JOURNAL OF FUNCTIONAL FOODS 14 (2015) 395–406

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The promoting effects of sesamin on osteoblast differentiation of human mesenchymal stem cells

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ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 21 January 2015
Accepted 27 January 2015
Available online 26 February 2015

Keywords:
Sesamin
MSCs
p38
ERK
Osteoblast differentiation

ABSTRACT

Sesamin, a major lignan in sesame seeds and oils, is well known for its health promotion activity. However, its effects on bone cell functions are infrequently observed. In the study, the effects of sesamin on the differentiation of human mesenchymal stem cells (MSCs), osteoblasts, were examined. The differentiation of cells into osteoblasts suggested that sesamin (SE) in combination with osteogenic factors (OS) significantly increased calcium deposition. Although the osteoblastic differentiation markers including bone morphogenetic protein 2 (*BMP-2*), type I collagen (*COL1A1*) and alkaline phosphatase (*ALP*) of each cell donor showed different expressions both in gene type and time point, the increasing trends of *COL1A1* and *ALP* were observed within the condition of OS + SE being compared with OS treatment. Increased ALP activity in OS + SE treatment could mediate the stimulatory effect of sesamin on osteoblast differentiation. Increased phosphorylation of p38 and ERK were related to increasing of ALP activity and mineralization in OS + SE treatment. Hence, it is suggested that sesamin enhanced osteoblast differentiation via activation of those MAPK signalling cascades. All results present that sesamin benefits differentiation of osteoblast progenitors towards functional bone forming cells, osteoblast. Thereby, sesamin would be a potential food supplement promoting bone health.

1. Introduction

Sesame seed and oil from *Sesamum indicum* L. are commonly used as dietary supplements and seasoning. The plant is widely cultivated in Asian and African countries. The oil of the seeds has a high content of beneficial nutrients including proteins, fats, minerals and health-beneficial lignans, such as sesamin and sesamolin. Various pharmaceutical and physiological effects of sesame have been reported as stemming from its nutrients and bioactive compounds. Jeng, Hou, Wang, and Ping (2005) suggested that health benefits of sesame seeds may be attributed to its lignans, especially sesamin. Sesamin, a major lignan found in sesame seeds and oils, exhibits various

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http://dx.doi.org/10.1016/j.jff.2015.01.046
1756-4646/

biochemical actions, such as having modulatory effects on fatty acids (Umeda-Sawada, Fujiwara, Abe, & Seyama, 2003) and cholesterol metabolism (Wu, Kang, Wang, Jou, & Wang, 2006). Sesamin also has an inhibition effect on carcinogenesis (Harikumar et al., 2010) and an ability to protect neuronal cells against oxidative stress (Hamada et al., 2009). Recently, sesame seed lignans have been described in relation to their high physiological functions and possible use as ingredients in functional foods and nutraceuticals (Kamal-Eldin, Moazzami, & Washi, 2011). Boulbaroud, Mesfioui, Arfaoui, Ouichou, and el-Hessni (2008) as well as El Wakf, Hassan, and Gharib (2014) suggested that the sesame oil's recovery functions may come from its lignans, based on the bone loss conditions observed in ovariectomized rats. However, there is still little evidence concerning the molecular mechanism which sesame lignans have on bone cell differentiation and function.

