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SHORT COMMUNICATION

A novel peptide from the skin of amphibian *Rana limnocharis* with potency to promote skin wound repair

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ABSTRACT

In clinical trials, the healing of wounds remains a substantial physiological and financial incumbrance on patients. Therefore, the development of new drugs that can accelerate wound healing is vital. Based on genomic methods, we identified a new peptide (RL-RL10) with the amino acid sequence 'RLFKCWKKDS' from the skin of an amphibian frog species (*Rana limnocharis*). RL-RL10 promoted wound healing of human keratinocytes (HaCaT) in a concentration-dependent manner. RL-RL10 also had an effect on the migration and proliferation of HaCaT cells and promoted healing of a full-thickness wound in mice in a dose-dependent manner. In conclusion, we discovered RL-RL10 that promoted healing activity of cellular and animal wounds, thus providing a new peptide template for the development of novel wound-repairing drugs.

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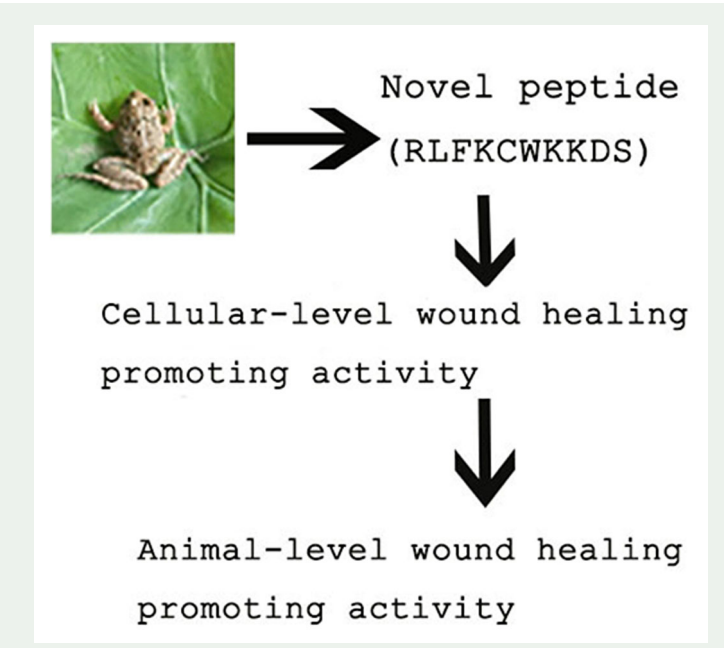

1. Introduction

Skin acts as a physical barrier between the body and external environment and participates in various critical physiological functions; Skin also protects tissues and organs from physical, mechanical, and chemical injury. However, the skin is highly vulnerable to damage and rupture (Heng 2011). In recent years, trauma cases have increased steadily, leading to significant clinical challenges in the treatment of chronic injury (Pereira et al. 2017). Currently, however, therapies and drug treatments for refractory skin injury remain unsatisfactory and new, more effective wound healing drugs are urgently required (Bian et al. 2018).

The skin of anuran amphibians is a rich source of compounds with a wide range of biological activity (Triana-Vidal et al. 2018; Spinelli et al. 2019; Zvejniece et al. 2019). *Rana limnocharis* is one of the most widely distributed species among Southeast Asian frogs. It occurs in all countries of Southeast Asia and much of the Asian region extending across western Japan, Taiwan, southwestern China, the Malay Peninsula, Nepal, the Philippines, Indonesia, India, and Pakistan (Liao et al. 2011). At present, only a few active peptides have been reported in *R. limnocharis* (Wang et al. 2016). It would be hopeful to find a variety of active peptides from this species. In the current study, we identified a new peptide (here in named RL-RL10) in the skin of *R. limnocharis* via genomic methods. Results showed that this peptide promoted cell scratch repair and wound healing in animal models. Therefore, this research provides a new template for the development of novel wound-healing drugs and therapeutics.

