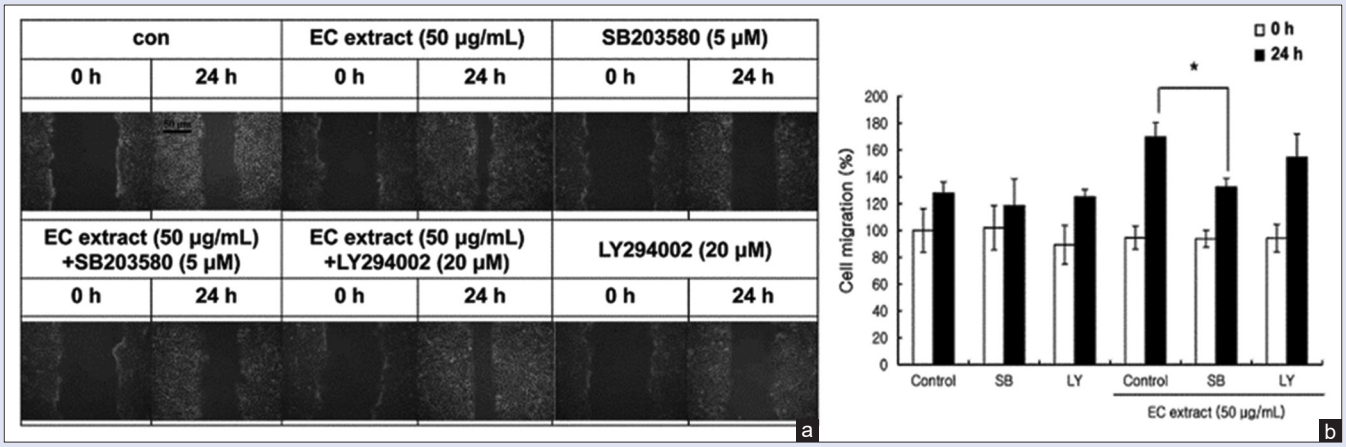

Figure 4: Effects of LY294002 and SB203580 on *Eucheuma cottonii* extract-mediated keratinocyte migration. HaCaT cells (5×10^5 cells/well) were seeded into a 6-well plate and serum starved for 24 h. Thereafter, wounds were made in cultures as described in "Materials and Methods." HaCaT cells were pretreated with LY294002 (20 μM) and SB203580 (5 μM) for 30 min, followed by addition of the *Eucheuma cottonii* extract (50 μg/mL) and were then incubated for 24 h. (a) Phase contrast images of wound widths were captured using a digital video camera. (b) Quantification of the cell migration rate is shown in the graph. HaCaT cells were maintained for 0 h and 24 h. Data represent the mean ± standard deviation of wound widths in 10 randomly chosen fields expressed as percentages of the control. *$P < 0.01$ compared to the *Eucheuma cottonii* extract-treated control

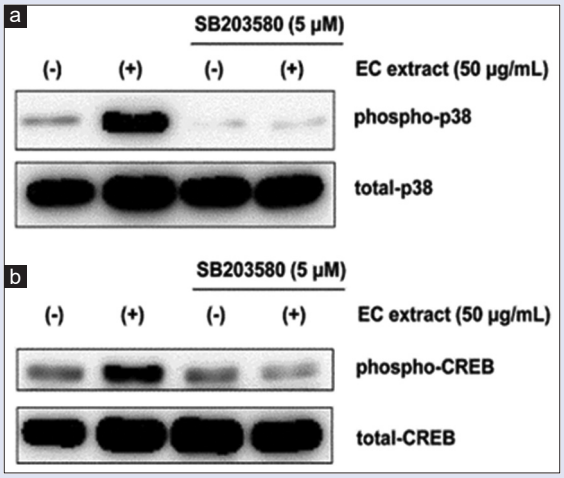

Figure 5: Effects of SB203580 on *Eucheuma cottonii* extract-mediated keratinocyte migration. After serum starvation, HaCaT cells were pretreated with SB203580 (5 μM) for 30 min, followed by the *Eucheuma cottonii* extract (50 μg/mL), and were then incubated for 24 h. Protein samples were analyzed by western blotting with antibodies specific for phospho-p38 mitogen-activated protein kinase (a) and phospho-cAMP response element-binding protein (b). Equal protein loading was confirmed by total p38 and cAMP response element-binding protein expression levels

scratch migration assay. In another study, LY294002 was shown to block insulin-induced keratinocyte migration.[30] However, in our study, LY294002 only blocked EC extract-induced keratinocyte migration slightly [Figure 4]. Thus, the EC extract had little influence on the Akt pathway.

