Regulation of the expression of stromelysin-2 by growth factors in keratinocytes: implications for normal and impaired wound healing

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Keratinocyte growth factor (KGF) has been implicated in wound re-epithelialization and branching morphogenesis of several organs. To determine whether KGF induces these effects via induction of matrix metalloproteinase expression we have analysed the effect of KGF on the expression of stromelysin-2 in cultured HaCaT keratinocytes. Here we show a strong induction of stromelysin-2 mRNA within 5–8 h of stimulation of these cells with KGF. The degree of induction was similar to that achieved by treatment with epidermal growth factor or tumour necrosis factor α , whereas the stimulatory effect of transforming growth factor β 1 was even stronger. To determine whether the induction of stromelysin-2 expression by growth factors and cytokines might be important for wound healing, we analysed the expres-

sion of this gene during the healing process of full-thickness excisional wounds in mice. Whereas stromelysin-2 mRNA could hardly be detected in unwounded skin, a biphasic induction was seen after injury and highest levels were found at days 1 and 5 after wounding. Hybridization *in situ* revealed the presence of stromelysin-2 mRNA in basal keratinocytes at the wound edge but not in the underlying mesenchymal tissue. During impaired wound healing as seen in glucocorticoid-treated mice, stromelysin-2 expression was significantly increased compared with untreated control mice. Taken together, these results suggest that correct regulation of this broad-spectrum metalloproteinase might be important for normal repair.

INTRODUCTION

Keratinocyte growth factor (KGF) is a member of the fibroblast growth factor family of mitogens and is characterized by its specificity for different types of epithelial cells [1]. KGF is a paracrine mediator of proliferation for a wide variety of epithelial cells, including keratinocytes [1], gastrointestinal epithelial cells [2], mammary epithelial cells [3], hepatocytes [2] and type II pneumocytes [4].

Recent studies *in vivo* have provided new insight into the function of KGF in these tissues. For example, inhibition of KGF receptor signalling in the epidermis of transgenic mice by targeted expression of a dominant-negative KGF receptor in keratinocytes resulted in epidermal atrophy and hair follicle abnormalities and caused a marked delay in wound re-epithelialization [5]. A similar approach revealed a role for KGF receptor signalling in lung branching morphogenesis and alveolar differentiation [6]. These findings relevant to lung development suggest that KGF is not only involved in proliferative processes but also in organ formation. This additional role for KGF was supported by organ culture studies *in vitro*, where addition of a neutralizing antibody against the mitogen inhibited branching morphogenesis of the seminal vesicles and the prostate [7,8]. The potent activity of KGF in wound re-epithelialization and the branching morphogenesis of several organs suggests that KGF might affect tissue remodelling by modulating the expression of matrix metalloproteinases (MMPs) or their inhibitors.

In this study we analysed the effect of KGF on the expression of stromelysin-2 (MMP-10), a member of the metalloproteinase gene family that is expressed in human keratinocytes but not in fibroblasts [9]. Compared with collagenase-I, which is capable of only cleaving interstitial fibrillar collagens (types I, II and III),

stromelysin-2 has a much broader substrate specificity, being able to degrade the protein core of proteoglycans, type IV and IX collagens, laminin-I, fibronectin and the globular domains of collagens I and III [10]. Furthermore this MMP can superactivate trypsin- or plasmin-activated procollagenase-I, generating an enzyme with an approx. 10-fold increase in specific activity relative to collagenase-I [9]. Recently, Saarialho-Kere et al. [11] demonstrated that stromelysin-2 mRNA is expressed by a distinct population of basal keratinocytes in acute and chronic human wounds. In normal, intact skin, however, no stromelysin-2 signal could be detected by hybridization *in situ*. Because KGF is highly induced in fibroblasts below the wound and at the wound edge in mice and humans [12,13] and because the presence of a functional KGF receptor in keratinocytes is essential for wound re-epithelialization [5], this mitogen, acting in a paracrine manner, might control the expression of the stromelysin-2 gene in basal keratinocytes.

MATERIALS AND METHODS

Animals

Balb/c mice were obtained from the animal care facility of the Max-Planck-Institut für Biochemie (Martinsried, Germany). The animal care facility was maintained by professionals who followed federal guidelines, and all procedures were approved by the Local Government of Bavaria.

