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TLR3 activation induces S100A7 to regulate keratinocyte differentiation after skin injury

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Human S100A7 (psoriasin) is highly expressed in psoriasis and other inflammatory diseases; however, the function of S100A7 in wound repair remains largely unknown. Here we demonstrated that skin injury increased the expression of S100A7. Damaged cells from wounded skin induced the expression of S100A7 via the activation of Toll-like receptor 3 (TLR3) followed by the activation of p38 MAPK. S100A7, in turn, acted on keratinocytes to induce the expression of terminal differentiation marker gene loricrin through the activation of p38 MAPK and caspase-1. The differentiation of keratinocytes induced by S100A7 resulted in skin stratification, thus efficiently promoting wound closure. Taken together, our results demonstrate that the activation of TLR3 accelerates wound closure via the induction of S100A7 to induce keratinocyte differentiation. These findings also provide new insights into the development of different forms of treatment with skin wounds.

TLR3, S100A7, caspase-1, keratinocyte differentiation, wound closure

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INTRODUCTION

An efficient re-epithelialization is required for wound repair (Eckert et al., 2002). In the skin, re-epithelialization is accomplished by keratinocyte proliferation, migration, stratification, and differentiation (Laplante et al., 2001; Li et al., 2014). Among these, the proliferation and differentiation of keratinocytes enable skin stratification, thus promoting wound closure and forming a physical barrier (Lee et al., 2006). Multiple factors are involved in the regulation of keratinocyte proliferation and differentiation, including calcium, epidermal growth factor and tumor necrosis factor (Jost et al., 2001). Although the activation of Toll-like receptors (TLRs) such as TLR3 and TLR4 has been shown to play critical roles in the response to tissue injury and subsequent tissue repair and regeneration (Larsen et al., 2007; Rakoff-Nahoum and Medzhitov, 2008; Chen et al., 2010, 2013), whether TLR activation would regulate these factors to induce keratinocyte differentiation after skin injury remains largely unknown.

Many genes encoding structural and regulatory proteins are critical for keratinocyte proliferation and differentiation. S100A family proteins that are characterized by two calcium-binding sites are one of them. The gene of S100A family is located on chromosome 1q21 which encodes many keratinocyte differentiation-related proteins such as involucrin, filaggrin and trichoyalin (Eckert et al., 2004). It has been shown that the expression of S100A8 and S100A9 is strongly induced within the first week after epidermal injury. The increased expression of S100A8 and S100A9 in wound-associ-

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ated keratinocytes is related to the formation of hyper-thickened epithelium (Thorey et al., 2001). Besides S100A8 and S100A9, S100A7 has been shown to enhance the expression of several differentiation marker genes in normal human keratinocytes (Hattori et al., 2014). All these suggest that S100A family proteins may induce epidermal keratinocyte differentiation during wound healing.

Recently it has been reported that the topical application of TLR2 agonist, macrophage-activating lipopeptide-2, accelerates wound healing in diabetic murine skin wounds (Deiters, 2004). In addition to TLR2, the activation of TLR3 by application of its agonist polyinosinic:polycytidylic acid (polyI:C) improves wound repair (Lin, 2012). Both of these two ligands promote wound healing via recruiting leukocytes to the wounds. However, whether activation of TLR3 would regulate S100A family proteins to induce keratinocyte differentiation is unclear.

Given the importance of TLR3 in the regulation of wound healing and the potential role of S100A7 in keratinocyte differentiation during skin stratification, we hypothesized that TLR3 activation may induce S100A7 to regulate keratinocyte differentiation for wound closure. In this study we showed that TLR3 activation induced S100A7 expression in keratinocytes, and further delineated a new mechanism by which TLR3 regulated wound repair.

RESULTS

Skin injury increases the expression of S100A7

S100A7 has been reported as an antibacterial agent on the skin surface; however, whether it would be involved in wound re-epithelialization during wound repair remains largely unknown. To address this question, full-thickness incisions were made on the dorsal skin of C57BL/6 wild-type mice as previously reported (Lai et al., 2012) and the expression of S100A7 was examined at day 3. Quantitative real-time PCR (qPCR) showed that S100A7 mRNA expression was markedly increased in skin wounds compared to normal skin (Figure 1A). Consistent with increased mRNA, the protein abundance of S100A7 was dramatically increased in skin wounds by western blot analysis (Figure 1B). In addition to the wounded skin, S100A7 expression was increased in the burned skin of mice compared to normal skin (Figure 1C). Altogether, these results demonstrate that skin injury increases S100A7 expression.

Damaged cells from wounds activate TLR3-p38 MAPK to induce S100A7 expression in keratinocytes

To determine what factor induces S100A7 expression after skin injury, the supernatants of normal and wounded skin homogenates were collected for keratinocyte stimulation as previously reported (Lai et al., 2012). Both supernatants from normal and wounded-skin homogenates induced the expression of S100A7 in murine epidermal keratinocytes (Figure 2A). Specifically, S100A7 expression induced by the supernatant from wounded-skin homogenate was higher than that induced by normal skin homogenate (Figure 2A). These data suggest that components from damaged tissue have higher potentials to induce S100A7 expression in keratinocytes.

In skin injury, tissue damage results in the rapid generation of abundant amounts of damaged cells, including necrotic cells and apoptotic keratinocytes. To test whether damaged keratinocytes could induce S100A7 expression in adjacent normal cells, cultured keratinocytes were irradiated by ultraviolet B (UVB) to induce apoptosis and cell death (UVR-cells). UVR-cells were then collected and added to stimulate normal neonatal human epidermal keratinocytes (NHEKs). S100A7 was significantly induced when normal NHEKs exposed to UVR-cells but not when exposed to equal amounts of dead, non-irradiated cells (Figure 2B).

