# Cancer as an overhealing wound: an old hypothesis revisited

*Matthias Schäfer and Sabine Werner*

Abstract | What is the relationship between the wound-healing process and the development of cancer? Malignant tumours often develop at sites of chronic injury, and tissue injury has an important role in the pathogenesis of malignant disease, with chronic inflammation being the most important risk factor. The development and functional characterization of genetically modified mice that lack or overexpress genes that are involved in repair, combined with gene-expression analysis in wounds and tumours, have highlighted remarkable similarities between wound repair and cancer. However, a few crucial differences were also observed, which could account for the altered metabolism, impaired differentiation capacity and invasive growth of malignant tumours.

Scar
A connective tissue replacement following the wounding of the dermis.

Keloid
An overgrowth of scar tissue beyond the original wound edge.

Stroma
A connective tissue component of an organ (or tumour), which includes fibroblasts, blood and lymphatic vessels, inflammatory cells and extracellular matrix.

Granulation tissue
A highly vascularized and cell-rich tissue that replaces the fibrin clot in a skin wound.

Sarcoma
A cancer that arises from mesenchymal cells.

*Institute of Cell Biology, ETH Zürich, Schafmattstrasse 18, 8093 Zürich, Switzerland. Correspondence to S.W. e-mail: Sabine.werner@cell.biol.ethz.ch*
doi:10.1038/nrm2455
Published online 16 July 2008

Injury to adult tissues initiates a sophisticated repair process that aims to restore the damaged body site. The different events that are involved in repair must be tightly regulated and synchronized to re-establish the integrity of the affected tissue and homeostasis in the whole organism. Defects in wound repair constitute a severe health problem that frequently affects aged individuals, patients with diabetes or immunosuppression, and patients who receive chemotherapy or radiotherapy[1]. These individuals often develop painful, non-healing ulcers. However, excessive healing causes the formation of hypertrophic scars and keloids. Malignant transformation is a particularly severe complication of non-healing ulcers, and the development of cancer in fibrotic tissue is a frequent event[2,3]. This was recognized in 1863 by Rudolf Virchow, who postulated that chronic irritation and previous injuries are a precondition for tumorigenesis[2]. This view is supported by a multitude of clinical observations that show that cancer is frequently the consequence of chronic inflammatory disease in different tissues and organs. Important examples are the increased risk of developing cancer in patients that suffer from chronic viral hepatitis, *Helicobacter pylori*-induced gastric inflammation, inflammatory bowel disease or from the skin-blistering disease recessive dystrophic epidermolysis bullosa[3].

These observations suggested that common cellular and molecular mechanisms are active in wounds and in cancer tissue. Indeed, Sir Alexander Haddow suggested that "tumor production is a possible overhealing" (REF. 4). In a classic publication, Harold Dvorak postulated that "tumors are wounds that do not heal" (REF. 5). Dvorak recognized that the composition of the tumour stroma strongly resembles the granulation tissue of healing skin wounds, which suggests that epithelial tumours promote the formation of their stroma by activating the wound-healing response of the host. However, in contrast to healing wounds, the process is not self-limiting in cancer tissue, resulting in uncontrolled cell proliferation, invasion and metastasis. This hypothesis has been strongly supported by experimental studies.

One of the earliest experimental findings that pointed to a connection between wound repair, chronic inflammation and cancer was the observation that tumours that have been induced in chickens by the Rous sarcoma virus only develop at the site of the viral injection. However, generation of wounds at other body sites of the virus-infected animal caused tumour development at the site of injury[6]. Interestingly, not only mechanical injury of virus-infected animals but also local application of pro-inflammatory growth factors caused tumour growth at the site of injury or growth factor application. By contrast, tumour development at the wound site was prevented by anti-inflammatory therapy[7]. These findings indicate that inflammation is responsible for the development of wound-induced tumours.

A correlation between wounding and tumour development was also observed in transgenic mice that express the *Jun* oncogene. These animals develop dermal fibrosarcomas following wounding[8]. Prompted by these exciting observations, researchers started to explore the parallels between wound healing and cancer at the cellular and molecular level. Recently, microarray technology was used to compare the genes expressed in wounds

Box 1 | **Molecular parallels between wound healing and cancer**

Microarray analyses have provided insights into the molecular similarities and differences between tissue repair and cancer:

- The genes that are regulated in fibroblasts following serum treatment encode proteins that are required for wound repair[85].
- The gene-expression pattern of serum-treated fibroblasts strongly resembles that of human carcinomas. Remarkably, tumours with a gene-expression pattern that is similar to the serum-activated programme of fibroblasts were significantly more likely to progress to metastasis and cause death. Thus, the presence of a gene-expression signature that is similar to the one in early wounds allows a prediction of poor prognosis[105]. In these tumours, the 'wound' genes are expressed by the tumour cells themselves and by stromal cells.
- Most of the genes that are expressed in a model of renal regeneration and repair and in renal cancer were concordantly regulated. The genes regulated in opposing directions in wounds and tumours (for example, upregulated in wounds and downregulated in tumours and vice versa) encode proteins that are required for morphogenesis or glucose metabolism[106]. The identification of the glucose metabolism genes reflects the fact that most tumours produce ATP through anaerobic glycolysis (a process known as the Warburg effect).
- Genes were identified that are differentially expressed in the hyperproliferative epithelium of healing skin wounds compared with normal epidermis, and most of them were regulated in a similar manner in epithelial skin cancer. Genes regulated in opposing directions in wounds and tumours are associated with the irreversible loss of the differentiation and growth control capacity and with the invasiveness of malignant epidermal cancer cells[107].

It will be interesting to compare the gene-expression pattern of wounds at a late stage of repair with that of tumours. This comparison might reveal further differences, as wounds heal at this stage, whereas cancers continue to grow and metastasize. The genes that are differentially expressed at this stage might provide important insights into the mechanisms that are involved in the shutdown of the repair response and could represent promising targets for tumour therapy.

and in tumours. These experiments revealed remarkable similarities between early wounds and cancer, but also important differences (BOX 1).

In this review, we describe the different phases of the wound-repair process and the parallels between wound repair and cancer at the cellular and molecular level. Owing to space limitations, we focus on processes and genes for which *in vivo* functions have been demonstrated in both wound healing and cancer. Because the literature on cancer is more extensive, we frequently refer to review articles that describe the role of a process or pathway, or describe the function of a gene in cancer.

## The different phases of wound repair

The wound-healing process in the skin involves three partially overlapping phases: blood clotting and inflammation, new tissue formation, and tissue remodelling (FIG. 1). Immediately after a deep injury, the formation of a platelet plug and a blood clot results in a temporary sealing of the wound. Inflammatory cells, which are subsequently attracted to the wound site, are important for the defence against invading bacteria. The inflammatory cells also produce growth factors, cytokines and proteinases, which are required for the phase of new tissue formation. This phase involves migration and hyperproliferation of keratinocytes at the wound edge, which finally leads to coverage of the wound with a new epidermis, a process called re-epithelialization. Concomitantly, repair of the injured dermis is initiated.

Blood-vessel sprouting occurs at the wound edge, and new vasculature develops. Fibroblasts migrate into the wound, where they proliferate and produce large amounts of extracellular matrix (ECM). Some fibroblasts differentiate into myofibroblasts, which are responsible for wound contraction and the deposition of additional matrix. The new tissue that forms at the wound site is called granulation tissue because of the granular appearance of the numerous capillaries. In the final phase, a transition from granulation tissue to mature scar tissue occurs. This is characterized by matrix remodelling and a reduction in cellularity. The scar tissue shows reduced mechanical stability and elasticity compared with uninjured skin, and it lacks appendages, including hair follicles, sebaceous glands and sweat glands. In the following sections, we describe in more detail the cellular and molecular mechanisms that are involved in the different phases of wound repair and the parallels to cancer (FIG. 2).

## A fibrin matrix in wounds and tumours

Blood clots consist predominantly of crosslinked fibrin (FIG. 3) and extravasated plasma fibronectin, but also include other components, such as the ECM proteins vitronectin and thrombospondins. This provisional matrix constitutes a protective barrier against invading microorganisms and protects the wound from severe water loss. It is also a reservoir of growth factors that are required during the later stages of healing and it provides a scaffold for the different cell types that are attracted to the wound site. The importance of this new matrix was demonstrated by the wound-healing defect that has been observed in fibrinogen-deficient mice[9]. By contrast, wounds healed normally in mice that lacked plasma fibronectin[10], which suggests that the fibronectin produced at the wound site is sufficient for normal healing.

A matrix of crosslinked fibrin and fibronectin is also a hallmark of most cancers[5] (FIG. 3). In this case, however, it does not result from mechanically damaged vessels but from hyperpermeability of tumour vessels, allowing the continuous release of plasma proteins and the deposition of a fibrin and fibronectin matrix. Wound and tumour vessels are initially immature and leaky owing to the presence of factors that induce vascular permeability, such as histamine and vascular endothelial growth factor A (VEGFA). However, this leakiness is only transient in acute skin wounds, although it might persist in chronic venous ulcers. Several days after healing, most of the vessels have lost their permeability. Thus, deposition of the fibrin and fibronectin matrix is an acute and transient event in normal wound repair, but a chronic event during tumour growth.

## The inflammatory phase of wound repair

Within minutes of wounding, inflammatory cells are attracted by complement activation, degranulation of platelets and products of bacterial degradation. Neutrophils arrive first, followed by mast cells and monocytes that subsequently differentiate into tissue macrophages. The invasion of these cells is facilitated

Warburg effect
The observation that most cancer cells predominantly produce energy by anaerobic glycolysis, which results in lactate formation.

Keratinocyte
The epithelial cell of the skin.

Epidermis
The outer, protective, non-vascular layer of the skin that covers the dermis.

Re-epithelialization
Regeneration of the injured epidermis in a skin wound.

