INTEGRIN-MEDIATED SIGNALING IN OSTEOBLASTS ADHERING TO IMPLANT MATERIALS

A. Krause and E. Cowles#, Department of Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT 06030. #Department of Biology, Eastern Connecticut State University, Willimantic, CT 06226

Introduction: Long-term stability of implants depends on stimulation of osteoblast proliferation and differentiation leading to bone ingrowth or osseointegration. Previous studies have shown that receptors for extracellular matrix (ECM) proteins, integrins, are important for osteoblast activity and bone formation. We hypothesized that the same signal pathways which are stimulated by cell attachment to the ECM and promote osteoblast function, should be activated by those implant materials that lead to successful osseointegration. Therefore, integrin-mediated adhesion, intracellular signaling and cell proliferation were compared in primary osteoblasts attached to Tivanium (TIV, Ti6Al4V), fibronectin (FN) and poly-L-lysine (PLL). FN was chosen as an ECM substrate because it is the first ECM protein produced by osteoblasts *in vivo* during early bone formation and we have previously shown that osteoblasts use the fibronectin integrin receptor, $\alpha_5\beta_1$, to bind TIV[1].

The mechanism by which integrins transmit signals intracellularly is through integrin binding and clustering which results in the formation of focal adhesion sites. Intracellular signals are propagated by the association of proteins, such as FAK (focal adhesion kinase), with other components in the focal adhesion site. FAK, a tyrosine kinase, binds to the β_1 integrin cytoplasmic tail and initiates a signaling cascade of autophosphorylation. Intermediate steps lead to the transient phosphorylation of mitogen-activated protein kinases (MAPK). MAPK translocates to the nucleus where its nuclear target proteins include fos and jun, members of the AP-1 transcription complex, which control cell proliferation and gene expression.

Methods: Primary rat osteoblasts were isolated by sequential digestion of 20 day fetal rat calvaria by 0.1% hyaluronidase and 0.2 % collagenase. The third fraction was previously shown in our laboratory and by others to consist primarily of osteoblast progenitors and early osteoblasts. These osteoblasts were added to disks of TIV (prepared by Zimmer in the same manner as orthopaedic implants[1]), FN and PLL for various time points and then analyzed for FAK and MAPK phosphorylation, and c-fos and c-jun protein and mRNA levels by Western and Northern blots. Equal amounts of protein or RNA was added to each lane in Western and Northern blots, respectively. Western blots were performed on cell lysates with antibodies to FAK and MAPK and with an antibody which recognizes phosphotyrosine or active MAP kinase (Promega, Madison, WI). The number of cells which had adhered at 30 min to 24 h was measured. Phalloidin staining of the cells' cytoskeleton was performed at 4 and 24 h. Cell proliferation was analyzed by counting cells at 1, 2, 4 and 6 days in F-12 medium with 1% bovine serum albumin. Serum was not used due to the presence of proteins which could activate integrins.

Results: Adhesion assays demonstrated that osteoblasts rapidly attached to PLL and more slowly to FN and TIV. Adhesion to TIV was less (by approximately 50%) than on FN. Phalloidin-stained cells had well-defined actin filament bundles and focal adhesion sites at 4 h on TIV and FN, unlike cells on PLL. By 24 h, all cells were spread with extensive cytoskeletons. FAK and MAPK phosphorylation was elevated by 60 min on FN, 120 min on TIV but not on PLL. On PLL, no phosphorylated MAPK was apparent in osteoblasts, even at 24 h. Northern blot of c-fos and c-jun MNRA revealed a 2-fold increase by 30 min followed by a decrease at 120 min in cells FN and TIV. Both messages remained elevated on PLL. Protein levels were similar on TIV and FN with little expression on PLL. Cell proliferation peaked and plateaued on day 2 on FN, while cells on TIV peaked on day 4. Cell numbers declined on PLL.

Discussion: We have demonstrated that the same signaling pathway is activated by osteoblasts that bind to FN and TIV. Although more cells adhered to PLL, MAPK was not phosphorylated. Attachment to PLL is through ionic interactions, while attachment to FN and TIV is mediated by integrins. The pattern of c-fos and c-jun induction also differed on TIV and FN compared to PLL. Cell cytoskeletal organization and proliferation was enhanced on FN and TIV but not on PLL. Therefore, FN and TIV were very similar in the activation of specific integrin-mediated cell signaling molecules and cell proliferation, in the absence of serum, compared to PLL. The same signal pathways which are stimulated by cell attachment to the ECM and promote osteoblast survival and function, appear to be activated by TIV, which contributes to the success of TIV in promoting osseointegration.

References: 1. Gronowicz,G and McCarthy, MB. 1996, Response of human osteoblasts to implant materials: Integrin-mediated adhesion, J.Orthop Res 14:878-887.

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