Our previous observation has shown that damaged cells can activate TLR3 to initiate inflammatory response after skin in-

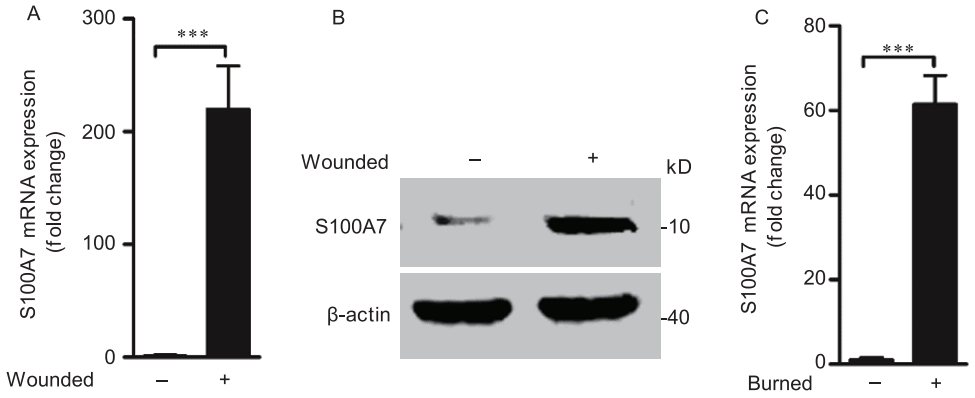

Figure 1 Skin injury increases epidermal S100A7 expression. A, Quantification of S100A7 mRNA expression in day-3 skin wounds by quantitative real-time PCR (qRCR). B, Immunoblot of S100A7 in skin extracts taken from 2 mm skin surrounding wound edges. C, Quantification of S100A7 mRNA expression of burned skin in mice. ***, $P<0.001$. P values were determined by unpaired two-tailed t tests. Data are the means±SE and representative of three independent experiments.

