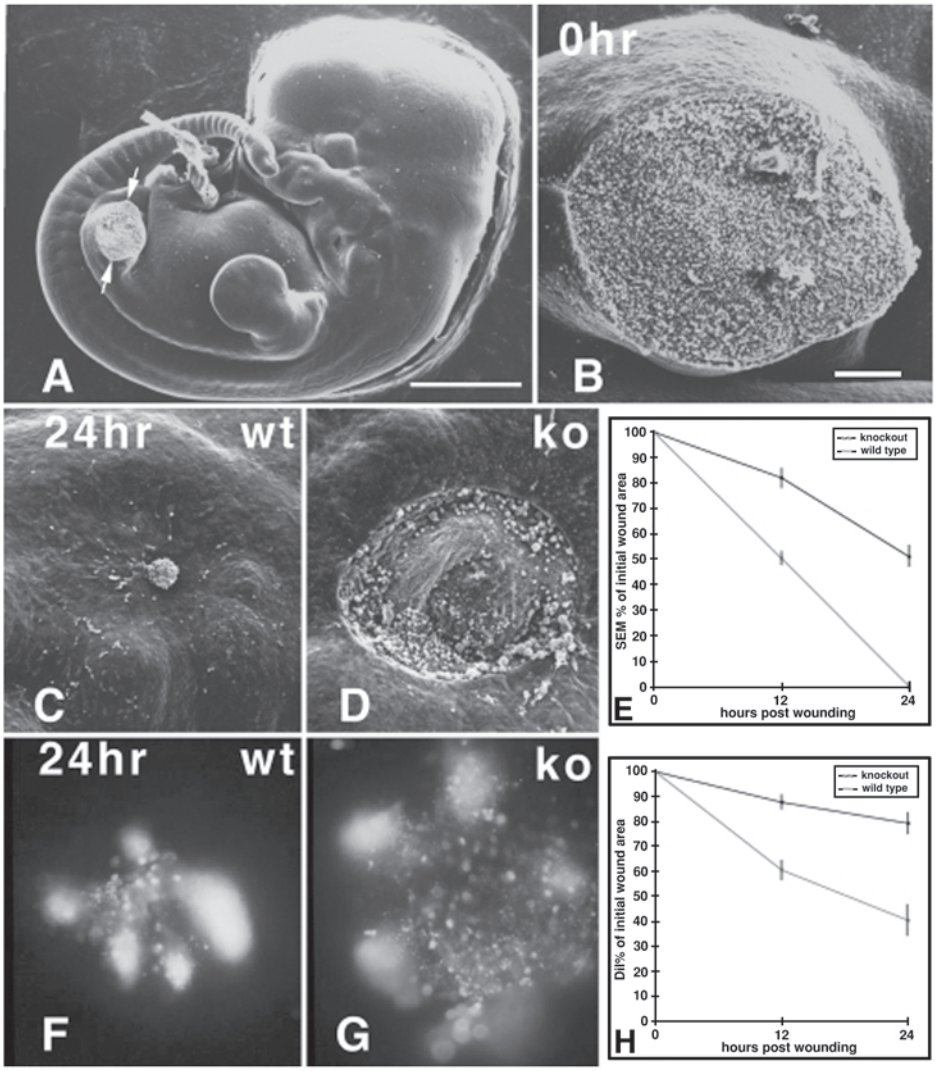

Fig. 3. Wound closure in wild-type and vimentin-deficient embryos. **(A)** Scanning electron micrograph of embryonic d 11.5 mouse embryo with left hind limb bud amputated to leave oval-shaped excisional wound (arrows). **(B)** Higher magnification detail of this 0-h wound. **(C)** After 24 h the wild-type wound is closed. **(D)** By contrast, at 24 h postwounding the vimentin-deficient wound is still open. **(E)** Graphic representation of wound closure (reepithelialization + connective-tissue contraction) as measured from scanning electron micrographs. Shaded and solid symbols indicate area measurements in wild-type and vimentin-deficient embryos, respectively, during the 24-h culture period. Error bars are SEMs. **(F)** Twenty-four hours postwounding, the wild-type controls have significantly contracted their DiI-marked wound mesenchyme.**(G)** By contrast, at the same time point, the vimentin-deficient wound has barely contracted. **(H)** Graphic representation of connective-tissue contraction component of wound closure as measured from DiI-marked specimens. Shaded and solid symbols indicate area measurements in wild-type and vimentin-deficient embryos, respectively, during the 24-h culture period. Error bars are SEMs. Scale bars: (A) 1 mm; (B, C, D, F, G) 100 mm. (Figure courtesy of Dr. Paul Martin. Reprinted from ref. 78 by permission. Copyright 2000 Company of Biologists Ltd.)

moter ***(83)***. Cre-mediated recombination of loxP sites flanking the target gene thus results in temporally and spatially restricted knockout of the target gene. In a different approach, Wang et al. ***(84)*** have used topical application of antiprogestin to induce expression of target genes. This method

works via skin-specific expression of a fusion protein, under the control of the loricrin promoter, containing a truncated progesterone receptor fused to the yeast GAL4 transcription factor. Thus, by engineering a GAL4-binding domain, normally absent in mammalian cells, upstream of the target gene, transcription can be activated in a tissue-specific and temporally controlled manner. Use of these types of systems promises to yield extremely valuable data on the actions of many genes crucial to wound repair, but formerly impossible to study.

Acknowledgments

We are particularly grateful to Prof. Anita Roberts, NIH Bethesda; Prof. Junji Takeda, Osaka University Medical School; and Dr. Paul Martin, University College London, for kindly providing **Figs. 1–3**. We also thank Prof. Manfred Blessing and Dr. Gillian Ashcroft for critical reading of the manuscript, and our coworkers for providing unpublished data. Work in Sabine Werner's laboratory is supported by the ETH Zurich, the Swiss National Science Foundation (grant no. 31-61358.00), the German Ministry for Education and Research (BMBF), and the Stiftung Verum.