Dermis
The connective tissue layer of the skin that is located below the epidermis.

Complement
A group of more than 20 serum proteins, some of which can be serially activated and participate in a cascade that results in cell lysis.

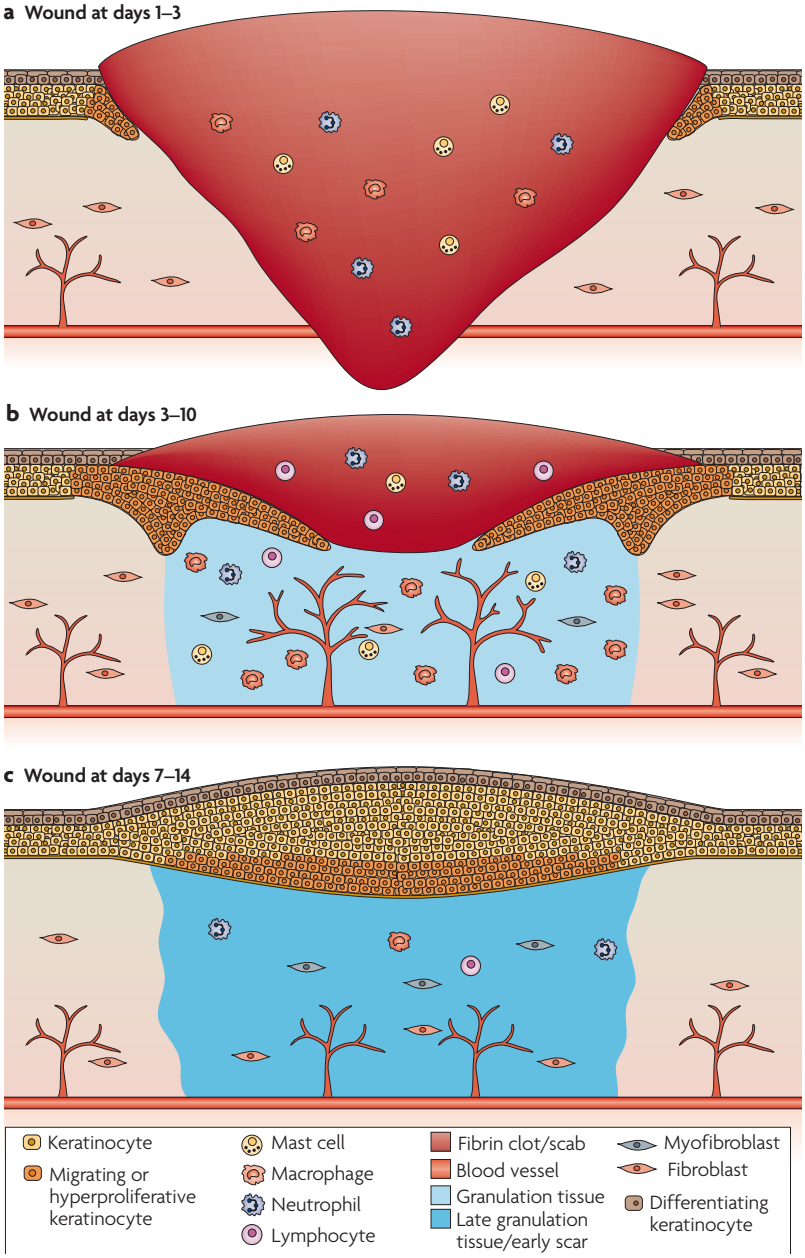



Figure 1 | **The different phases of skin wound repair. a** | The inflammatory phase. One to three days after injury the wound is filled with a clot and with inflammatory cells (neutrophils, mast cells, macrophages and lymphocytes) that have been attracted to the wound site. **b** | The phase of new tissue formation. Three to ten days after wounding, macrophages are abundant in the wound tissue and new blood vessels form. Fibroblasts migrate, proliferate and deposit extracellular matrix, and some of them differentiate into myofibroblasts. The new tissue that fills the wound is known as granulation tissue. At the wound edge, keratinocytes from the injured epidermis and hair follicles migrate along the injured dermis and above the provisional matrix, and their rate of proliferation is increased. **c** | The phase of tissue remodelling. One to two weeks after injury, wound re-epithelialization is completed, the cellular density of the granulation tissue gradually decreases and the extracellular matrix is remodelled. This results in the formation of a scar with reduced tensile strength and a lack of appendages.

by the vasodilation and enhanced vascular permeability that is associated with early wounds. This phenomenon has been attributed, at least in part, to mast-cell-derived histamine[11]. Although early studies have suggested that inflammatory cells, especially macrophages, are essential for the healing process[12], recent experiments in mice that lack different inflammatory cells have questioned this hypothesis[13].

In the absence of mast cells, neutrophil influx and early wound closure were reduced, but the overall healing process was not severely affected[11]. Neutrophils and macrophages are crucial for defence against invading bacteria through their phagocytic function and through their capacity to secrete toxic mediators, such as proteases and reactive oxygen species (ROS), that destroy these microorganisms[14]. However, in the absence of infection, these cells might inhibit rather than enhance the healing process. For example, loss of the PU.1 transcription factor (which results in the loss of functional neutrophils and macrophages) resulted in enhanced wound healing and reduced scarring[15]. Neutrophils seem to be deleterious to efficient repair, as re-epithelialization was enhanced in neutrophil-depleted mice[16]. This is consistent with clinical observations that demonstrate that persistent inflammation and abundance of neutrophils is a hallmark of chronic non-healing wounds[14]. The most likely mechanism by which neutrophils retard the healing process is through the production of toxic molecules, for example ROS, which damage the cells of the wound site and thereby retard the healing process. The toxicity of ROS is reflected by the severe endothelial cell damage and haemorrhage that is seen in wounds of mice that lack the ROS-detoxifying enzyme peroxiredoxin-6 (REF. 17).

***The role of inflammation in cancer.*** The abundance of inflammatory cells is also a hallmark of cancer, and their important roles in cancer development and progression have been demonstrated in numerous studies[18,19]. Of particular importance are macrophages. In inflamed non-cancerous tissues, macrophages are predominantly of the M1 phenotype. These cells produce high levels of ROS and inflammatory cytokines, resulting in potent antimicrobial, immunostimulatory and tumour cytotoxic functions. In established tumours, tumour-derived cytokines frequently induce the differentiation of macrophages to the M2 phenotype. These cells stimulate angiogenesis and ECM breakdown through the production of angiogenic growth factors and matrix metalloproteinases (MMPs), thereby promoting tumorigenesis[20]. Secretion of MMPs and other proteinases by macrophages at the invading front of a tumour also enhances cancer-cell motility and invasion[21]. It will be interesting to determine whether alterations in the differentiation of macrophages affect the wound-healing process, as suggested by a recent wound-healing study with interleukin-10-knockout mice[22]. In addition, it would also be interesting to determine whether abnormal macrophage differentiation is associated with an altered risk of tumorigenesis at the wound site.

It seems likely that inflammatory cells are directly involved in malignant transformation. They are a potent source of the mediators that perpetuate the inflammation, and they release ROS and reactive nitrogen species[20]. These reactive molecules can directly damage DNA and modify the proteins that are involved in DNA repair,

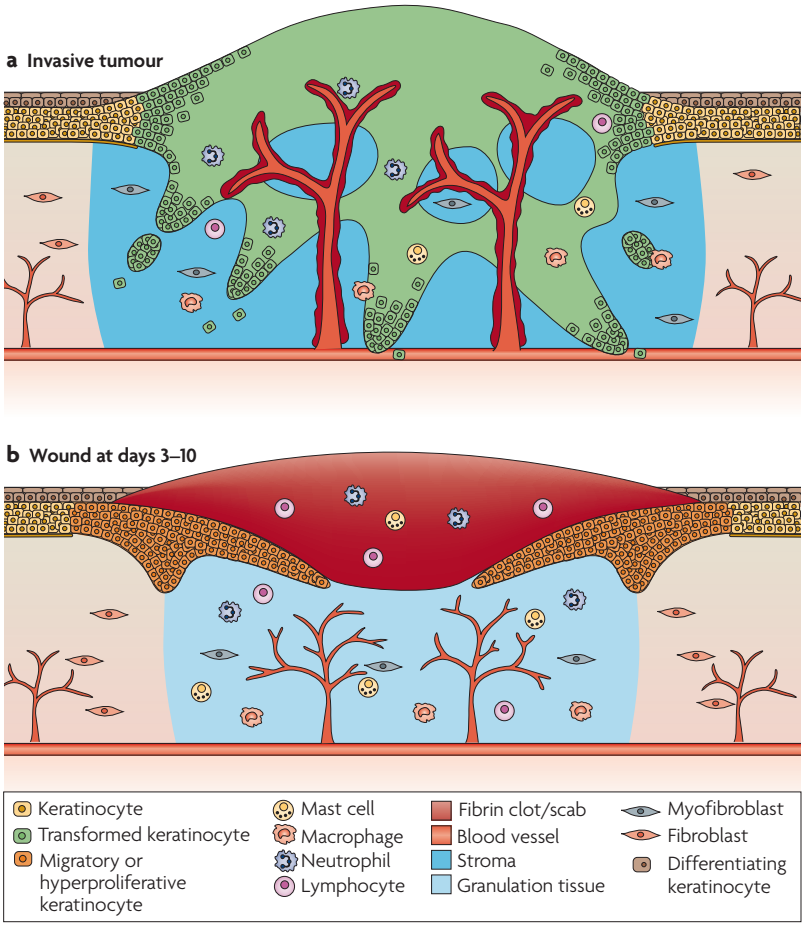



Figure 2 | **Cellular parallels between a tumour and a skin wound.** Schematic representation of an epithelial cancer (**a**) and a skin wound in the phase of new tissue formation 3–10 days after wounding (**b**). Note that both types of tissue are characterized by the presence of a fibrin clot, inflammatory cells (neutrophils, macrophages, mast cells and lymphocytes), newly formed blood vessels, and a large number of fibroblasts and myofibroblasts. These are components of the wound granulation tissue, which strongly resembles the tumour stroma. In addition, migrating and proliferating keratinocytes are present in the wound and in the cancer tissue. The main difference between tumours and wounds is the invasive growth of the transformed keratinocytes (which fill the tumour).