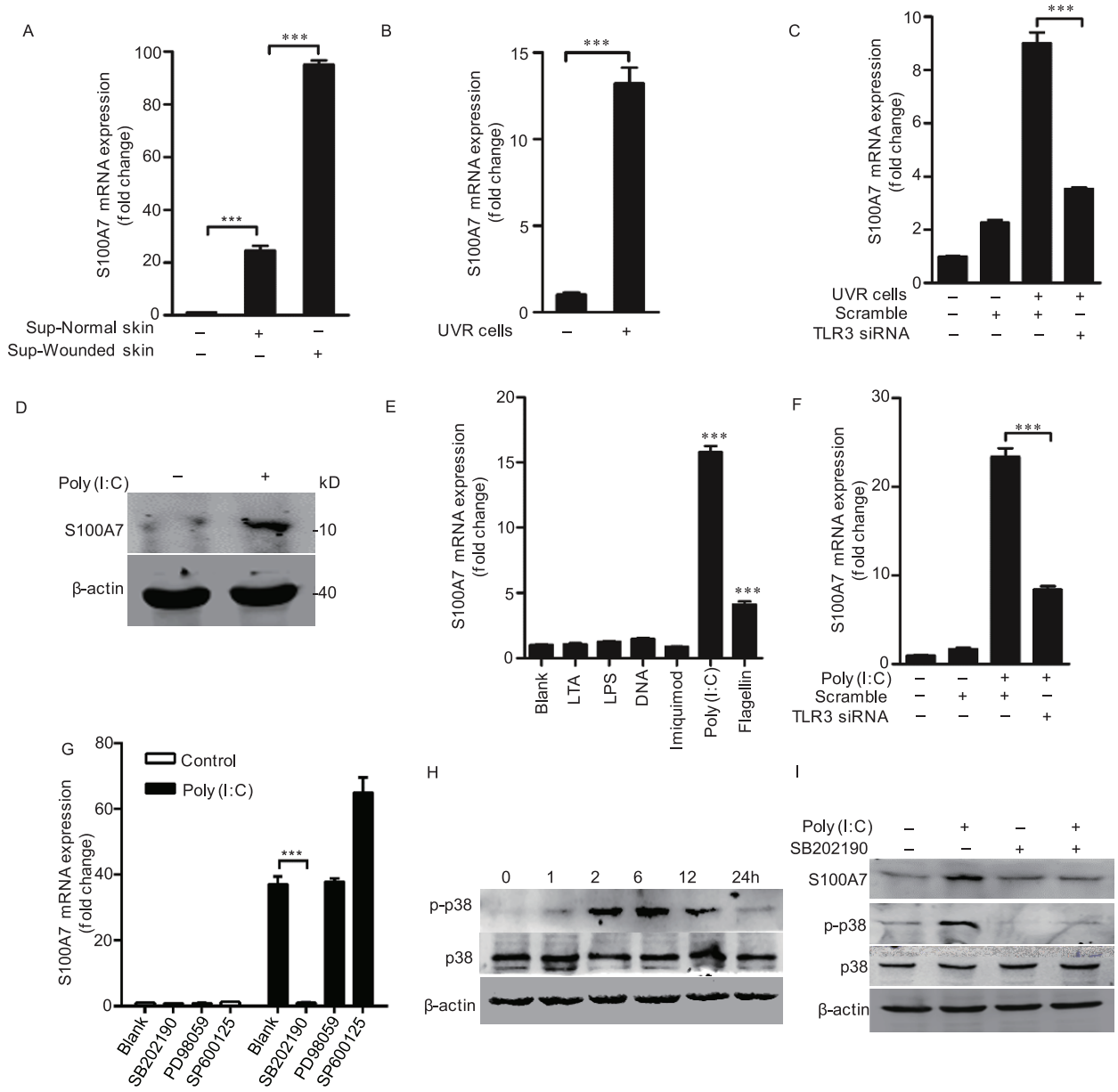

Figure 2 Damaged cells from wounds and poly(I:C) activate TLR3-p38 MAPK to induce S100A7 in keratinocytes. A, Quantification of S100A7 mRNA expression in murine keratinocytes stimulated by the supernatants of normal and wounded skin homogenates. 2 mm skin surrounding day-3 wound edges was taken for homogenization. B, S100A7 mRNA expression in NHEKs induced by UVB-irradiated dead keratinocytes (UVR cells). C, Quantification of S100A7 mRNA expression in NHEKs induced by UVR cells before and after TLR3 was silenced. D, Immunoblot of S100A7 in NHEKs treated with 5 μg mL^{-1} poly(I:C) by using S100A7-specific antibody. E, Quantification of S100A7 mRNA expression in NHEKs stimulated with TLR2 ligand (LTA), TLR4 ligand (LPS), TLR9 ligand (DNA), TLR3 liand (Poly(I:C)), TLR7 ligand (Imiquimod) and TLR5 ligand (Flagellin). F, Quantification of S100A7 mRNA expression in NHEKs induced by poly(I:C) before and after TLR3 was silenced. G, Quantification of S100A7 mRNA expression in NHEKs treated with 5 μg mL^{-1} poly(I:C) in the absence or presence of SB202190 (p38 MAPK inhibitor, 10 μmol L^{-1}), SP600125 (JNK inhibitor, 15 μmol L^{-1}) and PD98059 (ERK inhibitor, 10 μmol L^{-1}). H, Immunoblot of phospho-p38 MAPK in NHEKs treated with 5 μg mL^{-1} poly(I:C) at different time points. I, Immunoblot of S100A7 and phospho-p38 MAPK in NHEKs treated with 5 μg mL^{-1} poly(I:C) with or without SB202190 for 1 h. ***, P<0.001. P values were analyzed by unpaired two-tailed t tests (B), one-way ANOVA (A, C, E and F) and two-way ANOVA (G). Data are the means±SE and representative of two independent experiments.

jury (Lai et al., 2009). To evaluate whether the activation of TLR3 would be also required for S100A7 expression after skin injury, we checked the expression of S100A7 in NHEKs after TLR3 was silenced. TLR3 silencing significantly decreased the expression of S100A7 induced by UVR-cells in NHEKs (Figure 2C). Moreover, TLR3 ligand, poly(I:C), induced the expression of S100A7 in keratinocytes (Figure 2D, 2E and Figure S1A in Supporting Information) but not in macrophages and fibroblasts (Figure S1B in Supporting Information). Other TLR ligands such as LTA, LPS, DNA, imiquimod and flagellin did not induce S100A7 expression or had a minimal effect on the induction of S100A7 in ker-

atinocytes (Figure 2E). Furthermore, the induction of S100A7 by poly(I:C) was dependent on the activation of TLR3 as silencing TLR3 by siRNA significantly inhibited poly(I:C)-induced S100A7 expression (Figure 2F).

To further determine TLR3-mediated downstream signaling pathway, we used multiple inhibitors to treat NHEK in the presence or absence of poly(I:C). Among these inhibitors, only p38 mitogen-activated protein kinase (MAPK) inhibitor, SB202190, inhibited poly(I:C)-induced S100A7 expression (Figure 2G and data not shown). In addition, poly(I:C) induced the phosphorylation of p38 MAPK in a time-dependent manner (Figure 2H) and this induction was inhibited by p38 MAPK inhibitor SB202190 (Figure 2I). Consistent with this, the expression of S100A7 induced by poly(I:C) was also inhibited by p38 MAPK inhibitor (Figure 2I). All these data demonstrate that the expression of S100A7 is dependent on the activation of TLR3-p38 MAPK signaling pathway in keratinocytes.

Poly(I:C) and S100A7 induce keratinocyte differentiation

Next we determined the function of poly(I:C) and S100A7 in keratinocytes. We observed that poly(I:C) changed the morphology of keratinocytes to differentiated status (Figure S1C in Supporting Information) . Consistent with this, poly(I:C) significantly induced the expression of the terminal differentiation marker gene loricrin (LOR), but not other differentiation genes such as filaggrin (FLG) and involucrin (IVL), in a time-dependent manner in keratinocytes (Figure 3A). In addition to mRNA, poly(I:C) induced the production of loricrin protein in keratinocytes (Figure 3B).

Since TLR3 ligand poly(I:C) was observed to induce S100A7 expression, we hypothesized that the induction of loricrin by poly(I:C) might be via S100A7. To test this, we first stimulated keratinocytes with S100A7 and then evaluated the expression of loricrin. Similar to poly(I:C), S100A7 significantly induced the expression of loricrin mRNA in keratinocytes (Figure 3C and Figure S2A in Supporting Information). In line with the increased mRNA expression, loricrin protein was increased in keratinocytes stimulated by S100A7 (Figure 3D). Moreover, the induction of loricrin by poly(I:C) was dependent on the expression of S100A7 as silencing S100A7 markedly decreased poly(I:C)-induced loricrin (Figure 3E). Taken together, these data demonstrate that S100A7 induced by poly(I:C) increases the expression