References

1. Clark, R. A. F. (1996) Wound repair: Overview and general considerations, in *The Molecular and Cellular Biology of Wound Repair, 2nd ed.* (Clark, R. A. F., ed.) Plenum, New York, pp. 3-50.
2 Ornitz, D. M. and Itoph, N. (2001) Fibroblast growth factors. *Genome Biol.* 2, 3005.1-3005.12.
3. Werner, S. (1998) The role of fibroblast growth factors in skin morphogenesis and wound repair, in *Epithelial Morphogenesis in Development and Disease* (Birchmeier, C. and Birchmeier, W., eds.), Harwood Academic, GMBH, Chur, Switzerland, p. 233.
4. Johnson, D. E. and Williams, L. T. (1993) Structural and functional diversity in the FGF receptor multigene family. *Adv. Cancer Res.* **60,** 1-41.
5 Rosenquist, T. A. and Martin, G. R. (1996) Fibroblast growth factor signalling in the hair growth cycle: expression of the fibroblast growth factor receptor and ligand genes in the murine hair follicle. *Dev. Dyn.* **205,** 379-386.
6. Abraham, L. A. and Klagsbrun, M. (1996) Modulation of wound repair by members of the fibroblast growth factor family, in *The Molecular and Cellular Biology of Wound Repair, 2nd ed.* (Clark, R. A. F., ed.), Plenum, New York, pp. 195–248.
7 Miki, T., Bottaro, D. P., Fleming, T. P., Smith, C. L., Burgess, W. H., Chan, A. M., and Aaronson, S. A. (1992) Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. *Proc. Natl. Acad. Sci. USA* **89,** 246–250.
8 Werner, S., Peters, K. G., Longaker, M. T., Fuller-Pace, F., Banda, M., and Williams, L. T. (1992) Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proc. Natl. Acad. Sci. USA* **89,** 6896–6900.
9 Marchese, C., Chedid, M., Dirsch, O. R., Csaky, K. G., Santanelli, F., Latini, C., LaRochelle, W. J., Torrisi, M. R., and Aaronson, S. A. (1995) Modulation of keratinocyte growth factor and its receptor in re-epithelialising human skin. *J. Exp. Med.* **182,** 1369–1376.
10 Ueno, H., Colbert, H., Escobedo, J. A., and Williams, L. T. (1991) Inhibition of PDGF b-receptor by co-expression of a truncated receptor. *Science* **252,** 844–848.
11 Honegger, A. M., Schmidt, A., Ullrich, A., and Schlessinger, J. (1990) Evidence for epidermal growth factor (EGF)-induced intermolecular autophosphorylation of the EGF receptors in living cells. *Mol. Cell Biol.* **10,** 4035–4044.
12 Kashles, O., Yarden, Y., Fischer, R., Ullrich, A., and Schlessinger, J. (1991) A dominant negative mutation suppresses the function of normal epidermal growth factor receptors by heterodimerisation. *Mol. Cell Biol.* **11,** 1454–1463.
13. Mathieu, M., Chatelain, E., Ornitz, D., Bresnick, J., Mason, I., Kiefer, P., and Dickson, C. (1995) Receptor binding and mitogenic properties of mouse fibroblast growth factor 3: modulation of response by heparin. *J. Biol. Chem.* 41, **24,** 197–24,203.
14 Yamasaki, M., Miyake, A., Tagashira, S., and Itoh, N. (1996) Structure and expression of the rat mRNA encoding a novel member of the fibroblast growth factor family. *J. Biol. Chem.* **271,** 15,918–15,921.
15 Werner, S., Smola, H., Liao, X., Longaker, M. T., Krieg, T., Hofschneider, P. H., and Williams, L. T. (1994) The function of KGF in epithelial morphogenesis and wound re-epithelialisation. *Science* **266,** 819–822.
16 Guo, L., Degenstein, L., and Fuchs, E. (1996) Keratinocyte growth factor is required for hair development but not for wound healing. *Genes Dev.* **10,** 165–175.
17 Beer, H. D., Florence, C., Dammeier, J., McGuire, L., Werner, S., and Duan, D. R. (1997) Mouse fibroblast growth factor 10: cDNA cloning, protein characterization, and regulation of mRNA expression. *Oncogene* **15,** 2211–2218.
18 Barrandon, Y. and Green, H. (1987) Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-a and epidermal growth factor. *Cell* **50,** 1131–1137.

19 Schultz, G. S., White, M., Mitchell, R., Brown, G., Lynch, J., Twardzik, D. R., and Todaro, G. J. (1987) Epithelial wound healing enhanced by transforming growth factor-a and vaccinia growth factor. *Science* **235,** 350–352.
20 Mann, G. B., Fowler, K. J., Gabriel, A., Nice, E. C., Williams, R. L., and Dunn, A. R. (1993) Mice with a null mutation of the TGFa gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* **73,** 249–261.
21 Luetteke, N. C., Qiu, T. H., Peiffer, R. L., Oliver, P., Smithies, O., and Lee, D. C. (1993) TGFa deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* **73,** 263–278.
22 Marikovsky, M., Breuing, K., Liu, P. Y., Eriksson, E., Higashiyama, S., Farber, P., Abraham, J., and Klagsbrun, M. (1993) Appearance of heparin-binding EGF-like growth factor in wound fluid as a response to injury. *Proc. Natl. Acad. Sci. USA* **90,** 3889–3893.
23 Sibilia, M. and Wagner, E. F. (1995) Strain-dependent epithelial defects in mice lacking the EGF receptor. *Science* **269,** 234–238.