Reactive oxygen species (ROS). Molecules or ions that are formed by the incomplete one-electron reduction of oxygen. ROS include singlet oxygen, superoxides, peroxides, hydroxyl radicals and hypochlorous acid.

PU.1
A member of the ETS family of transcription factors that is required for the development of multiple haematopoietic lineages.

Peroxiredoxins
A family of six thiol proteins that detoxify hydrogen peroxide, lipid hydroperoxides and — in the case of peroxiredoxin-6 — also peroxinitrite.

cell-cycle checkpoint control or apoptosis. Therefore, they are probably responsible, at least in part, for the enhanced frequency of malignant transformation that is seen in chronically inflamed tissue, and for the accelerated malignant progression of tumours with a large inflammatory infiltrate[23].

Recently, ROS have also been shown to modify the activity of myeloid-derived suppressor cells (MDSCs). These cells are abundant in tumours and they strongly inhibit anti-tumour immunity[24]. In combination with nitric oxide, MDSC-derived ROS contribute to the generation of peroxynitrite. This aggressive molecule causes the nitration of various proteins on Tyr, including the T-cell receptor CD8; this modification alters antigen recognition and thereby induces T-cell tolerance[25]. MDSCs also induce tumour immunity through additional mechanisms, and they are directly involved in tumour angiogenesis. MDSC expansion and activation is triggered by various pro-inflammatory mediators; this might be an additional mechanism by which inflammation promotes malignancy[24]. It will be interesting to determine whether MDSCs also accumulate in wounded tissue, whether they affect the outcome of the healing process, and whether their enhanced and/or prolonged accumulation is associated with an enhanced risk of carcinogenesis.

Because of the pro-tumorigenic role of inflammatory cells, the inhibition of inflammation is a promising strategy for cancer prevention. Such treatments not only ameliorate chronic inflammatory disease but can also protect against certain cancers and/or reduce their progression[26], without significantly reducing the healing process[27]. One possibility to reduce inflammation is the use of non-steroidal anti-inflammatory drugs that target cyclooxygenases. Cyclooxygenase-2 is frequently overexpressed in many cancers and is functionally involved in the pathogenesis of tumours. Therefore, it represents an important target for pharmaceutical intervention[28]. Likewise, enhancing the expression of ROS-detoxifying enzymes — for example, by the activation of the cytoprotective transcription factor nuclear factor erythroid-derived-2-like-2 (NRF2), which regulates many of these genes — might limit inflammation in wounded skin[29] and also the development of cancer, as demonstrated in animal experiments[30]. Ongoing clinical studies with NRF2 activators will reveal the usefulness of this approach[26].

## Wound re-epithelialization

The release of mitogens and chemoattractants by degranulating platelets and inflammatory cells initiates new tissue formation. This begins 1–2 days after injury by the migration of keratinocytes from the epidermis at the wound edge and from injured appendages. Keratinocytes at these epidermal fronts move forward between the injured dermis and the fibrin clot. They attach initially to the exposed collagen and subsequently to the newly deposited matrix proteins of the granulation tissue. To allow efficient migration, keratinocytes at the front rearrange their actin cytoskeleton and extend lamellipodia, but they lose both their cell–cell contacts and the hemidesmosomes that mediate attachment to the basement membrane. In addition, keratinocytes at the front alter the expression of integrin receptors to allow attachment to new substrates, and they express various proteases to allow the degradation of connective tissue[31]. These events are reminiscent of the developmental process of epithelial–mesenchymal transition (EMT). However, full EMT does not occur in skin wounds, as keratinocytes at the wound edge retain some intercellular junctions and they continue to express epidermal keratins but not vimentin. In larger wounds, keratinocyte migration is followed by hyperproliferation of these cells in order to replenish the injured area with new epithelial cells.

***Wound re-epithelialization and tumour growth.*** Enhanced migration and proliferation of transformed epithelial cells is also a principal characteristic of carcinomas, in which cellular alterations strongly resemble

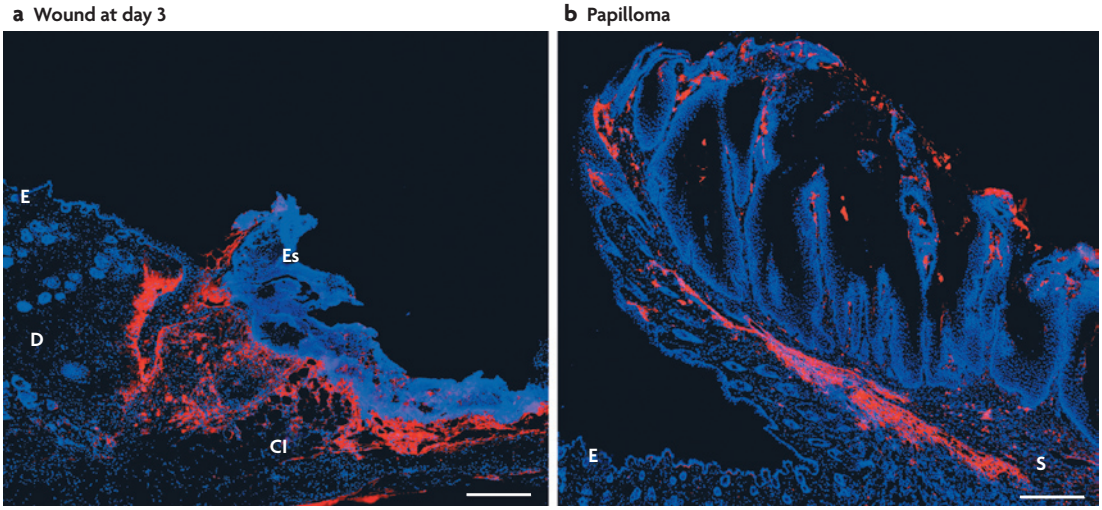



Figure 3 | **Fibrin deposition in wounds and tumours.** Fibrin is an important component of the early stroma of wounds and cancers. In wounds, the fibrin clot is formed following the release of fibrinogen from mechanically injured vessels. In tumours, fibrinogen is released from the hyperpermeable (uninjured) microvasculature. The released fibrinogen is rapidly clotted and transglutaminated to crosslinked fibrin, and this matrix is important for the initiation of the wound-healing response[5]. This response is transient and self-limited in wounds, but chronic in cancer. To demonstrate fibrin deposition in wounds and tumours, sections from a 3-day full-thickness excisional mouse skin wound (**a**) and a cutaneous murine papilloma (**b**) were analysed by immunofluorescence for the presence of fibrin or fibrinogen (red). Nuclei were counterstained with Hoechst 33342 (blue). Note the presence of fibrin in the wound granulation tissue and in the tumour stroma. Scale bar, 300 μm. Cl, clot; D, dermis; E, epidermis; Es, eschar; S, stroma.

Angiogenesis
The sprouting of new vessels from pre-existing vessels.

Matrix metalloproteinases
(MMPs). Zinc-dependent endopeptidases that cleave different extracellular matrix proteins and also growth factors, chemokines, cell-surface receptors and other proteins.

Myeloid-derived suppressor cells
(MDSCs). Heterogeneous mixture of immature myeloid cells that are potent inhibitors of anti-tumour immunity. In mice they are generally defined by the markers CD11b and GR1.

Cyclooxygenases
Enzymes responsible for the formation of prostaglandins, prostacyclins and thromboxanes.

NRF2
A Leu-zipper transcription factor that activates the expression of a battery of cytoprotective genes.

Lamellipodium
A flattened projection from the cell surface, generally associated with cell migration.

Epithelial–mesenchymal transition
(EMT). A developmental programme in which epithelial cells lose cell–cell adhesion, acquire a fibroblast-like morphology and increase their motility.

Carcinoma
A cancer that arises from epithelial cells.

STAT3
A protein that transduces the signal from activated cytokine or growth-factor receptors to the nucleus.

Psoriasis
An inflammatory skin disease that is associated with keratinocyte hyperproliferation and abnormal differentiation.

SMAD3
A signalling protein that is activated by the type I transforming growth factor-β receptor, and which transduces the signal from the plasma membrane to the nucleus.

those seen in healing wounds. Recent functional studies identified genes that are required for migration and proliferation of both wound keratinocytes and carcinoma cells. For example, β1 integrins are important for wound re-epithelialization, especially for keratinocyte migration[32]. Loss of these proteins in breast cancer cells strongly impaired the initiation and maintenance of mammary tumour growth *in vivo*. In this situation, however, β1 integrins were predominantly required for efficient cell proliferation[33]. Activation of plasminogen, which degrades the fibrin matrix (BOX 2) and activates MMPs, is required for the migration of wound keratinocytes and for the invasive growth of cancer cells. Thus, plasminogen-deficient mice had strongly impaired re-epithelialization[34], and this process was completely inhibited following the treatment of wounds with a broad-spectrum MMP inhibitor[35]. Likewise, the importance of efficient proteolytic degradation of matrix proteins for tumour growth has also been demonstrated[36].