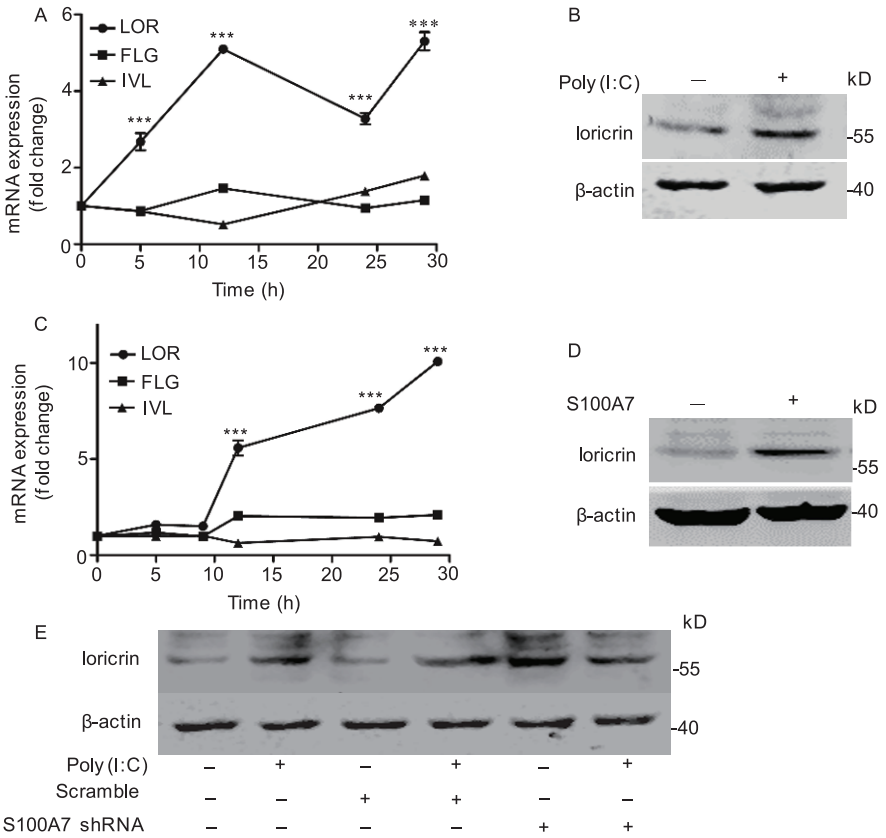

Figure 3 Poly(I:C) and S100A7 induce loricrin expression in keratinocytes. A, Quantification of LOR, FLG and IVL mRNA expression in NHEKs stimulated by 5 μg mL^{-1} poly(I:C) for different times. B, Immunoblot of loricrin protein production in NHEKs treated with 5 μg mL^{-1} poly(I:C) for 24 h. C, Quantification of LOR, FLG and IVL mRNA expression in NHEKs stimulated by 30 ng mL^{-1} S100A7 for different times. D, Immunoblot of loricrin protein production in NHEKs treated with 30 ng mL^{-1} S100A7 for 24 h. E, Immunoblot of loricrin in NHEKs treated with poly(I:C) (5 μg mL^{-1}) before and after S100A7 was silencing. ***, P<0.001. P-values were determined by two-way ANOVA. Data are the means±SE and representative of two independent experiments.

of terminal differentiation gene loricrin, resulting in cell differentiation.

S100A7 activates p38 MAPK and caspase-1 to induce loricrin

Having known that S100A7 can induce loricrin expression in keratinocytes, we next explored the mechanism by which S100A7 induced loricrin. After the screening, we also found that p38 MAPK inhibitor SB202190, but not other MAPK inhibitors, markedly inhibited S100A7-indued p38 MAPK phosphorylation and loricrin expression (Figure S2B in Supporting Information, Figure 4A and B). As an inducer of S100A7, poly(I:C) also induced loricrin expression and this induction was inhibited by p38 MAPK inhibitor (Figure 4C and Figure S2C in Supporting Information). In addition to p38 MAPK, S100A7 induced the expression of caspase-1 in a time-dependent manner (Figure 4D and Figure S2D in Supporting Information). The induction of caspase-1 by S100A7 was dependent on the activation of p38 MAPK as the inhibition of p38 MAPK blocked the production of S100A7-induced caspase-1 (Figure 4A). Moreover, the activation of caspase-1 was required for poly(I:C) and S100A7 to induce the expression of loricrin as the inhibition of caspase-1 activity by its inhibitor abrogated the effect of poly(I:C) and S100A7 on the induction of loricrin (Figure 4E, F and Figure S2E in Supporting Information). Furthermore, the induction of caspase-1 by poly(I:C) was dependent on S100A7 as silencing S100A7 by shRNA blocked the expression of caspase-1 induced by poly(I:C) (Figure 4G and Figure S2F in Supporting Information). Taken together, these data demonstrate that S100A7 induces loricrin expression via the activation of p38 MAPK and Caspase-1.

S100A7 accelerates wound closure

Having established the involvement of S100A7 in the regulation of keratinocyte differentiation, we next set out to determine the physiological relevance of S100A7 during wound healing. It is known that keratinocyte differentiation leads

