Bioinformatizing Osteoblast Adhesion: A Critical View of Peptide Arrays

Milani, R.[1], Zambuzzi, W.F.[1], Diks, S.[2], Peppelenbosch, M.P.[2], Aoyama, H.[1], Ferreira, C.V.[1], Galembeck, E.[1]

[1]Department of Biochemistry, University of Campinas, Campinas, Brazil;

[2]Department of Cell Biology, Groningen University Medical Centre, Groningen, The Netherlands.

Although essential and critical to perfect bone development and healing, osteoblast adhesion is a poorly characterized process when considering molecular aspects. It involves several signaling pathways controlling cell adhesion, spreading and proliferation. The disruption of these cellular events can be related to many bone diseases. Therefore, understanding the protein interactions present in these pathways can provide us checkpoints to assess defective relationships and their importance through the overall network, bringing direct intervention spots to light. Pre-osteoblasts (MC3T3-E1) were seeded on polystyrene surface and the cell lysate was collected 2 h later, an adequate period for the adhesion of these cells, as demonstrated by images from fluorescence microscopy that reveal round-shaped (spreading) cells. Next, we evaluated the *in vitro* phosphorylation of peptide arrays exhibiting the majority of Phospho.ELM-deposited protein sequences from MC3T3-E1 lysate. We have developed a novel analysis pipeline to visualize phosphorylation events in cell lysates and correlate them with signaling cascades, and applied it to scan how they affect osteoblast adhesion. Our results show several signaling pathways implicated in cellular events, mainly cytoskeleton rearrangement by activation of protein kinase C/cofilin and signal transduction upon integrin activation leading to focal adhesion kinase activities, such as FAK and Src. In conclusion, our data highlighted that PKC, FAK and Src kinases might be useful molecular markers to predict the quality of osteoblast adhesion. Besides, this work provided a huge amount of information for building a database of "pathway hotspots", enabling researchers to evaluate crucial interactions between new biomaterials and host cells in order to predict a better performance of them when implanted into whole bone.

Keywords: *analysis pipeline, bone development, kinome, osteoblast adhesion, protein array*

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