***Regulators of re-epithelialization and tumour growth.*** Several mitogens stimulate both wound re-epithelialization and the growth of carcinomas. The chemokine stromal-cell-derived factor-1 (SDF1) is a mitogen for keratinocytes and is expressed in endothelial cells and fibroblasts of normal and wounded skin[37,38], and also in tumours[39,40]. Myofibroblast-derived SDF1 promotes the proliferation of mammary carcinoma cells that express its high-affinity receptor CXCR4, and it might also contribute to cancer growth through the regulation of angiogenesis by attracting endothelial progenitor cells[40]. Although the role of SDF1 in wound re-epithelialization remains to be identified, other keratinocyte mitogens have been identified as crucial regulators of this process. These include hepatocyte growth factor (HGF)[41] and members of the epidermal growth factor (EGF) family[42]. These mitogens are also frequently overexpressed in cancer and stimulate tumour-cell proliferation and invasion[43,44]. Therefore, they represent targets for therapeutic intervention (Supplementary information S1 (table)).

One of the main signalling proteins that is activated by the HGF and EGF receptors, and also by several cytokine receptors, is signal transducer and activator of transcription-3 (STAT3). This protein promotes both wound repair and carcinogenesis[45]. Mice that lack STAT3 in the epidermis suffer from strongly reduced wound re-epithelialization, and they are resistant to carcinogen-induced skin-cancer development. However, mice that express a constitutively active form of STAT3 in the epidermis develop skin cancers with a shorter latency; the number and malignancy of the tumours was also increased. Skin wounding in these mice resulted in strong hyperthickening of the regenerated epidermis, and the phenotype shared histological features with psoriasis[45].

In contrast to these positive regulators of epithelial repair, transforming growth factor-β (TGFβ) and its downstream effector SMAD3 are negative regulators of wound re-epithelialization[46,47]. This signalling pathway also inhibits proliferation of many carcinoma cells, at least in the early stages of tumorigenesis[48].

Remarkably, divergent functions of growth factors in wound healing and cancer have also been reported. For example, the expression of a dominant-negative mutant of fibroblast growth factor (FGF) receptor-2-IIIb in the

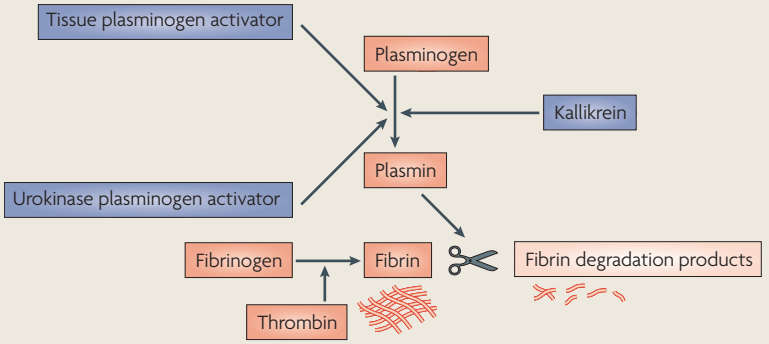
epidermis of transgenic mice caused impaired wound re-epithelialization[49]. Unexpectedly, mice that lacked this receptor in the epidermis were more susceptible to chemically induced skin carcinogenesis, demonstrating a tumour-suppressive effect of this type of receptor[50]. Therefore, wound re-epithelization and growth of epithelial tumours are not always regulated by the same factors, and it will be important to identify the molecular mechanisms underlying these differences.

***EMT: wounds versus tumours.*** The main difference between the migration of wound keratinocytes and cancer cells is the complete EMT that is frequently seen in cancer cells. Cancer cells often lose all cell–cell contacts, acquire a fibroblast-like morphology and express mesenchymal marker proteins. These processes resemble those that are activated during early embryogenesis and following injury of some epithelial tissues. Therefore, it seems likely that cancer cells reactivate a latent behavioural programme that is usually confined to embryonic development and tissue repair, although in cancer cells it occurs in an exaggerated and uncontrolled manner. The acquisition of the mesenchymal cell phenotype is of crucial importance for invasive growth and metastasis[51]. Most interestingly, the tumour cells that undergo EMT have recently been shown to acquire the properties of cancer stem cells[52]. Once settled in another tissue, the metastasizing cancer cells might re-establish their epithelial phenotype, a process that is termed mesenchymal–epithelial transition. The histology of the resulting metastasis frequently resembles that of the primary tumour.

Induction of EMT in cancer cells is achieved by a combination of stromal factors, such as HGF, TGFβ, tumour-necrosis factor-α and MMPs, and by mutations in the genome of cancer cells, for example, activating mutations in the *RAS* proto-oncogene[53,54]. This finding might explain why non-transformed wound keratinocytes only undergo partial EMT.

Box 2 | **The production and degradation of fibrin**

Insoluble fibrin is produced by the cleavage of soluble fibrinogen by the Ser proteinase thrombin, resulting in the formation of a fibrin clot. The clot provides a scaffold for the migration of different cell types into the wound. Degradation of fibrin is achieved by plasmin-mediated cleavage, allowing the replacement of the clot by mature granulation tissue and/or tumour stroma. The Ser proteinase plasmin is activated by different plasminogen activators, including tissue plasminogen activator, urokinase plasminogen activator and kallikrein.




Following the completion of wound repair, keratinocytes revert from their mesenchymal-like phenotype to the epithelial phenotype; they return to their normal proliferation rate and redifferentiate to restore the epidermal barrier. The mechanisms that are responsible for the shutdown of epidermal repair remain to be identified. These might include the formation of new integrin-dependent cell–matrix interactions and the upregulation or activation of inhibitors of epithelial cell growth, such as TGFβ. The identification of these mechanisms might help to develop new strategies for the limitation of cancer growth. The regulation of keratinocyte redifferentiation was addressed in a recent study that identified grainyhead-like transcription factor-3 as an important regulator of epidermal barrier function in normal and wounded skin[55]. In the future, it should be determined whether this factor and other key regulators of differentiation and barrier function are abnormally expressed in skin cancer.

## Formation of new vessels

Shortly after the onset of re-epithelialization, repair of the injured dermis is initiated. Formation of new blood vessels is crucial for the supply of oxygen and nutrients (FIG. 4a–c). Likewise, numerous studies have highlighted the essential role of neovascularization in the growth of tumours beyond a diameter of 2 mm and in metastasis[56]. Neovascularization in wounds and in tumours is mainly achieved by angiogenesis, the sprouting of new vessels from pre-existing vessels. In addition, bone-marrow-derived endothelial progenitor cells can also be incorporated into newly formed wound vessels, especially under ischaemic conditions[57]. The role of these cells in tumour vascularization might be even more important[56].

The dependency of tumour growth on angiogenesis was first proposed in 1971 by Judah Folkman, who suggested that inhibition of angiogenesis could be a powerful strategy for the treatment of cancer[58]. This finding provided the basis for the development of anti-angiogenic cancer drugs (Supplementary information S1 (table)).

Following the onset of blood-vessel sprouting in healing wounds, lymphangiogenesis is initiated in order to reconstruct the lymphatic vasculature[59]. This process is also a crucial step in tumorigenesis and is particularly important for metastasis[60]. Therefore, inhibition of lymphangiogenesis is a novel strategy for the inhibition of this process[61].

***VEGFs control angiogenesis in wounds and tumours.*** Angiogenesis and lymphangiogenesis are regulated by various growth factors, matrix molecules, proteinases and their inhibitors. Here we focus on growth factors, which have been particularly well studied in the context of wound and tumour angiogenesis.

VEGFA is a particularly important player in angiogenesis and lymphangiogenesis (FIG. 4a). VEGFA is upregulated in response to hypoxia by the activation of the transcription factor hypoxia-inducible factor-1α (HIF1α). The functional importance of VEGFA for cancer growth has been demonstrated in numerous

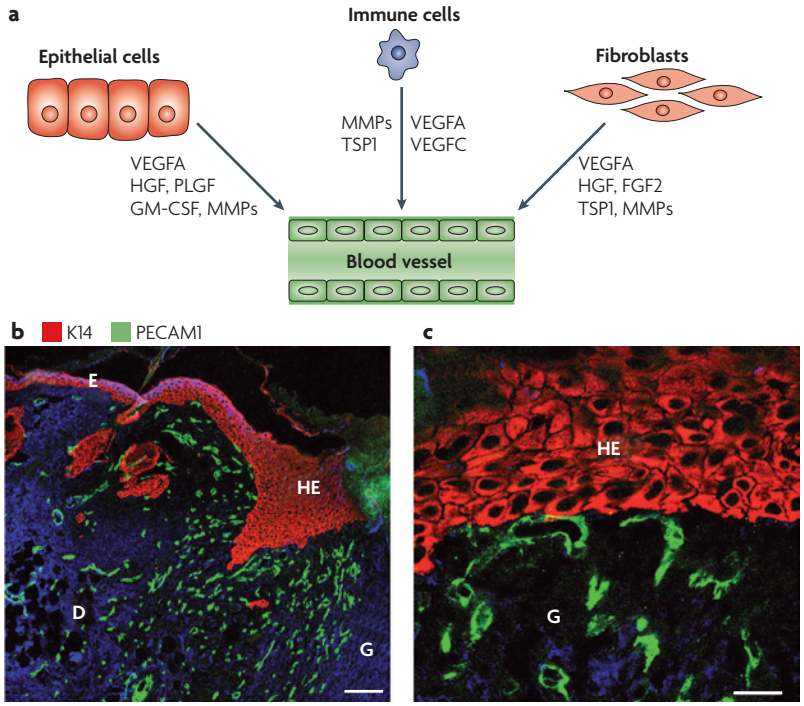



Figure 4 | **Angiogenesis in healing skin wounds. a** | The factors that are involved in angiogenesis are shown together with the cells that are the main producers of these factors in skin wounds and in tumours. Stimulation of angiogenesis is achieved by several growth factors (including vascular endothelial growth factor-A (VEGFA), VEGFC, placental growth factor (PLGF), hepatocyte growth factor (HGF), granulocyte–macrophage colony stimulating factor (GM-CSF), fibroblast growth factor-2 (FGF2)) and by matrix metalloproteinases (MMPs). By contrast, thrombospondins (TSPs) are negative regulators of this process. **b**, **c** | To visualize the new vasculature in healing skin wounds, sections from 5-day full-thickness mouse wounds were analysed by immunofluorescence with an antibody against the endothelial cell marker platelet endothelial cell adhesion molecule-1 (PECAM1; green). Keratinocytes were counterstained with an antibody against keratin-14 (K14; red) and nuclei were counterstained with Hoechst 33342 (blue). Scale bar, 20 µm (**b**) and 100 µm (**c**). D, dermis; E, epidermis; G, granulation tissue; HE, hyperproliferative wound epidermis. Figure parts **b** and **c** reproduced, with permission, from REF. 108.

