The bright and the dark sides of activin in wound healing and cancer

Maria Antsiferova* and Sabine Werner*

Department of Biology, Institute of Molecular Health Sciences, ETH Honggerberg, HPL E12, 8093, Zurich, Switzerland

*Authors for correspondence (maria.antsiferova@cell.biol.ethz.ch; sabine.werner@cell.biol.ethz.ch)

Journal of Cell Science 125, 3929–3937

doi: 10.1242/jcs.094789

Summary

Activin was initially described as a protein that stimulates release of follicle stimulating hormone from the pituitary, and it is well known for its important roles in different reproductive functions. In recent years, this multifunctional factor has attracted the attention of researchers in other fields, as new functions of activin in angiogenesis, inflammation, immunity, fibrosis and cancer have been discovered. Studies from our laboratory have identified activin as a crucial regulator of wound healing and skin carcinogenesis. On the one hand, it strongly accelerates the healing process of skin wounds but, on the other hand, it enhances scar formation and the susceptibility to skin tumorigenesis. Finally, results from several laboratories have revealed that activin enhances tumour formation and/or progression in some other organs, in particular through its effect on the tumour microenvironment, and that it also promotes cancer-induced bone disruption and muscle wasting. These findings provide the basis for the use of activin or its downstream targets for the improvement of impaired wound healing, and of activin antagonists for the prevention and treatment of fibrosis and of malignant tumours that overexpress activin. Here, we summarize the previously described roles of activin in wound healing and scar formation and discuss functional studies that revealed different functions of activin in the pathogenesis of cancer. The relevance of these findings for clinical applications will be highlighted.

Key words: Activin, Cancer, Tumour microenvironment, Wound healing

Introduction

In 1863, Rudolf Virchow had already recognized that “chronic irritation and inflammatory hyperplasia are predispositions for cancer development” (Virchow, 1863). A century later, Alexander Haddow suggested that “tumour production is a possible overhealing” (Haddow, 1974). Finally, Harold Dvorak postulated that “tumors are wounds that do not heal” (Dvorak, 1986). These observations suggested that common cellular and molecular mechanisms are active in wounds and in cancer, and this concept has been strongly supported during subsequent years by various experimental studies.

A hallmark of wounds and of carcinomas (cancer of epithelial cells) is the enhanced proliferation and migration of epithelial cells. In healing wounds, this allows efficient re-epithelialization of the injured body site and it is terminated when the wound is fully covered by a new epidermis. In a carcinoma, however, this process is not self-limiting and results in unlimited tumour growth and eventually metastasis (reviewed by Schäfer and Werner, 2008). During cutaneous wound repair, keratinocytes at the wound edge lose their cell–cell contacts, rearrange their actin cytoskeleton and express various proteases to degrade the basement membrane and interstitial connective tissue. These events are reminiscent of the epithelial–mesenchymal transition (EMT) that occurs during development and is also frequently seen in advanced-stage carcinomas. However, in skin wounds EMT is only partial and reversible – keratinocytes retain some intercellular contacts and continue to express epidermal keratins (reviewed by Schäfer and Werner, 2008). By contrast, cancer cells that undergo EMT often acquire a fibroblast-like morphology; they lose their cell–cell contacts and start expressing mesenchymal markers such as vimentin. Furthermore, matrix metalloproteinases and other proteolytic enzymes that are produced by epithelial and stromal cells are involved in the breakdown of extracellular matrix, which is essential for the migration of the cells at the wound edge, and also for increased cancer cell motility and invasion (reviewed by Arnoux et al., 2005).

Besides the similar behaviour of epithelial cells during wound healing and cancerogenesis, many similarities have also been found in the stroma of healing wounds and carcinomas. These include similarities in the cellular composition and also in the deposited extracellular matrix. Examples of the matrix alterations that are seen in wounds and in tumours are differences in collagen remodeling compared with that in normal tissues, and upregulation of various extracellular matrix molecules, such as tenascin-C and an embryonic splice variant of fibronectin. Furthermore, fibrinogen is released from injured vessels upon wounding or from chronically hyperpermeable vessels in tumours (reviewed by Werner and Grose, 2003; Egeblad et al., 2010). It is subsequently cleaved by thrombin to form fibrin, which polymerizes to form a clot. This matrix provides a favourable substrate that promotes cell migration and proliferation (Dvorak, 1986).

Inflammation is a central event in wound repair that protects the host from infection, as inflammatory cells release reactive oxygen species and proteases to defend bacteria. Furthermore, inflammatory cells are potent sources of various growth factors and cytokines that are required for new tissue formation (reviewed by Werner and Grose, 2003). In cancer, inflammation is essential for the generation of anti-tumour immunity and elimination of

tumour cells. However, during tumour growth, transformed cells frequently escape this immunosurveillance by generating an immunosuppressive and pro-tumorigenic microenvironment that involves the recruitment of regulatory T cells (Tregs; see glossary in Box 1) and myeloid-derived suppressor cells (Box 1). In addition, macrophages and neutrophils change their polarization to a phenotype called N2 (for neutrophils) and M2 (for macrophages). N2 neutrophils and M2 macrophages promote tissue remodelling and angiogenesis, and favour tumour progression (reviewed by Flavell et al., 2010). Neutrophils and macrophages produce large amounts of reactive oxygen and nitrogen species that are not only toxic for invading microorganisms, but also directly damage DNA and modify proteins involved in DNA repair, cell cycle control or apoptosis through covalent modifications of these macromolecules. Therefore, they contribute to the accumulation of further mutations and epigenetic alterations of tumour cells. In addition, reactive oxygen and nitrogen species induce epigenetic, and possibly genetic, changes in stromal cells, which further promote tumour growth and progression (reviewed by Egeblad et al., 2010; Schäfer and Werner, 2008). Through the production of angiogenic growth factors, inflammatory cells as well as tumour cells stimulate the formation of new blood vessels, which is essential for supplying the newly formed wound tissue or the tumour with oxygen and nutrients (reviewed in Hanahan and Weinberg, 2011; Schäfer and Werner, 2008).

Box 1. Glossary – Immune cells that are affected by activin

B cells: lymphocytes governing a humoral (antibody-mediated) immunity.
Dendritic cells: immune cells that process antigens and present them on their surface to other cells of the immune system, thereby initiating an immune response.
Dendritic epidermal T cells (DETCs): a subpopulation of T cells present in the murine epidermis; they express γδ T cell receptor and are involved in regulating epidermal homeostasis, inflammation, wound repair and tumour surveillance.
Langerhans cells: dendritic cells of the skin and mucosae contiguous with skin (mouth, foreskin, vagina).
Macrophages: phagocytic cells that engulf and digest cellular debris and pathogens; they acquire different activation states (e.g. M1 or M2) and function in innate and adaptive immunity, and in the regulation of inflammation and tissue repair.
Mast cells: resident immune cells populating most tissues, especially frequent in the skin and mucosae; they mainly function in allergic reactions and in response to helminthes, but are also involved in modulating inflammation, wound repair and carcinogenesis.
Myeloid-derived suppressor cells: heterogeneous population of immature myeloid cells that expands during cancer, inflammation and infection; they potently suppress natural killer (NK) and T cell responses and have been implicated in tumour-associated immune suppression.
Natural killer cells (NK cells): cytotoxic lymphocytes that are part of the innate immune system; in particular, they kill virus-infected cells and tumour cells.
Regulatory T cells (Tregs): a type of T cell that suppresses the immune system, maintains tolerance to self-antigens, downregulates autoimmune disease and inhibits anti-tumour immunity.
T cells: lymphocytes, providing cell-mediated immunity; they are subdivided in subsets (Th1, Th2, Tregs, γδ T cells, etc.), each with a distinct function.

