European Journal of BioMedical Research
www.frenchsciencespg.com
ISSN: 2428-5544

Expression of chloride channels in mouse skin incisional wounds

Jinhuan Yue[1], Nannan Yu[1], Qinhong Zhang[1,2*]

[1] Department of Acupuncture and Moxibustion, Heilongjiang University of Chinese Medicine, Harbin, China.
[2] Department of Biomedical Data Science, Stanford University, CA, 94305, USA

Short Communication

Abstract: This study aimed to determine the types of chloride channels (CLCs) expressed in mouse skin wounds. Twelve male C57BL/6 mice were randomly divided into the skin wound group (SWG) and normal unwounded group (NUG), with 6 mice in each group. A 5-mm lancet wound was made by cutting into the full-thickness skin in the SWG, and the skin was kept intact in the NUG. The expression of CLCs in both groups was evaluated using real-time PCR (RT-PCR) and western blotting (WB). The results of RT-PCR showed that nine CLCs, including CLC-1, CLC-2, CLC-3, CLC-4, CLC-5, CLC-6, CLC-7, CLC-kα, and CLC-kβ, were expressed in both groups. However, the WB results showed that only CLC-2, CLC-7, CLC-kα, and CLC-kβ were expressed in both groups. The results of this study indicate that CLC-2, CLC-7, CLC-kα, and CLC-kβ were expressed in intact and wounded mouse skin according to both RT-PCR and WB analyses.

Key words: Skin wound; wound healing; chloride channels.

Correspondence to: zhangqh0451@163.com

Received: February 8 2017; **Accepted:** February 12 2017; **Published:** February 15 2017

doi: http://dx.doi.org/10.18088/ejbmr.3.1.2016.pp4-5

Introduction

Endogenous electric fields play a very critical role in the process of wound healing (1-2). It has been reported that they can improve wound healing by directing cell migration (3). Previous studies have found that applying exogenous electric fields helped to stimulate the division, migration, and differentiation of variety of cells, such as neuronal cells (4–5), fibroblasts (6), skin epithelial cells (7), and others.

Previous studies have found that several ion fluxes (Na^+, Cl^-, K^+, Ca^{2+}, H^+) contribute to mouse skin wound currents (8). Of those ion fluxes, Cl^- flux contributes the most to the wound current (8). In addition, electroacupuncture can also improve wound healing in mouse skin by increasing Cl- flux(9). Chloride channels (CLCs) consist of nine channels, including CLC-1, CLC-2, CLC-3, CLC-4, CLC-5, CLC-6, CLC-7, CLC-kα, and CLC-kβ. However, no study has determined what types of CLCs contribute to wound healing in mouse skin. This study aimed to determine the exact types of CLCs present in mouse skin wounds.

Materials and Methods

Twelve male C57BL/6 mice (18-23 g) were randomly divided into the skin wound group (SWG) and normal unwounded group (NUG), with six mice in each group. The mice in the SWG were anaesthetised with an intraperitoneal administration of pentobarbital sodium (60 mg/kg) before the measurement. Then, the back hair was shaved and antisepticised with VEET Cream. A lancet wound (about 5 mm in length) was incised through the full thickness of the skin (8).

RT-PCR analysis

For the RNA expression analysis of the intact and wounded mouse skin, total RNA was extracted using TRIzol (Invitrogen) and reverse transcribed using a Revert Aid first-strand cDNA synthesis kit (AccuPower™ RocketScript RT Premix). RT-PCR was performed in 2X GreenStar qPCR Master Mix (Applied Bioneer Inc., Alameda, CA, USA) using Power SYBR Green (Applied Biosystems). Then, the target mRNA was quantified and normalised to the internal control reference (GAPDH). The RT-PCR cycle number (CT) was used to calculate the relative gene expression. The relative quantification of the genes was calculated using $2^{-\Delta\Delta}$CT. $^{\Delta}$CT=CT-target gene–CTGAPDH. $^{\Delta\Delta}$CT=$^{\Delta}$CT (Experiment) -$^{\Delta}$CT(Control). The primers used are listed in Table 1.

Western blot analysis

For western blotting, cells from the mouse skin were harvested and lysed with Laemelli buffer containing 120 mM Tris-HCl (pH-6.8), 20% glycerol, and 4% SDS. Then, equal amounts of protein were separated by SDS PAGE and transferred to a nitrocellulose membrane (Millipore). The membrane was then incubated with primary antibody (overnight at 4 °C) and secondary antibody (3 h at room temperature). The blots were developed with a chemiluminescent substrate (Millipore). Image J software was used to measure the bands of each marker. The primary antibodies are listed in Table 2.

Results

The RT-PCR results showed that all nine CLCs were expressed in both intact and wounded mouse skin. These CLCs included CLC-1, CLC-2, CLC-3, CLC-4, CLC-5, CLC-6, CLC-7, CLC-kα, and CLC-kβ (Figure 1). The WB results showed that four CLCs were expressed in both groups of mice. These positive CLCs included CLC-2, CLC-7, CLC-kα, and CLC-kβ (Figure 2).

Table 1. List of primer sequences used for RT-PCR analysis.