studies, and VEGFA antagonists have been approved for the treatment of different types of human cancer[62] (Supplementary information S1 (table)). Keratinocyte-specific deletion of the *Vegfa* gene in mice resulted in delayed wound healing owing to impaired angiogenesis. Furthermore, development of chemically induced skin cancers was almost completely inhibited[63]. Conversely, overexpression of VEGFA (the VEGF164 isoform) in the skin of transgenic mice enhanced wound angiogenesis[64]. In another study, accelerated Ras-induced skin carcinogenesis was observed in mice that overexpress VEGF120 (REF. 65). Surprisingly, VEGFA overexpression in the epidermis stimulated not only the growth of new blood vessels but also lymphangiogenesis in skin wounds and tumours, and the formation of lymphatic metastases[64,66].

Angiogenesis is also regulated by another VEGF family member — placental growth factor (PLGF; FIG. 4a). In PLGF-deficient mice, both wound and tumour angiogenesis were impaired[67], and overexpression of PLGF in the epidermis of healing-impaired diabetic mice accelerated angiogenesis and reverted the wound-healing defect[68]. Intradermal inoculation of melanoma cells in mice with overexpressed PLGF resulted in enhanced growth of melanomas compared with control mice[69].

A third member of this family, VEGFC, is a potent regulator of lymphangiogenesis (FIG. 4a). Adenoviral overexpression of VEGFC accelerated diabetic wound healing through the induction of angiogenesis and lymphangiogenesis[70]. When transgenic mice that overexpress VEGFC in the epidermis were subjected to a chemically induced skin carcinogenesis model, the enhanced levels of this growth factor induced lymphangiogenesis in sentinel lymph nodes and promoted metastasis to distant sites[71].

***Positive and negative regulation of angiogenesis.*** Other pro-angiogenic growth factors that are expressed in wounds and tumours include FGF2, HGF and granulocyte–macrophage colony stimulating factor[42] (FIG. 4a). These findings highlight the strong similarities between wound and tumour angiogenesis and provide an explanation for the impairment of wound repair by endogenous or exogenous angiogenesis inhibitors.

For example, wound healing was strongly impaired in mice that overexpress the matricellular protein thrombospondin-1 (TSP1) in the epidermis, owing to reduced angiogenesis and granulation tissue formation[72]. TSP1 overexpression in human squamous cell carcinoma cell lines also inhibited tumour growth in a mouse xenotransplant model as a result of reduced tumour angiogenesis and subsequent necrosis[73]. A second example is the chemokine IP10, which inhibits wound repair and tumorigenesis, particularly through the impairment of angiogenesis[74,75]. Similar results were obtained following treatment of mice with the anti-angiogenic polypeptides endostatin and vasostatin. Both peptides inhibited angiogenesis in skin wounds and tumours, and tumour growth was strongly affected[76,77,78]. By contrast, the overall healing process, including wound closure, was either not impaired or was only modestly affected in these studies. This might be different in human wounds, in which angiogenesis is more limiting. Indeed, impaired wound healing is one of the main side effects of anti-angiogenic cancer therapy using VEGF antagonists[79] (Supplementary information S1 (table)).

When the new granulation tissue is covered by a neo-epidermis, angiogenesis is inhibited and most of the newly formed vessels regress through apoptosis of endothelial cells. The underlying mechanisms have not been fully explored, but are likely to include a reduction in the levels of pro-angiogenic growth factors. Many of these factors, in particular VEGFA, are regulated by hypoxia and pro-inflammatory cytokines. The loss of these stimuli at later stages of healing might therefore cause vessel regression. Furthermore, reduced expression of MMPs might have a role in this process. MMPs are required for the degradation of the wound matrix, which is a prerequisite for vessel sprouting. Finally, the replacement of the provisional matrix by mature collagen and thus the loss of important attachment sites for the sprouting endothelial cells might further contribute to the reduction in vascular density.

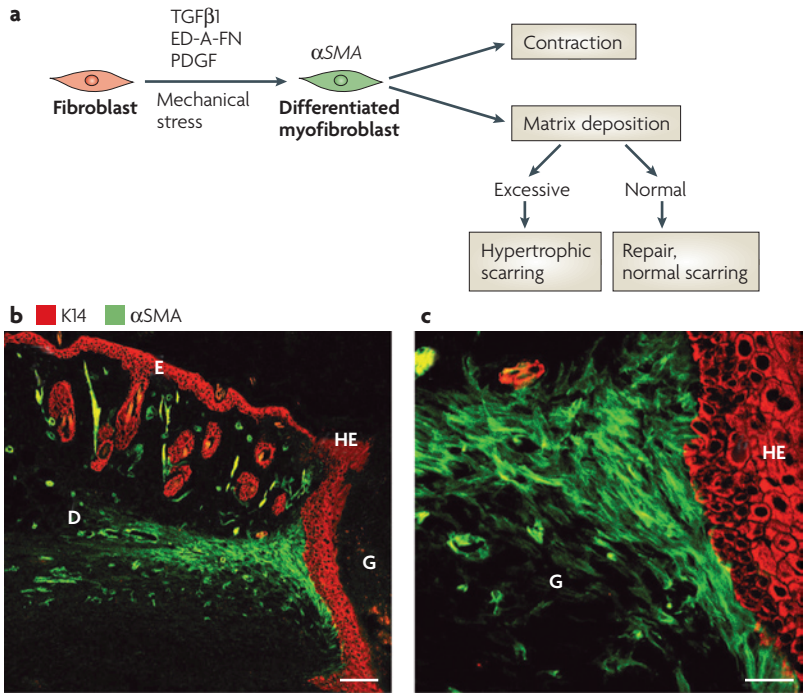



Figure 5 | **Myofibroblast differentiation in healing skin wounds. a** | Platelet-derived growth factor (PDGF), transforming growth factor-β1 (TGFβ1), and ED-A-FN (a splice variant of fibronectin), in combination with mechanical stress, induce the differentiation of fibroblasts into α-smooth muscle actin (α*SMA*)-expressing myofibroblasts, an event that occurs in wounds and in tumours. Myofibroblasts produce various matrix proteins and they are also crucial for wound contraction. Excessive myofibroblast differentiation and prolonged survival of these cells in skin wounds causes hypertrophic scarring. **b**, **c** | To visualize myofibroblasts in healing skin wounds, sections from 5-day full-thickness mouse wounds were analysed by immunofluorescence with an antibody against αSMA (green), a marker for myofibroblasts. Keratinocytes were counterstained with an antibody to keratin-14 (K14; red). D, dermis; E, epidermis; G, granulation tissue; HE, hyperproliferative wound epidermis. Scale bar, 20 μm (**b**) and 100 μm (**c**).

## Formation of fibrous tissue and scarring

Fibroblasts, which deposit large amounts of extracellular matrix, repopulate the wounded area in parallel to angiogenesis. Several days after injury a subset of wound fibroblasts differentiate into myofibroblasts (FIG. 5a–c), which are responsible for wound contraction and for the deposition of additional matrix proteins. During the tissue remodelling phase, the initial collagen type III of the granulation tissue is gradually dominated by collagen type I, and the resultant larger collagen fibrils are abnormally arranged in parallel bundles. These processes result in the formation of a scar that contains dense connective tissue of reduced tensile strength and elasticity compared with normal skin[31].

Fibroblasts are attracted to the wound from the adjacent dermis by factors that also stimulate their proliferation (see below). Furthermore, bone-marrow-derived cells populate the wound tissue and differentiate into collagen-producing fibroblasts[80]. Within the granulation tissue, fibroblasts produce growth factors that regulate wound re-epithelialization in a paracrine manner, including EGF and FGF family members, and HGF[81]. Fibroblasts also secrete proteinases to degrade the initial fibrin matrix (BOX 2) and produce most of the new ECM proteins. Following differentiation into myofibroblasts, the cells deposit large amounts of fibronectin, collagen type I and type III, proteoglycans and glycosaminoglycans (the so-called desmoplastic stroma). In addition, they secrete proteinases, which mobilize growth factors from the ECM and which allow the remodelling of the connective tissue. Following the completion of wound repair myofibroblasts undergo apoptosis, and the granulation tissue is progressively replaced by the acellular scar.

A prolonged presence of myofibroblasts at the wound site results in the formation of hypertrophic scars or even keloids. Interestingly, the presence of fibrotic lesions strongly increases the risk of cancer in many tissues, including lung tissue and breast tissue, through the disruption of cell polarity, stimulation of cell proliferation and myofibroblast-induced inflammation and angiogenesis[82]. These findings further highlight the relationship between exaggerated healing and cancer.

***The role of (myo)fibroblasts in cancer.*** The presence of large numbers of fibroblasts and myofibroblasts is a hallmark of carcinomas, particularly at the advanced stage. In fact, carcinomas provoke a desmoplastic response, possibly as a defensive mechanism to isolate the tumour. The newly formed connective tissue then becomes an important component of the tumour microenvironment[26,40], which is crucial for tumour progression. It has been shown that physical matrix remodelling by (myo)fibroblasts allows the collective invasion of squamous-cell carcinoma cells in an organotypic culture model. This suggests that tumour fibroblasts can enable cancer-cell invasion, even if these cancer cells do not undergo EMT[83].