24 Miettinen, P. J., Berger, J. E., Meneses, J., Phung, Y., Pedersen, R. A., Werb, Z., and Derynck, R. (1995) Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* **376,** 337–341.
25 Murillas, R., Larcher, F., Conti, C. J., Santos, M., Ullrich, A., and Jorcano, J. L.(1995) Expression of a dominant negative mutant of epidermal growth factor receptor in the epidermis of transgenic mice elicits striking alterations in hair follicle development and skin structure. *EMBO J.* **14,** 5216–5223.
26 Bikfalvi, A., Klein, S., Pintucci, G., and Rifkin, D. B. (1997) Biological roles of fibroblast growth factor-2. *Endocr. Rev.* **18,** 26–45.
27 Ortega, S., Ittmann, M., Tsang, S. H., Ehrlich, M., and Basilico, C. (1998) Neuronal defects and delayed wound healing in mice lacking fibroblast growth factor 2. *Proc. Natl. Acad. Sci. USA* **95,** 5672–5677.
28 Gibran, N. S., Isik, F. F., Heimbach, D. M., and Gordon, D. (1994) Basic fibroblast growth factor in the early human burn wound. *J. Surg. Res.* **56,** 226–234.
29 McGee, G. S., Davidson, J. M., Buckley, A., Sommer, A., Woodward, S. C., Aquino, A. M., Barbour, R., and Demetriou, A. A. (1988) Recombinant basic fibroblast growth factor accelerates wound healing. *J. Surg. Res.* **45,** 145–153.
30 Hebda, P. A., Klingbeil, C. K., Abraham, J. A., and Fiddes, J. C. (1990) Basic fibroblast growth factor stimulation of epidermal wound healing in pigs. *J. Invest. Dermatol.* **95,** 626–631.
31 Tsuboi, R. and Rifkin, D. B. (1990) Recombinant basic fibroblast growth factor stimulates wound healing in healing-impaired db/db mice. *J. Exp. Med.* **172,** 245–251.
32 Broadley, K. N., Aquino, A. M., Woodward, S. C., Buckley-Sturrock, A., Sato, Y., Rifkin, D. B., and Davidson, J. M. (1989) Monospecific antibodies implicate basic fibroblast growth factor in normal wound repair. *Lab. Invest.* **61,** 571–575.
33. Massagué, J. (1990) The transforming growth factor-b family. *Annu. Rev. Cell Biol.* **6,** 597–641.
34 Massague, J. and Wotton, D. (2000) Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J.* **19,** 1745–1754.
35. Roberts, A. B. and Sporn, M. B. (2001) Transforming growth factor-β, in *The Molecular and Cellular Biology of Wound Repair,* 2nd ed. (Clark, R. A. F., ed.), Plenum, New York, pp. 275–308.
36 Levine, J. H., Moses, H. L., Gold, L. I., and Nanney, L. B. (1993) Spatial and temporal patterns of immunoreactive TGF-β1, β2, β3 during excisional wound repair. *Am. J. Pathol.* **143,** 368–380.
37. Frank, S., Madlener, M., and Werner, S. (1996) Transforming growth factors β1, β2, and β3 and their receptors are differentially regulated during normal and impaired wound healing. *J. Biol. Chem.* 271, **10,** 188–10,193.
38 Shah, M., Foreman, D. M., and Ferguson, M. W. J. (1994) Neutralising antibody to TGF-β1,2 reduces cutaneous scarring in adult rodents. *J. Cell Sci.* **107,** 1137–1157.
39 Shah, M., Foreman, D. M., and Ferguson, M. W. J. (1995) Neutralisation of TGF-b1 and TGF-b2 or exogenous addition of TGF-β3 to cutaneous rat wounds reduces scarring. *J. Cell Sci.* **108,** 985–1002.
40 Brown, R. L., Ormsby, I., Doetschman, T. C., and Greenhalgh, D. G. (1995) Wound healing in the transforming growth factor-b1-deficient mouse. *Wound Rep. Reg.* **3,** 25–36.
41 Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N., and Doetschman, T. (1992) Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflammatory disease. *Nature* **359,** 693–699.
42 Kulkarni, A. B., Huh, C.-G., Becker, D., Geiser, A., Lyght, M., Flanders, K. C., Roberts, A. B., Sporn, M. B., Ward, J. M., and Karlsson, S. (1993) Transforming growth factor β1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA* **90,** 770–774.
43 Letterio, J. J., Geiser, A. G., Kulkarni, A. B, Roche, N. S., Sporn, M. B., and Roberts, A. B. (1994) Maternal rescue of transforming growth factor-β1 null mice. *Science* **264,** 1936–1938.
44 Greenhalgh, D. G. and Gamelli, R. L. (1987) Is impaired wound healing caused by infection or nutritional depletion? *Surgery* **102,** 306–312.
45 Reed, B. R. and Clark, R. A. (1985) Cutaneous tissue repair: practical implications of current knowledge. II. *J. Am. Acad. Dermatol.* **13,** 919–941.
46 Crowe, M. J., Doetschman, T., and Greenhalgh, D. G. (2000) Delayed wound healing in immunodeficient

TGF-β 1 knockout mice. *J. Invest. Dermatol.* **115,** 3–11.
47 Shah, M., Revis, D., Herrick, S., Baillie, R., Thorgeirson, S., Ferguson, M., and Roberts, A. (1999) Role of elevated plasma transforming growth factor-β1 levels in wound healing. *Am. J. Pathol.* **154,** 1115–1124.
48 Massague, J. (1998) TGF-β signal transduction. *Annu. Rev. Biochem.* **67,** 753–791.
49 Derynck, R., Zhang, Y., and Feng, X. H. (1998) Smads: transcriptional activators of TGF-β responses. *Cell* **95,** 737–740.