2. Results and discussion

2.1. Discovery of RL-RL10

Here, we obtained a 261-bp cDNA sequence encoding a peptide precursor from the skin of *R. limnocharis*. The precursor was 54 amino acid residues in length (Figure S1A) and showed similarity to previously identified antimicrobial peptides from frogs, including Odorranain-H-RA1, Esculentin-2a-02, and Esculentin-2a-03 (Figure S1B) based on a BLASTp search in the NCBI database (Vineeth Kumar et al. 2019). These peptides shared highly conserved sequences and could be divided into four parts: i.e., signal peptide, acidic segment, enzyme cleavage site, and the highly variable mature peptide. The mature peptide of RL-RL10 showed a linear motif, which was quite different from the cyclic motif of Odorranain-H-RA1, Esculentin-2a-02, and Esculentin-2a-03, which showed no obvious similarity with other bioactive frog peptides. Thus, the peptide was considered novel and was named RL-RL10, as per our previous reports (Song et al. 2019). Besides, the theoretical molecular weight of RL-RL10 was predicted as 1310.58 Da (PeptideMass: https://web.expasy.org/peptide_mass/).

2.2. RL-RL10 promoted HaCaT cell scratch closure

Keratinocyte migration and proliferation are important during the healing of cutaneous wounds. Pioneer keratinocytes exhibit early migration to a wound area and form a neo-epithelial layer to cover the wound for timely repair (Schultz et al. 2011). We performed an *in-vitro* cell scratch assay to investigate the effects of RL-RL10 on keratinocyte proliferation and migration. As illustrated in Figure S2A-B, the background healing rates of HaCaT cells 12 h and 24 h after plating were 19% and 39%, respectively. After the addition of RL-RL10 (100 nM), however, the healing rate increased significantly to 29% and 51% in the 12-h and 24-h groups, respectively (Figure S2A-B). As shown in Figure S2B, RL-RL10 significantly promoted wound healing both time and concentration dependently.

2.3. RL-RL10 promoted HaCaT cell proliferation and migration

Keratinocytes are highly important cells during the proliferation phase of wound healing (Schultz et al. 2011). MTT assay was performed to detect the effects of RL-RL10 on cell proliferation *in vitro*. As illustrated in Figure S2C, RL-RL10 promoted HaCaT proliferation in a concentration-dependent manner, which, in turn, contributed to the wound healing potency. A transwell experiment was performed on HaCaT cells using RL-RL10 at different concentrations (25, 50, 100 nM). Subsequently, the cells travelling to the bottom chamber were observed by microscopy. As illustrated in Figure S3A, RL-RL10 promoted the migration of HaCaT cells in a concentration-dependent manner. Results demonstrated that RL-RL10 (25, 50, 100 nM) significantly increased HaCaT cell migration compared with the vehicle (0 pM) at 24 h (Figure S3B), consistent with the above results. These observations suggest that RL-RL10 promoted cell migration in a concentration-dependent manner (Figure S3).

2.4. RL-RL10 promoted full-thickness wound healing in mice

As RL-RL10 significantly promoted cell scratch repair *in vitro*, we assumed that RL-RL10 could promote wound repair *in vivo*. We used the full-thickness wound model in mice in here. Compared with that of the negative control, RL-RL10 (100 nM) had a significant accelerating effect on repair of the residual wound area on post-injury days 4, 6, and 8 (Figure S4A). In addition, compared with the negative control, KFX (10 mg/ml) showed significant recovery activity (Figure S4B). Thus, results indicated that the rate of wound-healing was enhanced when the dose of RL-RL10 increase from 25 to 50 nM at the animal level. Based on histological analysis, mice topically treated with RL-RL10 (100 nM) showed a significant increase in the regeneration of epidermis and dermis compared with the saline group, as well as better granulation tissue contraction and thinner neo-epidermis formation (Figure S5A-C). On day 8, ideal re-epithelialization and well-formed granulation tissue were observed in both the RL-RL10-treated and KFX-treated (10 mg/ml) groups (Figure S5A). The newly formed thinner epidermis covered almost the entire wound area and the granulation tissue was well structured. In contrast, mice treated with saline formed a thicker hyper-proliferative wound epidermis and disorderly formed granulation tissue. Granulation and neo-epidermis tissue thickness were also measured (Figure S5B-C). Both KFX and RL-RL10 application resulted in the appearance of granulation tissue and markedly thinner epidermis than that in the saline group. Thus, RL-RL10 significantly accelerated the healing of skin wounds both dose and time dependently. Moreover, RL-RL10 accelerated the healing of skin wounds with less scarring.