There are several studies indicating that p38 MAPK is related to keratinocyte migration. Hypoxia was reported to regulate keratinocyte migration through p38 MAPK activation.[31] Moreover, the extract of *Centella asiatica*, a medicinal plant containing madecassoside and madecassic acid, was found to activate p38 MAPK that is involved in keratinocyte migration.[32] Our data also showed that the EC extract strongly induced p38 MAPK phosphorylation [Figure 3b]. Another study showed that SB203580 inhibited p38 MAPK-induced keratinocyte migration.[31] As shown in Figure 4, SB203580 downregulated EC extract-induced keratinocyte migration. After we identified that EC extract provokes keratinocyte migration via p38 MAPK, we attempted to examine the downstream pathway of p38 MAPK. Although the relationship between p38 MAPK and keratinocyte migration has been demonstrated, downstream molecules of p38 MAPK has not been studied in keratinocytes till date. Interleukin-6 induces fibroblast migration through p38 MAPK and its downstream molecule CREB.[25] Therefore, we hypothesized that EC extract induces keratinocyte migration through the p38 MAPK/CREB pathway. As shown in Figure 3b, EC-extract induced CREB activation. Moreover, EC extract-induced phosphorylation of CREB was blocked by SB203580 [Figure 5b]. These data demonstrated that CREB plays a key role in EC extract-induced keratinocyte migration.

In summary, our results indicated that EC extract promotes keratinocyte migration. We demonstrated that p38 MAPK and CREB are involved in EC extract-induced keratinocyte migration. Thus, EC extract could be considered as a new treatment option for wound healing.

Acknowledgements

The author thank the Chung-Ang University Research for support.

Financial support and sponsorship

This research was supported by the Chung-Ang University Research Scholarship grants in 2019.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ahmed AB, Adel M, Karimi P, Peidayesh M. Pharmaceutical, cosmeceutical, and traditional applications of marine carbohydrates. Adv Food Nutr Res 2014;73:197-220.
2. Cheung RC, Ng TB, Wong JH. Marine peptides: Bioactivities and applications. Mar Drugs 2015;13:4006-43.
3. Matanjun P, Mohamed S, Muhammad K, Mustapha NM. Comparison of cardiovascular protective effects of tropical seaweeds, *Kappaphycus alvarezii*, *Caulerpa lentillifera*, and

Sargassum polycystum, on high-cholesterol/high-fat diet in rats. J Med Food 2010;13:792-800.