50 Ashcroft, G. S. and Roberts, A. B. (2000) Loss of Smad3 modulates wound healing. *Cytokine Growth Factor Rev.* **11,** 125–131.
51 Christian, J. L. and Nakayama, T. (1999) Can't get no SMADisfaction: Smad proteins as positive and negative regulators of TGF-beta family signals. *Bioessays* **21,** 382–390.
52 Weinstein, M., Yang, X., Li, C., Xu, X., Gotay, J., and Deng, C. X. (1998) Failure of egg cylinder elongation and mesoderm induction in mouse embryos lacking the tumor suppressor smad2. *Proc. Natl. Acad. Sci. USA* **95,** 9378–9383.
53 Yang, X., Castilla, L. H., Xu, X., Li, C., Gotay, J., Weinstein, M., Liu, P. P., and Deng, C. X. (1999) Angiogenesis defects and mesenchymal apoptosis in mice lacking SMAD5. *Development* **126,** 1571–1580.
54 Ashcroft, G. S., Yang, X., Glick, A. B., Weinstein, M., Letterio, J. L., Mizel, D. E.,Anzano, M., Greenwell-Wild, T., Wahl, S. M., Deng, C., and Roberts, A. B. (1999) Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat. Cell Biol.* **1,** 260–266.
55 Hocevar, B. A., Brown T. L., and Howe, P. H. (1999) TGF-beta induces fibronectin synthesis through a c-Jun *N*-terminal kinase-dependent, Smad4-independent pathway. *EMBO J.* **18,** 1345–1356.
56 Matzuk, M. M., Kumar, T. R., Vassalli, A., Bickenbach, J. R., Roop, D. R., Jaenisch, R., and Bradley, A. (1995) Functional analysis of activins during mammalian development. *Nature* **374,** 354–356.
57 Matzuk, M. M., Lu, N., Vogel, H., Sellheyer, K., Roop, D. R., and Bradley, A. (1995) Multiple defects and perinatal death in mice deficient in follistatin. *Nature* **374,** 360–363.
58. Hübner, G., Hu, Q., Smola, H., and Werner, S. (1996) Strong induction of activin expression after injury suggests an important role of activin in wound repair. *Dev. Biol.* **173,** 490–498.
59 Munz, B., Smola, H., Engelhardt, F., Bleuel, K., Brauchle, M., Lein, I., Evans, L. W., Huylebroeck, D., Balling, R., and Werner, S. (1999) 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.* **18,** 5205–5215.
60 Lyons, K. M., Pelton, R. W., and Hogan, B. L. (1989) Patterns of expression of murine Vgr-1 and BMP-2a RNA suggest that transforming growth factor-beta-like genes coordinately regulate aspects of embryonic development. *Genes Dev.* **3,** 1657–1668.
61 Blessing, M., Schirmacher, P., and Kaiser, S. (1996) Overexpression of bone morphogenetic protein-6 (BMP-6) in the epidermis of transgenic mice: inhibition or stimulation of proliferation depending on the pattern of transgene expression and formation of psoriatic lesions. *J. Cell Biol.* **135,** 227–239.
62 Kaiser, S., Schirmacher, P., Philipp, A., Protschka, M., Moll, I., Nicol, K., and Blessing, M. (1998) Induction of bone morphogenetic protein-6 in skin wounds: delayed reepitheliazation and scar formation in BMP-6 overexpressing transgenic mice. *J. Invest. Dermatol.* **111,** 1145–1152.
63 Paquet, P. and Pierard, G. E. (1996) Interleukin-6 and the skin. *Int. Arch. Allergy Immunol.* **109,** 308–317.
64 Sato, M., Sawamura, D., Ina, S., Yaguchi, T., Hanada, K., and Hashimoto, I. (1999) In vivo introduction of the interleukin 6 gene into human keratinocytes: induction of epidermal proliferation by the fully spliced form of interleukin 6, but not by the alternatively spliced form. *Arch. Dermatol. Res.* **291,** 400–404.
65 Grossman, R. M., Krueger, J., Yourish, D., Granelli-Piperno, A., Murphy D. P., May, L. T., Kupper, T. S., Sehgal, P. B., and Gottlieb, A. B. (1989) Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. *Proc. Natl. Acad. Sci. USA* **86,** 6367–6371.
66 Gallucci, R. M., Simeonova, P. P., Matheson, J. M., Kommineni, C., Guriel, J. L., Sugawara, T., and Luster, M. I. (2000) Impaired cutaneous wound healing in interleukin-6-deficient and immunosuppressed mice. *FASEB J.* **14,** 2525–2531.
67 Bromberg, J. (2001) Activation of STAT proteins and growth control. *Bioessays* **23,** 161–169.
68 Takeda, K., Noguchi, K., Shi, W., Tanaka, T., Matsumoto, M., Yoshida, N., Kishimoto, T., and Akira, S. (1997) Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. *Proc. Natl. Acad. Sci. USA* **94,** 3801–3804.
69 Sano, S., Itami, S., Takeda, K., Tarutani, M., Yamaguchi, Y., Miura, H., Yoshikawa, K., Akira, S., and Takeda, J. (1999) Keratinocyte-specific ablation of Stat3 exhibits impaired skin remodeling, but does not affect skin morphogenesis. *EMBO J.* **18,** 4657–4668.
70 Devalaraja, M. N. and Richmond, A. (1999) Multiple chemotactic factors: fine control or redundancy? *Trends Pharmacol. Sci.* **20,** 151–156.
71 Engelhardt, E., Toksoy, A., Goebeler, M., Debus, S., Brocker, E. B., and Gillitzer, R. (1998) Chemokines IL-8, GROalpha, MCP-1, IP-10, and Mig are sequentially and differentially expressed during phase-specific infiltration of leukocyte subsets in human wound healing. *Am. J. Pathol.* **153,** 1849–1860.

