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Calcitonin-like peptide from Ciona intestinalis stimulates osteoblast alkaline phosphatase activity and mineralization in MC3T3-E1 cells

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This study examined the effects of Ciona intestinalis calcitonin-like peptide (CCLP) on osteoblast differentiation and mineralization in the culture system of MC3T3-E1 cells. The primary structures of the CCLP containing Cys-Asp-Gly-Val-Ser-Thr-Cys-Trp-Leu-His-Glu-Leu-Gly-Asn-Ser-Val-His-Ala-Thr-Ala-Gly-Gly-Lys-Gln-Asn-Val-Gly-Phe-Gly-Pro-NH2 was synthesized automatically using the solid phase method with fluorenylmethoxycarbonyl (Fmoc) resin. Pre-osteoblast MC3T3-E1 cells were cultured with various concentrations of CCLP (7.5, 15, and 30 μM) during the osteoblast differentiation period. To examine osteoblast differentiation, alkaline phosphatase (ALP) activity was determined by reading the absorbance at 405 nm using a spectrophotometer, and mineralization was evaluated by staining with Alizalin red S. Moreover, the expression of differentiation markers such as ALP, osteocalcin (OSC), and osteopontin (OPN) were measured using RT-PCR and Western blot analysis. The results showed that CCLP did not exhibit any cytotoxic effect on MC3T3-E1 cells even at the highest concentration (30 μM) at 2 and 5 days. CCLP also enhanced MC3T3-E1 cells proliferation, differentiation, and mineralization demonstrated by the increased expression of several osteoblast phenotype markers such as ALP, and Alizarin red S staining. In addition, the CCLP induced mitogen-activated protein kinase (MAPK) pathway in MC3T3-E1 cells. These results suggest that CCLP exerts positive effects on osteoblast differentiation and may represent a potential target for pharmaceutical development.

Biography

Van-Tinh Nguyen received his PhD from the Department of Biomedical Engineering, Pukyong National University, Korea. He graduated in 2012 from the University of Chosun and his current research interests include the isolation, safety and bioavailability of bioactive materials; development of marine-integrated cells and tissue regenerative biomedical substances.

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