Bone is an ossified tissue playing a variety of roles in human physiology, including protection, structural stabilization, physical movement, as a haematopoietic reservoir, mineral homeostasis, and in blood pH regulation. In adult life, bone continuously repairs itself through a mechanism of bone resorption by osteoclasts and bone formation by osteoblasts. This repairing mechanism is called the bone remodelling process. This process is tightly regulated by bone cells themselves (Matsuo & Irie, 2008) and also by several environmental factors such as cytokines, growth factors and hormones (Qu, Harkonen, Monkkonen, & Vaananen, 1999). In situations where bone repair is not functioning correctly, bone disorders may develop, which can either increase bone volume or cause a loss of bone. Osteoblasts, bone-forming cells, originate from progenitors called mesenchymal stem cells or MSCs. Mesenchymal stem cells have, for many years, been of interest as a promising cellular therapeutic approach (Gimble, Guilak, & Bunnell, 2010) including cartilage repair (Johnson et al., 2012) and bone regeneration (Dawson, Kanczler, Tare, Kassem, & Oreffo, 2014). In addition, the osteogenic induction of MSCs is a good model for osteoblast differentiation research and useful in the examination of the effects of interested agents on bone cell metabolism. Under *in vitro* conditions, MSCs can be stimulated to differentiate into osteoblasts by chemical factors, specifically β-glycerophosphate, ascorbic acid and dexamethasone. During osteoblast cell differentiation, the cell phase is divided into three stages: (1) cell proliferation, (2) extracellular matrix formation, and finally (3) mineralization. Bone formation-related genes up-regulate differently at each time point of differentiation. Hence, they can be used as markers for osteoblast differentiation. For instance, bone morphogenetic protein-2 (BMP-2), a member of the transforming growth factor-β (TGF-β) superfamily, is an important growth factor triggering osteoblast differentiation through the BMPs-Smad signalling pathway (Miyazawa, Shinozaki, Hara, Furuya, & Miyazono, 2002; Ryoo, Lee, & Kim, 2006). Type I collagen (COL1), a structural protein, supports both cell proliferation and matrix formation which occur in the early phase of differentiation, whereas initiation of the mineral deposition process can be reflected by higher expression of alkaline phosphatase (ALP), an early marker of mineralization. This study is interested in the effects of sesamin on the osteogenic differentiation capacity of human MSCs. The results represented that sesamin under osteogenic-induced MSC conditions enhanced osteoblast differentiation of MSCs as evidenced by an increase of ALP activity and as a consequence of the mineralization process. Therefore, sesamin may have a potential for food supplementation maintaining bone integrity and preventing bone loss.

2. Materials and methods

2.1. *Cell isolation and culture*

The non-pathological appearance of human trabecular bone was obtained from arthroplasty surgery (of 18–45 year olds) with the patients' consent and approval by the research ethics committee, faculty of medicine, Chiang Mai University (Ethical approved number ORT-12-1089-FB). Cell isolation and characterization was applied from previous descriptions (Sanchez-Guijo et al., 2009). Bone pieces were washed with a serum-free Dulbecco's modified Eagle's medium, DMEM (Gibco, Grand Island, NY, USA) containing 100 units/ml of penicillin and 100 μg/ml of streptomycin (Gibco, Grand Island, NY, USA). Cells within the bone pieces were aspirated with a syringe in order to flush the cells out of the bone cavity. The bone pieces were discarded, and the cell suspension was centrifuged at 1660 *g* for 5 min. The cell pellet was re-suspended and cultured in a 25 cm^2 culture flask in a 10% foetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), supplemented with DMEM in a humidified incubator with 5% CO_2 at 37 °C. Two days later, the medium was changed in order to remove un-adherent cells, and the media were changed every 3 days. When cells were at 80% confluence, cells were trypsinized and expanded into a 75 cm^2 culture flask (passage 0). The cells were repeatedly expanded until enough cells were available for assay in each experiment. Cells in the same passage were used for all experiments.

2.2. *Cell characterization*

Adherent cell culture was used for immunophenotypic analysis. Cells were trypsinized and centrifuged to obtain a cell pellet (5×10^5). The cells were pre-incubated with 10% human AB serum for 30 min at 4 °C. The cells were probed with fluorescent-conjugated antibodies, specifically CD34, CD105, CD45 and CD90 at 4 °C for 30 min. Antibodies were purchased from Biolegend, Sandiago, CA, USA. The cells were washed twice with 1% BSA-PBS and readied for analysis by a flow cytometer.

2.3. *Sesamin treatment*

Cells at passage 4 were seeded at a density of 3×10^4 cells/cm^2 in 24 well plates unless indicated and left until confluence. Sesamin was prepared as previously described (Phitak et al., 2012). The dosage amount of sesamin used in the study was based on a previous report which stated that sesamin amounts between 0.3 and 20 μg/ml is not toxic to the lineage of osteoblast cells or their progenitors. In accordance, this study used a minimum effective dose of 5 μg/ml (Wanachewin, Boonmaleerat, Pothacharoen, Reutrakul, & Kongtawelert, 2012). Thus, cells were exposed to 5 μg/ml sesamin (SE) with or without osteogenic factors (OS) containing 50 μg/ml ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA), 0.1 mM