72 Luster, A. D., Cardiff, R. D., MacLean, J. A., Crowe, K., and Granstein, R. D. (1998) Delayed wound healing and disorganized neovascularization in transgenic mice expressing the IP-10 chemokine. *Proc. Assoc. Am. Physicians* **110,** 183–196.
73 Fivenson, D. P., Faria, D. T., Nickoloff, B. J., Poverini, P. J., Kunkel, S., Burdick, M., and Streiter, R. M. (1997) Chemokine and inflammatory cytokine changes during chronic wound healing. *Wound Rep. Reg.* **5,** 310–322.
74 Cacalano, G., Lee, J., Kikly, K., Ryan, A. M., Pitts-Meek, S., Hultgren, B., Wood, W. I., and Moore, M. W. (1994) Neutrophil and B cell expansion in mice that lack the murine IL-8 receptor homolog. *Science* **265,** 682–684.
75 Devalaraja, R. M., Nanney, L. B., Qian, Q., Du, J., Yu, Y., Devalaraja, M. N., and Richmond, A. (2000) Delayed wound healing in CXCR2 knockout mice. *J. Invest. Dermatol.* **115,** 234–244.
76 Martin, P. (1997) Wound healing-aiming for perfect skin regeneration. *Science* **276,** 75–81.
77 Liechty, K. W., Kim, H. B., Adzick, N. S., and Crombleholme, T. M. (2000) Fetal wound repair results in scar formation in interleukin-10-deficient mice in a syngeneic murine model of scarless fetal wound repair. *J. Pediatr. Surg.* **35,** 866–872; discussion: 872,873.
78 Eckes, B., Colucci-Guyon, E., Smola, H., Nodder, S., Babinet, C., Krieg, T., and Martin, P. (2000) Impaired wound healing in embryonic and adult mice lacking vimentin. *J. Cell Sci.* **113,** 2455–2462.
79 McCluskey, J. and Martin, P. (1995) Analysis of the tissue movements of embryonic wound healing-DiI studies in the limb bud stage mouse embryo. *Dev. Biol.* **170,** 102–114.
80 Brock, J., McCluskey, J., Baribault, H., and Martin, P. (1996) Perfect wound healing in the keratin 8 deficient mouse embryo. *Cell. Motil. Cytoskel.* **35,** 358–366.
81 Rajewsky, K., Gu, H., Kuhn, R., Betz, U. A., Muller, W., Roes, J., and Schwenk, F. (1996) Conditional gene targeting. *J. Clin. Invest.* **98,** 600–603.
82 Indra, A. K., Warot, X., Brocard, J., Bornert, J. M., Xiao, J. H., Chambon, P., and Metzger, D. (1999) Temporally-controlled site-specific mutagenesis in the basal layer of the epidermis: comparison of the recombinase activity of the tamoxifen- inducible Cre-ER(T) and Cre-ER(T2) recombinases. *Nucleic Acids Res.* **27,** 4324–4327.
83 Vasioukhin, V., Degenstein, L., Wise, B., and Fuchs, E. (1999) The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. *Proc. Natl. Acad. Sci. USA* **96,** 8551–8556.
84 Wang, X. J., Liefer, K. M., Tsai, S., O'Malley, B. W., and Roop, D. R. (1999) Development of gene-switch transgenic mice that inducibly express transforming growth factor beta1 in the epidermis. *Proc. Natl. Acad. Sci. USA* **96,** 8483–8488.
85 Guo, L., Degenstein, L., Dowling, J., Yu, Q.-C., Wollman, R., Perman, B., and Fuchs, E. (1995) Gene targeting of BPAG1: abnormalities in mechanical strength and cell migration in stratified epithelia and neurologic degeneration. *Cell* **81,** 233–243.
86 Abbott, R. E., Corral, C. J., MacIvor, D. M., Lin, X., Ley, T. J., and Mustoe, T. A. (1998) Augmented inflammatory responses and altered wound healing in cathepsin G-deficient mice. *Arch. Surg.* **133,** 1002–1006.
87 Kaya, G., Rodriguez, I., Jorcano, J. L., Vassalli, P., and Stamenkovic, I. (1997) Selective suppression of CD44 in keratinocytes of mice bearing an antisense CD44 transgene driven by a tissue-specific promoter disrupts hyaluronate metabolism in the skin and impairs keratinocyte proliferation. *Genes Dev.* **11,** 996–1007.
88 Hansen, L. A., Alexander, N., Hogan, M. E., Sundberg, J. P., Dlugosz, A., Threadgill, D. W., Magnuson, T., and Yuspa, S. H. (1997) Genetically null mice reveal a central role for epidermal growth factor receptor in the differentiation of the hair follicle and normal hair development. *Am. J. Pathol.* **150,** 1959–1975.
89 Bugge, T. H., Kombrinck, K. W., Flick, M. J., Daugherty, C. C., Danton, M. J., and Degen, J. L. (1996) Loss of fibrinogen rescues mice from the pleiotropic effects of plasminogen deficiency. *Cell* **87,** 709–719.
90 Bezerra, J. A., Carrick, T. L., Degen, J. L., Witte, D., and Degen, S. J. F. (1998) Biological effects of targeted inactivation of hepatocyte growth factor-like protein in mice. *J. Clin. Invest.* **101,** 1175–1183.
91 Huang, X., Griffiths, M., Wu, J., Farese, R.V. Jr., and Sheppard, D. (2000) Normal development, wound healing, and adenovirus susceptibility in beta5-deficient mice. *Mol. Cell. Biol.* **20,** 755–759.
92 Wojcik, S. M., Bundman, D. S., and Roop, D. R. (2000) Delayed wound healing in keratin 6a knockout mice. *Mol. Cell. Biol.* **20,** 5248–5255.
