MOLECULAR AND CELLULAR MECHANISMS OF TISSUE REPAIR

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In our daily life we are frequently exposed to mechanical, chemical and physical insults, which may damage the skin as well as various internal tissues and organs. Rapid repair of these injuries is crucial for the restoration of organ function and for the protection of the body from invading microorganisms. The different events involved in repair must be tightly regulated and synchronized to re-establish the integrity of the affected tissue and the homeostasis in the whole organism. Defects in tissue repair constitute a severe health problem and are frequently seen in aged individuals, in patients with diabetes or immunosuppression, and in those receiving chemo- or radiotherapy (reviewed by Gurtner *et al.*, 2008). Many of these patients suffer from chronic non-healing ulcers, which strongly affect their quality of life and cause a significant burden to the health care system. On the other hand, wound healing can also be excessive, resulting in the formation of hypertrophic scars and keloids. These common fibrotic skin lesions constitute not only a cosmetic problem, but also cause functional impairment due to the lack of appendages (hairs, sebaceous and sweat glands) in scar and keloid tissue.

If a tissue is chronically injured, normal and efficient repair can no longer occur and functional tissue is gradually replaced by non-functional connective tissue. The resulting fibrosis causes severe organ dysfunction and even organ failure as seen for example in fibrosis of the lung, liver and kidney, but also in atherosclerosis. A particularly severe consequence of chronic tissue injury is the development of cancer at the site of damage, which mainly results from the chronic inflammation. This is highlighted by the increased cancer risk of patients suffering from chronic viral hepatitis, *Helicobacter Pylori*-induced gastric inflammation, inflammatory bowel disease or from the skin blistering disease *Recessive Dystrophic Epidermolysis Bullosa*. These findings

suggested that similar molecular and cellular mechanisms may underlie tissue repair and cancer, and recent studies have provided strong evidence for this hypothesis (reviewed by Schäfer and Werner, 2008). In this review I will summarize current knowledge on tissue repair, using wound repair of the skin as an example. In addition, I will report on studies from our laboratory addressing the roles and mechanisms of action of fibroblast growth factors in the wound repair process.

The different phases of wound repair

Injury of the skin in adult mammals initiates a series of events, which aim to reconstitute the wounded tissue. These events are divided into three partially overlapping phases – the inflammatory phase, the phase of new tissue formation and the phase of tissue remodeling.

Figure 1:

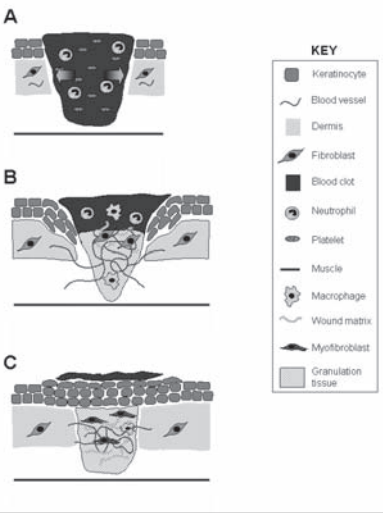
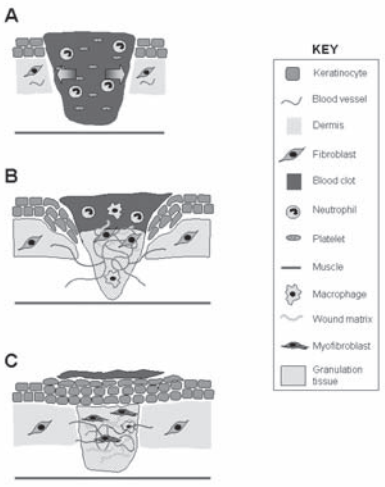


Figure 1: Schematic representation of different stages of wound repair. *A: 12–24 hours after injury the wounded area is filled with a blood clot. Neutrophils have invaded into the clot. B: At days 3–7 after injury, the majority of neutrophils have undergone apoptosis. Instead, macrophages are abundant in the wound tissue at this stage of repair. Endothelial cells migrate into the clot; they proliferate and form new blood vessels. Fibroblasts migrate into the wound tissue, where they proliferate and deposit extracellular matrix. The new tissue is called granulation tissue. Keratinocytes proliferate at the wound edge and migrate down the injured dermis and above the provisional matrix. C: 1–2 weeks after injury the wound is completely filled with granulation tissue. Fibroblasts have transformed into myofibroblasts, leading to wound contraction and collagen deposition. The wound is completely covered with a neoepidermis. Reprinted from Werner and Grose (2003), Physiol. Rev.* ***83****: 835–870, Fig. 1, with permission from the American Physiological Society.*