Glucocorticoid treatment of mice

For each experiment 11 female Balb/c mice (3 months old) were injected subcutaneously at 9:00 h daily with 0.2 or 1 mg of

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; EGF, epidermal growth factor; IL-1 β , interleukin 1 β ; KGF, keratinocyte growth factor; MMP, matrix metalloproteinase; TGF, transforming growth factor; TNF α , tumour necrosis factor α .

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dexamethasone/kg body weight for 5 days. Seven control mice were injected with PBS. Three dexamethasone-treated mice and three control mice were subsequently injected with dexamethasone or PBS for another 2–5 days but left unwounded. The other mice were wounded as described below. During the wound-healing period the daily injection of the glucocorticoid or of PBS was continued.

Wounding and preparation of wound tissue

To assess stromelysin-2 expression during wound healing, six full-thickness wounds were created on each animal and skin biopsy specimens from four animals were obtained 1, 3, 5, 7 and 13 days after injury. To study the effect of dexamethasone on stromelysin-2 expression, wounds from four dexamethasone-treated mice and four control mice were analysed at days 2 and 5 after injury. Mice were anaesthetized with a single intraperitoneal injection of Avertin. The hair on the backs of these mice was cut with fine scissors and the back was subsequently wiped with 70% (v/v) ethanol. Six full-thickness wounds (6 mm in diameter, 3–4 mm apart) were made on the backs of these mice by excising the skin and panniculus carnosus. The wounds were allowed to dry to form a scab. The complete wound, including the scab and 2 mm of the epithelial margins, was excised at each time point. A similar amount of skin from the backs of three non-wounded animals was used as a control. In every experiment, the wounds from four animals (24 wounds) and the non-wounded back skin from three animals, were combined, frozen immediately in liquid nitrogen and stored at -70°C until used for RNA isolation.

Histology

Glucocorticoid-treated mice and control mice were wounded as described above. Animals were killed at day 5 after injury. Complete wounds were isolated from the middle of the back, bisected, fixed overnight in 4% (w/v) paraformaldehyde and embedded in paraffin. Sections (6 μm) from the middle of the wound were stained with haematoxylin/eosin.

RNA isolation and RNase protection analysis

Isolation of total cellular RNA and RNase protection analysis were performed as described [12]. Briefly, DNA probes were cloned into the transcription vectors pBluescript II KS(+) or pBluescript II SK(+) (Stratagene) and linearized. An anti-sense transcript was synthesized *in vitro* by using T3 or T7 RNA polymerases and [^{32}P]rUTP (800 Ci/mmol). RNA samples were hybridized at 42°C overnight with 100 000 c.p.m. of the labelled anti-sense transcript. Hybrids were digested with RNases A and T1 for 1 h at 30°C . Under these conditions, every single mismatch is recognized by the RNases. Thus cross-reaction of the individual probes with other stromelysins can be excluded. In addition, AT-rich sequences at the ends of the riboprobes that do not efficiently hybridize to the corresponding RNA are sometimes partly degraded, resulting in slightly smaller protected fragments, as seen in Figures 1, 2, 3 and 5. Protected fragments were separated on 5% (w/v) acrylamide/8M urea gels and analysed by autoradiography. All protection assays were performed with at least two different sets of RNA from independent cell-culture or wound-healing experiments.

DNA templates

A full-length human stromelysin-2 cDNA [14] cloned into the transcription vector pBluescript II SK(+) (Stratagene) was used. Linearization by *Bbs*I yielded a 271 bp template corresponding

to nt 1473–1743 of the human stromelysin-2 cDNA. A 227 bp murine stromelysin-2 fragment was cloned as described below.

