Integrin Alpha2 and Beta1 Subunits Mediate Osteoblast Response to Surface Roughness and Chemistry

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STATEMENT OF PURPOSE: Titanium (Ti) surface microstructure promotes osteoblast differentiation and increase osteogenic local factors over what is observed in cultures grown on tissue culture polystyrene (TCPS) surfaces. There are a number of mechanisms that may contribute to the differential response of osteoblasts to surface features of their substrate. Cells on Ti rougher surfaces express a different integrin profile than cells on TCPS, notably exhibiting increased levels of $\alpha2\beta1$. Targeted knockdown of either the $\alpha2$ or $\beta1$ integrin blocks the stimulatory effects of Ti surface microstructure on osteoblast differentiation, indicating that it is mediated at least in part through $\alpha2\beta1$ signaling. Surface chemistry is another important factor that could affect osteoblast differentiation. The aim of the present study was to determine if osteoblast differentiation on Ti surfaces mediated by integrin $\alpha2$ and $\beta1$ subunits is a surface roughness response, surface chemistry response or a combination of both.

METHODS: Integrin $\alpha2$ shRNA sequences were designed and cloned into a pSuppressorNeo vector containing a U6 promoter. MG63 cells were transfected and selected with G418. Silencing was assessed by Western blot analysis. ITG$\alpha2-$shRNA exhibited a consistent 70% reduction in the $\alpha2$ integrin subunit. Lentiviral transduction particles containing shRNA sequences specific to the integrin $\beta1$ were added to MG63 cells at 7.5 MOI. Transduced cells were selected with puromycin. Integrin $\beta1$ subunit was reduced 65% compared to control. MG63, ITG$\beta1$-shRNA or ITG$\alpha2$-shRNA cells were growth on tissue culture polystyrene (TCPS) or on Ti microstructured substrates with different surface topographies and surface chemistries. Titanium sand blasted and acid etched surfaces (SLA [Ra=2.20 μm]) and SLA surfaces sputter-coated with amorphous carbon (SLA-aC [Ra=2.40 μm]) were used as experimental surfaces; TCPS surfaces were used as control. At confluence, cell number, alkaline phosphatase specific activity and levels of osteocalcin, osteoprotegerin (OPG) and vascular endothelial growth factor-A (VEGF) were determined. Data were calculated as means±SEM for N=6 independent cultures for each variable. Statistical significance was determined using ANOVA followed by Bonferroni's modification of Student's t-test.

RESULTS: MG63 cells decreased in cell number on SLA surfaces and increased alkaline phosphatase, osteocalcin, OPG and VEGF levels in comparison with TCPS. Amorphous carbon-coated SLA surfaces (SLA-aC) did not affect osteoblast differentiation when compared with SLA. Integrin $\alpha2$ silencing dramatically increased cell number and decreased alkaline phosphatase activity, osteocalcin, OPG and VEGF levels on SLA surfaces in comparison with MG63 cells. However, there was no effect of integrin $\alpha2$ silencing in any of these parameters on SLA-aC or TCPS surfaces. Integrin $\beta1$ silencing increased cell number on all surfaces tested. Alkaline phosphatase activity, osteocalcin, OPG and VEGF levels were greatly decreased in ITG$\beta1$-shRNA cells on SLA and SLA-aC when compared to MG63 cells.

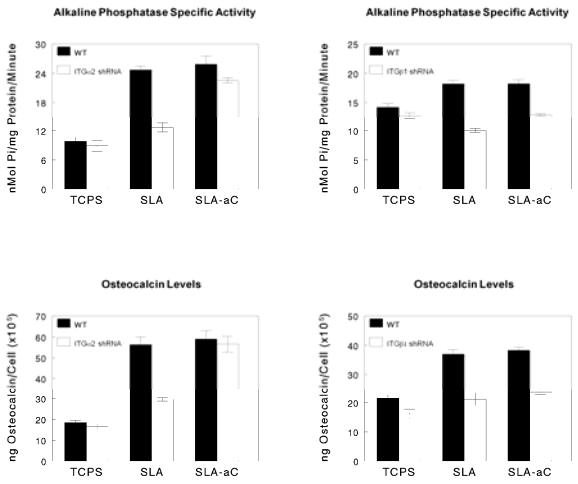

Figure 1: Effect of ITG$\alpha2$ and ITG$\beta1$ silencing on surface roughness and chemistry. (A) Alkaline phosphatase activity and osteocalcin levels on ITG$\alpha2$; and (B) ITG$\beta1$ silenced cells.

DISCUSSION: This study examined the effect of surface chemistry on substrates with the same roughness on osteoblast differentiation. Osteoblast differentiation was not affected by surface chemistry. Lack of osteoblast differentiation in integrin $\beta1$ silenced cells on surfaces with similar roughness but different chemistries suggests a major role of integrin $\beta1$ subunit in roughness recognition. Meanwhile, integrin $\alpha2$ silenced cells were affected dramatically on titanium surfaces and slightly affected on SLA-aC surfaces, indicating that integrin $\alpha2$ subunit may be specific for titanium surfaces.

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