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MODIFICATION OF TRANSCRIPTIONAL ACTIVITY AND REGULATION DRIVES OSTEOBLAST RESPONSE TO HIV AND ITS TREATMENT *IN VITRO*

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This oral abstract is a combination of data presented within Posters [103](#) and [104](#) (see abstracts below).

ABSTRACT 103

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HIV-1 PROTEASE INHIBITORS SELECTIVELY INDUCE GENE EXPRESSION ALTERATIONS ASSOCIATED WITH REDUCED CALCIUM DEPOSITION IN PRIMARY HUMAN OSTEOBLASTS

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HIV infected patients are at increased risk of decreased bone mineral density. Some studies have implicated antiretroviral therapy as a contributor to the decreased bone mineral density seen in treated HIV-1 patients. In this study we explore the interactions between protease inhibitors (PI) and primary human osteoblast gene expression, highlighting a group of dysregulated genes which are potentially key factors in reducing bone formation. Runx2 mRNA expression, calcium deposition and alkaline phosphatase activity (ALP) activity decreased significantly in human osteoblast cultures after exposure to the PIs nelfinavir (NFV) and indinavir (IDV). Saquinavir (SQV), ritonavir (RTV), indinavir (IDV) or nelfinavir (NFV) exposure induced significant changes in

genotypic expression as assessed by gene-chip microarray analysis. The altered genes from each group were compared to each other and a list of 8 upregulated and 13 downregulated genes only after NFV and IDV exposure was identified. This set includes TIMP-3 which has been previously demonstrated to be involved in osteoblast differentiation and extracellular matrix development processes. Silencing TIMP3 mRNA expression using siRNA duplexes enhanced calcium deposition and ALP activity significantly, even after exposure to NFV and IDV. Our data suggests a link between reduced osteoblastic phenotype and a group of 21 altered genes following NFV and IDV treatment, and also suggests TIMP-3 may be involved in the PI induced inhibition of osteoblast function.

ABSTRACT 104

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HIV PROTEINS SUPPRESS OSTEOBLAST FUNCTION VIA MODULATION OF RUNX-2 AND PPAR γ TRANSCRIPTION FACTOR ACTIVITY

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AIMS: Decreased bone mineral density (BMD) has been associated with HIV infection, however the exact molecular mechanism of decreased BMD remains to be elucidated. RUNX-2 is a transcription factor that drives the development and maintenance of an osteoblastic phenotype in mesenchymal stem cells (MSCs) and osteoblasts (OBs), while PPAR γ is associated with pro-adipogenic development in MSCs. In this study we have examined *in vitro* the effect of exposure to HIV proteins on osteoblast function and on the expression and activity of PPAR γ and RUNX-2.

METHODS: An immature human osteoblast (hOB) cell line (PromoCell) was cultured and treated with HIV p55-gag, HIV gp120 and HTLV env (100 ng/ml, 24 h) (NIH Aids Reagent Program). HTLV-1 env, was used to control for non-specific viral effects. Cells were analysed for calcium deposition and alkaline phosphatase activity (ALP) using quantitative alizarin red staining and p-NNP assay respectively. Real-Time PCR with gene specific primers was used to analyse mRNA levels of RUNX-2, and PPAR γ . RUNX-2 and PPAR γ activity were assessed using a commercially available assay (Active Motif).

RESULTS: Exposure of hOBs to p55-gag and gp120 significantly altered osteoblast phenotype, as evidenced by reduced calcium deposition of hOBs by $30 \pm 10\%$ and $29 \pm 8\%$ and a reduction in ALP activity ($11 \pm 5\%$ and $19 \pm 0.1\%$ respectively $P=0.05$). Both p55-gag and gp120 significantly reduced RUNX-2 mRNA levels ($60 \pm 14\%$ and 35% respectively $P=0.05$), however no significant changes in PPAR γ mRNA levels were observed. The activity of RUNX-2 was reduced by both p55-gag and gp120 ($38 \pm 5\%$ and $25 \pm 0.8\%$ respectively $P=0.05$), while gp120 significantly increased PPAR γ activity ($179 \pm 50\%$, $P=0.05$).

CONCLUSION: Our findings suggest that exposure to HIV proteins decreases bone formation in osteoblasts and this change in phenotype is paralleled by alterations in RUNX-2 and PPAR γ transcription factor activity in osteoblasts. Alterations of these activities may provide a molecular mechanism that contributes to HIV-associated reduction in BMD.

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