Tumour fibroblasts are derived from the mesenchyme that surrounds the tumour, but a contribution of bone-marrow cells to tumour-associated fibroblasts and myofibroblasts has also been shown[84]. Finally, myofibroblasts might also derive from epithelial cells through EMT[82]. Treatment of mouse mammary epithelial cells with MMP3 resulted in phenotypic alterations that were associated with EMT. MMP3 upregulated the small GTPase Rac1B, which in turn stimulated the production of ROS through mitochondrial activation. The enhanced levels of ROS then induced EMT and invasiveness through the induction of vimentin and other genes that are expressed by myofibroblasts[54]. These results highlight a novel function of ROS in tumorigenesis and provide evidence for a role of EMT in the desmoplastic response of a tumour, in addition to its role in invasion and metastasis.

In general, the processes that lead to the formation of the desmoplastic tumour stroma are highly similar to those in healing wounds, although they operate continuously over a long period of time in cancer tissue, but only over several days in a wound. Therefore, it is not surprising that the molecular mechanisms underlying fibroblast function in tumours and wounds are similar. This has been highlighted by the gene-expression signatures of malignant tumours and of skin wounds, which resemble the signature of serum-treated fibroblasts[85,86].

***Factors involved in fibroblast activation and scarring.*** Transient hypoxia at the wound site has been shown to activate HIF1α, which in turn induces the production of heat-shock protein-90α (HSP90α). HSP90α is released from fibroblasts and stimulates their migration in an autocrine manner[87]. HSP90α is also secreted by cancer cells and enhances their invasiveness[88].

A main fibroblast mitogen is platelet-derived growth factor (PDGF) (FIG. 5a). Following wounding, PDGF is released from degranulating platelets. It is also produced by several cell types in the wound tissue, and it is highly expressed in many types of tumours, especially by the tumour cells[89]. In some cancers, the extent of PDGF overexpression increases with tumour progression. Cancer-cell-derived PDGF mainly targets stromal cells, which might explain the presence of large stromal areas in many advanced carcinomas. Consistent with this assumption, deletion of the PDGF receptor-β gene in fibroblasts restricted the migration and proliferation of these cells *in vitro* and reduced their survival under stress conditions[90]. In addition to its paracrine action in carcinomas, PDGF stimulates growth of mesenchymal tumours in an autocrine manner. Therefore, inhibitors of PDGF receptor signalling are used in the clinic for the treatment of these tumours[89] (Supplementary information S1 (table)).

The therapeutic effect of PDGF inhibitors is not restricted to the reduction of tumour-cell growth, but also to the inhibition of pericyte recruitment and thus to the formation of mature blood vessels. Inhibition of PDGF receptor signalling also reduces the high interstitial fluid pressure seen in many tumours, and therefore enhances drug uptake *in vivo*[91]. As expected, PDGF inhibitors affect the wound-healing process, as demonstrated by impaired healing in mice that are treated with the PDGF inhibitor imatinib mesylate (Gleevec)[92] (Supplementary information S1 (table)). However, imatinib mesylate was identified as an anti-fibrotic agent[93], demonstrating the necessity for the tight regulation of PDGF expression and activity in healing skin wounds. Thus, PDGF is beneficial under situations of impaired healing, as demonstrated by its positive effect on the healing of diabetic ulcers[94]. However, excessive levels of PDGF can lead to fibroplasia and also facilitate the growth of malignant tumours.

Another main regulator of fibroplasia is TGFβ, which is released by platelets following wounding. Subsequently, TGFβ is produced by many cell types in the healing wound. It inhibits re-epithelialization (see above), but stimulates proliferation of fibroblasts and matrix deposition. Furthermore, TGFβ, in combination with extra-domain-A (ED-A) fibronectin and mechanical tension, is the most important inducer of myofibroblast differentiation[95] (FIG. 5a). Therefore, it is a key regulator of the scarring response, as demonstrated in several *in vivo* studies in which neutralizing antibodies against TGFβ1 and TGFβ2, or the loss of the TGFβ signalling protein SMAD3, inhibited the scarring of mouse wounds[96,47]. This is because of a direct effect of TGFβ on fibroblasts and because of its anti-inflammatory role.

Pericyte
A mesenchymal cell that is associated with the wall of small blood vessels.

The effect of TGFβ on fibroplasia is mediated, at least in part, through β-catenin, as the pro-mitogenic effect of TGFβ is lost in β-catenin-deficient fibroblasts. Loss of β-catenin in skin wounds resulted in a reduced wound size owing to a reduction in the number of fibroblasts[97]. Conversely, transgenic mice, which inducibly express a stabilized form of β-catenin in mesenchymal cells, developed hyperplastic skin wounds after injury and spontaneous aggressive fibromatosis[98]. This is also relevant in humans, as somatic mutations in the β-catenin gene that lead to the stabilization of the protein are frequent in aggressive fibromatosis — an invasive, but non-metastasizing tumour that is considered to be an exaggerated healing response[99].

The important role of TGFβ in cancer is well documented and a topic of many ongoing studies. It frequently exerts a tumour-suppressive function in early carcinogenesis through a direct effect on epithelial tumour cells. However, at later stages it can also exert a tumour-promoting role. In epithelial tissues this is often accompanied by a decreased responsiveness of the cancer cells to TGFβ-mediated growth inhibition and the concomitant upregulation of TGFβ expression, which activates the stroma[48]. However, TGFβ can also exert a tumour-suppressive effect through fibroblasts, as demonstrated by the oncogenic tendency of mice that lack the TGFβ type II receptor in these cells. These mice developed spontaneous epithelial tumours in several organs owing to upregulation of epithelial mitogens, such as HGF and TGFα[100,101]. It seems possible that TGFβ type-II-receptor-deficiency in fibroblasts also affects the process of wound re-epithelialization in a similar manner.

Although TGFβ is generally considered to be the most important regulator of scarring, additional factors contribute to the fibrotic response. This is highlighted by the observation that knockdown of the extracellular matrix glycophosphoprotein osteopontin in mouse wounds strongly reduces inflammation and scarring[102]. The mechanisms of action of osteopontin have not been fully explored, but the potent anti-inflammatory effect is probably responsible for the inhibition of scarring. The role of osteopontin in wound repair is remarkable, because osteopontin is overexpressed in many human cancers, and this correlates with poor prognosis[103]. Osteopontin regulates important features of cancer cells, including migration, proliferation and invasion. Therefore, it represents a potential target for therapeutic intervention in metastatic cancer. Most importantly, osteopontin targeting has the potential to inhibit tumorigenesis without perturbing the wound-healing process. The identification of additional molecules with similar properties will be a major challenge for the future.

## Conclusions

The early hypothesis that "tumours are wounds that do not heal" (REF. 5) has been verified in a large number of cellular and molecular studies. These revealed that tumours, in particular carcinomas, activate the latent wound-healing programme of the host but in an exaggerated and prolonged manner. Consistent with this assumption, most of the genes that orchestrate the

wound-healing process are also important regulators of cancer growth and progression. However, there are important differences between both processes, which result from mutations and epigenetic changes in the tumour cells themselves and possibly also in the stroma. It is possible that epigenetic changes, such as DNA methylation and histone modifications[104], also occur following tissue injury. This exciting question should be addressed in the future, as such changes could possibly affect the healing process and also influence the risk of malignant transformation.

As a consequence of mutations and epigenetic changes, tumours alter their metabolism, lose their differentiation capacity, invade adjacent non-cancerous tissue and metastasize to distant sites. Because inflammation in tumours is not self-limiting, continuous activation of the wound-healing programme occurs, resulting in chronic inflammation that is accompanied by the production of high levels of ROS. Increased levels of ROS cause further genomic alterations, resulting in the accumulation of mutations and the perpetuation of tumour growth. In addition, they contribute to the development of tumour-induced T-cell tolerance.

Owing to enhanced inflammation in chronic skin wounds and other chronically inflamed tissues, ROS-induced mutagenesis is likely to be increased. Continuous cell proliferation in chronic lesions might further lead to the multiplication of cells with a potentially dangerous cancer mutation, thus increasing the risk that an already mutated cell accumulates additional deleterious mutations. Therefore, it will be a major challenge for the future to reduce chronic inflammation and subsequent ROS production, to improve the healing capacity of chronic wounds and to identify the factors that control the termination of the wound-healing process. These factors are likely to be important targets for the development of drugs that limit the growth of malignant tumours.


