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Tenofovir modulates Semaphorin 4D signaling and regulates bone homeostasis, which can be counteracted by dipyridamole and adenosine A2A receptor.

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Poster # 13

INTRODUCTION

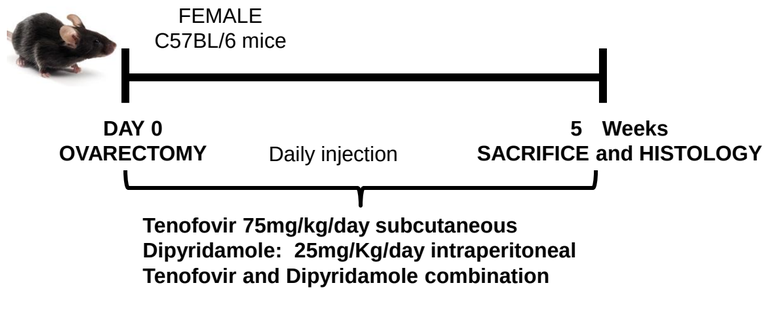
Communication between osteoclast and osteoblast is essential for bone homeostasis. Semaphorins (Sema) are a family of proteins associated with neuronal development and guidance. Sema4D/CD100 was first identified as a regulator of immune response when it was shown to mediate cell–cell interactions in the immune synapse, as well as regulation of the immune response in resting T cells and NK cells. It has been recently found that loss of CD100 expression plays an important role in dysfunctional immunity in HIV infection. Sema4D is secreted by osteoclasts in the presence of RANKL and binds to its receptor PlexinB1 on osteoblasts to inhibit their differentiation and function by activating RhoA/ROCK.

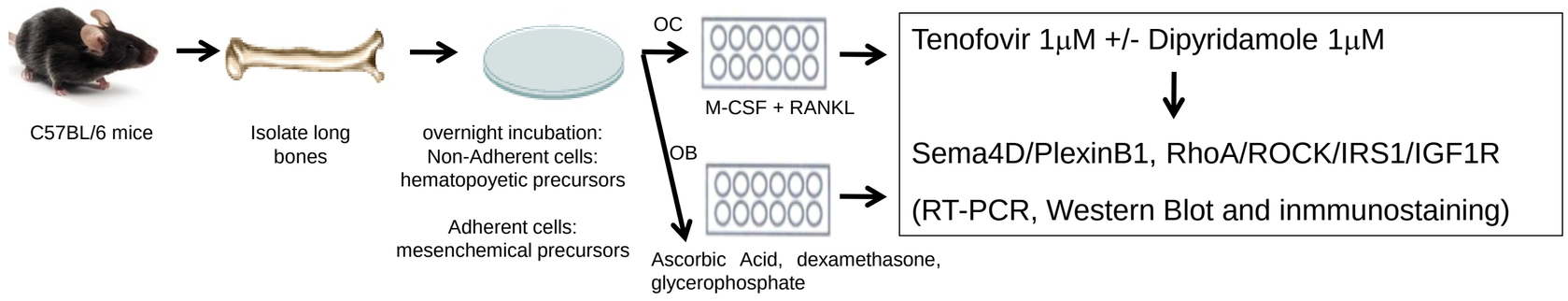
Adenosine A2AR activation inhibits Sema4D-mediated osteoclast activation and diminishes inflammatory osteolysis.

Osteopenia has been associated with HIV infection. Tenofovir, one of the most commonly used antivirals in HIV, increases in bone catabolism markers and decreased bone mineral density (BMD). We have recently described that treatment with agents that increase local adenosine concentrations, like dipyridamole, might prevent bone loss following tenofovir treatment both *in vitro* and *in vivo*.

Therefore, tenofovir might be activating Sema4D signaling to alter bone turnover.