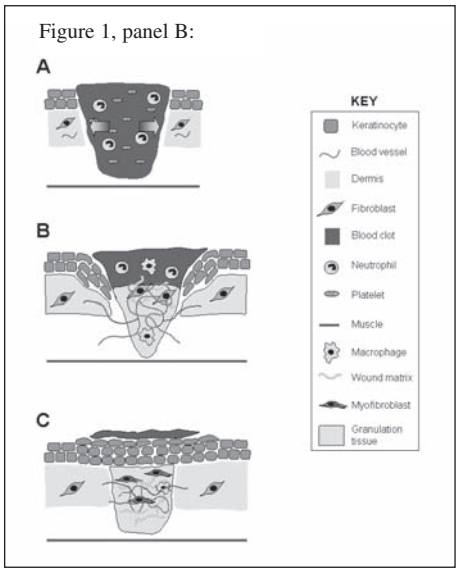
They are initiated upon injury of blood vessels, resulting in the release of growth factors, cytokines, hormones and low molecular weight mediators from the serum and from degranulating platelets. In addition, vessel injury causes formation of a blood clot, which provides a provisional sealing and protects the body from severe water loss but also from invading bacteria and other microorganisms. In addition, the clot serves as a matrix for cells that invade the wound from the adjacent normal tissue or from the circulation.

Fig.1, panel A:



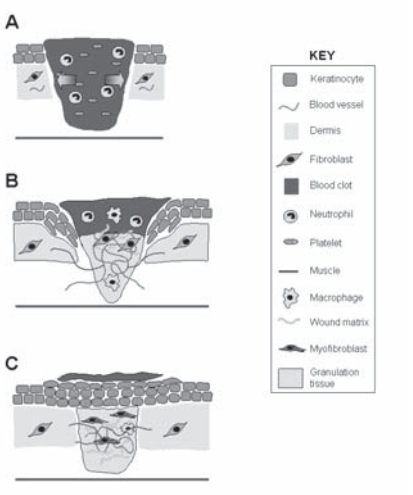
Within minutes of injury, various inflammatory cells invade the wound. Neutrophils arrive first due to their abundance in the circulation, followed by monocytes and lymphocytes. These cells secrete a broad spectrum of cytokines and growth factors, which attract cells from the wound edge and from the circulation. Cytokines also stimulate proliferation of cells at the wound site and their survival in this rather harsh environment. In addition to cytokines, inflammatory cells secrete proteinases and reactive oxygen species as a defense against contaminating bacteria, and they are responsible for the phagocytosis of cell debris. Although these activities are generally beneficial, enhanced numbers or prolonged presence of inflammatory cells at the wound site is deleterious due to the continuous production of toxic reactive oxygen species (reviewed by Leibovich and Martin, 2005). Therefore, prolonged inflammation – as seen for example upon bacterial infection – frequently results in severe tissue damage, which delays the healing process and eventually causes

excessive scarring or even malignant transformation of cells at the wound site (reviewed by Schäfer and Werner, 2008).



The second phase of wound healing, the formation of new tissue, is initiated by the migration of keratinocytes of the injured epidermis and hair follicles, followed by proliferation of these cells at the wound edge. This process, which is called reepithelialization, is responsible for efficient coverage of the wound with a new epidermis. Upon completion of the reepithelialization process keratinocytes re-differentiate to restore the barrier function of the skin. The latter is important for the prevention of water loss and for the protection of the skin from chemical and physical insults as well as from invading microorganisms. In parallel to the reepithelialization process, repair of the injured dermis, the connective tissue layer of the skin, occurs. This is achieved by migration and proliferation of fibroblasts, which produce large amounts of extracellular matrix proteins. A large percentage of the fibroblasts at the wound site differentiate into myofibroblasts, which are crucial for wound contraction. Massive sprouting of blood vessels at the wound edge leads to the formation of a new vascular network, which is essential for the support of the new tissue with oxygen and nutrients. In parallel, the lymphatic vasculature is also restored and nerve sprouting occurs at the wound edge. The resulting wound tissue that replaces the lost dermis is called granulation tissue because of the granular appearance of the large number of capillaries.

