Osteoblast morphological changes is autocrine-Sonic hedgehog-dependent in a bio-inspired three-dimensional in vitro model

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INTRODUCTION: Three-dimensional in vitro model has facilitated the analysis of cell physiology under conditions that more closely resemble an in vivo-like niche compared to conventional two-dimensional cell culture. Furthermore, it has led to significant progress in understanding reciprocal and adaptive interactions among cells and the 3D-matrix models.
OBJECTIVE: Our purpose was to evaluate the intracellular mechanisms in response to bio-inspired three-dimensional in vitro model by using peptide array.
MATERIAL AND METHODS: MC3T3-E1 pre-osteoblast cells were cultured up to 10 days and the biological samples were collected to perform peptide array (Pepchip). Pepchip results were validated either by using western blotting or treating the cells with Shh inhibitor.
RESULTS AND DISCUSSION: In this work, we have shown that osteoblast cultured onto bio-inspired three-dimensional in vitro model (Matrigel) curiously presented tremendous morphological changes up to 10 days in culture. As the molecular mechanisms responsible for guiding osteoblast morphological changes remain unclear, we decided to explore this biological model to identify intracellular events correlating with this process, mainly looking for protein signalling network. In order to fully access the protein signalling network, we generated phosphoproteome profiles employing peptide arrays exhibiting over 1000 kinase peptide consensus substrates and used the obtained profiles to generate comprehensive overviews of the changes in cellular signalling associated with osteoblast morphological changes in this three-dimensional in vitro model. As these profiles contain a strong hedgehog signal transduction fingerprint we investigated the role of hedgehog signalling in this context and observed that hedgehog is active, necessary and sufficient to promote osteoblasts morphological changes.
CONCLUSION: Taken the results into account we conclude that the critical role of hedgehog in bone homeostasis may be partially mediated by its action in formation and maintenance of this tissue.

Keywords: Osteoblast; Sonic Hedgehog; 3D matrices; Biomaterials; Bioengineering.

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