Taken together, these parallels suggest that cancers activate a latent pre-existing wound-healing programme of the host, but do so in an exaggerated and uncontrolled manner. Consistently, many genes that orchestrate the wound repair process, including genes encoding various growth factors, cytokines, matrix molecules and transcription factors, are also important regulators of carcinogenesis, and the presence of a 'wound healing signature' is a hallmark of highly malignant cancers (Chang et al., 2004). One of these wound-induced genes encodes the growth and differentiation factor activin A (Hübner et al., 1999), which has been recently shown to play an important role in skin carcinogenesis (Antsiferova et al., 2011). In this Commentary, we will summarize the current knowledge on the functions of activin in wound healing and cancer. Finally, the possibility of targeting activin in cancer therapy will be addressed.

Structure of activins and their signalling mechanisms

Activins belong to the transforming growth factor (TGF)-β superfamily of growth and differentiation factors. They are homo- or hetero-dimeric proteins, consisting of two β subunits that are crosslinked by a disulfide bond (Fig. 1). Like other TGF-β family members, they are produced as inactive precursors, which are cleaved in the endoplasmic reticulum. In contrast to TGF-β, however, the mature activin dimers are released in an active form that is not inhibited by binding to the pro-peptide. Nevertheless, there is evidence that these dimers still associate with the pro-peptide in the extracellular environment, which is important for their interaction with the extracellular matrix (reviewed by Harrison et al., 2011; Gold and Risbridger, 2012). Four distinct activin genes are present in mammals, encoding the activin subunits βA, βB, βC and βE (Fig. 1). These subunits dimerize to form the most abundant activin variant, activin A (βAβA), as well as additional homo- or hetero-dimers (reviewed by Gold and Risbridger, 2012).

Like other members of the TGF-β superfamily, activins exert their biological effects through activation of transmembrane receptors with serine/threonine kinase activity. The initial step of activin action is their binding to a type II activin receptor (ActRII or ActRIIB), which leads to the recruitment, phosphorylation and activation of a type I activin receptor. The major type I activin receptor is ActRIB, also known as Alk4, but ActRIC (Alk7) can also mediate activin signalling. ActRIA (Alk2) had been shown to bind activin, but it is predominantly a receptor for bone-morphogenetic proteins (reviewed by Tsuchida et al., 2008). Once phosphorylated by the type II receptor, and thereby activated, the type I receptor kinase phosphorylates the receptor-Smad proteins (R-Smads), SMAD2 and SMAD3. Access of the R-Smads to the type I receptors is facilitated by auxillary proteins, such as Smad anchor for receptor activation (SARA). Upon phosphorylation, R-Smads multimerize with the co-Smad SMAD4 and translocate to the nucleus, where they control the expression of activin target genes (Fig. 1). In addition to this canonical Smad pathway, other signalling pathways can be activated by activin, including the extracellular-signal-regulated kinase (ERK), p38 (MAPK14) and c-Jun N-terminal kinases (JNK) mitogen-activated protein kinase (MAPK) pathways (reviewed by ten Dijke and Hill, 2004; Chen et al., 2006). Although most of the functions of activins appear to be mediated through the canonical Smad pathway, MAPK signalling also controls some of the functions of activins, such as stimulation of keratinocyte migration (Zhang

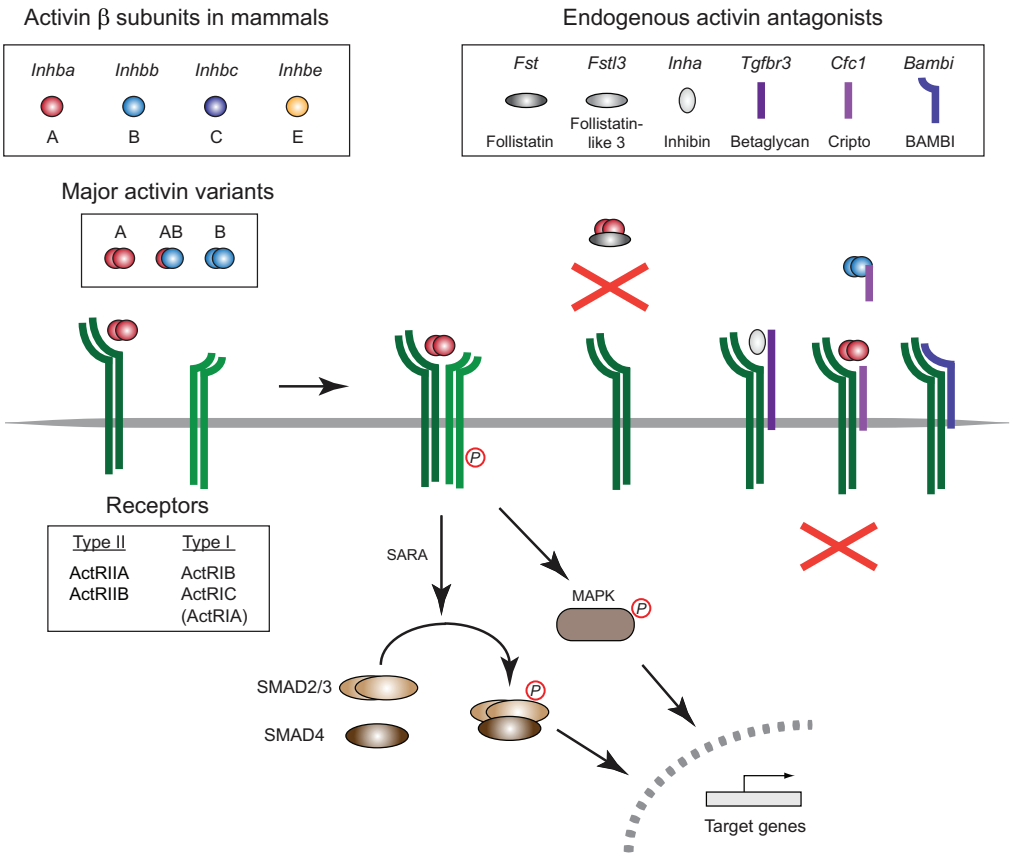

Fig. 1. Schematic representation of activin signalling and its control by antagonists. The four mammalian activin subunits are shown on the left, which are encoded by the *Inhba*, *Inhbb*, *Inhbc* and *Inhbe* genes. These subunits can form homo- or hetero-dimers, including the major activin variants shown. These bind and activate a type II receptor, resulting in recruitment and phosphorylation of a type I receptor. Downstream signal transduction occurs via Smad proteins as well as via MAPK pathways. Smad anchor for receptor activation (SARA) facilitates activation of the Smad pathway. A number of soluble and transmembrane proteins, including betaglycan in combination with its binding partner inhibin as well as cripto and BAMBI, that are known to inhibit activin signalling, are shown on the top right.

et al., 2011). Interestingly, the same signalling pathways are also used by TGF-β, explaining some of the overlapping functions of both cytokines, e.g. in the regulation of several immune functions (reviewed by Flavell et al., 2010). However, activin and TGF-β can also have very different functions. The underlying mechanisms remain to be determined, but different signalling strength and duration, differential use of Smad2 versus Smad3 or differential activation of the non-canonical MAPK signalling pathways might be involved.

Several proteins have been described that limit the availability and functional activity of activins. These include the secreted glycoproteins follistatin and follistatin-like protein 3 (also known as follistatin-related gene protein), which directly bind activin and thereby inhibit activation of the signalling receptors by activin. Additional activin antagonists are the secreted inhibin proteins, which are heterodimers of an activin-β chain and the inhibin α subunit. Upon binding to the transmembrane proteoglycan betaglycan, inhibins compete with activins for receptor binding and activation. Finally, activin receptor signalling can be inhibited by the transmembrane proteins cripto (also known as cryptic protein) and BAMBI (Fig. 1) (reviewed in Harrison et al., 2005).

