The Effect of Cellular Spreading Area and Shape on Apoptosis and Differentiation of Osteoblasts

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Abundant evidences has shown that extracellular matrix (ECM) plays critical roles in regulating proliferation and differentiation of cell. In vivo, osteoblasts located on the surface of trabecular bone finally differentiate into osteocytes after being embedded within the mineralized matrix. In addition, osteoblasts closely set on the trabecular bone, but mature osteocytes sparsely distribute inside bone minerals, which implies that the major parts of osteoblasts enter into apoptosis. According to the phenomenon in the transition from osteoblasts to osteocytes, we have confidence to hypothesize that the constraints of extracellular matrix which control cell spreading geometry can affect cell physiological properties, e.g. apoptosis and differentiation.

In this study, osteoblastic cells (MC-3T3) were cultured on a micropatterned surface to make sure that only one cell was seeded onto an geometry-designed island. Four kinds of island shapes with decreasing circularity were designed, i.e., circle, circle with short, medium or long branches. The area of every island shape varied as 314, 615, 1256 square micrometers. At 3, 6, 12, 24 or 48 hours after cell seeding, the apoptosis were detected using TUNEL assay kits. At one or five days after cell seeding, immunofluorescent staining was adopted to measure the expression of alkaline phosphatase (ALP), collagen I (COL I), osteocalcin (OCN), three makers of differentiation for osteoblasts.

The experimental results showed that, the actual spreading area of osteoblasts stained by DiI seeded on the micropatterned islands coincided with the designed area. The results of apoptosis assay implied that the percentage of apoptotic osteoblasts decreased along with the increase of islands' area. For different kinds of islands with the same area, cellular apoptosis was significantly reduced in osteoblasts with longer branches comparing with circular islands. The observations for osteoblastic differentiation demonstrated that the differentiation was reduced with the increase of spreading area. Circular shape promotes the expression of differentiation markers but the shape with branches decreased the differentiation even with same area. The above results reveal the spreading area and shape regulate osteoblastic differentiation and apoptosis, especially the spreading shape plays more critical roles.

In future study, we have confidence that our quantitative research will not only help to give insight into the apoptosis and differentiation mechanism of osteoblasts, but also make a breakthrough in the dynamic of cytoskeleton and focal adhesion related to the cell-matrix interaction.

References:

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