PCR amplification

Single-stranded cDNA was synthesized from 5 μg of total RNA isolated from full-thickness wounds of Balb/c mice 5 days after injury, by using oligo(dT) as a primer. With an aliquot of this cDNA as a template, a murine stromelysin-2 cDNA probe was cloned by PCR with the following primers (the underlined sequences represent *Eco*RI and *Bam*HI restriction sites respectively): mST-2 5', 5'-CGGAATTCCTTGCTCAGCAATACCT-AGA-3' [the last 20 nucleotides corresponding to positions 8–27 of a known rat stromelysin-2 cDNA fragment (EMBL Sequence Data Library, accession number X64020)]; mST-2 3', 5'-CGGGATCCAAGGTACTGAAGCCACCAACGT-3' (the last 22 nucleotides corresponding to positions 198–219 of the rat stromelysin-2 cDNA fragment). Amplification was performed in a 50 μl reaction volume by 30 cycles (1 min at 94°C , 90 s at 45°C , 90 s at 72°C) in a Perkin Elmer GeneAmp PCR System 9600 followed by incubation with 2 units of Klenow enzyme (Boehringer Mannheim Biochemicals) at 37°C for 1 h. The resulting 227 nt fragment was purified by gel electrophoresis on a 2% (w/v) NuSieve Agarose (FMC BioProducts) gel and cloned into an *Sma*I-digested pBluescript II KS(+) transcription vector (Stratagene). The cloned fragment was sequenced by standard procedures and analysed by sequence comparison in the GenBank nucleic acid sequence data library.

Hybridization *in situ*

Anti-sense and sense riboprobes were made by transcription *in vitro* with T3 or T7 RNA polymerases and [^{35}S]-labelled rUTP, in which the murine stromelysin-2 cDNA fragment described above was used as a template. Hybridization *in situ* was performed on 6 μm paraffin sections as described by Wilkinson et al. [15]. After development, sections were counterstained with haematoxylin/eosin.

Cell culture

The human keratinocyte cell line HaCaT [16] was used for all tissue culture experiments. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) fetal calf serum. For stromelysin-2 induction experiments they were grown to confluency without changing the medium and rendered quiescent by a 16 h incubation in serum-free DMEM. Cells were then incubated in fresh DMEM containing purified growth factors [10 ng/ml KGF/20 ng/ml epidermal growth factor (EGF)/1 ng/ml transforming growth factor β 1 (TGF- β 1)] or cytokines [300 i.u./ml tumour necrosis factor α (TNF α)/100 i.u./ml interleukin 1 β (IL-1 β)] for various periods. Aliquots of cells were harvested before and at different time points after treatment with these reagents and used for RNA isolation. Serum and DMEM were purchased from Gibco/BRL; growth factors and cytokines were from Boehringer Mannheim Biochemicals.

RESULTS

Induction of stromelysin-2 mRNA expression in cultured human keratinocytes by KGF

Given the cell-type-specific and site-specific expression of stromelysin-2 and the KGF receptor *in vivo*, we speculated that KGF might regulate stromelysin-2 expression. To test this possibility, confluent, serum-starved HaCaT keratinocytes were

Figure 1 Induction of stromelysin-2 mRNA by KGF in cultured keratinocytes

HaCaT keratinocytes were rendered quiescent by serum starvation. They were subsequently stimulated with 10 ng/ml KGF for 1, 2.5, 4 or 8 h or with 10% (v/v) fetal calf serum for 5 h as indicated. Total cellular RNA (30 µg) from these cells was analysed by RNase protection analysis for stromelysin-2 mRNA expression. tRNA (50 µg) was used as a negative control. The gel was exposed for 4 days.

Figure 2 Induction of stromelysin-2 mRNA by TGF-β1 and TNF-α in cultured keratinocytes

Cells were rendered quiescent by serum starvation and stimulated with 1 ng/ml TGF-β1 or 300 i.u./ml TNF-α for different time periods, as indicated. Total cellular RNA (20 µg) from these cells was analysed for stromelysin-2 mRNA expression. The gel was exposed for 3 days.

treated with KGF for different periods and analysed for stromelysin-2 mRNA expression by RNase protection analysis. RNase protection assays were performed under conditions where a single base mismatch could be detected. Thus cross-reaction of the probe with other stromelysins could be excluded. Whereas stromelysin-2 mRNA was barely detectable in control HaCaT keratinocytes, expression of this gene was significantly induced after the addition of KGF (Figure 1). Enhanced levels of stromelysin-2 mRNA were detectable within 2.5 h of stimulation with KGF, and maximal stimulation was reached after 8 h of exposure. The same temporal pattern of stromelysin-2 induction was seen consistently in two independent experiments.