The role of activin in wound healing

Initially, activins were described as reproductive hormones, but more recently important functions of activins in development, tissue homeostasis and repair have been identified (reviewed by Werner and Alzheimer, 2006). Studies from our laboratory have revealed an important role of activin in skin biology. Initially, we showed that expression of the activin βA and βB subunits strongly increases in keratinocytes and stromal cells after skin injury in mice and humans [(Hübner et al., 1996) and our unpublished data]. In addition to activin, all known activin receptors are present in normal and wounded skin, but their expression levels do not change after injury (Hübner et al., 1996). Serum growth factors and pro-inflammatory cytokines are able to induce the expression of activin βA mRNA in cultured fibroblasts and keratinocytes (Hübner and Werner, 1996), suggesting a possible role of the pro-inflammatory wound environment in the induction of activin expression upon tissue injury. Interestingly, upregulation of activin βA was also observed at the wound margin after fin amputation in zebrafish (Jaźwińska et al., 2007), demonstrating that activin, which is highly conserved throughout the animal kingdom (Tiedemann et al., 1992), is an evolutionary old player in the wound repair programme.

To gain further insight into the function of activin in the skin, we generated transgenic mice that overexpress the activin βA subunit in keratinocytes under the control of a keratin 14 promoter (Munz et al., 1999). These mice release the strongly diffusible activin A into the immediate environment and even into the blood stream as revealed by high serum levels of activin A (Antsiferova et al., 2011). They are characterized by epidermal hyperthickening and fibrosis in the tail skin (Munz et al., 1999). Interestingly, wound healing was strongly accelerated in these mice due to enhanced reepithelialization and granulation tissue formation (Munz et al., 1999). A stimulatory effect of activin B on wound healing has also been demonstrated: it was shown that recombinant activin B accelerates wound closure and reepithelialization in mice through stimulation of the RhoA–JNK signalling pathway (Zhang et al., 2011). The important role of activin in re-epithelialization was

confirmed in loss-of-function studies. For instance, expression of a dominant-negative ActRIB mutant in keratinocytes of transgenic mice caused delayed wound re-epithelialization (Bamberger et al., 2005), whereas keratinocyte-specific deletion of follistatin in mice accelerated this process (Antsiferova et al., 2009). Similarly, inhibition of activin signalling in a zebrafish wound model resulted in a complete block of fin regeneration through the inhibition of cell migration and proliferation (Jaźwińska et al., 2007). Interestingly, however, the positive effect of activin on keratinocyte proliferation in wounded skin (our unpublished data) appears to be indirect and mediated through the stroma, because activin does not stimulate, but rather inhibits proliferation of cultured keratinocytes (Seishima et al., 1999). Finally, activin has been shown to regulate the expression of sensory neuropeptides, including calcitonin gene-related peptide (CGRP), following skin injury (Cruise et al., 2004). This is likely to be of functional importance, as sensory innervation is required for vascular regeneration and survival of ischaemic tissues (Kjartansson and Dalsgaard, 1987). A schematic representation of the direct and indirect activities of activin during wound repair is shown in Fig. 2.

However, we also found negative consequences of activin A overexpression. For instance, the healed wounds of activin transgenic mice had a much larger scar area (our unpublished data). Furthermore, the late granulation tissue and the resulting scar tissue were characterized by a higher cellular density compared with that in control mice as well as by the presence of keratinocytic cysts (Fig. 3). Interestingly, when endogenous activin signalling was blocked by overexpression of follistatin in keratinocytes of transgenic mice, a contrasting phenotype was observed. These follistatin-overexpressing mice, which release large amounts of follistatin into wound tissue, were characterized by thinning of the epidermis and the dermis, and a severe delay in wound repair. However, their scarring response was reduced, resulting in an aesthetical improvement of the healed wounds (Wankell et al., 2001).

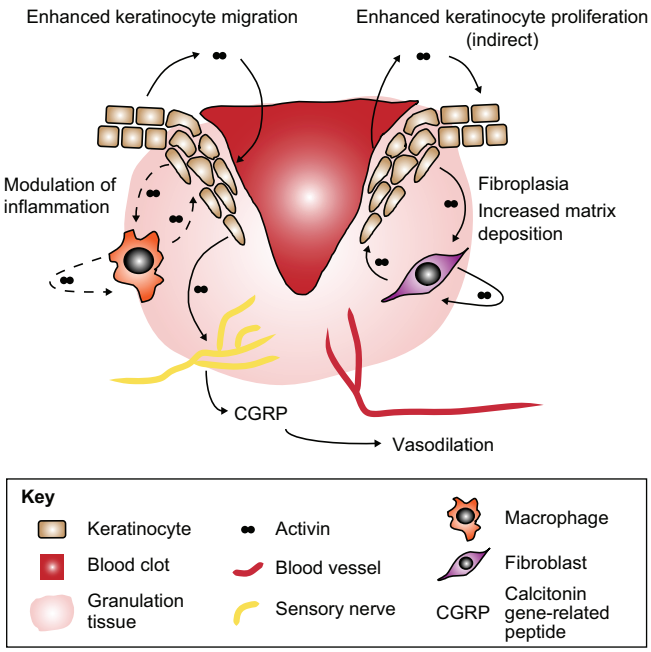

Fig. 2. Schematic representation of possible mechanisms of the action of activin in wound healing. Functions that have been demonstrated experimentally in wounds are indicated with arrows. These include stimulation of re-epithelialization and granulation tissue formation as well as upregulation of calcitonin gene-related peptide (CGRP) in sensory neurons, which subsequently promotes vasodilation. In addition, in vitro experiments and in vivo studies not involving wound healing have revealed a potent effect of activin on different types of immune cells. Therefore, activin-induced alterations in immune cells might also contribute to its potent stimulatory effect on wound healing (indicated by dashed arrows).

Taken together, the results described above demonstrate that activins and follistatin are important regulators of skin homeostasis and wound repair, but the signalling pathways that are used by activin to control wound repair and the responsible activin target genes remain largely unknown. Furthermore, the cell types that are affected by activin in healing wounds have only partly been identified (Fig. 2).

The role of activin in scar formation and fibrosis

The enhanced scar formation observed in activin-A-overexpressing transgenic mice (our unpublished data), and the reduced scar formation seen in follistatin-overexpressing transgenic mice (Wankell et al., 2001), suggested that activin has a role in the pathogenesis of hypertrophic scars and keloids (scars that grow beyond the boundaries of the original wound). Indeed, immunostaining of active-phase hypertrophic scars showed that myofibroblasts (fibroblasts with contractile properties resembling smooth muscle cells) as well as dendritic cells (see glossary in Box 1) express activin A. Increased expression of activin was also found in keloids, when compared with that in normal skin, in particular in basal keratinocytes of the epidermis (Mukhopadhyay et al., 2007). In addition, in vitro studies showed that fibroblasts derived from keloids or from active-phase hypertrophic scars produce significantly more activin A than normal fibroblasts. Furthermore, when exogenous activin was added to fibroblasts that had been isolated from normal skin, hypertrophic scars or keloids, it stimulated their proliferation and the production of key extracellular matrix components, such as collagen I and fibronectin (Fumagalli et al., 2007; Mukhopadhyay et al., 2007).

In addition to the skin, activin overexpression is a hallmark of fibrotic disorders of various other organs, including liver, pancreas, kidney and lung (reviewed by Werner and Alzheimer, 2006). This has potential therapeutic implications, as follistatin, owing to its effect of limiting activin-mediated fibrosis, might be considered for the treatment of organ fibrosis. For example, recombinant follistatin attenuated liver fibrosis in a rat model of CCl_4-induced liver injury (Patella et al., 2006) and lung fibrosis in a rat model of bleomycin-induced lung injury (Aoki et al., 2005). Interestingly, follistatin not only blocked the action of activin itself, but also inhibited TGF-β-induced collagen production, indicating that this function of TGF-β is at least in part mediated through the activation of an autocrine loop that involves activin (Wada et al., 2004). Taken together, these results identify activin as a potent pro-fibrotic factor in different tissues and organs, whereas follistatin is found to counteract this effect.