93 Di Colandrea, T., Wang, L., Wille, J., D'Armiento, J., and Chada, K. K. (1998) Epidermal expression of collagenase delays wound-healing in transgenic mice. *J. Invest. Dermatol.* **111,** 1029–1033.
94 Bullard, K. M., Lund, L., Mudgett, J. S., Mellin, T. N., Hunt, T. K., Murphy, B., Ronan, J., Werb, Z., and Banda, M. J. (1999) Impaired wound contraction in stromelysin-1-deficient mice. *Ann. Surg.* **230,** 260–265.
95 Atit, R. P., Crowe, M. J., Greenhalgh, D. G., Wenstrup, R. J., and Ratner, N. (1999) The Nf1 tumor suppressor regulates mouse skin wound healing, fibroblast proliferation, and collagen deposited by fibroblasts. *J. Invest. Dermatol.* **112,** 835–842.
96 Lee, P. C., Salyapongse, A. N., Bragdon, G. A., Shears, L. L. 2nd, Watkins, S. C., Edington, H. D., and Billiar, T. R. (1999) Impaired wound healing and angiogenesis in eNOS-deficient mice. *Am. J. Physiol.* **277,** H1600–H1608.
97 Yamasaki, K., Edington, H. D., McClosky, C., Tzeng, E., Lizonova, A., Kovesdi, I., Steed, D. L., and Billiar,

T. R. (1998) Reversal of impaired wound repair in iNOS-deficient mice by topical adenoviral-mediated iNOS gene transfer. *J. Clin. Invest.* **101,** 967–971.
98 Liaw, L., Birk, D. E., Ballas, C. B., Whitsitt, J. S., Davidson, J. M., and Hogan, B. L. (1998) Altered wound healing in mice lacking a functional osteopontin gene (spp1). *J. Clin. Invest.* **101,** 1468–1478.
99 Dougherty, K. M., Pearson, J. M., Yang, A. Y., Westrick, R. J., Baker, M. S., and Ginsburg, D. (1999) The plasminogen activator inhibitor-2 gene is not required for normal murine development or survival. *Proc. Natl. Acad. Sci. USA* **96,** 686–691.
100 Connolly, A. J., Suh, D. Y., Hunt, T. K., and Coughlin, S. R. (1997) Mice lacking the thrombin receptor, PAR1, have normal skin wound healing. *Am. J. Pathol.* **151,** 1199–1204.
101 Romer, J., Bugge, T. H., Pyke C., Lund, L. R., Flick, M. J., Degen, J. L., and Dano, K. (1996) Impaired wound healing in mice with a disrupted plasminogen gene. *Nat. Med.* **2,** 287–292.
102 Subramaniam, M., Saffarpour, S., Van-de-Water, L., Frenette, P. S., Mayadas, T. N., Hynes, R. O., and Wagner, D. D. (1997) Role of endothelial selectins in wound repair. *Am. J. Pathol.* **150,** 1701–1709.
103 Andersen, B., Weinberg, W. C., Rennekampff, O., et al. (1997) Functions of the POU domain genes Skn-1a/i and Tst-1/Oct-6/SCIP in epidermal differentiation. *Genes Dev.* **11,** 1873–1884.
104 Ashcroft, G. S., Lei, K., Jin, W., Longenecker, G., Kulkarni, A. B., Greenwell-Wild, T., Hale-Donze, H., McGrady, G., Song, X. Y., and Wahl, S. M. (2000) Secretory leukocyte protease inhibitor mediates non-redundant functions necessary for normal wound healing. *Nat. Med.* **6,** 1147–1153.
105 Echtermeyer, F., Streit, M., Wilcox-Adelman, S., Saoncella, S., Denhez, F., Detmar, M., and Goetinck, P. F. (2001) Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. *J. Clin. Invest.* **107,** R9–R14.
106 Forsberg, E., Hirsch, E., Frohlich, L., Meyer, M., Ekblom, P., Aszodi, A., Werner, S., and Fassler, R. (1996) Skin wounds and severed nerves heal normally in mice lacking tenascin-C. *Proc. Natl. Acad. Sci. USA* **93,** 6594–6599.
107. Peterson, J. J., Rayburn, H. B., Lager, D. J., Raife, T. J., Kealey, G. P., Rosenberg, R. D., and Lentz, S. R.4.4. CXCR2 Null Mice Show Multiple Defects in Wound Healing
108. Raife, T. J, Lager, D. J., Peterson, J. J., Erger, R. A., and Lentz, S. R. (1998) Keratinocyte-specific expression of human thrombomodulin in transgenic mice: effects on epidermal differentiation and cutaneous wound healing. *J. Invest. Med.* **46,** 127–133.
109. Streit, M., Velasco, P., Riccardi, L., Spencer, L., Brown, L.F., Janes, L., Lange-Asschenfeldt, B., Yano, K., Hawighorst, T., Iruela-Arispe, L., and Detmar, M. (2000) Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. *EMBO J.* **19,** 3272–3282.
110. Kyriakides, T. R., Tam, J. W., and Bornstein, P. (1999) Accelerated wound healing in mice with a disruption of the thrombospondin 2 gene. *J. Invest. Dermatol.* **113,** 782–787.
111. Carmeliet, P., Schoonjans, L., Kieckens, L., Ream, B., Degen, J., Bronson, R., De Vos, R., van den Oord, J. J., Collen, D., and Mulligan, R. C. (1994) Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* **368,** 419–424.
112. Bugge, T. H., Flick, M. J., Danton, M. J., Daugherty, C. C., Romer, J., Dano, K., Carmeliet, P., Collen, D., and Degen, J. L. (1996) Urokinase-type plasminogen activator is effective in fibrin clearance in the absence of its receptor or tissue-type plasminogen activator. *Proc. Natl. Acad. Sci. USA* **93,** 5899–5904.