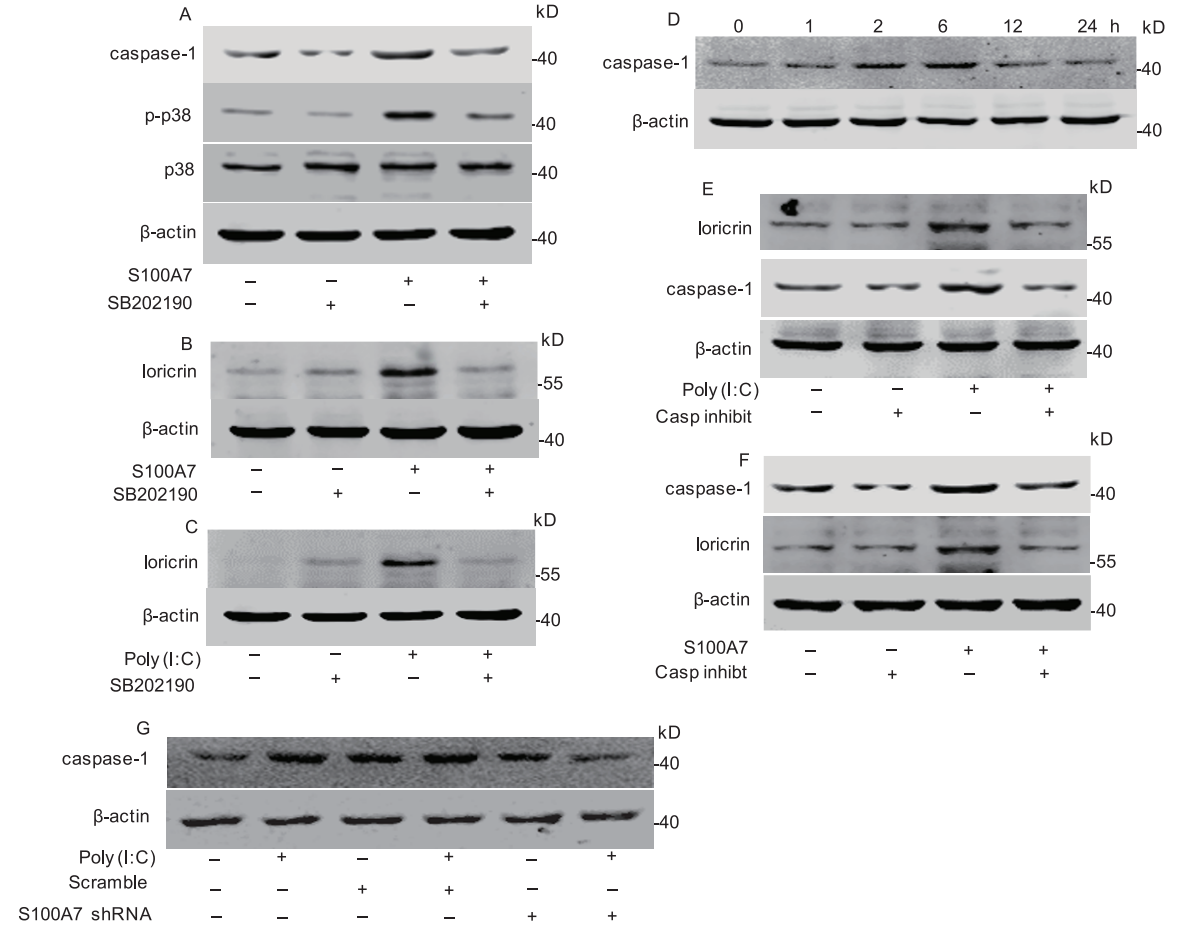

Figure 4 S100A7 activates p38 MAPK and caspase-1 to induce loricrin. A, Immunoblot of phospho-p38 MAPK and caspase-1 in NHEKs treated with S100A7 (30 ng mL^{-1}) in the presence or absence of SB202190 (10 μmol L^{-1}) for 2 h. B and C, Immunoblot of loricrin in NHEKs treated with S100A7 (30 ng mL^{-1}) or poly(I:C) (5 μg mL^{-1}) with or without SB202190 (10 μmol L^{-1}) for 24 h. D, Immunoblot of caspase-1 in NHEKs treated with S100A7 (30 ng mL^{-1}) for different times. E and F, Immunoblot of loricrin and caspase-1 in NHEKs treated with poly(I:C) (5 μg mL^{-1}) or S100A7 (30 ng mL^{-1}) with or without caspase-1 inhibitor I (20 μmol L^{-1}). G, Immunoblot of caspase-1 in NHEKs treated with poly(I:C) (5 μg mL^{-1}) before and after S100A7 was silenced. Data are representative of two independent experiments.

to skin stratification and the appropriate stratification is required for wound closure (Laplante et al., 2001). We thereby hypothesized that S100A7 might be involved in skin stratification during wound repair. To test this, 500 ng S100A7 was intradermally injected into mouse dorsal skin before wounding. The application of S100A7 significantly increased epidermal stratification, re-epithelialization in a time-dependent manner compared to control mice (Figure 5A), thus accelerating wound healing (Figure 5B and C). Consistently, the expression of loricrin was increased in day-7 skin wounds accompanied with quicker epidermal stratification (Figure 5D). All these data demonstrate that S100A7 induces skin stratification to accelerate wound closure.

DISCUSSION

After skin injury, re-epithelialization is one of the most essential part for wound repair, as the tissue's primary objective is to quickly reestablish barrier function (Martin, 1997; Epstein et al., 1999). It is known that the proliferation, migration and differentiation of epidermal keratinocytes are precisely