The role of activin in cancer development

Owing to the parallels between wound healing and cancer, it is of particular interest to study the role of activin in the pathogenesis of different malignancies. Interestingly, depending on the tissue type, activin can exert either pro- or anti-tumorigenic effects in different types of cancer. For instance, activins have a growth-inhibitory effect on breast, liver, prostate and pancreatic carcinoma cells and

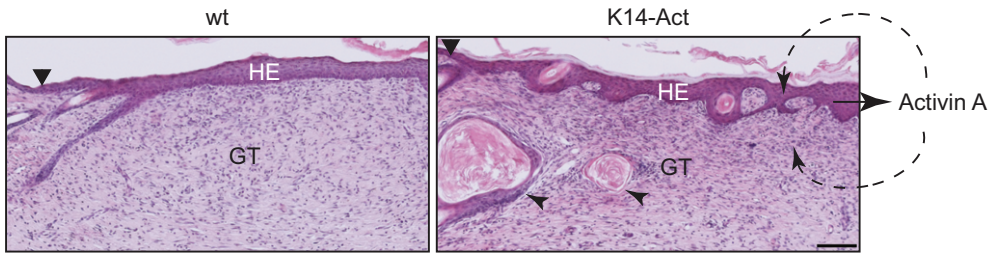

Fig. 3. Activin overexpression leads to keratinocytic cysts and high cell density in the granulation tissue of healed wounds. Representative microphotographs of haematoxylin- and eosin-stained tissue specimens from 14-day-old full-thickness excisional wounds of wild-type (wt) and K14-activin transgenic (K14-Act) mice are shown. Note the keratinocytic cysts in the granulation tissue (indicated by arrowheads) and the high cell density, which is reflected by the multiple nuclei (blue). The transgene-derived activin is secreted by basal keratinocytes (arrow); it acts in an autocrine manner on keratinocytes and in a paracrine manner on stromal cells (dashed arrows). Triangle, wound edge; GT, Granulation tissue; HE, Hyperproliferative wound epidermis. Scale bar: 100 μm.

on pituitary adenoma cells. Accordingly, to allow malignant progression, these cancer cells and tissues acquire resistance to activin by downregulating the expression of activin receptors. Tissues whose growth is promoted by activin include the testis and ovary; here, activin acts as a mitogen for the tumour cells (reviewed by Risbridger et al., 2001).

In addition to a direct effect on tumour cells, several studies indicate that activin can also affect tumorigenesis by altering the tumour microenvironment. A possible target of activin is the vasculature system (Fig. 4). Thus, in xenograft models of neuroblastoma, activin has been suggested to indirectly inhibit tumour growth by inhibiting angiogenesis (Panopoulou et al., 2005). Consistent with this hypothesis, activin A inhibited several functions of cultured endothelial cells that are crucial for tumorigenesis, such as proteolytic activity, migration and proliferation (McCarthy and Bicknell, 1993; Panopoulou et al., 2005). Moreover, activin inhibited angiogenesis in the chorioallantoic membrane assay (Breit et al., 2000). By contrast, activin A supported inflammatory corneal angiogenesis (Poulaki et al., 2004), indicating that it might also have pro-angiogenic effects under certain circumstances. Another study revealed a pro-tumorigenic role of activin B in a xenograft model of clear cell renal cell carcinoma, most likely through an as-yet-unidentified component of the tumour microenvironment (Wacker et al., 2009).

Multiple in vitro studies have provided evidence that activin is involved in the regulation of inflammation and immune function (reviewed by Werner and Alzheimer, 2006; Hedger et al., 2011). These activities could considerably influence cancer development and progression in vivo (Fig. 4). Interestingly, activin can exert pro- or anti-inflammatory effects. A pro-inflammatory activity is suggested by the pro-migratory effect of activin on monocytes (Petraglia et al., 1991). Furthermore, activin stimulated the production of pro-inflammatory cytokines in human peripheral blood mononuclear cells (Yamashita et al., 1993) and in rat bone-marrow-derived macrophages (Nüsing and Barsig, 1999). However, anti-inflammatory effects of activin have also been demonstrated. For example,1 it antagonized interleukin (IL)-6 activity in different cell types, and inhibited the processing of IL-1β to the mature cytokine in human macrophage cell lines (reviewed by Werner and Alzheimer, 2006). It has been suggested that activin exerts a pro-inflammatory effect early in inflammation, but once the inflammatory response is ongoing, it might act to oppose the inflammatory signalling and the production of inflammatory mediators (reviewed by Hedger et al., 2011).

Several recent studies have revealed that activin not only controls the development of immune cells in bone marrow and thymus, but also exerts more subtle immunomodulatory activities (reviewed by Hedger et al., 2011). For example, activin A was found to be important for dendritic cell (DC) function by acting as a chemotactic signal for immature DCs in mice and humans (Salogni et al., 2009). Activin is produced by DCs during maturation in vitro and it enhanced their antigen uptake during their maturation (Scutera et al., 2008). Furthermore, activin induced the differentiation of circulating and skin-resident

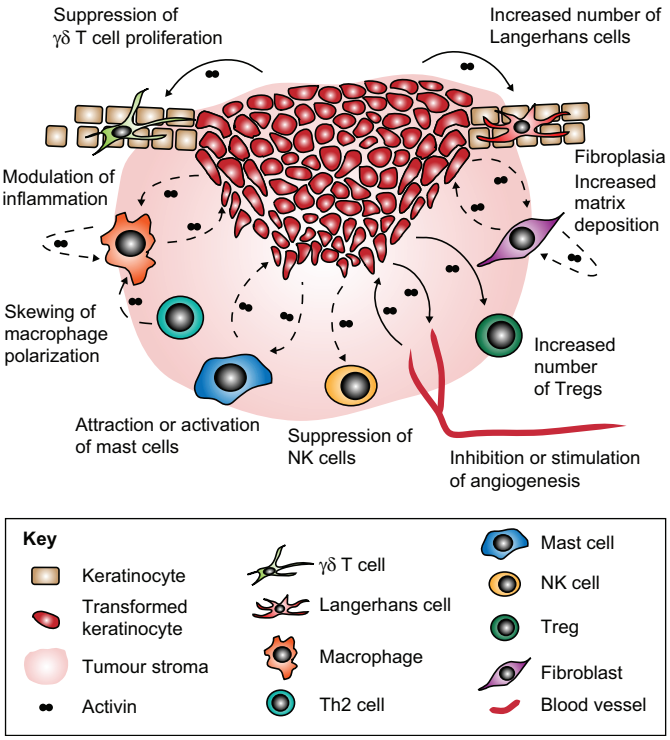

Fig. 4. Overview of possible mechanisms by which activin can contribute to tumorigenesis. Functions that have been demonstrated experimentally in cancer are indicated with arrows. These include alterations in angiogenesis, an increase in the number of Tregs and Langerhans cells and suppression of γδ T cell proliferation. Hypothetical functions, based on the results from other experimental systems, are shown with dashed arrows. These include stimulation of fibroblast proliferation and matrix deposition by these cells, modulation of inflammation, alteration of macrophage polarization, attraction and activation of mast cells and suppression of natural killer cells.

precursor cells into Langerhans cells (see glossary in Box 1) (Musso et al., 2008). The in vivo relevance of this finding is reflected in the strongly reduced number of Langerhans cells in transgenic mice that overexpress follistatin in keratinocytes (Stoitzner et al., 2005). Thus, activin appears to positively regulate the recruitment and development of dendritic cells and their antigen uptake. However, in another study, activin attenuated cytokine and chemokine production by stimulated DCs (Robson et al., 2008), thereby inhibiting the ability of DC to activate T cells (see glossary in Box 1). This indicates that activin can also have an immunosuppressive function through its activity on DCs. Therefore, the effect of activin on DCs might depend on the activation status of these cells.

It has been further demonstrated that activin A is preferentially produced by a Th2 subpopulation of T cells upon their activation (Ogawa et al., 2006). These types of helper T cells are generally involved in humoral immune responses known to correlate with enhanced tumour growth and progression (Tan and Coussens, 2007). In addition to its effect on T cells, modulation of B cell (see glossary in Box 1) function by activin has also been reported (reviewed by Ogawa and Funaba, 2011). This function is likely to be relevant for cancer biology, as B cells have been shown to be required for establishing the chronic inflammatory states that promote de novo carcinogenesis (de Visser et al., 2006). Furthermore, activin induced migration and maturation of mast cells (see glossary in Box 1) (Funaba et al., 2003), and these immune cells can also contribute to malignant progression in skin (Coussens et al., 1999) and pancreatic cancer (Soucek et al., 2007).