Induction of stromelysin-2 mRNA expression in cultured human keratinocytes by serum growth factors and proinflammatory cytokines

Because other peptide growth factors or cytokines might also induce stromelysin-2 expression in HaCaT keratinocytes, we performed analogous induction experiments with TNF-α, EGF, IL-1β and TGF-β1. In accordance with the findings of Windsor et al. [9], who investigated stromelysin-2 expression in primary human foreskin keratinocytes in response to some of these factors, we observed no induction after the addition of IL-1β, whereas the addition of EGF or TNF-α resulted in a significant induction of stromelysin-2 mRNA expression with kinetics strongly resembling those of the KGF-induced response (EGF, results not shown; TNF-α, Figure 2). Finally, we examined the

Figure 3 Kinetics of stromelysin-2 mRNA expression during the healing process of excisional full-thickness wounds in Balb/c mice

(A) Total cellular RNA (20 µg) isolated from normal and wounded mouse back skin was analysed by RNase protection assay with a ³²P-labelled riboprobe complementary to mRNA encoding murine stromelysin-2. The times after injury are indicated at the top. The gel was exposed for 4 days. (B) The degree of stromelysin-2 mRNA induction at different stages of the repair process as assessed by laser scanning densitometry of the autoradiograms. Each bar indicates the mean ± S.E.M. for triplicate determinations (with RNA species from three independent wound-healing experiments).

effect of TGF-β1 on the expression of stromelysin-2 mRNA in HaCaT keratinocytes. Interestingly, the stimulatory effect on stromelysin-2 was even stronger than that observed for KGF, EGF or TNF-α. The kinetics of this inductive response was again similar to that described for the other peptide growth factors (Figure 2).

Stromelysin-2 mRNA expression is strongly up-regulated during wound healing in Balb/c mice

We next determined whether the observed induction of the expression of stromelysin-2 mRNA *in vitro* is physiologically relevant during the repair of normally healing cutaneous wounds. We used a specific RNase protection assay to determine the time course of stromelysin-2 expression during cutaneous wound repair. For this purpose we cloned a 227 bp fragment of the murine stromelysin-2 cDNA that was highly similar to the corresponding regions of rat and human stromelysin-2 cDNAs (98% and 92% respectively). Using this probe we determined the levels of stromelysin-2 mRNA in samples isolated from unwounded back-skin and from excisional wounds harvested 12 h, 1 day, 3 days, 5 days, 7 days and 13 days after injury to Balb/c mice. Stromelysin-2 mRNA was hardly detected in unwounded skin, but expression of this MMP was markedly induced early after injury. This induction occurred in two phases (Figures 3A and 3B). After a first wave of induction, which led to 15-fold and 44-fold elevated stromelysin-2 mRNA levels 12 and 24 h after injury, stromelysin-2 mRNA levels subsided transiently around day 3 after injury. At 5 days after injury, however, stromelysin-2 mRNA levels increased again. At this time point, stromelysin-2 mRNA levels were 26-fold higher than in non-wounded skin. At the end of the proliferative phase of

Figure 4 Localization of stromelysin-2 mRNA in wounded skin by hybridization *in situ*

Full-thickness excisional wounds were made on the backs of Balb/c mice. Mice were killed 12 h (A) and 5 days (B) after injury. Wounds were isolated, bisected, fixed in 4% (w/v) paraformaldehyde, embedded in paraffin and sectioned. Sections (6 μ m) were hybridized with a 35 S-labelled riboprobe complementary to the coding sequence of murine stromelysin-2. They were coated with autoradiography emulsion (NBT), exposed for 3 weeks and counterstained with haematoxylin/eosin. Silver grains appear as black dots. Magnification $\times 132$. Abbreviations: E, epidermis at the wound edge; D, dermis; G, granulation tissue; ES, eschar.

wound repair (13 days after injury) stromelysin-2 mRNA levels had dropped to near-basal levels. This same biphasic pattern of stromelysin-2 expression was detected with different sets of RNAs from three independent wound-healing experiments.