2.5. RL-RL10 showed no direct antimicrobial activity, but weak antioxidant activity

RL-RL10 demonstrated no direct killing effect on gram-positive bacterial strains, gram-negative bacterial strains, or fungal strain at the maximum concentration of 100 nM (Table S1). For antioxidant reactions, RL-RL10 showed no scavenging activity against free radical DPPH (Figure S6A); however, at concentrations of 25 nM to 100 nM, RL-RL10 showed scavenging activity against free radical $ABTS^{+}$ (Figure S6B).

2.6. RL-RL10 showed no acute toxicity against mice

In the acute toxicity test, no lethal effects were observed on mice after a single intraperitoneal injection of RL-RL10 at doses of 10, 25, and 50 μmol/kg after 24 h (Table S2).

3. Conclusions

In this study, we identified a new 10-amino-acid-residue peptide (RL-RL10) from the skin of *R. limnocharis* by genomic methods. The identified amino acid sequence was 'RLFKCWKKDS'. Of note, RL-RL10 exhibited strong wound-healing-promoting activity *in vitro* and *in vivo*. RL-RL10 showed no antimicrobial activity or acute toxicity but did

exhibit weak antioxidant activity. Our study thus provides a new peptide template for research on novel wound-healing drugs and medicinal therapy.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Bian W, Meng B, Li X, Wang S, Cao X, Liu N, Yang M, Tang J, Wang Y, Yang X. 2018. OA-GL21, a novel bioactive peptide from Odorrana andersonii, accelerated the healing of skin wounds. Biosci Rep. 38(3). pii: BSR20180215.

Heng MC. 2011. Wound healing in adult skin: aiming for perfect regeneration. Int J Dermatol. 50(9):1058–1066.

Liao WB, Lu X, Shen YW, Hu JC. 2011. Age structure and body size of two populations of the rice frog Rana limnocharis from different altitudes. Italian J Zool. 78(2):215–221.

Pereira SG, Moura J, Carvalho E, Empadinhas N. 2017. Microbiota of chronic diabetic wounds: Ecology, impact, and potential for innovative treatment strategies. Front Microbiol. 8:1791.

Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. 2011. Dynamic reciprocity in the wound microenvironment. Wound Repair Regen. 19(2):134–148.

Song Y, Wu C, Zhang X, Bian W, Liu N, Yin S, Yang M, Luo M, Tang J, Yang X. 2019. A short peptide potentially promotes the healing of skin wound. Biosci Rep. 39(3).

Spinelli R, Aimaretti FM, López JA, Siano AS. 2019. Amphibian skin extracts as source of bioactive multi-target agents against different pathways of Alzheimer's disease. Nat Prod Res. :1–4.

Triana-Vidal LE, Castro MS, Pires Junior OR, Alvares ACM, de Freitas SM, Fontes W, Vargas JAG, Zuniga-Baos JA, Correia Batista IF, Grellier P, et al. 2018. Dendropsophin 1, a novel antimicrobial peptide from the skin secretion of the endemic Colombian frog Dendropsophus columbianus. Nat Prod Res. 32(12):1383–1389.

Vineeth Kumar T, Asha R, George S. 2019. Identification and functional characterisation of Esculentin-2 HYba peptides and their C-terminally amidated analogs from the skin secretion of an endemic frog. Nat Prod Res. :1–5.

Wang Y, Zhang Y, Lee WH, Yang X, Zhang Y. 2016. Novel peptides from skins of amphibians showed broad-spectrum antimicrobial activities. Chem Biol Drug Des. 87(3):419–424.

Zvejniece L, Dambrova M, Smits G. 2019. Total synthesis and *in vivo* evaluation of 8-deoxypumiliotoxin 193H. Nat Prod Res. :1–7.