Treatment of mouse macrophages with activin A markedly induced the expression of arginase-1 (Ogawa et al., 2006), a marker for M2 macrophages that are involved in tissue repair processes (Loke et al., 2007) (see glossary in Box 1). By contrast, it decreased the interferon (IFN)-γ-induced expression of inducible nitric oxide (NO) synthase, which is a marker for inflammatory M1 macrophages (Ogawa et al., 2006). This indicates that activin A is involved in polarization of macrophages towards an M2 phenotype – a phenomenon that is also frequently associated with a tumour-promotive microenvironment (Mantovani et al., 2002). However, a recent study on human macrophages demonstrated the opposite effect; activin was preferentially released by M1 macrophages and contributed to M1 polarization (Sierra-Filardi et al., 2011). As macrophages represent a dynamic cell population and are able to repolarize under the appropriate cytokine conditions (Gratchev et al., 2006), it has been suggested that activin acts as a switch between these polarization states (Sierra-Filardi et al., 2011).

Finally, activin A attenuated several functions of human natural killer (NK) cells (see glossary in Box 1), such as IFN-γ production, proliferation and phenotypic maturation (Robson et al., 2009), further suggesting it has immunosuppressive properties. Taken together, activin affects various types of immune cells in a manner that supports tumorigenesis and impairs anti-tumour immunity.

Role of activin in malignant progression

Besides regulating tumour development, activin might also affect malignant progression of existing tumours. This hypothesis is based on the observed suppression of experimentally induced multiple-organ metastases of small cell lung cancer cells upon treatment of tumour-bearing mice with recombinant follistatin (Ogino et al., 2008). The mechanisms underlying this effect have not been investigated in detail, but the authors of that study speculate that inhibition of angiogenesis by follistatin is responsible for the observed effects. Thus, contradictory to data obtained in neuroblastoma (see above), activin or other follistatin-binding partners, including several bone morphogenetic proteins, appear to have pro-angiogenic activities under these conditions. Moreover, clinical data support a possible role for activin or follistatin in the metastatic spread of tumours and show that circulating levels of activin in breast and prostate cancer patients are associated with the presence of bone metastases. Interestingly, there was a significant correlation between activin levels and the number of metastatic lesions in these patients (Incorvaia et al., 2007; Leto et al., 2006). Therefore, activin might even promote the progression of tumours whose growth was initially inhibited by activin, possibly through alterations in the tumour microenvironment.

Taken together, multiple lines of evidence, including those from in vitro data and clinical observations, suggest that activin has an important role in the regulation of tumour growth and progression through direct and indirect mechanisms. However, studies using in vivo mouse models of cancer, in which activin, its signalling components, or activin antagonists are genetically manipulated, are very scarce. Results from these experiments are summarized in Table 1. Importantly, most in vivo data were obtained with xenograft models in immunocompromized hosts, which in light of the immunomodulatory functions of activin, might be problematic. Thus different approaches are required to decipher the role of activin in cancer in the context of a functioning immune system, and the example of skin cancer is discussed in the following section.

A functional role of activin in skin cancer

The observation that activin-overexpressing mice show hyperproliferation of wound keratinocytes and the presence of keratinocytic cysts in the scar tissue (Fig. 3) prompted us to speculate that activin might have a role in the pathogenesis of skin cancer. Indeed, experiments performed with these transgenic mice revealed that activin overexpression in keratinocytes dramatically increased the susceptibility to skin carcinogenesis induced by the application of the mutagen 7,12-dimethylbenz(a)anthracene (DMBA) followed by 20 applications of the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Antsiferova et al., 2011). Interestingly, we found that the pro-tumorigenic effect of activin is not cell-autonomous, but mediated through its action on stromal cells. Several types of immune cells were affected in these mice during DMBA-TPA-induced tumorigenesis. Most importantly, activin inhibited the proliferation of epidermal γδ T cells (dendritic epidermal T cells; see Box 1) in vitro and in the hyperproliferative, TPA-treated epidermis in vivo. This lead to an almost complete loss of these cytotoxic tumour-protective cells after several TPA applications. This loss probably resulted in an increased survival of transformed keratinocytes with a subsequent increase in tumour load (Antsiferova et al., 2011).

Besides epidermal γδ T cells, other cells are also affected by activin overexpression during DMBA-TPA-induced carcinogenesis in mice (Fig. 3). For example, the number of Langerhans cells was increased after chronic TPA treatment in the presence of high levels of activin (Antsiferova et al., 2011). This finding is in line with previous reports that demonstrate that activin A induces the differentiation of Langerhans cells from circulating and skin-resident precursor cells (Musso et al., 2008), and that transgenic

Table 1. Summary of functional roles of activin in cancer

Type of tumour	Animal model	Result	Reference
Prostate cancer	Xenograft model; activin overexpressing human prostate cancer cell line	Suppression of tumorigenicity	(Zhang et al., 1997)
Plasmacytoma	Plasmacytoma cell line; in vivo skin transplantation model, treatment with activin A	Reduced formation of plasmacytoma tumours	(Shoham et al., 2001)
Neuroblastoma	Xenograft model; activin A overexpressing human neuroblastoma cell line	Inhibition of neuroblastoma growth and angiogenesis	(Panopoulou et al., 2005; Schramm et al., 2005)
Mammary carcinoma	Xenograft model; activin-A-overexpressing human mammary carcinoma cells	Faster tumour growth	(Krneta et al., 2006)
Oesophageal squamous cell carcinoma	Xenograft model; activin-A-overexpressing human oesophageal carcinoma cell line	Enhanced tumorigenesis due to enhanced proliferation	(Yoshinaga et al., 2008)
Renal cell carcinoma	Xenograft model; knockdown of activin B in renal cell carcinoma cell line	Reduced tumour growth	(Wacker et al., 2009)
Myeloma	Engraftment of human multiple myeloma cell line into severe combined immunodeficiency (SCID) mice; treatment with a soluble activin decoy receptor	Inhibition of tumour growth	(Vallet et al., 2010)
Gastric cancer	Xenograft model; activin-A-overexpressing gastric carcinoma cell line	Decreased tumour growth and tumour angiogenesis	(Kaneda et al., 2011)
Skin cancer (epithelial)	Transgenic mice overexpressing activin A	Enhanced tumorigenesis and malignant progression	(Antsiferova et al., 2011)

Results of functional studies in mice are summarized. The overview is restricted to data obtained with activin, results obtained with inhibin, follistatin or other activin inhibitors are not considered.

mice overexpressing follistatin in keratinocytes have a strongly reduced number of Langerhans cells (Stoitzner et al., 2005). As these cells promote skin carcinogenesis that is induced by DMBA and TPA, by metabolizing DMBA into a more mutagenic compound and thereby increasing DNA damage (Modi et al., 2012), their accumulation in the epidermis of activin-overexpressing mice could further contribute to the pro-tumorigenic phenotype.

Finally, αβ T cells, in particular FOXP3-positive Tregs, which are potent suppressors of anti-tumour immunity (reviewed by Tang and Bluestone, 2008), accumulated in the epidermis of activin-overexpressing mice during chemical carcinogenesis (Antsiferova et al., 2011). Consistent with this finding, activin A has been shown previously to promote the TGF-β-induced conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ Tregs in vitro and in vivo in activin-overexpressing mice (Huber et al., 2009). The induction of this T cell subtype by activin A has also been confirmed in a mouse model of allergic airway disease (Semitekolou et al., 2009). An important role of Tregs in the pathogenesis of human skin cancer appears probable, as they have been shown to infiltrate human squamous cell carcinomas and to be at least partly responsible for the evasion of the host immune response by these tumours (Clark et al., 2008).