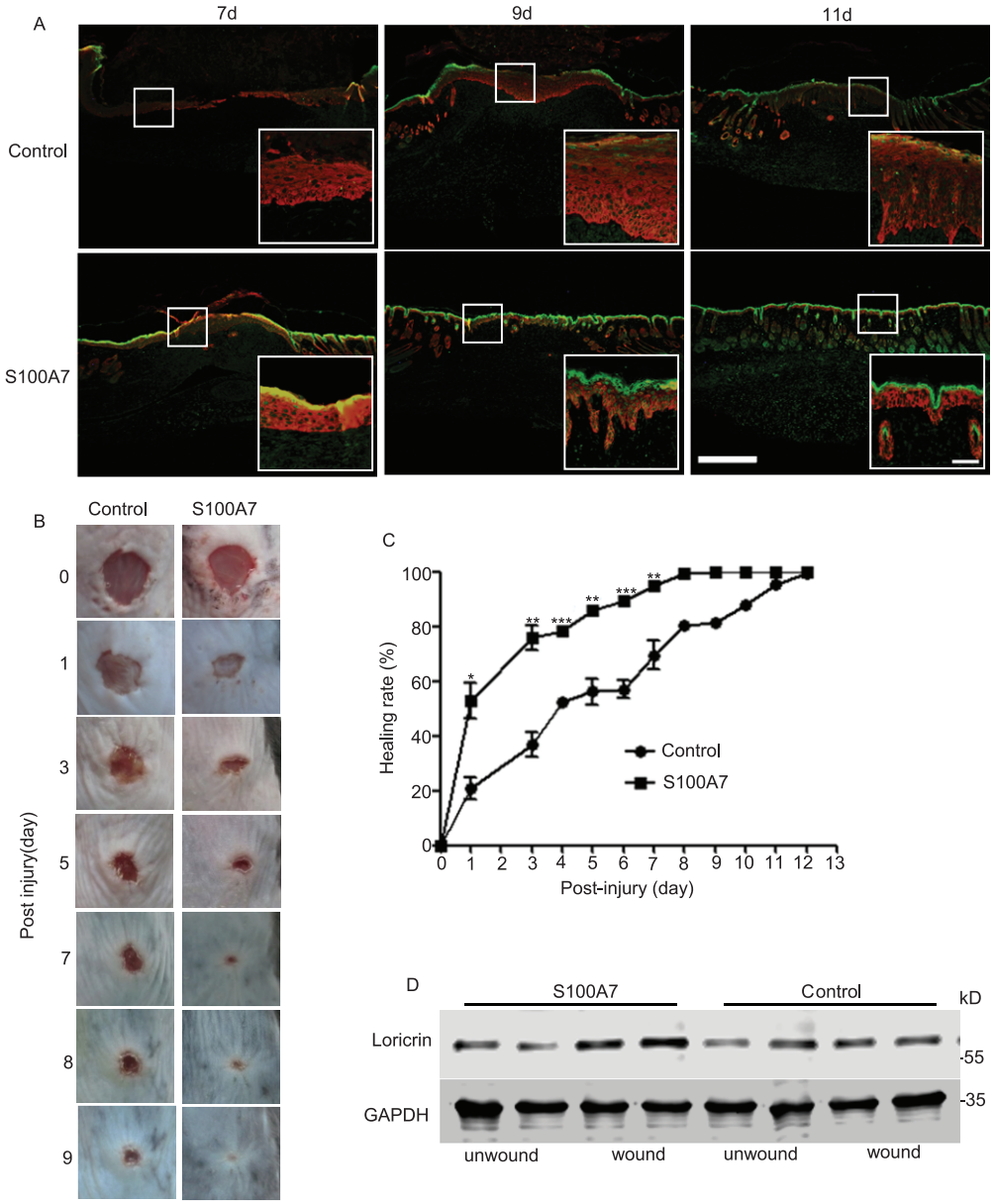

Figure 5 (Colour online) S100A7 accelerates wound closure *in vivo*. A, Immunofluorescent staining of skin wounds with or without S100A7 treatment at indicated times by K14 and Loricrin antibodies. Long scale bar represents 500 μm, and short scale bar represents 50 μm. Squares designate region of 200× magnification shown in inset. B, Photographs of healing wounds in mice intradermally injected with or without S100A7. C, Wound healing of mice treated as in (A). D, Immunoblot of loricrin in skin wounds of mice treated with or without S100A7 at day 7. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. *P*-values were determined by two-way ANOVA. Data are the means±SE and representative of two independent experiments.

controlled to ensure the successful wound re-epithelialization (Gurtner et al., 2008); however, whether the activation of TLRs would be involved in this process was not clear. Here we observed that TLR3 activation might regulate epidermal repair through the induction of S100A7 to control keratinocyte differentiation during wound repair. Our results show that damaged cells from skin wounds activate TLR3 to induce S100A7 expression. S100A7, in turn, induces keratinocytes differentiation by the activation of p38 MAPK and caspase-1, events that we show have a major role in promoting skin stratification and wound re-epithelialization. Thus, the identification of S100A7 as a stimulus for keratinocyte differentiation, and the elucidation of its mechanism of action, provides the crucial information for understanding epidermal homeostasis and wound repair.

Recently Lin et al. has reported that topical application with TLR3 ligand poly(I:C) markedly enhanced re-epithelialization, granulation and neovascularization, thus promoting wound healing (Lin et al., 2012). They pointed out that poly(I:C) exerted these functions by increasing neutrophil and macrophage recruitment, which is consistent with our previous observation in skin wounds (Lai et al., 2009). However, wound re-epithelialization is accomplished by keratinocyte proliferation, migration and differentiation, not leukocyte recruitment. It thereby raised possibility that poly(I:C) might regulate keratinocyte proliferation and differentiation to promote re-epithelialization. Here we confirmed this hypothesis and showed that stimulating keratinocytes with poly(I:C) *in vitro* directly induced the differentiation of keratinocytes, and this induction was dependent on S100A7 expression, suggesting that poly(I:C) promotes wound re-epithelialization via the induction of S100A7.