Figure 1, panel C:



The third phase of wound repair is the tissue remodelling phase. At this stage, the epidermis returns to its normal thickness through re-differentiation of keratinocytes. Many cells in the granulation tissue undergo apoptosis, including most of the inflammatory cells, a large number of endothelial cells and also myofibroblasts. However, persistence of the latter is frequently observed, resulting in the formation of hypertrophic scars and keloids. Extensive remodeling of the extracellular matrix occurs, and the provisional matrix of the granulation tissue is gradually replaced by a new collagen-rich dermal matrix. However, the resulting larger collagen fibrils are abnormally arranged in parallel bundles. These processes result in formation of a scar with a dense connective tissue, which is characterized by reduced tensile strength and elasticity compared to normal skin. In addition, the scar tissue lacks all skin appendages – hair follicles, sebaceous glands and sweat glands.

Surprisingly, wounds in mammalian embryos until the beginning of the third trimester do not form a scar, and it is a major challenge for current research to investigate the differences between wound healing in embryos and adults (reviewed by Martin 1997). The knowledge about these processes may pave the way for the development of strategies to inhibit scarring in adults and to allow full tissue regeneration.

Molecular mechanisms of wound repair

Recent studies have identified a variety of genes and their products, which orchestrate the healing process. In particular, microarray analy-

ses of murine and human wounds have revealed a characteristic profile of gene expression at the wound site (Thorey *et al.* 2001; Cole *et al.* 2001; Cooper *et al.* 2001), which resembles the gene expression pattern of serum-treated fibroblasts (Iyer *et al.*, 1999). Most interestingly, the gene-expression pattern of serum-treated fibroblasts as well as of skin wounds is highly similar to that of malignant carcinomas. Remarkably, the tumors with a gene expression pattern similar to the one observed in wounds were characterized by a higher malignancy with an increased rate of metastasis and a higher mortality rate of the affected patients. Therefore, the presence of a «wound signature» allows a prediction of poor prognosis in several types of human carcinomas (Chang *et al*., 2004).

Due to these interesting results, it is of major importance to functionally characterize the genes that are expressed in healing wounds and in cancers. In our laboratory, a large number of wound-regulated genes were identified, which encode growth factors, cytokines, matrix proteins, enzymes, transcription factors and others. The major challenge is the functional analysis of these genes with particular emphasis on their roles in wound repair. Of particular importance for the analysis of *in vivo* gene function are genetically modified mouse models that allow the overexpression or knockout of genes in different tissues and organs. The use of such models for wound healing studies, which was pioneered in our laboratory, has provided important insight into the function of many wound-regulated genes in the healing process (reviewed by Grose and Werner, 2004). Here I will report on the role of fibroblast growth factors (FGFs) in skin morphogenesis, repair and disease, since this growth factor family has been studied in detail in our laboratory during the past fifteen years.

Fibroblast growth factors: Expression and function in normal and wounded skin

FGFs comprise a family of 22 polypeptides, which regulate migration, proliferation, differentiation and survival of a wide variety of cell types. They exert their functions through binding to four different transmembrane tyrosine kinase receptors, designated FGF receptors (FGFR) 1–4. Further complexity of the system is achieved by alternative splicing in

the extracellular domains of FGFR1, FGFR2 and FGFR3. Of particular importance is alternative splicing in the third immunoglobulin-like domain, which generates IIIb and IIIc variants of each receptor that are characterized by different ligand binding specificities (reviewed by Ornitz and Itoh, 2001). For example, the IIIb splice variant of FGFR2 (FGFR2-IIIb) is a high affinity receptor for FGF7, FGF10, and FGF22, whereas the IIIc variant (FGFR2-IIIc) binds another set of FGF ligands (Zhang *et al.*, 2006).

When we first addressed the function of FGFs in wound repair, we studied their expression in mouse excisional skin wounds at different stages after injury. These studies revealed that several FGFs are upregulated in response to wounding, in particular FGF7. This growth factor is mainly expressed by fibroblasts in the dermis at the wound edge and in the granulation tissue (Werner *et al.*, 1992) as well as by intraepithelial γδ T cells (Jameson *et al.*, 2002), whereas its only high-affinity receptor (FGFR2-IIIb) is expressed on keratinocytes of the epidermis and hair follicles (Werner *et al.*, 1992).