In summary, in a chemically induced mouse skin cancer model, activin modulated several immune cell types in a manner that favours the development and progression of tumours (as summarized in Fig. 4). Therefore, the role of activin in other cancers should also be addressed by taking into account its immuno-modulatory action.

Conclusions and perspectives

A major question for the future is the relevance of the data obtained from the transgenic activin-overexpressing mice for the pathogenesis of human skin cancer. In support of a role for activin in the pathogenesis of skin cancer in humans, we have also found that activin is overexpressed in human basal and squamous cell carcinomas of the skin (Antsiferova et al., 2011). It is currently under investigation whether this observation correlates with similar immunological alterations, as seen in the mouse tumours, and whether activin overexpression affects the clinical outcome. This is likely to be the case, as strong activin overexpression correlates with a poor prognosis in patients with head and neck carcinomas (Chang et al., 2010). Likewise, the role of activin in normal and impaired wound healing in humans will have to be determined. As activin is also upregulated in acute human wounds of healthy adult volunteers (our own unpublished data) and is overexpressed in human hypertrophic scars and keloids (see above), activin is likely to have similar roles in wound healing and scar formation in mice and humans. Therefore, a temporally limited activation of activin, for example by application of the protein or by activation of downstream signalling pathways, might help to accelerate the acute phase of wound healing. However, activin activity will need to be reduced at the later stages of wound repair, for instance by application of follistatin or more specific activin antagonists that have recently been developed (Makanji et al., 2011), to reduce the scarring response.

The multiple tumour-promotive and immunomodulatory properties of activin also make it an interesting potential target for cancer therapy. This is an especially attractive prospect, as activin antagonists have recently shown efficacy in the treatment of cancer-associated morbidity and mortality. Here, a soluble form of the activin type IIB receptor consisting of its ligand-binding domain fused to an immunoglobulin Fc domain significantly reduced cancer-induced muscle wasting and cachexia and increased the survival of mice inoculated with carcinoma cells (Benny Klimek et al., 2010; Zhou et al., 2010). Furthermore, a human ActRIIA–Fc fusion protein (ACE-011) is currently in phase IIa clinical trials in multiple myeloma patients, as it was shown to stimulate bone formation and to block the formation of osteolytic lesions in animal models of myeloma and breast cancer (Chantry et al., 2010). Therefore, we are facing the exciting possibility that inhibition of activin action could be used for the treatment of certain types of cancer, as well as of cancer-induced morbidity. Although the inhibitory effect of activin antagonists on wound healing needs to be considered, this side effect might well be outweighed in most patients by the potential benefit.

Taken together, the example of activin presented here demonstrates that orchestrators of wound repair can have an equally important role in tumorigenesis. Therefore, the identification of factors involved in the healing response is also of major relevance for cancer research and might lead to possible new means of therapeutic intervention.

Funding

The activin research in our laboratory is funded by the Swiss Cancer League [grant number KFS-2822-08-2011 to S.W.].

References

Antsiferova, M., Klatte, J. E., Bodó, E., Paus, R., Jorcano, J. L., Matzuk, M. M., Werner, S. and Kögel, H. (2009). Keratinocyte-derived follistatin regulates epidermal homeostasis and wound repair. *Lab. Invest.* **89**, 131-141.

Antsiferova, M., Huber, M., Meyer, M., Piwko-Czuchra, A., Ramadan, T., MacLeod, A. S., Havran, W. L., Dummer, R., Hohl, D. and Werner, S. (2011). Activin enhances skin tumourigenesis and malignant progression by inducing a pro-tumourigenic immune cell response. *Nat Commun* **2**, 576.

Aoki, F., Kurabayashi, M., Hasegawa, Y. and Kojima, I. (2005). Attenuation of bleomycin-induced pulmonary fibrosis by follistatin. *Am. J. Respir. Crit. Care Med.* **172**, 713-720.

Arnoux, V., Come, C., Kusewitt, D. F., Hudson, L. and Savagner, P. (2005). Cutaneous wound reepithelialization: a partial and reversible EMT. In *Rise and Fall of Epithelial Phenotype: Concepts of Epithelial-Mesenchymal Transition* (ed. P. Savagner), pp. 111-134. New York: Springer.

Bamberger, C., Schärer, A., Antsiferova, M., Tychsen, B., Pankow, S., Müller, M., Rülicke, T., Paus, R. and Werner, S. (2005). Activin controls skin morphogenesis and wound repair predominantly via stromal cells and in a concentration-dependent manner via keratinocytes. *Am. J. Pathol.* **167**, 733-747.

Benny Klimek, M. E., Aydogdu, T., Link, M. J., Pons, M., Koniaris, L. G. and Zimmers, T. A. (2010). Acute inhibition of myostatin-family proteins preserves skeletal muscle in mouse models of cancer cachexia. *Biochem. Biophys. Res. Commun.* **391**, 1548-1554.

Breit, S., Ashman, K., Wilting, J., Rössler, J., Hatzi, E., Fotsis, T. and Schweigerer, L. (2000). The N-myc oncogene in human neuroblastoma cells: down-regulation of an angiogenesis inhibitor identified as activin A. *Cancer Res.* **60**, 4596-4601.

Chang, H. Y., Sneddon, J. B., Alizadeh, A. A., Sood, R., West, R. B., Montgomery, K., Chi, J. T., van de Rijn, M., Botstein, D. and Brown, P. O. (2004). Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. *PLoS Biol.* **2**, E7.

Chang, K. P., Kao, H. K., Liang, Y., Cheng, M. H., Chang, Y. L., Liu, S. C., Lin, Y. C., Ko, T. Y., Lee, Y. S., Tsai, C. L. et al. (2010). Overexpression of activin A in oral squamous cell carcinoma: association with poor prognosis and tumor progression. *Ann. Surg. Oncol.* **17**, 1945-1956.

Chantry, A. D., Heath, D., Mulivor, A. W., Pearsall, S., Baud'huin, M., Coulton, L., Evans, H., Abdul, N., Werner, E. D., Bouxsein, M. L. et al. (2010). Inhibiting activin-A signaling stimulates bone formation and prevents cancer-induced bone destruction in vivo. *J. Bone Miner. Res.* **25**, 2633-2646.

Chen, Y. G., Wang, Q., Lin, S. L., Chang, C. D., Chuang, J. and Ying, S. Y. (2006). Activin signaling and its role in regulation of cell proliferation, apoptosis, and carcinogenesis. *Exp. Biol. Med. (Maywood)* **231**, 534-544.

Clark, R. A., Huang, S. J., Murphy, G. F., Mollet, I. G., Hijnen, D., Muthukuru, M., Schanbacher, C. F., Edwards, V., Miller, D. M., Kim, J. E. et al. (2008). Human squamous cell carcinomas evade the immune response by down-regulation of vascular E-selectin and recruitment of regulatory T cells. *J. Exp. Med.* **205**, 2221-2234.

Coussens, L. M., Raymond, W. W., Bergers, G., Laig-Webster, M., Behrendtsen, O., Werb, Z., Caughey, G. H. and Hanahan, D. (1999). Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* **13**, 1382-1397.

Cruise, B. A., Xu, P. and Hall, A. K. (2004). Wounds increase activin in skin and a vasoactive neuropeptide in sensory ganglia. *Dev. Biol.* **271**, 1-10.

de Visser, K. E., Eichten, A. and Coussens, L. M. (2006). Paradoxical roles of the immune system during cancer development. *Nat. Rev. Cancer* **6**, 24-37.

Dvorak, H. F. (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N. Engl. J. Med.* **315**, 1650-1659.

Egeblad, M., Nakasone, E. S. and Werb, Z. (2010). Tumors as organs: complex tissues that interface with the entire organism. *Dev. Cell* **18**, 884-901.

Flavell, R. A., Sanjabi, S., Wrzesinski, S. H. and Licona-Limón, P. (2010). The polarization of immune cells in the tumour environment by TGFbeta. *Nat. Rev. Immunol.* **10**, 554-567.