Expression of stromelysin-2 mRNA is abundant in a distinct population of basal keratinocytes at the migrating front of the epidermis in wounds of Balb/c mice

Having characterized the time course of stromelysin-2 expression after cutaneous injury, we determined the spatial distribution of stromelysin-2 mRNA in wounded skin. For this purpose we hybridized sections of 12 h, 3-day and 5-day wounds from Balb/c mice with an anti-sense RNA probe generated from the murine stromelysin-2 cDNA fragment (Figure 4). At 12 h after injury, stromelysin-2 mRNA was seen at highest levels in a distinct population of basal keratinocytes at the edge of the wound (Figure 4A), but no expression was detected in the

Figure 5 Decreased re-epithelialization and elevated levels of stromelysin-2 mRNA during wound healing in glucocorticoid-treated mice

Balb/c mice were injected daily with 0.2 mg of dexamethasone/kg body weight (low dose Dex) or with 1.0 mg of dexamethasone/kg body weight (high dose Dex) as described in the Materials and methods section. Mice injected with PBS (ctrl) were used as a control. Full-thickness wounds were made on the backs of control mice (A, Ctr) and glucocorticoid-treated mice (B, Dex). Mice were killed at day 5 after injury. Wounds were isolated, bisected, fixed in 4% (w/v) paraformaldehyde, and embedded in paraffin. Sections from the middle of the wound were stained with haematoxylin/eosin. Magnification $\times 66$. Abbreviations: E, epidermis; D, dermis; ES, eschar; G, granulation tissue; M, muscle layer. (C) RNA was isolated from non-wounded and wounded skin (2 days after wounding) of control mice and dexamethasone-treated mice. Total RNA (20 μ g) was analysed for the presence of stromelysin-2 mRNA. tRNA (50 μ g) served as a negative control. The gel was exposed for 5 days.

epithelium of unwounded skin away from the migrating border. After formation of an extensive hyperproliferative epithelium, i.e. at days 3 and 5 after injury, stromelysin-2 mRNA expression

was still confined to the basal layer of the migrating epithelium (day 3, results not shown; day 5, Figure 4B).

Induction of stromelysin-2 mRNA expression after injury is enhanced in glucocorticoid-treated mice

To determine further the expression pattern of stromelysin-2 during repair processes, we analysed the expression of this MMP during impaired wound healing. Because the inhibition of wound healing by glucocorticoids is a well-established phenomenon, we analysed the effect of glucocorticoid treatment on stromelysin-2 expression during wound repair in mice. Dexamethasone was injected daily at 9:00 h over a period of 5 days before wounding and for 2–5 days after wounding. To verify that dexamethasone impaired healing, wounds were analysed histologically at day 5 after injury. At that time an abundance of granulation tissue was clearly evident in PBS-treated mice, whereas in dexamethasone-treated mice granulation tissue formation was hardly detectable. Furthermore, and relevant to our experiments, re-epithelialization was markedly delayed in glucocorticoid-treated mice (Figures 5A and 5B).

RNA was isolated from non-wounded skin and from wounded skin of glucocorticoid-treated and control mice at days 2 and 5 after injury and analysed for stromelysin-2 expression by RNase protection analysis. As shown in Figure 5(C) for 2-day wounds, dexamethasone treatment resulted in a markedly enhanced expression level of stromelysin-2 mRNA in normal skin and even more prominently at day 2 after injury. At day 5 after injury, stromelysin-2 mRNA levels were still higher in the wounds of glucocorticoid-treated mice than in those of control mice (results not shown). These results demonstrate that glucocorticoid treatment modulates the normal induction of stromelysin-2 expression after cutaneous injury.

DISCUSSION

Cutaneous wound healing is a complex process involving re-epithelialization, granulation tissue formation and tissue remodelling [17]. Growth factors and cytokines are key players in the regulation of these events, mediating the co-ordinated action of keratinocytes, fibroblasts, endothelial cells and inflammatory cells. In a previous study we demonstrated that KGF receptor signalling is required for normal wound re-epithelialization [5], a process involving keratinocyte migration and proliferation. KGF stimulates both processes *in vitro* [1,18] and might also do so *in vivo*. During wound healing, keratinocyte migration starts immediately after injury and is confined to cells at the wound edge, whereas proliferation begins approx. 24 h after injury in a population of keratinocytes adjacent to but distal from the wound edge [11,19]. The migration of keratinocytes during re-epithelialization is preceded by their detachment from the underlying basement membrane and is accompanied by successive degradation of the wound clot, of damaged dermal components, and finally of the granulation tissue. All these remodelling processes require the degradation of a large variety of proteins and involve various proteinases, including the family of zinc-dependent MMPs, which together can degrade essentially all extracellular matrix components (reviewed in [20]).