Figure 2:

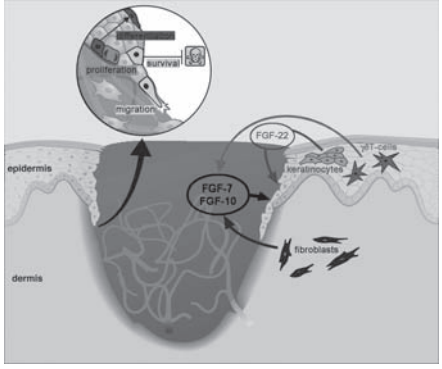


Figure 2: Cartoon to illustrate the functions of FGFs in a healing skin wound. *Upon injury, dermal fibroblasts and γδ T cells secrete FGF7 and FGF10; suprabasal keratinocytes express FGF22. In a paracrine or autocrine manner, respectively, they activate keratinocytes at the wound edge and stimulate reepithelialization. On the cellular level, these FGFs exert multiple effects on keratinocytes as shown enlarged in the upper left circle. They induce keratinocyte proliferation, migration and differentiation, and protect keratinocytes against ROS-induced apoptosis. Reprinted from Steiling and Werner (2003), Curr. Opin. Biotechnol.* ***14****: 533–537, Fig. 1, with permission from Elsevier Publishers.*

Interestingly, the upregulation of FGF7 was much less pronounced in wounds of genetically diabetic mice and of glucocorticoid-treated mice, which are both characterized by a severe delay in wound repair (Werner *et al.*, 1994a; Brauchle *et al.*, 1995). This finding suggested that upregulation of FGF7 expression after injury is important for normal repair. To determine the function of FGF7 and its receptor for the wound healing process, we generated transgenic mice expressing a dominant-negative FGFR2-IIIb mutant in keratinocytes. This mutant inhibits the activation of the endogenous receptors by its ligands (e.g. FGF7) when expressed in excess compared to the endogenous receptors. These mice were then subjected to full-thickness excisional wounding. Interestingly, wound reepithelialization was strongly delayed in these animals, demonstrating that activation of FGFR2-IIIb is crucial for this process (Werner *et al.*, 1994b). Surprisingly, however, wound healing abnormalities were not observed in FGF7 knockout mice, suggesting functional redundancy among different FGFs and possibly FGF receptors (Guo *et al.*, 1996). Indeed, expression studies revealed that other FGFR2-IIIb ligands, FGF10 and FGF22, are also expressed in normal and wounded skin (Beer *et al.*, 1997; Nakatake *et al.*, 2001; Beyer *et al.*, 2003), and together with FGF7 they are likely to orchestrate the reepithelialization process via their receptors that are present on keratinocytes. Therefore, there is obviously functional redundancy among different FGF family members at the wound site. The reepithelialization defect in mice lacking FGF7- and FGF10-producing γδ T cells as well as recent studies from our laboratory using keratinocyte-specific FGF receptor knockout mice support this hypothesis (Jameson et al., 2002 and our own unpublished data) (summarized in Fig. 2). Interestingly, the important role of these FGFs and their receptors is not restricted to the wound repair process of the skin, but is also relevant for repair of other epithelial tissues and organs (reviewed by Steiling and Werner, 2003b). One important example is the liver as demonstrated by the impaired regeneration of this organ in mice expressing a dominant-negative FGFR2-IIIb mutant in hepatocytes (Steiling *et al.*, 2003).

Mechanisms of FGF action in epithelial repair processes

Due to the important functions of FGFR2-IIIb ligands in epithelial repair processes, we identified and functionally characterized genes,