Fumagalli, M., Musso, T., Vermi, W., Scutera, S., Daniele, R., Alotto, D., Cambieri, I., Ostorero, A., Gentili, F., Caposio, P. et al. (2007). Imbalance between activin A and follistatin drives postburn hypertrophic scar formation in human skin. *Exp. Dermatol.* **16**, 600-610.

Funaba, M., Ikeda, T., Ogawa, K., Murakami, M. and Abe, M. (2003). Role of activin A in murine mast cells: modulation of cell growth, differentiation, and migration. *J. Leukoc. Biol.* **73**, 793-801.

Gold, E. and Risbridger, G. (2012). Activins and activin antagonists in the prostate and prostate cancer. *Mol. Cell. Endocrinol.* **359**, 107-112.

Gratchev, A., Kzhyshkowska, J., Köthe, K., Muller-Molinet, I., Kannookadan, S., Utikal, J. and Goerdt, S. (2006). Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. *Immunobiology* **211**, 473-486.

Haddow, A. (1974). Addendum to "molecular repair, wound healing, and carcinogenesis: tumor production a possible overhealing"? *Adv. Cancer Res.* **20**, 343-366.

Hanahan, D. and Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* **144**, 646-674.

Harrison, C. A., Gray, P. C., Vale, W. W. and Robertson, D. M. (2005). Antagonists of activin signaling: mechanisms and potential biological applications. *Trends Endocrinol. Metab.* **16**, 73-78.

Harrison, C. A., Al-Musawi, S. L. and Walton, K. L. (2011). Prodomains regulate the synthesis, extracellular localisation and activity of TGF-β superfamily ligands. *Growth Factors* **29**, 174-186.

Hedger, M. P., Winnall, W. R., Phillips, D. J. and de Kretser, D. M. (2011). The regulation and functions of activin and follistatin in inflammation and immunity. *Vitam. Horm.* **85**, 255-297.

Huber, S., Stahl, F. R., Schrader, J., Lüth, S., Presser, K., Carambia, A., Flavell, R. A., Werner, S., Blessing, M., Herkel, J. et al. (2009). Activin a promotes the TGF-beta-induced conversion of CD4+CD25- T cells into Foxp3+ induced regulatory T cells. *J. Immunol.* **182**, 4633-4640.

Hübner, G. and Werner, S. (1996). Serum growth factors and proinflammatory cytokines are potent inducers of activin expression in cultured fibroblasts and keratinocytes. *Exp. Cell Res.* **228**, 106-113.

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.

Hübner, G., Alzheimer, C. and Werner, S. (1999). Activin: a novel player in tissue repair processes. *Histol. Histopathol.* **14**, 295-304.

Incorvaia, L., Badalamenti, G., Rini, G., Arcara, C., Fricano, S., Sferrazza, C., Di Trapani, D., Gebbia, N. and Leto, G. (2007). MMP-2, MMP-9 and activin A blood levels in patients with breast cancer or prostate cancer metastatic to the bone. *Anticancer Res.* **27**, 1519-1525.

Jaźwińska, A., Badakov, R. and Keating, M. T. (2007). Activin-betaA signaling is required for zebrafish fin regeneration. *Curr. Biol.* **17**, 1390-1395.

Kaneda, H., Arao, T., Matsumoto, K., De Velasco, M. A., Tamura, D., Aomatsu, K., Kudo, K., Sakai, K., Nagai, T., Fujita, Y. et al. (2011). Activin A inhibits vascular endothelial cell growth and suppresses tumour angiogenesis in gastric cancer. *Br. J. Cancer* **105**, 1210-1217.

Kjartansson, J. and Dalsgaard, C. J. (1987). Calcitonin gene-related peptide increases survival of a musculocutaneous critical flap in the rat. *Eur. J. Pharmacol.* **142**, 355-358.

Krneta, J., Kroll, J., Alves, F., Prahst, C., Sananbenesi, F., Dullin, C., Kimmina, S., Phillips, D. J. and Augustin, H. G. (2006). Dissociation of angiogenesis and tumorigenesis in follistatin- and activin-expressing tumors. *Cancer Res.* **66**, 5686-5695.

Leto, G., Incorvaia, L., Badalamenti, G., Tumminello, F. M., Gebbia, N., Flandina, C., Crescimanno, M. and Rini, G. (2006). Activin A circulating levels in patients with bone metastasis from breast or prostate cancer. *Clin. Exp. Metastasis* **23**, 117-122.

Loke, P., Gallagher, I., Nair, M. G., Zang, X., Brombacher, F., Mohrs, M., Allison, J. P. and Allen, J. E. (2007). Alternative activation is an innate response to injury that requires CD4+ T cells to be sustained during chronic infection. *J. Immunol.* **179**, 3926-3936.

Makanji, Y., Walton, K. L., Chan, K. L., Gregorevic, P., Robertson, D. M. and Harrison, C. A. (2011). Generation of a specific activin antagonist by modification of the activin A propeptide. *Endocrinology* **152**, 3758-3768.

Mantovani, A., Sozzani, S., Locati, M., Allavena, P. and Sica, A. (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* **23**, 549-555.

McCarthy, S. A. and Bicknell, R. (1993). Inhibition of vascular endothelial cell growth by activin-A. *J. Biol. Chem.* **268**, 23066-23071.

Modi, B. G., Neustadter, J., Binda, E., Lewis, J., Filler, R. B., Roberts, S. J., Kwong, B. Y., Reddy, S., Overton, J. D., Galan, A. et al. (2012). Langerhans cells facilitate epithelial DNA damage and squamous cell carcinoma. *Science* **335**, 104-108.

Mukhopadhyay, A., Chan, S. Y., Lim, I. J., Phillips, D. J. and Phan, T. T. (2007). The role of the activin system in keloid pathogenesis. *Am. J. Physiol. Cell Physiol.* **292**, C1331-C1338.

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.

Musso, T., Scutera, S., Vermi, W., Daniele, R., Fornaro, M., Castagnoli, C., Alotto, D., Ravanini, M., Cambieri, I., Salogni, L. et al. (2008). Activin A induces Langerhans cell differentiation in vitro and in human skin explants. *PLoS ONE* **3**, e3271.

Nüsing, R. M. and Barsig, J. (1999). Induction of prostanoid, nitric oxide, and cytokine formation in rat bone marrow derived macrophages by activin A. *Br. J. Pharmacol.* **127**, 919-926.

Ogawa, K. and Funaba, M. (2011). Activin in humoral immune responses. *Vitam. Horm.* **85**, 235-253.

Ogawa, K., Funaba, M., Chen, Y. and Tsujimoto, M. (2006). Activin A functions as a Th2 cytokine in the promotion of the alternative activation of macrophages. *J. Immunol.* **177**, 6787-6794.

Ogino, H., Yano, S., Kakiuchi, S., Muguruma, H., Ikuta, K., Hanibuchi, M., Uehara, H., Tsuchida, K., Sugino, H. and Sone, S. (2008). Follistatin suppresses the production of experimental multiple-organ metastasis by small cell lung cancer cells in natural killer cell-depleted SCID mice. *Clin. Cancer Res.* **14**, 660-667.

Panopoulou, E., Murphy, C., Rasmussen, H., Bagli, E., Rofstad, E. K. and Fotsis, T. (2005). Activin A suppresses neuroblastoma xenograft tumor growth via antimitotic and antiangiogenic mechanisms. *Cancer Res.* **65**, 1877-1886.

Patella, S., Phillips, D. J., Tchongue, J., de Kretser, D. M. and Sievert, W. (2006). Follistatin attenuates early liver fibrosis: effects on hepatic stellate cell activation and hepatocyte apoptosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **290**, G137-G144.

Petraglia, F., Sacerdote, P., Cossarizza, A., Angioni, S., Genazzani, A. D., Franceschi, C., Muscettola, M. and Grasso, G. (1991). Inhibin and activin modulate human monocyte chemotaxis and human lymphocyte interferon-gamma production. *J. Clin. Endocrinol. Metab.* **72**, 496-502.