Gene	Forward primer	Reverse primer
CLC-1	5'-GGATTCTTTGCAGCCACATT-3'	5'- AGCTGGGAGTTCCTTCAGGT-3'
CLC-2	5'-CCACCTTCTTCGCTGTTAGG-3'	5'-TTCTTCATCACGGTTCCACA-3'
CLC-3	5'-CCTGGCTGCTGATGTTATGA-3'	5'-TCTCTTCTGAGGGCAAATCC-3'
CLC-4	5'-CTTCCCTTTCATCCTGCTTG-3'	5'-CCGCAATAACCTCCAACACT-3'
CLC-5	5'-ATCGTGGTGGACATCTTCC-3'	5'-TTGGTTTGCCATCTGTGCTA-3'
CLC-6	5'-TGCCTGGATTGCTGAACTTT-3'	5'-CCCATCACGACGAAGAAAC-3'
CLC-7	5'-GTCACCTCACACTCGCTCAC-3'	5'-CCTACATCCTCCACCACAGG-3'
CLC- kα	5'-GAGTGGATGTGCCCTTTGAC-3'	5'-GGTGTAGCGATTGGTCTTGA-3'
CLC- kβ	5'-CCTCTCACTTCTCCGTCTGG-3'	5'-TGGTCTCCTGTTCATTGTTGA-3'
GAPDH	5'-AAGGCTGTGGGCAAGG-3'	5'-TGGAGGAGTGGGTGTCG-3'

Table 2. List of Antibodies used.

Antibody	Cat.No.	Company
Anti-ACTb	PAB340Mi01	Cloud-Clone Corp.
Anti-CLC-2	BS7915	Bioworld Technology,Inc.
Anti-CLC-3	MABN490	Bioss
Anti-CLC-4	C15079	Assay Biotechnology Company, Inc.
Anti-CLC-5	BS7632	Bioworld Technology,Inc.
Anti-CLC-7	C15082	Assay Biotechnology Company, Inc.
Anti-CLC- kα	LS-C332792	LifeSpan BioSciences, Inc.
Anti-CLC- kβ	Ab66460	Abcam

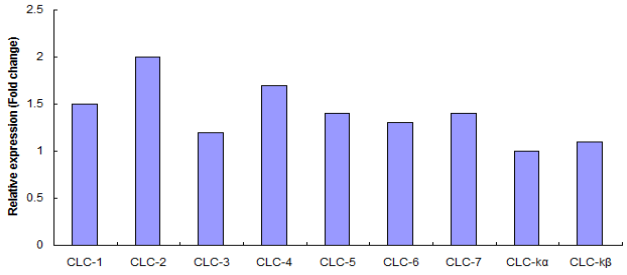

Figure 1. Expression of CLCs by RT-PCR test.

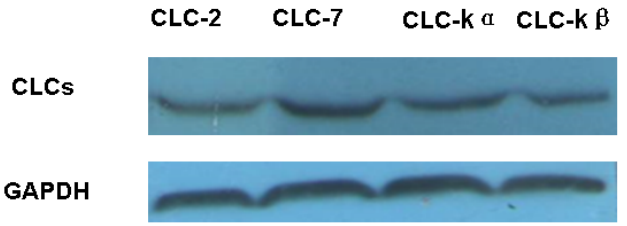

Figure 2. Expression of CLCs by western blot test.

Discussion

It has been found that endogenous currents often play a very important role in the process of wound healing. Based on this evidence, other studies have demonstrated that exogenous electric fields can help to improve wound healing.

A previous study reported that Cl^- flux contributed the most to the skin wound current (8). The results of this study also indicted that Cl^- flux increased significantly after mouse skin was incised compared with that in normal mice skin. However, no study has determined what types of CLCs are expressed using both RT-PCR and WB analyses.

In this study, we found using RT-PCR that all nine CLCs, including CLC-1, CLC-2, CLC-3, CLC-4, CLC-5, CLC-6, CLC-7, CLC-kα, and CLC-kβ, were expressed in both intact and wounded mouse skin. However, the WB results indicated that only four CLCs were expressed. These CLCs included CLC-2, CLC-7, CLC-kα, and CLC-kβ.

In summary, four CLCs were expressed in both intact and wounded mouse skin. In future studies, we will explore the effect of Bangci electroacupuncture therapy on these four CLCs in wounded mouse skin and explore the mechanism involved.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 81303045).

References

1. Zhao M, Song B, Pu J, Wada T, Reid B, et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature 2006; 442(7101): 457–60.
2. Zhao M, Forrester JV, McCaig CD. A small, physiological electric field orients cell division. Proc Natl Acad Sci USA 1999; 96: 4942–6.
3. Zhao M. Electrical fields in wound healing–an overriding signal that directs cell migration. Semin Cell Dev Biol 2009; 20(6): 674–82.
4. Hinkle L, McCaig CD, Robinson KR. The direction of growth of differentiating neurons and myoblasts from frog embryos in an applied electric field. J Physiol (Lond) 1981; 314: 121–35.
5. Patel N, Poo MM. Orientation of neurite growth by extracellular electric fields. J Neurosci 1982; 2: 483–96.
6. Robinson KR. The responses of cells to electric fields: a review. J Cell Biol 1985; 101(6): 2023–7.
7. Ojingwa JC, Isseroff RR. Electrical stimulation of wound healing. J Invest Dermatol 2003; 121: 1–12.
8. Sun Z, Yue J, Zhang Q. Ionic Components of Wound Current at Mouse Skin Incisional Wounds. Eur J Bio Med Res 2015: 1(1):3-7.
9. Yue JH, Sun ZR, Zhang QH. Effect of electroacupuncture Bangci on chloride flux at mouse skin incisional wounds. Eur J Bio Med Res 2017: 3(1):1-3.