1. Reed, B. R. & Clark, R. A. Cutaneous tissue repair: practical implications of current knowledge. II. *J. Am. Acad. Dermatol.* **13**, 919–941 (1985).
2. Virchow, R. Virchow, R. *Aetiologie der neoplastischen Geschwulste/Pathogenie der neoplastischen Geschwulste*. (Verlag von August Hirschwald, Berlin, Germany, 1863).
3. Dunham, L. J. Cancer in man at site of prior benign lesion of skin or mucous membrane: a review. *Cancer Res.* **32**, 1359–1374 (1972).
4. Haddow, A. Molecular repair, wound healing, and carcinogenesis: tumor production a possible overhealing? *Adv. Cancer Res.* **16**, 181–234 (1972).
5. Dvorak, H. F. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N. Engl. J. Med.* **315**, 1650–1659 (1986).
**In this paper, H. Dvorak formulated his famous hypothesis that tumours are wounds that do not heal, and he describes the underlying cellular basis.**
6. Dolberg, D. S., Hollingsworth, R., Hertle, M. & Bissell, M. J. Wounding and its role in RSV-mediated tumor formation. *Science* **230**, 676–678 (1985).
**The first demonstration that tumours preferentially grow at sites of wounding in retrovirus-infected chickens.**
7. Martins-Green, M., Boudreau, N. & Bissell, M. J. Inflammation is responsible for the development of wound-induced tumors in chickens infected with Rous sarcoma virus. *Cancer Res.* **54**, 4334–4341 (1994).
8. Schuh, A. C., Keating, S. J., Monteclaro, F. S., Vogt, P. K. & Breitman, M. L. Obligatory wounding requirement for tumorigenesis in v-*jun* transgenic mice. *Nature* **346**, 756–760 (1990).
9. Drew, A. F., Liu, H., Davidson, J. M., Daugherty, C. C. & Degen, J. L. Wound-healing defects in mice lacking fibrinogen. *Blood* **97**, 3691–3698 (2001).
10. Sakai, T. *et al.* Plasma fibronectin supports neuronal survival and reduces brain injury following transient focal cerebral ischemia but is not essential for skin-wound healing and hemostasis. *Nature Med.* **7**, 324–330 (2001).
11. Weller, K., Foitzik, K., Paus, R., Syska, W. & Maurer, M. Mast cells are required for normal healing of skin wounds in mice. *FASEB J.* **20**, 2366–2368 (2006).
12. Leibovich, S. J. & Ross, R. The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am. J. Pathol.* **78**, 71–100 (1975).
13. Martin, P. & Leibovich, S. J. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol.* **15**, 599–607 (2005).
**Summarizes the positive and negative effects of inflammation on the wound-repair process.**
14. Eming, S. A., Krieg, T. & Davidson, J. M. Inflammation in wound repair: molecular and cellular mechanisms. *J. Invest. Dermatol.* **127**, 514–525 (2007).
15. Martin, P. *et al.* Wound healing in the *PU.1* null mouse—tissue repair is not dependent on inflammatory cells. *Curr. Biol.* **13**, 1122–1128 (2003).
16. Dovi, J. V., He, L. K. & DiPietro, L. A. Accelerated wound closure in neutrophil-depleted mice. *J. Leukoc. Biol.* **73**, 448–455 (2003).
17. Kumin, A. *et al.* Peroxiredoxin 6 is required for blood vessel integrity in wounded skin. *J. Cell Biol.* **179**, 747–760 (2007).
18. de Visser, K. E., Eichten, A. & Coussens, L. M. Paradoxical roles of the immune system during cancer development. *Nature Rev. Cancer* **6**, 24–37 (2006).
19. Balkwill, F., Charles, K. A. & Mantovani, A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* **7**, 211–217 (2005).
**References 18 and 19 summarize the current knowledge on the role of inflammation in cancer development and progression.**
20. Allavena, P., Sica, A., Garlanda, C. & Mantovani, A. The yin-yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol. Rev.* **222**, 155–161 (2008).
21. Condeelis, J. & Pollard, J. W. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* **124**, 263–266 (2006).
**Summarizes the different functions of macrophages in cancer that are also relevant for the wound-healing process.**
22. Eming, S. A. *et al.* Accelerated wound closure in mice deficient for interleukin-10. *Am. J. Pathol.* **170**, 188–202 (2007).
23. Hussain, S. P., Hofseth, L. J. & Harris, C. C. Radical causes of cancer. *Nature Rev. Cancer* **3**, 276–285 (2003).
24. Marigo, I., Dolcetti, L., Serafini, P., Zanovello, P. & Bronte, V. Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol. Rev.* **222**, 162–179 (2008).
25. Nagaraj, S. *et al.* Altered recognition of antigen is a mechanism of $CD8^+$ T cell tolerance in cancer. *Nature Med.* **13**, 828–835 (2007).
26. Albini, A. & Sporn, M. B. The tumour microenvironment as a target for chemoprevention. *Nature Rev. Cancer* **7**, 139–147 (2007).
27. Muller-Decker, K., Hirschner, W., Marks, F. & Furstenberger, G. The effects of cyclooxygenase isozyme inhibition on incisional wound healing in mouse skin. *J. Invest. Dermatol.* **119**, 1189–1195 (2002).
28. Subbaramaiah, K. & Dannenberg, A. J. Cyclooxygenase 2: a molecular target for cancer prevention and treatment. *Trends Pharmacol. Sci.* **24**, 96–102 (2003).
29. Braun, S. *et al.* 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 (2002).
30. Yu, X. & Kensler, T. Nrf2 as a target for cancer chemoprevention. *Mutat. Res.* **591**, 93–102 (2005).
31. Martin, P. Wound healing—aiming for perfect skin regeneration. *Science* **276**, 75–81 (1997).
32. Grose, R. *et al.* A crucial role of β 1 integrins for keratinocyte migration *in vitro* and during cutaneous wound repair. *Development* **129**, 2303–2315 (2002).
33. White, D. E. *et al.* Targeted disruption of β1-integrin in a transgenic mouse model of human breast cancer reveals an essential role in mammary tumor induction. *Cancer Cell* **6**, 159–170 (2004).
34. Romer, J. *et al.* Impaired wound healing in mice with a disrupted plasminogen gene. *Nature Med.* **2**, 287–292 (1996).
35. Lund, L. R. *et al.* Functional overlap between two classes of matrix-degrading proteases in wound healing. *EMBO J.* **18**, 4645–4656 (1999).
36. Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nature Rev. Cancer* **2**, 161–174 (2002).
37. Florin, L., Maas-Szabowski, N., Werner, S., Szabowski, A. & Angel, P. Increased keratinocyte proliferation by JUN-dependent expression of PTN and SDF-1 in fibroblasts. *J. Cell Sci.* **118**, 1981–1989 (2005).
38. Avniel, S. *et al.* Involvement of the CXCL12/CXCR4 pathway in the recovery of skin following burns. *J. Invest. Dermatol.* **126**, 468–476 (2006).
39. Ceradini, D. J. *et al.* Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nature Med.* **10**, 858–864 (2004).
40. Orimo, A. *et al.* Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335–348 (2005).
**Describes the important role of carcinoma-associated fibroblasts for tumour growth and the underlying mechanisms of action.**
41. Chmielowiec, J. *et al.* c-Met is essential for wound healing in the skin. *J. Cell Biol.* **177**, 151–162 (2007).
42. Werner, S. & Grose, R. Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* **83**, 835–870 (2003).
43. Benvenuti, S. & Comoglio, P. M. The MET receptor tyrosine kinase in invasion and metastasis. *J. Cell Physiol.* **213**, 316–325 (2007).
44. Sibilia, M. *et al.* The epidermal growth factor receptor: from development to tumorigenesis. *Differentiation* **75**, 770–787 (2007).
45. Sano, S., Chan, K. S. & Digiovanni, J. Impact of Stat3 activation upon skin biology: a dichotomy of its role between homeostasis and diseases. *J. Dermatol. Sci.* **50**, 1–14 (2008).
46. Amendt, C., Schirmacher, P., Weber, H. & Blessing, M. Expression of a dominant negative type II TGF-β receptor in mouse skin results in an increase in carcinoma incidence and an acceleration of carcinoma development. *Oncogene* **17**, 25–34 (1998).