In this study we focused on stromelysin-2 (MMP-10). This member of the MMP family is characterized by its broad substrate specificity [20] and its specific expression in epithelial cells, including basal keratinocytes [9,11]. In view of the role of KGF as a paracrine mediator in wound re-epithelialization and epithelial morphogenesis, it seemed tempting to speculate that dermally derived KGF might also influence the expression of

stromelysin-2, and this hypothesis was confirmed by the results presented here. Expression of metalloproteinases is usually regulated at the mRNA level. Indeed, preliminary evidence from our laboratory suggests that the elevated stromelysin-2 mRNA levels seen after KGF treatment correlate with increased proteolytic activity as determined by casein zymography (results not shown).

Previously, Putnins et al. [21] reported that KGF regulates protease production in epithelial cells, specifically by inducing the expression of MMP-9 and plasminogen activator activity in a pig epithelial culture model. Unlike our results, protease expression in the pig cells required contact with polycarbonate membranes, whereas KGF induction of stromelysin-2 expression in HaCaT cells did not require growth on precoated culture dishes.

We further demonstrate that expression of stromelysin-2 in HaCaT cells was also induced in response to EGF and TNF α . These findings agree with those of Windsor et al. [9], who demonstrated that transcription of the stromelysin-2 gene is induced by EGF, TGF α and TNF α in cultured human foreskin keratinocytes. Additionally, we provide evidence that TGF- β 1, a peptide growth factor known to enhance matrix accumulation in fibroblasts by a variety of mechanisms including the inhibition of MMP induction, strongly activates expression of the matrix-degrading enzyme stromelysin-2 in HaCaT keratinocytes. Previously, TGF- β 1 was shown to slightly stimulate the expression of 92 and 72 kDa gelatinases (MMP-9 and MMP-2 respectively) in human keratinocytes [22]. The rates of induction for these MMPs, however, were much lower (one-half to one-fifth) than those reported here for stromelysin-2.

Whereas EGF, KGF and TGF α are strong inducers of keratinocyte migration and proliferation [18,23,24], TGF- β 1 acts as an inhibitor of both processes *in vitro* [24,25]. However, during wound healing, TGF- β 1 stimulates wound re-epithelialization presumably via induction of keratinocyte migration [26,27]. Furthermore all of these factors are present in wounds (reviewed in [28]) as a result of blood vessel disruption (EGF, TGF α and TGF- β 1) and/or of secretion by mesenchymal, epithelial or inflammatory cells (TGF- β 1, KGF and TNF α). Therefore growth factor-mediated activation of stromelysin-2 expression in keratinocytes might also be of biological importance *in vivo*.

Our analysis of stromelysin-2 expression during cutaneous wound repair after acute injury in Balb/c mice revealed kinetics characterized by two distinct peaks of expression followed by a progressive return to basal levels. Furthermore, our hybridization studies *in situ* indicate that stromelysin-2 expression is most abundant in a small number of basal keratinocytes at the leading edge of the migrating epithelium throughout both the inflammatory and the proliferative phases of the healing process. Thus the expression of stromelysin-2 in the epithelium is not only a feature of chronic wounds: it also occurs during normal wound healing.

In view of the mounting evidence for a well-defined and important role for keratinocyte-specific MMPs during normal wound healing, it seemed possible that wound-healing disorders might be associated with a defect in the control of the proteolytic activity of these enzymes. Indeed, our data demonstrate that the expression level of stromelysin-2 during wound healing in glucocorticoid-treated mice is considerably higher than that observed in untreated control animals. Preliminary results from our laboratory have also demonstrated aberrant kinetics of stromelysin-2 expression during cutaneous wound repair in genetically diabetic (*db/db*) mice, which are characterized by a markedly delayed wound-healing process [29]. Interestingly, this impaired repair process seems to be associated with a delay in

stromelysin-2 induction and a significantly prolonged expression of this gene (results not shown). These results suggest that excess proteolysis resulting from enhanced or prolonged MMP activity might at least in part underlie the wound-healing disorders found in these animals. Such a hypothesis is further supported by findings that fluid from chronic wounds contains significantly more gelatinolytic activity than that from acute wounds [30,31].

In conclusion, our results suggest that a temporally and spatially correct regulation of stromelysin-2 expression is important for the normal repair process.

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