113. Inada, R., Matsuki, M., Yamada, K., Morishima, Y., Shen, S., Kuramoto, N., Yasuno, H., Takahashi, K., Miyachi, Y., and Yamanishi, K. (2000) Facilitated wound healing by activation of the transglutaminase 1 gene. *Am. J. Pathol.* **157,** 1875–1882.
114. Jang, Y. C., Tsou, R., Gibran, N. S., and Isik, F. F. (2000) Vitronectin deficiency is associated with increased wound fibrinolysis and decreased microvascular angiogenesis in mice. *Surgery* **127,** 696–704.
115. Rodriguez-Puebla, M.L., de Marval, P.L., LaCava, M., Moons, D.S., Kiyokawa, H., Conti, C.J. (2002). Cdk4 deficiency inhibits skin tumor development but does not affect normal keratinocyte proliferation. *Am J Pathol.* **161,** 405–411.
116.Kretz, M., Euwens, C., Hombach, S., et al. (2003). Altered connexin expression and wound healing in the epidermis of connexin-deficient mice. *J Cell Sci.* **116,** 3443–3552.
117. Chen, C.C., Mo, F.E., Lau, L.F. (2001). The angiogenic factor Cyr61 activates a genetic program for wound healing in human skin fibroblasts. *J Biol Chem.* **276,** 47329–37.
118. D'Souza, S.J., Vespa, A., Murkherjee, S., Maher, A., Pajak, A., Dagnino, L. (2002). E2F-1 is essential for normal epidermal wound repair. *J Biol Chem.* **277,** 10626–10632.
119. Miller, D.L., Ortega, S., Bashayan, O., Basch, R., Basilico, C. (2000). Compensation by fibroblast growth factor 1 (FGF1) does not account for the mild phenotypic defects observed in FGF2 null mice. *Mol Cell Biol.* **20,** 2260–2268.
120. Drew, A.F., Liu, H., Davidson, J.M., Daugherty, C.C., Degen, J.L. (2001). Wound-healing defects in mice lacking fibrinogen. *Blood.* **97,** 3691–3698.
121. Sakai, T., Johnson, K.J., Murozono, M., et al. (2001). Plasma fibronectin supports neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. *Nat Med.* **7,** 324–330.
122. Muro, A.F., Chauhan, A.K., Gajovic, S., Iaconcig, A., Porro, F., Stanta, G., Baralle, F.E. (2003). Regulated

splicing of the fibronectin EDA exon is essential for proper skin wound healing and normal lifespan. *J Cell Biol.* **162,** 149–160.
123. Wankell, M., Munz, B., Hubner, G., Hans, W., Wolf, E., Goppelt, A., Werner, S. (2001). Impaired wound healing in transgenic mice overexpressing the activin antagonist follistatin in the epidermis. *EMBO J.* **20,** 5361–5372.
124. Grose, R., Werner, S., Reichardt, H., et al. (2002). A role for endogenous glucocorticoids in wound repair. *EMBO Reports.* **3,** 575–582.
125. Mann, A., Breuhahn, K., Schirmacher, P., Blessing, M. (2001). Keratinocyte-derived Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) accelerates wound healing: Stimulation of keratinocyte proliferation, granulation tissue formation, and vascularization. *J Invest Dermatol.* **117,** 1382–1390.
126. Toyoda, M., Takayama, H., Horiguchi, N., et al. (2001). Overexpression of hepatocyte growth factor/scatter factor promotes vascularization and granulation tissue formation in vivo. *FEBS Lett.* **509,** 95–100.
127. Mack, J.A., Abramson, S.R., Ben, Y., et al. (2003). Hoxb13 knockout adult skin exhibits high levels of hyaluronan and enhanced wound healing. *FASEB J.* **17,** 1352–1354.
128. Chen, J., Diacovo, T.G., Grenache, D.G., Santoro, S.A., Zutter, M.M. (2002). The alpha(2) integrin subunit-deficient mouse: a multifaceted phenotype including defects of branching morphogenesis and hemostasis. *Am J Pathol.* **161,** 337–344.
129. Grose, R., Hutter, C., Bloch, W., et al. (2002). A crucial role of ___1 integrins for keratinocyte migration in vitro and during cutaneous wound repair. *Development.* **129,** 2303–2315.
130. Lin, Z.Q., Kondo, T., Ishida, Y., Takayasu, T., Mukaida, N. (2003). Essential involvement of IL-6 in the skin wound-healing process as evidenced by delayed wound healing in IL-6-deficient mice. *J Leukoc Biol.* **73,** 713–721.
131. Li, G., Gustafson-Brown, C., Hanks, S.K., et al. (2003). c-Jun is essential for organization of the epidermal leading edge. *Dev Cell.* **4,** 865–877.
132. Zenz, R., Scheuch, H., Martin, P., et al. (2003). c-Jun regulates eyelid closure and skin tumor development through EGFR signaling. *Dev Cell.* **4,** 879–889.
133. Grose, R., Harris, B.S., Cooper, L., Topilko, P., Martin, P. (2002). The immediate early genes krox-24 and krox-20 are rapidly upregulated following wounding in the embryonic and adult mouse. *Dev Dyn.* **223,** 371–378.
134. Low, Q.E., Drugea, I.A., Duffner, L.A., et al. (2001). Wound healing in MIP-1alpha(-/-) and MCP-1(-/-) mice. *Am J Pathol.* **159,** 457–463.
135. Mohan, R., Chintala, S.K., Jung, J.C., et al. (2002). Matrix metalloproteinase gelatinase B (MMP-9) coordinates and effects epithelial regeneration. *J Biol Chem.* **277,** 2065–2072.