which are regulated by FGF7 in keratinocytes. The results revealed that this growth factor regulates various genes that encode proteins involved in migration and proliferation of keratinocytes (reviewed by Braun *et al.*, 2004). Interestingly, some of the FGF target genes encode proteins that regulate the cellular redox homeostasis. These include the enzyme peroxiredoxin 6, which detoxifies hydrogen peroxide and organic peroxides (Frank *et al.*, 1997), as well as the NF-E2-related factor 2 (Nrf2) transcription factor, which induces the expression of a battery of genes involved in the cellular stress response (Braun et *al.*, 2002). The identification of these genes as targets of FGF7 is of particular interest, since FGF7 is clinically used in cancer patients for the protection from chemo- and radiotherapy-induced oral mucositis, a condition associated with painful inflammation and ulceration of the mucous membranes lining the digestive tract (reviewed by Finch and Rubin, 2004). Thus, FGF7 promotes survival of epithelial cells under conditions that are associated with enhanced oxidative stress. In our laboratory, a protective effect of FGF7 was also demonstrated for keratinocytes of the skin. We found that FGF7 protected keratinocytes of the epidermis and hair follicles from the toxicity of chemotherapeutic agents or UV irradiation *in vitro* and *in vivo* (Braun *et al.*, 2006). These results encourage the use of FGF7 for skin protection from chemical and physical insults. Our recent results strongly suggest that the identified target genes of FGF7 are at least in part responsible for this protective effect. Thus, peroxiredoxin 6 was shown to be an important regulator of wound repair, and it also protects keratinocytes from the toxicity of UVA and UVB irradiation (Kümin *et al.*, 2006, 2007). Similar findings were obtained for the Nrf2 transcription factor (Braun *et al.*, 2002; Kawachi *et al.*, 2008). Most interestingly, Nrf2 also protected the skin from chemically-induced skin carcinogenesis through induction of enzymes that detoxify the carcinogen as well as reactive oxygen species that are produced in the inflamed skin (auf dem Keller *et al.*, 2006). These results not only highlight the parallels between wound healing and cancer at the molecular level but also demonstrate that the identification of genes that are important for wound repair may also be relevant for cancer research. These genes and their products are likely to be important targets for the improvement of wound healing defects as well as for cancer prevention and/or therapy.

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REFERENCES

Auf dem Keller U., Huber M., Beyer T. A., Kümin A, Siemes C., Braun S., Bugnon P., Mitropoulos V., Johnson D. A., Johnson J. A., Hohl D., Werner S. 2006. Nrf transcription factors in keratinocytes are essential for skin tumor prevention but not for wound healing. *Mol Cell Biol* **26**: 3773–3784.

Beer H. D., Florence C., Dammeier J. McGuire L., Werner S., Duan D. R. 1997. Mouse fibroblast growth factor 10: cDNA cloning, protein characterization and regulation of mRNA expression. *Oncogene* **15**: 2211–2218.

Beyer T. A., Werner S., Dickson C., Grose R. 2003. Fibroblast growth factor 22 and its potential role during skin development and repair. *Exp Cell Res* **287**: 228–236.

Brauchle M., Fässler R., Werner S. 1995. Suppression of keratinocyte growth factor expression by glucocorticoids in vitro and during wound healing. *J Invest Dermatol* **105**: 579–584.

Braun S., Hanselmann C. Gassmann M. G., auf dem Keller U., Born-Berclaz C., Chan K., Kan Y. W., Werner S. 2002. Nrf2 transcription factor, a novel target of keratinocyte growth factor action which regulates gene expression and inflammation in healing skin wounds. *Mol Cell Biol* **22**: 5492–5505.

Braun S., auf dem Keller U., Steiling H., Werner S. 2004. Fibroblast growth factors in epithelial repair and cytoprotection. *Philos Trans R Soc London B Biol Sci* **359**: 753–757.

Braun M., Krampert M., Bodo E., Kümin A., Born-Berclaz C., Paus R., Werner S. 2006. Keratinocyte growth factor protects epidermis and hair follicles from cell death induced by UV irradiation, chemotherapeutic or cytotoxic agents. *J Cell Sci* **119**: 4841–4849.

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., Brown P. O. 2004. Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumours and wounds. *PLoS Biol* **2**: E7.

Cole J., Tsou R., Wallace K. Gibran N., Isik F. 2001. Early gene expression profile of human skin to injury using high-density cDNA microarrays. *Wound Repair Regen* **9**: 360–370.

Cooper L., Johnson C., Burslem F., Martin P. 2001. Wound healing and inflammation genes revealed by array analysis of macrophageless PU.1 null mice. *Genome Biol* **6**: R5.

Finch P.W., Rubin J.S. 2004. Keratinocyte growth factor/fibroblast growth factor 7, a homeostatic factor with therapeutic potential for epithelial protection and repair. *Adv Cancer Res* **91**: 69–136.

Frank S., Munz B., Werner S. 1997. The human homologue of a bovine non-selenium glutathione peroxidase is a novel keratinocyte growth factor-regulated gene. *J Biol Chem* **271**: 24337–24340.

Guo L., Degenstein L., Fuchs E. 1996. Keratinocyte growth factor is required for hair development but not for wound healing. *Genes Dev* **10**: 165–175.

