ACTIN FILAMENTS IN RESPONSE TO CHEMICAL OSTEOGENESIS SUPPLEMENTS ALTER THE MULITCELLULAR BEHAVIOR OF OSTEOCYTIC SPHEROIDS

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Introduction

Osteocytes are the most abundant cells in the bone to play an important role as control tower for bone remodeling. Our group has developed 3D osteocytic spheroids reconstructed by human mesenchymal stem cells (MSCs) [1, 2]. In this study, we investigated the multicellular behavior of the osteocytic spheroids in response to chemically induced osteogenic supplements (OS).

Method

We fabricated scaffold-free spheroids using human MSCs (Riken BRC, Japan). 2500 cells were subcultured in each well of U-bottom ultra-low attachment dish (Thermo Fisher, USA). As chemical OS, 50 µM ascorbic acid, 10 mM b-glycerophosphate, and 100 nM dexamethasone were added in high glucose DMEM (Gibco, USA). The spheroids were cultured for 2 or 7 days with or without the OS to measure their projected area. The samples were then collected and stained with Alexa Fluor 488 Phalloidin (Invitrogen, USA) and Hoechst 33342 (Invitrogen, USA).

Results and Discussion

As shown in Fig. 1(A) and (B), the sizes of the spheroids were decreased time-dependently and further shrunk by addition of the OS. We also conducted a fusion experiment of two spheroids as well as collagen embedding experiment of the spheroids in response to the OS. The results showed that the OS delayed the fusion speed of the two spheroids and dissociation speed of the spheroids embedded in the collagen matrix. To reveal the mechanism, we observed the actin filaments (F-actin) in the spheroid using the LSM880 (Carl Zeiss, Germany) with the Airyscan detector. From the super-resolution images of the F-actin in the spheroids in Fig. 1(C) – (H), we first found out that the F-actin on the surface of the spheroids was more tightly generated in the presence of the OS when compared to the one in the absence of the OS. The tight F-actin generated the greater tension to squeeze the spheroid, resulting in the smaller size. Furthermore, the tight F-actin played a role as a barrier in the fusion experiment and collagen embedding experiment, which delays the fusion speed and dissociation speed by capturing and disturbing the cells inside the spheroid to break up the barrier. In this study, we firstly reported the importance of the F-actin in the spheroids to modulate the multicellular behaviors of the spheroid model. Moreover, the three different experiments conducted in this study might become useful *in vitro* experiments to anticipate and measure the multicellular behaviors of the 3D culture model such as organoids.

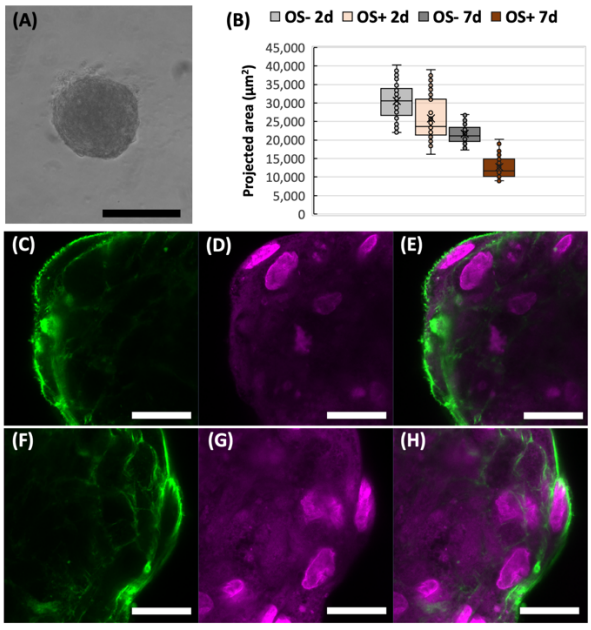

Figure 1: (A) Spheroid reconstructed by human mesenchymal stem cells after 2-day culture in the presence of chemical osteogenic supplements (OS). A black bar indicates 200 µm. (B) Evaluation of projected area of the spheroids after 2- and 7-day incubation in the presence or absence of the OS. Fluorescence image of actin filaments (F-actin) and nuclei in the spheroid; (C), (F) F-actin, (D), (G) nuclei, and (E), (H) merge image of the spheroid without or with the OS, respectively. White bars indicate 20 µm.

References

1. Kim et al, Sci Rep, 11, 13204, 2021.
2. Kim et al, Biochem Biophys Res Commun, 622, 79-85, 2022.

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