TLR activation triggers inflammatory responses in injury and trauma, thus either impairing or promoting the healing process (Dasu and Rivkah Isseroff, 2012). For example, wound healing in *Tlr2*$^{-/-}$ diabetic mice was markedly quicker than that in wild-type diabetic mice (Dasu et al., 2010). However, Deiters et al showed that the application of TLR2 agonist accelerates wound healing in diabetic mice (Deiters et al., 2004). Moreover, the deficiency of TLR4 in normal mice leads to impaired early stage skin wound closure due to the decreased expression of IL-1β and IL-6 (Dasu and Rivkah Isseroff, 2012; Chen et al., 2013). One may assume that the timing of the activation of specific TLRs under specific circumstances may be a critical element in determining whether the activation of TLRs impairs or improves wound healing (Dasu and Rivkah Isseroff, 2012). Alternatively, the route of presentation of TLR agonist (endogenous or exogenous ligands) and its access to cells bearing the receptor would initiate different innate responses (Dasu and Rivkah Isseroff, 2012). Furthermore, in sterile conditions TLRs are activated by endogenous ligands such as hyaluronan and double-stranded RNA that are released from necrotic cells (Scaffidi et al., 2002; Jiang et al., 2005; Tian et al., 2007; Lai et al., 2009). Whether these ligands initiate appropriate inflammatory response to regulate keratinocyte proliferation and differentiation for wound repair or induce excessive production of inflammatory cytokines to impair wound healing needs further investigation.

Multiple factors including all-trans retinoic acid, calcium and UV have been reported to be involve in the induction of S100A7 (Hoffmann et al., 1994; Zouboulis et al., 1996; Eckert et al., 2004). However, which factors in skin wounds would induce the expression of S100A7 was unclear. Our previous observation has shown that skin injury increased the expression of IL-17 and IL-22, and then IL-17 and IL-22 induce S100A7 expression in keratinocytes (Nakajima et al., 2011; Lai et al., 2012). Here we show that damaged cells from skin wounds activated TLR3 to directly induce S100A7 expression. Moreover, our previous study shows that damaged cells also activate TLR3 to initiate inflammatory response after skin injury (Lai et al., 2009). Therefore, another possibility will be that damaged cells activate TLR3 to recruit IL-17- or IL-22-producing cells to wounds and then release IL-17 and IL-22 to induce S100A7 expression.

It is well-known that calcium plays an important role in the formation of the cornfield envelop and stratum corneum barrier by inducing the terminal differentiation of keratinocytes (Watt, 1989; Nemes et al., 1999). As calcium binding protein, S100A family proteins have been shown to be induced by calcium and then regulate cell differentiation during tissue remodeling and repair (Hoffmann et al., 1994; Voss et al., 2011, 2012). However, the underlying mechanism was not fully understood. Here we revealed that S100A7 that was induced by dead cells around wounds regulated the terminal differentiation marker gene loricrin expression, thus leading to keratinocyte differentiation. The induction of loricrin by S100A7 was via the activation of p38 MAPK, an important factor involved in the differentiation of keratinocyte (Efimova et al., 2003; Ivanova and Dagnino, 2007). Although previous reports have shown that the activation of caspase-3 and caspase-14 led to normal loss of nuclei in differentiated keratinocyte (Weil et al., 1999; Eckhart et al., 2000), here we found that S100A7 activated caspase-1, but not caspase-3 and caspase-14, to induce keratinocytes differentiation, suggesting that a specific signal cascade is activated by S100A7 for keratinocyte differentiation.

In conclusion, our findings suggest that the induction of S100A7 by TLR3 activation in skin wounds is necessary for wound closure. Specifically, we find that S100A7 exerts its effect by inducing keratinocyte differentiation and skin stratification via the activation of p38 MAPK and caspase-1. These findings reveal a new mechanism by which TLR3 activation regulates wound repair, and provide a potential therapeutic strategy for the treatment of skin wounds.

MATERIALS AND METHODS

Reagents

Caspase-1 inhibitor I and antibodies including loricrin and caspase-1 were purchased from Santa Cruz Biotechnology (USA). Antibodies directed against phospho-p38 MAPK and p38 MAPK were purchased from Cell Signaling Technology (Beverly, USA). The antibody for S100A7 and soluble recombinant human S100A7 were purchased from Sino Biological Inc (Beijing). The antibody against β-actin and inhibitors including SB202190, SP600125 and PD98059 were purchased from Sigma-Aldrich (USA). Poly(I:C) was purchased from InvivoGen (France).

Mice

All mice (C57BL/6) were housed and bred in specific pathogen-free conditions in the animal facilities of East China Normal University. All animal experiments were approved by East China Normal University Animal Care and Use Committee.

Cutaneous injury *in vivo*

For incisional wounds, full-thickness incisions were made on the dorsal skin of C57BL/6 wild-type mice by 8 mm biopsy punches. For burned wounds, a 8 mm-diameter iron block was preheated in boiling water for 10 min, and stuck to the dorsal skin of mice for 5 s. 2 mm of skin around wound edges or unwounded skin were taken at day 3 for protein extraction and RNA isolation as described previously (Lai et al., 2012). For S100A7 treatment, the dorsal skin of mice were intradermally injected with 100 μL phosphate buffer saline (PBS) or 100 μL S100A7 (5 ng μL^{-1}) for twice (6 and 24 h) before full-thickness incisions. The wounds were photographed every other days and the healing area was calculated by Image J.