Grose R., Werner S. 2004. Wound healing studies in transgenic and knockout mice. *Mol Biotechnol* **28**: 147–166.

Gurtner G.C., Werner S., Barrandon Y., Longaker M.T. 2008. Wound repair and regeneration. *Nature 453*: 314–321.

Iyer V.R., Eisen M.B., Ross D.T., Schuler G., Moore T., Lee J.C., Trent J.M., Staudt L.M., Hudson J. Jr, Boguski M.S., Lashkari D., Shalon D., Botstein D., Brown P.O. 1999. The transcriptional program in the response of human fibroblasts to serum. *Science* **283**: 83–87.

Jameson J., Ugarte K., Chen N., Yachi P., Fuchs E., Boismenu R., Havran W.L. 2002. A role for skin dammadelta T cells in wound repair. *Science* **296**: 747–749.

Kawachi Y., Xu X., Taguchi S., Sakurai H., Nakamura Y., Ishii Y., Fujisawa Y., Furuta J., Takahashi T., Itoh K., Yamamoto M., Yamazaki F., Otsuka F. 2008. Attenuation of UVB-induced sunburn reaction and oxidative DNA damage with no alterations in UVB-induced skin carcinogenesis. *J Invest Dermatol* **128**: 1773–1779.

Kümin A., Huber C., Rülicke T., Wolf E., Werner S. 2006. Peroxiredoxin 6 is a potent cytoprotective enzyme in the epidermis. *Am J Pathol* **169**: 1194–1205.

Kümin A., Schäfer M., Epp N., Bugnon P., Born-Berclaz C., Oxenius A., Klippel A., Bloch W., Werner S. 2007. Peroxiredoxin 6 is required for blood vessel integrity in wounded skin. *J Cell Biol* **179**: 747–760.

Leibovich S.J., Martin P. 2005. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol* **15**: 599–607.

Martin P. 1997. Wound healing – aiming for perfect skin regeneration. *Science* **276**: 75–81.

Nakatake Y. Hoshikawa M., Asaki T., Kassy Y., Itoh N. 2001. Identification of a novel fibroblast growth factor, FGF-22, preferentially expressed in the inner root sheath of the hair follicle. *Biochim Biophys Acta* **1517**: 228–236.

Ornitz D., Itoh N. 2001. Fibroblast growth factors. *Genome Biol* **2**: REVIEWS3005.

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

Steiling H., Werner S. 2003. Fibroblast growth factors: Key players in epithelial morphogenesis, repair and cytoprotection. *Curr Opin Biotechnol* **14**: 533–537.

Steiling H., Wüstefeld T., Bugnon P., Brauchle M., Fässler R., Teupser D., Thiery J., Gordon J.I., Trautwein C., Werner S. 2003. Fibroblast growth factor receptor signalling is crucial for liver homeostasis and regeneration. *Oncogene* **22**: 4380–4388.

Thorey I.S., Roth J., Regenbogen J., Halle J.P., Bittner M., Vogl T., Kaesler S., Bugnon P., Reitmaier B., Durka S., Graf A., Wöckner M., Rieger N., Konstantinov A., Wolf E., Goppelt A., Werner S. 2001. The Ca2+-binding proteins S100A8 and S100A are encoded by novel injury-regulated genes. *J Biol Chem* **276**: 35818–35825.

Werner S., Peters K.G., Longaker M.T., Fuller-Pace F., Banda M.J., Williams L.T. 1992. Large induction of keratinocyte growth factor expression in the dermis during wound healing. *Proc Natl Acad Sci USA* **89**: 6896–6900.

Werner S., Breeden M., Hübner G. Greenhalgh D.G., Longaker M.T. 1994a. Induction of keratinocyte growth factor expression is reduced and delayed during wound healing in the genetically diabetic mouse. *J Invest Dermatol* **103**: 469–473.

Werner S., Smola H.,Liao X., Longaker M.T., Krieg T., Hofschneider P.H., Williams LT. 1994b. The function of KGF in morphogenesis of epithelium and reepithelialization of wounds. *Science* **266**: 819.822.

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

Zhang X., Ibrahimi O.A., Olsen S.K., Umemori H., Mohammadi M., Ornitz D.M. 2006. Receptor specificity of the fibroblast growth factor family. The complete mammalian FGF family. *J Biol Chem* **281**: 15694–15700.