47. Ashcroft, G. S. *et al.* Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nature Cell Biol.* **1**, 260–266 (1999).
48. Wakefield, L. M. & Roberts, A. B. TGF-β signaling: positive and negative effects on tumorigenesis. *Curr. Opin. Genet. Dev.* **12**, 22–29 (2002).
49. Werner, S. *et al.* The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science* **266**, 819–822 (1994).
**The first study to use transgenic mice to study wound repair.**
50. Grose, R. *et al.* The role of fibroblast growth factor receptor 2b in skin homeostasis and cancer development. *EMBO J.* **26**, 1268–1278 (2007).
51. Tse, J. C. & Kalluri, R. Mechanisms of metastasis: epithelial-to-mesenchymal transition and contribution of tumor microenvironment. *J. Cell Biochem.* **101**, 816–829 (2007).
52. Mani, S. A. *et al.* The epithelial–mesenchymal transition generates cells with properties of stem cells. *Cell* **133**, 704–715 (2008).
53. Janda, E. *et al.* Ras and TGFβ cooperatively regulate epithelial cell plasticity and metastasis: dissection of Ras signaling pathways. *J. Cell Biol.* **156**, 299–314 (2002).
54. Radisky, D. C. *et al.* Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. *Nature* **436**, 123–127 (2005).
55. Ting, S. B. *et al.* A homolog of *Drosophila* grainy head is essential for epidermal integrity in mice. *Science* **308**, 411–413 (2005).
56. Ferrara, N. & Kerbel, R. S. Angiogenesis as a therapeutic target. *Nature* **438**, 967–974 (2005).
57. Tepper, O. M. *et al.* Adult vasculogenesis occurs through *in situ* recruitment, proliferation, and tubulization of circulating bone marrow-derived cells. *Blood* **105**, 1068–1077 (2005).
58. Folkman, J. Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* **285**, 1182–1186 (1971).
**In this paper, J. Folkman formulates the important hypothesis that angiogenesis is a prerequisite for tumour growth.**
59. Paavonen, K., Puolakkainen, P., Jussila, L., Jahkola, T. & Alitalo, K. Vascular endothelial growth factor receptor-3 in lymphangiogenesis in wound healing. *Am. J. Pathol.* **156**, 1499–1504 (2000).
60. Saharinen, P., Tammela, T., Karkkainen, M. J. & Alitalo, K. Lymphatic vasculature: development, molecular regulation and role in tumor metastasis and inflammation. *Trends Immunol.* **25**, 387–395 (2004).
61. Liersch, R. & Detmar, M. Lymphangiogenesis in development and disease. *J. Thromb. Haemost.* **98**, 304–310 (2007).
62. Ferrara, N. Role of vascular endothelial growth factor in physiologic and pathologic angiogenesis: therapeutic implications. *Semin. Oncol.* **29**, 10–14 (2002).
63. Rossiter, H. *et al.* Loss of vascular endothelial growth factor A activity in murine epidermal keratinocytes delays wound healing and inhibits tumor formation. *Cancer Res.* **64**, 3508–3516 (2004).
64. Hong, Y. K. *et al.* VEGF-A promotes tissue repair-associated lymphatic vessel formation via VEGFR-2 and the α1β1 and α2β1 integrins. *FASEB J.* **18**, 1111–1113 (2004).
65. Larcher, F., Murillas, R., Bolontrade, M., Conti, C. J. & Jorcano, J. L. VEGF/VPF overexpression in skin of transgenic mice induces angiogenesis, vascular hyperpermeability and accelerated tumor development. *Oncogene* **17**, 303–311 (1998).
66. Hirakawa, S. *et al.* VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis. *J. Exp. Med.* **201**, 1089–1099 (2005).
67. Carmeliet, P. *et al.* Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nature Med.* **7**, 575–583 (2001).
68. Cianfarani, F. *et al.* Placenta growth factor in diabetic wound healing: altered expression and therapeutic potential. *Am. J. Pathol.* **169**, 1167–1182 (2006).
69. Marcellini, M. *et al.* Increased melanoma growth and metastasis spreading in mice overexpressing placenta growth factor. *Am. J. Pathol.* **169**, 643–654 (2006).
70. Saaristo, A. *et al.* Vascular endothelial growth factor-C accelerates diabetic wound healing. *Am. J. Pathol.* **169**, 1080–1087 (2006).
71. Hirakawa, S. *et al.* VEGF-C-induced lymphangiogenesis in sentinel lymph nodes promotes tumor metastasis to distant sites. *Blood* **109**, 1010–1017 (2007).
72. Streit, M. *et al.* Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. *EMBO J.* **19**, 3272–3282 (2000).
73. Streit, M. *et al.* Overexpression of thrombospondin-1 decreases angiogenesis and inhibits the growth of human cutaneous squamous cell carcinomas. *Am. J. Pathol.* **155**, 441–452 (1999).
74. Luster, A. D., Cardiff, R. D., MacLean, J. A., Crowe, K. & Granstein, R. D. Delayed wound healing and disorganized neovascularization in transgenic mice expressing the IP-10 chemokine. *Proc. Assoc. Am. Physicians* **110**, 183–196 (1998).
75. Arenberg, D. A. *et al.* Interferon-γ-inducible protein 10 (IP-10) is an angiostatic factor that inhibits human non-small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. *J. Exp. Med.* **184**, 981–992 (1996).
76. O'Reilly, M. S. *et al.* Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* **88**, 277–285 (1997).
77. Bloch, W. *et al.* The angiogenesis inhibitor endostatin impairs blood vessel maturation during wound healing. *FASEB J.* **14**, 2373–2376 (2000).
78. Lange-Asschenfeldt, B. *et al.* The angiogenesis inhibitor vasostatin does not impair wound healing at tumor-inhibiting doses. *J. Invest. Dermatol.* **117**, 1036–1041 (2001).
79. Kamba, T. & McDonald, D. M. Mechanisms of adverse effects of anti-VEGF therapy for cancer. *Br. J. Cancer* **96**, 1788–1795 (2007).
80. Opalenik, S. R. & Davidson, J. M. Fibroblast differentiation of bone marrow-derived cells during wound repair. *FASEB J.* **19**, 1561–1563 (2005).
81. Werner, S. & Smola, H. Paracrine regulation of keratinocyte proliferation and differentiation. *Trends Cell Biol.* **11**, 143–146 (2001).
82. Radisky, D. C., Kenny, P. A. & Bissell, M. J. Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? *J. Cell Biochem.* **101**, 830–839 (2007).
83. Gaggioli, C. *et al.* Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. *Nature Cell Biol.* **9**, 1392–1400 (2007).
84. Direkze, N. C. *et al.* Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. *Cancer Res.* **64**, 8492–8495 (2004).
85. Iyer, V. R. *et al.* The transcriptional program in the response of human fibroblasts to serum. *Science* **283**, 83–87 (1999).
86. Cooper, L., Johnson, C., Burslem, F. & Martin, P. Wound healing and inflammation genes revealed by array analysis of 'macrophageless' PU.1 null mice. *Genome Biol.* **6**, R5 (2005).
87. Li, W. *et al.* Extracellular heat shock protein-90α: linking hypoxia to skin cell motility and wound healing. *EMBO J.* **26**, 1221–1233 (2007).
88. Eustace, B. K. *et al.* Functional proteomic screens reveal an essential extracellular role for hsp90 α in cancer cell invasiveness. *Nature Cell Biol.* **6**, 507–514 (2004).
89. Ostman, A. PDGF receptors-mediators of autocrine tumor growth and regulators of tumor vasculature and stroma. *Cytokine Growth Factor Rev.* **15**, 275–286 (2004).
90. Gao, Z. *et al.* Deletion of the PDGFR-β gene affects key fibroblast functions important for wound healing. *J. Biol. Chem.* **280**, 9375–9389 (2005).
91. Pietras, K. *et al.* STI571 enhances the therapeutic index of epothilone B by a tumor-selective increase of drug uptake. *Clin. Cancer Res.* **9**, 3779–3787 (2003).
92. Rajkumar, V. S. *et al.* Platelet-derived growth factor-β receptor activation is essential for fibroblast and pericyte recruitment during cutaneous wound healing. *Am. J. Pathol.* **169**, 2254–2265 (2006).
93. Distler, J. H. *et al.* Imatinib mesylate reduces production of extracellular matrix and prevents development of experimental dermal fibrosis. *Arthritis Rheum.* **56**, 311–322 (2007).
94. Steed, D. L. Clinical evaluation of recombinant human platelet-derived growth factor for the treatment of lower extremity ulcers. *Plast. Reconstr Surg.* **117**, S143–S149 (2006).
95. Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C. & Brown, R. A. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nature Rev. Mol. Cell Biol.* **3**, 349–363 (2002).
96. Shah, M., Foreman, D. M. & Ferguson, M. W. Neutralisation of TGF-β 1 and TGF-β 2 or exogenous addition of TGF-β 3 to cutaneous rat wounds reduces scarring. *J. Cell Sci.* **108**, 985–1002 (1995).
97. Cheon, S. S. *et al.* β-catenin regulates wound size and mediates the effect of TGF-β in cutaneous healing. *FASEB J.* **20**, 692–701 (2006).
98. Cheon, S. S. *et al.* β-Catenin stabilization dysregulates mesenchymal cell proliferation, motility, and invasiveness and causes aggressive fibromatosis and hyperplastic cutaneous wounds. *Proc. Natl Acad. Sci. USA* **99**, 6973–6978 (2002).
99. Alman, B. A., Li, C., Pajerski, M. E., Diaz-Cano, S. & Wolfe, H. J. Increased β-catenin protein and somatic APC mutations in sporadic aggressive fibromatoses (desmoid tumors). *Am. J. Pathol.* **151**, 329–334 (1997).
100. Bhowmick, N. A. *et al.* TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* **303**, 848–851 (2004).
101. Stover, D. G., Bierie, B. & Moses, H. L. A delicate balance: TGF-β and the tumor microenvironment. *J. Cell Biochem.* **101**, 851–861 (2007).
102. Mori, R., Shaw, T. J. & Martin, P. Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. *J. Exp. Med.* **205**, 43–51 (2008).
103. El-Tanani, M. K. *et al.* The regulation and role of osteopontin in malignant transformation and cancer. *Cytokine Growth Factor Rev.* **17**, 463–474 (2006).
104. Esteller, M. Epigenetics in cancer. *N. Engl. J. Med.* **358**, 1148–1159 (2008).
105. Chang, H. Y. *et al.* Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. *PLoS Biol.* **2**, E7 (2004).
**Reports on the parallels in the gene-expression pattern between malignant tumours and serum-treated fibroblasts.**
106. Riss, J. *et al.* Cancers as wounds that do not heal: differences and similarities between renal regeneration/repair and renal cell carcinoma. *Cancer Res.* **66**, 7216–7224 (2006).
107. Pedersen, T. X. *et al.* Laser capture microdissection-based *in vivo* genomic profiling of wound keratinocytes identifies similarities and differences to squamous cell carcinoma. *Oncogene* **22**, 3964–3976 (2003).
108. Thorey, I. S. *et al.* Transgenic mice reveal novel activities of growth hormone in wound repair, angiogenesis, and myofibroblast differentiation. *J. Biol. Chem.* **279**, 26674–26684 (2004).



### Acknowledgements
We are grateful to M. Detmar, ETH Zürich, Switzerland, P. Martin, University of Bristol, UK, and A. Knuth, University of Zürich, Switzerland, for helpful suggestions and critical comments on the manuscript; to U. Scheier for help with the figures and the references; and to I. Thorey for providing the micrographs shown in Figure 4 and 5. Work in the laboratory of S.W. is supported by the ETH Zürich, the Swiss National Science Foundation (grant 3100A0-109340/1), Oncosuisse (grant OCS-02017-02-2007), and the European Union (grant Ulcertherapy). M.S. is supported by an EMBO postdoctoral fellowship.


### DATABASES
**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
*Jun*
**OMIM:**
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
recessive dystrophic epidermolysis bullosa
**UniProtKB:** http://ca.expasy.org/sprot
CXCR4 | cyclooxygenase-2 | FGF2 | HGF | HIF1α | IP10 | MMP3 | NRF2 | osteopontin | PLGF | SDF1 | STAT3 | TGFβ1 | TGFβ2 | TSP1 | VEGFA | VEGFC

### FURTHER INFORMATION
**Sabine Werner's homepage:**
http://www.cell.biol.ethz.ch/people/werner

### SUPPLEMENTARY INFORMATION
**See online article:** S1 (table)

**ALL LINKS ARE ACTIVE IN THE ONLINE PDF**