Cell culture

Neonatal human epidermal keratinocytes (NHEKs) (Cascade Biologics, USA) were cultured in serum-free EpiLife medium (Cascade Biologics) containing 0.6 mmol L^{-1} Ca^{2+}, 1× Epilife® defined growth supplement (EDGS), 50 U mL^{-1} penicillin, and 50 μg mL^{-1} streptomycin under standard culture conditions.

shRNA preparation and siRNA

Oligonucleotides encoding human S100A7 (Table 1) were designed and synthesized. Blast search was performed by using the National Center for Biotechnology Information (NCBI) database to ensure that the shRNA constructs were targeting only human S100A7. The oligonucleotides which contained *Hpa* I and *Xho* I restriction sites were annealed and cloned into the pLL3.7 vector as the manufacturer described. The detailed method was described as previously reported (Lai et al., 2012). 3 pairs of siRNA targeting TLR3 were purchased from GenePharma (Table 2) company and transfected into NHEK cells as the manufacturer described.

Quantitative real-time PCR

Total RNA was isolated from wounded or unwounded mouse skin or cells by using RNAiso Plus (TaKaRa, Japan). 500 ng total RNA was used for reverse transcription by PrimeScript® RT reagent Kit (TaKaRa). Quantitative Real-time PCR was performed in a Stratagene Mx3005P Multiplex Quantitative PCR System (Agilent Technologies, USA). Quantitative real-time PCR specific primers as shown in Table 3 were used to evaluate gene expression. The comparative ΔΔCT method was used to determine the quantification of gene expression. The target gene expression in the test samples was normalized to the endogenous reference GAPDH level and reported them as the fold difference relative to GAPDH gene expression.

Immunoblot

2 mm skin taken from mouse wound edge 3 days after aseptic injury or cells were lysed by using RIPA buffer (pH 7.4) containing protease inhibitor cocktail (Roche, Switzerland),

Table 1 S100A7 shRNA oligonucleotides

	5′	STEMP	Loop	STEMP	3′
S100A7-shRNA					
S100A7-shRNA-1F	T	GTTTCACAAAT-ACACCAGACGT-GATGACA	TTCAAGAGA	TGTCAT-CACGTCTGGTG-TATTTGTGAAAC	TTTTTTC
S100A7-shRNA-1R	TCGAGAAAAAA	GTTTCACAAAT-ACACCAGACGT-GATGACA	TCTCTTGAA	TGTCAT-CACGTCTGGTG-TATTTGTGAAAC	A
S100A7-shRNA-2F	T	AAGCCTGCTGAC-GATGATGAAGGA-GAACT	TTCAAGAGA	AGTTCTCCTTCAT-CATCGTCAGCA-GGCTT	TTTTTTC
S100A7-shRNA-2R	TCGAGAAAAAA	AAGCCTGCTGAC-GATGATGAAGGA-GAACT	TCTCTTGAA	AGTTCTCCTTCAT-CATCGTCAGCA-GGCTT	A

Table 2 TLR3 siRNA oligonucleotides

	Forward (3′–5′)	Reverse (3′–5′)
Negative control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
h-TLR3-1757	GUCCCAUUUAUUUCCUAAATT	UUUAGGAAAUAAAUGGGACTT
h-TLR3-2025	GCGCUUUAAUCCCUUUGAUTT	AUCAAAGGGAUUAAAGCGCTT
h-TLR3 -2658	GGAGAUUCCAGAUUAUAAATT	UUUAUAAUCUGGAAUCUCCTT

Table 3 qPCR and RT-PCR primers

	Forward (3′–5′)	Reverse (3′–5′)
hFLG-(filaggrin)	TGGATCACTTGGATATAGACCACAAC	GCTTGAGCCAACTTGAATACCAT
hIVL-(involucrin)	GGTCCAAGACATTCAACCAGCC	TCTGGACACTGCGGGTGGTTAT
hLOR-(loricrin)	GGGCACCGATGGGCTTAG	GGTAGGTTAAGACATGAAGGATTTGC
hS100A7	GCTGACGATGATGAAGGAGAACT	GCGAGGTAATTTGTGCCCTTT
hGAPDH	CTTAGCACCCCTGGCCAAG	TGGTCATGAGTCCTTCCACG
mS100A7	AGCCATACTACATCACAGA	TACAGGAACTCATCAAAGC
hcaspase-1	TCACTGCTTCGGACATGACTACA	GGAACGTGCTGTCAGAGGTCTT
m18S	CATTCGAACGTCTGCCCTATC	CCTGCTGCCTTCCTTGGA
mS100A7(RT)	CCCTGGAGGAGTTGAAAG	CCGGTGGAACTGGAGATG

and then were sonicated on ice-cold water for 15 min. Protein concentrations of the extracts were measured by BCA™ Protein Assay Kit (Pierce, USA) and equal amount of total protein from each sample was separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to nitrocellulose membrane followed by probing with the indicated antibodies.

Immunofluorescence

5 μm paraffin-embedded tissue sections were dewaxed and pretreated with antigen retrieval solution. The sections were stained with the first antibodies (K14, Abcam; and Loricrin, Abcam, UK) and the secondary antibodies (Alexa Fluor 488 and 594 conjugate, Life Technologies, USA) and then mounted in ProLong Gold antifade reagent with 4,6-diamidino-2-phenylindole (DAPI) (Invitrogen, USA).

Statistical analysis

All data are presents as mean±SE, and Student's *t*-test was used to assess the significant difference between two groups. One-way or Two-way ANOVA was used for analyzing significant difference of multiple groups. For all statistical tests, we considered $P<0.05$ to be statistically significant.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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SUPPORTING INFORMATION

Figure S1 Poly(I:C) induces keratinocyte differentiation and the expression of S100A7 in keratinocytes, but not in macrophages and fibroblasts.

Figure S2 S100A7 activates p38 MAPK and caspase-1 to induce loricrin.

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