Poulaki, V., Mitsiades, N., Kruse, F. E., Radetzky, S., Iliaki, E., Kirchhof, B. and Joussen, A. M. (2004). Activin a in the regulation of corneal neovascularization and vascular endothelial growth factor expression. *Am. J. Pathol.* **164**, 1293-1302.

Risbridger, G. P., Schmitt, J. F. and Robertson, D. M. (2001). Activins and inhibins in endocrine and other tumors. *Endocr. Rev.* **22**, 836-858.

Robson, N. C., Phillips, D. J., McAlpine, T., Shin, A., Svobodova, S., Toy, T., Pillay, V., Kirkpatrick, N., Zanker, D., Wilson, K. et al. (2008). Activin-A: a novel dendritic cell-derived cytokine that potently attenuates CD40 ligand-specific cytokine and chemokine production. *Blood* **111**, 2733-2743.

Robson, N. C., Wei, H., McAlpine, T., Kirkpatrick, N., Cebon, J. and Maraskovsky, E. (2009). Activin-A attenuates several human natural killer cell functions. *Blood* **113**, 3218-3225.

Salogni, L., Musso, T., Bosisio, D., Mirolo, M., Jala, V. R., Haribabu, B., Locati, M. and Sozzani, S. (2009). Activin A induces dendritic cell migration through the polarized release of CXC chemokine ligands 12 and 14. *Blood* **113**, 5848-5856.

Schäfer, M. and Werner, S. (2008). Cancer as an overhealing wound: an old hypothesis revisited. *Nat. Rev. Mol. Cell Biol.* **9**, 628-638.

Schramm, A., von Schuetz, V., Christiansen, H., Havers, W., Papoutsi, M., Wilting, J. and Schweigerer, L. (2005). High activin A-expression in human neuroblastoma: suppression of malignant potential and correlation with favourable clinical outcome. *Oncogene* **24**, 680-687.

Scutera, S., Riboldi, E., Daniele, R., Elia, A. R., Fraone, T., Castagnoli, C., Giovarelli, M., Musso, T. and Sozzani, S. (2008). Production and function of activin A in human dendritic cells. *Eur. Cytokine Netw.* **19**, 60-68.

Seishima, M., Nojiri, M., Esaki, C., Yoneda, K., Eto, Y. and Kitajima, Y. (1999). Activin A induces terminal differentiation of cultured human keratinocytes. *J. Invest. Dermatol.* **112**, 432-436.

Semitekolou, M., Alissafi, T., Aggelakopoulou, M., Kourepini, E., Kariyawasam, H. H., Kay, A. B., Robinson, D. S., Lloyd, C. M., Panoutsakopoulou, V. and Xanthou, G. (2009). Activin-A induces regulatory T cells that suppress T helper cell immune responses and protect from allergic airway disease. *J. Exp. Med.* **206**, 1769-1785.

Shoham, T., Sternberg, D., Brosh, N., Krupsky, M., Barda-Saad, M. and Zipori, D. (2001). The promotion of plasmacytoma tumor growth by mesenchymal stroma is antagonized by basic fibroblast growth factor induced activin A. *Leukemia* **15**, 1102-1110.

Sierra-Filardi, E., Puig-Kröger, A., Blanco, F. J., Nieto, C., Bragado, R., Palomero, M. I., Bernabéu, C., Vega, M. A. and Corbí, A. L. (2011). Activin A skews macrophage polarization by promoting a proinflammatory phenotype and inhibiting the acquisition of anti-inflammatory macrophage markers. *Blood* **117**, 5092-5101.

Soucek, L., Lawlor, E. R., Soto, D., Shchors, K., Swigart, L. B. and Evan, G. I. (2007). Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat. Med.* **13**, 1211-1218.

Stoitzner, P., Stössel, H., Wankell, M., Hofer, S., Heufler, C., Werner, S. and Romani, N. (2005). Langerhans cells are strongly reduced in the skin of transgenic mice overexpressing follistatin in the epidermis. *Eur. J. Cell Biol.* **84**, 733-741.

Tan, T. T. and Coussens, L. M. (2007). Humoral immunity, inflammation and cancer. *Curr. Opin. Immunol.* **19**, 209-216.

Tang, Q. and Bluestone, J. A. (2008). The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nat. Immunol.* **9**, 239-244.

ten Dijke, P. and Hill, C. S. (2004). New insights into TGF-beta-Smad signalling. *Trends Biochem. Sci.* **29**, 265-273.

Tiedemann, H., Lottspeich, F., Davids, M., Knöchel, S., Hoppe, P. and Tiedemann, H. (1992). The vegetalizing factor. A member of the evolutionarily highly conserved activin family. *FEBS Lett.* **300**, 123-126.

Tsuchida, K., Nakatani, M., Uezumi, A., Murakami, T. and Cui, X. (2008). Signal transduction pathway through activin receptors as a therapeutic target of musculoskeletal diseases and cancer. *Endocr. J.* **55**, 11-21.

Vallet, S., Mukherjee, S., Vaghela, N., Hideshima, T., Fulciniti, M., Pozzi, S., Santo, L., Cirstea, D., Patel, K., Sohani, A. R. et al. (2010). Activin A promotes multiple myeloma-induced osteolysis and is a promising target for myeloma bone disease. *Proc. Natl. Acad. Sci. USA* **107**, 5124-5129.

Virchow, R. (1863). *Aetiologie der neoplastischen Geschwulste/Pathogenie der neoplastischen Geschwulste*. Berlin: Verlag von August Hirschwald.

Wacker, I., Sachs, M., Knaup, K., Wiesener, M., Weiske, J., Huber, O., Akçetin, Z. and Behrens, J. (2009). Key role for activin B in cellular transformation after loss of the von Hippel-Lindau tumor suppressor. *Mol. Cell. Biol.* **29**, 1707-1718.

Wada, W., Kuwano, H., Hasegawa, Y. and Kojima, I. (2004). The dependence of transforming growth factor-beta-induced collagen production on autocrine factor activin A in hepatic stellate cells. *Endocrinology* **145**, 2753-2759.

Wankell, M., Munz, B., Hübner, G., Hans, W., Wolf, E., Goppelt, A. and Werner, S. (2001). Impaired wound healing in transgenic mice overexpressing the activin antagonist follistatin in the epidermis. *EMBO J.* **20**, 5361-5372.

Werner, S. and Grose, R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiol. Rev.* **83**, 835-870.

Werner, S. and Alzheimer, C. (2006). Roles of activin in tissue repair, fibrosis, and inflammatory disease. *Cytokine Growth Factor Rev.* **17**, 157-171.

Yamashita, N., Nakajima, T., Takahashi, H., Kaneoka, H., Mizushima, Y. and Sakane, T. (1993). Effects of activin A on IgE synthesis and cytokine production by human peripheral mononuclear cells. *Clin. Exp. Immunol.* **94**, 214-219.

Yoshinaga, K., Yamashita, K., Mimori, K., Tanaka, F., Inoue, H. and Mori, M. (2008). Activin a causes cancer cell aggressiveness in esophageal squamous cell carcinoma cells. *Ann. Surg. Oncol.* **15**, 96-103.

Zhang, M., Liu, N. Y., Wang, X. E., Chen, Y. H., Li, Q. L., Lu, K. R., Sun, L., Jia, Q., Zhang, L. and Zhang, L. (2011). Activin B promotes epithelial wound healing in vivo through RhoA-JNK signaling pathway. *PLoS ONE* **6**, e25143.

Zhang, Z., Zheng, J., Zhao, Y., Li, G., Batres, Y., Luo, M., Wan, M. and Ying, S. (1997). Overexpression of activin A inhibits growth, induces apoptosis, and suppresses tumorigenicity in an androgen-sensitive human prostate cancer cell line LNCaP. *Int. J. Oncol.* **11**, 727-736.

Zhou, X., Wang, J. L., Lu, J., Song, Y., Kwak, K. S., Jiao, Q., Rosenfeld, R., Chen, Q., Boone, T., Simonet, W. S. et al. (2010). Reversal of cancer cachexia and muscle wasting by ActRIIB antagonism leads to prolonged survival. *Cell* **142**, 531-543.