4. Sudirman S, Hsu YH, He JL, Kong ZL. Dietary polysaccharide-rich extract from *Eucheuma cottonii* modulates the inflammatory response and suppresses colonic injury on dextran sulfate sodium-induced colitis in mice. PLoS One 2018;13:e0205252.
5. Shamsabadi FT, Khoddami A, Fard SG, Abdullah R, Othman HH, Mohamed S. Comparison of tamoxifen with edible seaweed (*Eucheuma cottonii* L.) extract in suppressing breast tumor. Nutr Cancer 2013;65:255-62.
6. Anderson NS, Dolan TC, Rees DA. Carrageenans. VII. Polysaccharides from *Eucheuma spinosum* and *Eucheuma cottonii*. The covalent structure of L-carrageenan. J Chem Soc Perkin 1 1973;19:2173-6.
7. Beer HD, Gassmann MG, Munz B, Steiling H, Engelhardt F, Bleuel K, *et al.* Expression and function of keratinocyte growth factor and activin in skin morphogenesis and cutaneous wound repair. J Investig Dermatol Symp Proc 2000;5:34-9.
8. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature 2008;453:314-21.
9. Singer AJ, Clark RA. Cutaneous wound healing. N Engl J Med 1999;341:738-46.
10. Raja, Sivamani K, Garcia MS, Isseroff RR. Wound re-epithelialization: Modulating keratinocyte migration in wound healing. Front Biosci 2007;12:2849-68.
11. Yamaguchi Y, Yoshikawa K. Cutaneous wound healing: An update. J Dermatol 2001;28:521-34.
12. Chrissouli S, Pratsinis H, Velissariou V, Anastasiou A, Kletsas D. Human amniotic fluid stimulates the proliferation of human fetal and adult skin fibroblasts: The roles of bFGF and PDGF and of the ERK and Akt signaling pathways. Wound Repair Regen 2010;18:643-54.
13. Lima MH, Caricilli AM, de Abreu LL, Araújo EP, Pelegrinelli FF, Thirone AC, *et al.* Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: A double-blind placebo-controlled clinical trial. PLoS One 2012;7:e36974.
14. Costa M, Marchi M, Cardarelli F, Roy A, Beltram F, Maffei L, *et al.* Dynamic regulation of ERK2 nuclear translocation and mobility in living cells. J Cell Sci 2006;119:4952-63.
15. He M, Xue ZM, Li J, Zhou BQ. Breviscapine inhibits high glucose-induced proliferation and migration of cultured vascular smooth muscle cells of rats via suppressing the ERK1/2 MAPK signaling pathway. Acta Pharmacol Sin 2012;33:606-14.
16. Matsubayashi Y, Ebisuya M, Honjoh S, Nishida E. ERK activation propagates in epithelial cell sheets and regulates their migration during wound healing. Curr Biol 2004;14:731-5.
17. Shono T, Kanetake H, Kanda S. The role of mitogen-activated protein kinase activation within focal adhesions in chemotaxis toward FGF-2 by murine brain capillary endothelial cells. Exp Cell Res 2001;264:275-83.
18. Anand-Apte B, Zetter BR, Viswanathan A, Qiu RG, Chen J, Ruggieri R, *et al.* Platelet-derived growth factor and fibronectin-stimulated migration are differentially regulated by the Rac and extracellular signal-regulated kinase pathways. J Biol Chem 1997;272:30688-92.
19. Lee S, Kim MS, Jung SJ, Kim D, Park HJ, Cho D. ERK activating peptide, AES16-2M promotes wound healing through accelerating migration of keratinocytes. Sci Rep 2018;8:14398.
20. Yu J, Peng H, Ruan Q, Fatima A, Getsios S, Lavker RM. MicroRNA-205 promotes keratinocyte migration via the lipid phosphatase SHIP2. FASEB J 2010;24:3950-9.
21. Li W, Nadelman C, Henry G, Fan J, Muellenhoff M, Medina E, *et al.* The p38-MAPK/SAPK pathway is required for human keratinocyte migration on dermal collagen. J Invest Dermatol 2001;117:1601-11.
22. Harper EG, Alvares SM, Carter WG. Wounding activates p38 map kinase and activation transcription factor 3 in leading keratinocytes. J Cell Sci 2005;118:3471-85.
23. Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol 1988;106:761-71.
24. Jeong YM, Park WJ, Kim MK, Baek KJ, Kwon NS, Yun HY, *et al.* Leucine-rich glioma inactivated 3 promotes HaCaT keratinocyte migration. Wound Repair Regen 2013;21:634-40.
25. Nishikai-Yan Shen T, Kanazawa S, Kado M, Okada K, Luo L, Hayashi A, *et al.* Interleukin-6 stimulates Akt and p38 MAPK phosphorylation and fibroblast migration in non-diabetic but not diabetic mice. PLoS One 2017;12:e0178232.
26. Reinke JM, Sorg H. Wound repair and regeneration. Eur Surg Res 2012;49:35-43.
27. Turchi L, Chassot AA, Rezzonico R, Yeow K, Loubat A, Ferrua B, *et al.* Dynamic characterization of the molecular events during *in vitro* epidermal wound healing. J Invest Dermatol 2002;119:56-63.
28. Shibata S, Tada Y, Asano Y, Hau CS, Kato T, Saeki H, *et al.* Adiponectin regulates cutaneous wound healing by promoting keratinocyte proliferation and migration via the ERK signaling pathway. J Immunol 2012;189:3231-41.
29. Zhao X, Guan JL. Focal adhesion kinase and its signaling pathways in cell migration and angiogenesis. Adv Drug Deliv Rev 2011;63:610-5.
30. Liu Y, Petreaca M, Yao M, Martins-Green M. Cell and molecular mechanisms of keratinocyte function stimulated by insulin during wound healing. BMC Cell Biol 2009;10:1.
31. Jiang X, Guo X, Xu X, Teng M, Huang C, Zhang D, *et al.* Hypoxia regulates CD9-mediated keratinocyte migration via the P38/MAPK pathway. Sci Rep 2014;4:6304.
32. Singkhorn S, Tantisira MH, Tanasawet S, Hutamekalin P, Wongtawatchai T, Sukketsiri W. Induction of keratinocyte migration by ECa 233 is mediated through FAK/Akt, ERK, and p38 MAPK signaling. Phytother Res 2018;32:1397-403.