136. Frye, M., Gardner, C., Li, E.R., Arnold, I., Watt, F.M. (2003). Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. *Development.* **130,** 2793–2808.
137. Most, D., Efron, D.T., Shi, H.P., Tantry, U.S., Barbul, A. (2002). Characterization of incisional wound healing in inducible nitric oxide synthase knockout mice. *Surgery.* **132,** 866–876.
138. Ekstrand, A.J., Cao, R., Bjorndahl, M., et al. (2003). Deletion of neuropeptide Y (NPY) 2 receptor in mice results in blockage of NPY-induced angiogenesis and delayed wound healing. *Proc Natl Acad Sci USA.* **100,** 6033–6038.
139. Braun, S., Hanselmann, C., Gassmann, M.G., et al. (2002). Nrf2 Transcription Factor, a Novel Target of Keratinocyte Growth Factor Action Which Regulates Gene Expression and Inflammation in the Healing Skin Wound. *Mol Cell Biol.* **22,** 5492–5505.
140. Chan, J.C., Duszczyszyn, D.A., Castellino, F.J., Ploplis, V.A. (2001). Accelerated skin wound healing in plasminogen activator inhibitor-1-deficient mice. *Am J Pathol.* **159,** 1681–1688.
141. Chuang-Tsai, S., Sisson, T.H., Hattori, N., et al. (2003). Reduction in fibrotic tissue formation in mice genetically deficient in plasminogen activator inhibitor-1. *Am J Pathol.* **163,** 445–452.
142. Buetow, B.S., Crosby, J.R., Kaminski, W.E., et al. (2001). Platelet-derived growth factor B-chain of hematopoietic origin is not necessary for granulation tissue formation and its absence enhances vascularization. *Am J Pathol.* **159,** 1869–1876.
143. Chida, K., Hara, T., Hirai, T., et al. (2003). Disruption of protein kinase C eta results in impairment of wound healing and enhancement of tumor formation in mouse skin carcinogenesis. *Cancer Res.* **63,** 2404–2408.
144. Carmeliet, P., Moons, L., Luttun, A., et al. (2001). Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med.* 5:575–583.
145. Michalik, L., Desvergne, B., Tan, N.S., et al. (2001). Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. *J Cell Biol.* **154,** 799–814.
146. Tan, N.S., Michalik, L., Noy, N., et al. (2001). Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes and Dev.* **15,** 3263–3277.
147. Martin, P., D'Souza, D., Martin, J., et al. (2003). Wound healing in the PU.1 null mouse-tissue repair is not dependent on inflammatory cells. *Curr Biol.* **13,** 1122–1128.
148. Flanders, K.C., Sullivan, C.D., Fujii, M., et al. (2002). Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J Pathol.* **160,** 1057–1068.

149. Stepp, M.A., Gibson, H.E., Gala, P.H., Iet asl. (2002). Defects in keratinocyte activation during wound healing in the syndecan-1-deficient mouse. *J Cell Sci.* **115,** 4517–4531.
150. Cao, T., Grant, A.D., Gerard, N.P., Brain, S.D. (2001). Lack of a significant effect of deletion of the tachykinin neurokinin-1 receptor on wound healing in mouse skin. *Neuroscience.* **108,** 695–700.
151. Jameson, J., Ugarte, K., Chen, N., et al. (2002). A Role for Skin gamma delta T Cells in Wound Repair. *Science.* **296,** 747–749.
152. Gonzalez-Suarez, E., Samper, E., Ramirez, A., et al.. (2001). Increased epidermal tumors and increased skin wound healing in transgenic mice overexpressing the catalytic subunit of telomerase, mTERT, in basal keratinocytes. *EMBO J.* **20,** 2619–2630.
153. Luetteke, N.C., Qiu, T.H., Peiffer, R.L., Oliver, P., Smithies, O., Lee, D.C. (1993). TGF alpha deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell.* **73,** 263–278.
154. Mann, G.B., Fowler, K.J., Gabriel, A., Nice, E.C., Williams, R.L., Dunn, A.R. (1993). Mice with a null mutation of the TGF alpha gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell.* **73,** 249–261.
155. Kim, I., Mogford, J.E., Chao, J.D., Mustoe, T.A. (2001). Wound epithelialization deficits in the transforming growth factor-alpha knockout mouse. *Wound Repair* Regen. **9,** 386–390.
156. Koch, R.M., Roche, N.S., Parks, W.T., Ashcroft, G.S., Letterio, J.J., Roberts, A.B. (2000). Incisional wound healing in transforming growth factor-beta1 null mice. *Wound Repair Regen.* **8,** 179–191.
157. Yang, L., Chan, T., Demare, J., Iwashina, T., Ghahary, A., Scott, P.G., Tredget, E.E. (2001). Healing of burn wounds in transgenic mice overexpressing transforming growth factor-beta 1 in the epidermis. *Am J Pathol.* **159,** 2147–2157.
158. Chan, T., Ghahary, A., Demare, J., Yang, L., Iwashina, T., Scott, P., Tredget, E.E. (2002). Development, characterization, and wound healing of the keratin 14 promoted transforming growth factor-beta1 transgenic mouse. *Wound Repair Regen.* **10,** 177–187.
159. Amendt, C., Mann, A., Schirmacher, P., Blessing, M. (2002). Resistance of keratinocytes to TGFbeta-mediated growth restriction and apoptosis induction accelerates re-epithelialization in skin wounds. *J Cell Sci.* **115,** 2189–2198.
160. Mori, R., Kondo, T., Ohshima, T., Ishida, Y., Mukaida, N. (2002). Accelerated wound healing in tumor necrosis factor receptor p55-deficient mice with reduced leukocyte infiltration. *FASEB J.* **16,** 963–974

.