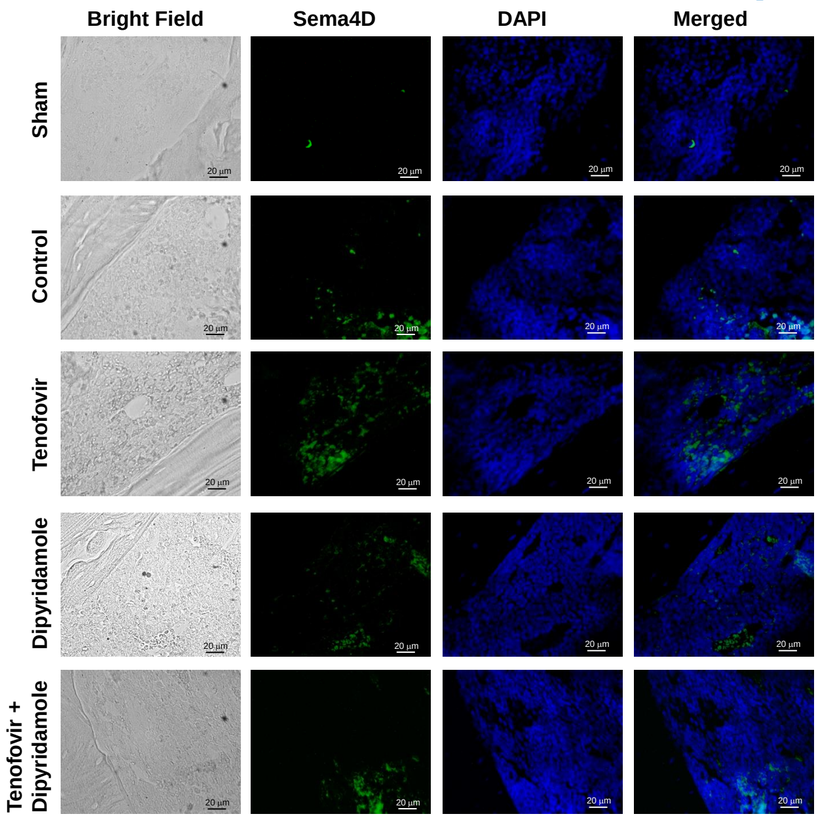
METHODS

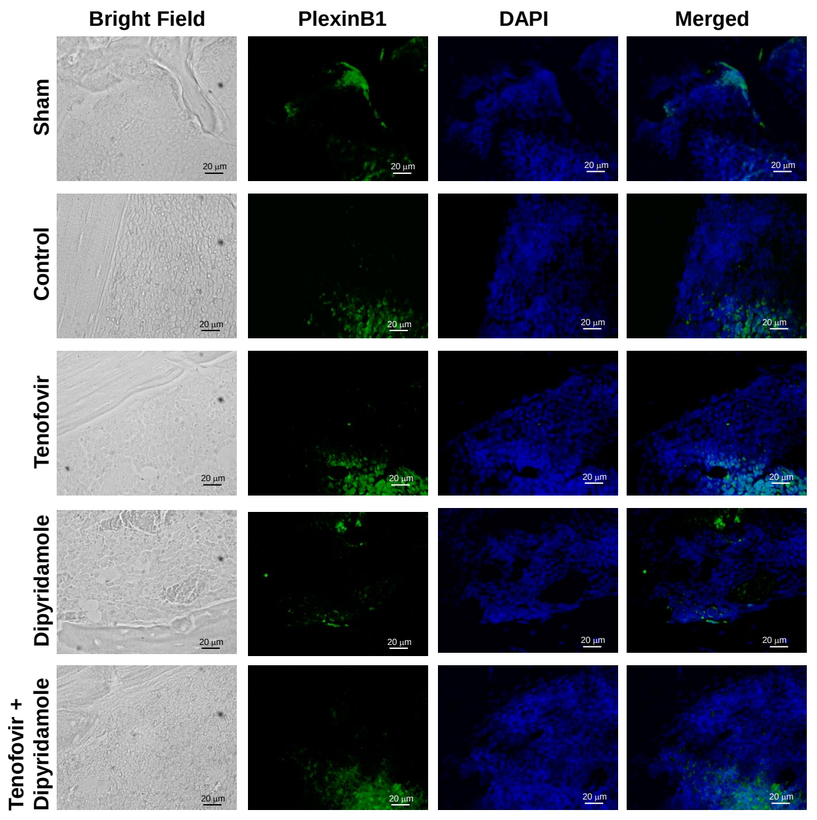




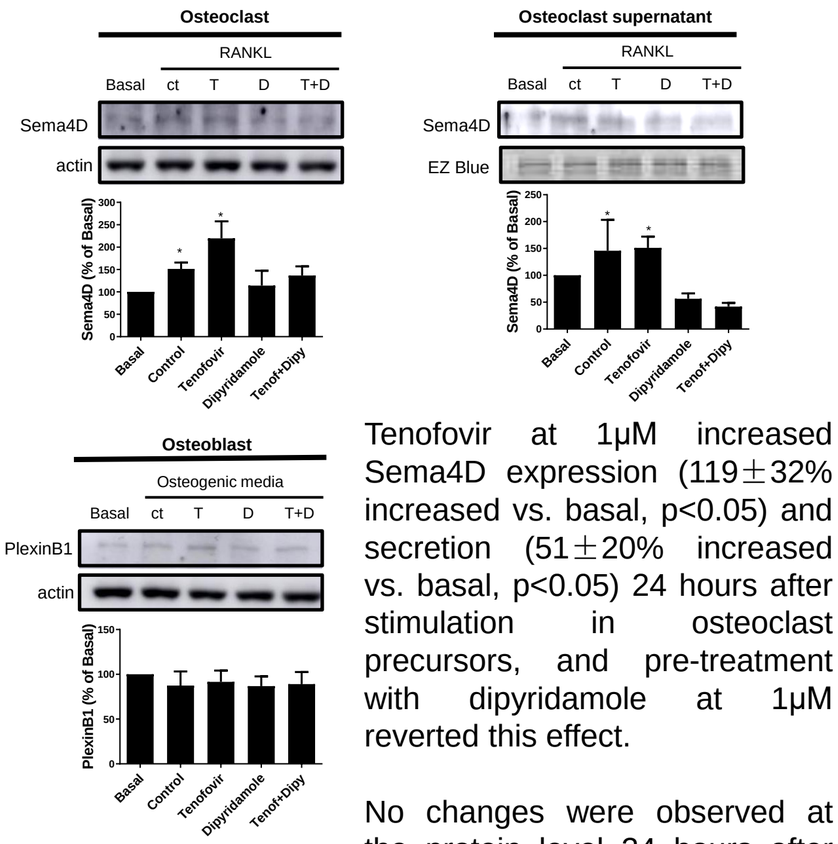
RESULTS

Tenofovir showed an increased expression of Sema4D both *in vivo* and *in vitro*





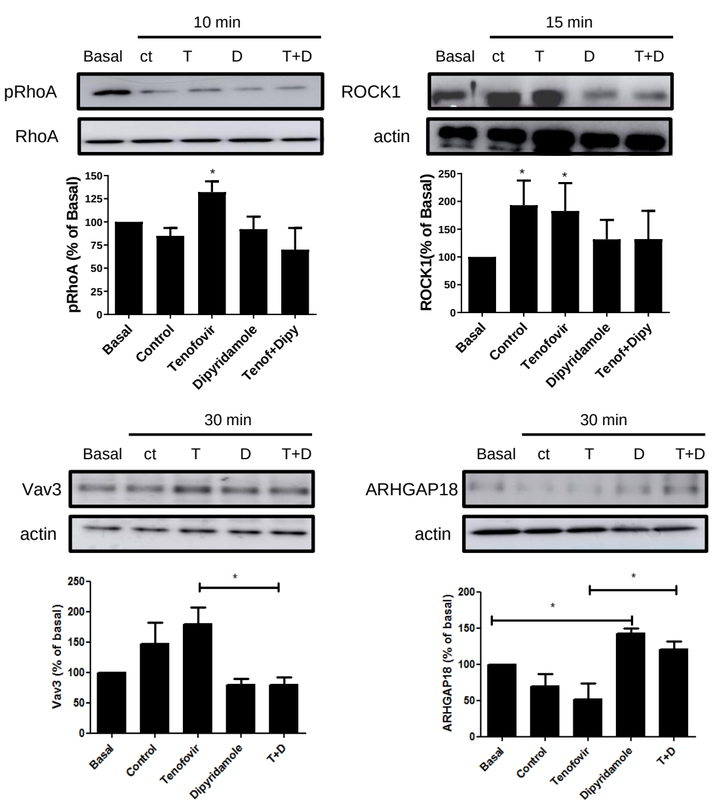
There was an increase in Sema4D-positive cells in tenofovir treated mice, that was reverted in the presence of dipyridamole. No changes were found on the number of PlexinB1-positive cells when mice were treated with tenofovir alone or in combination with dipyridamole.

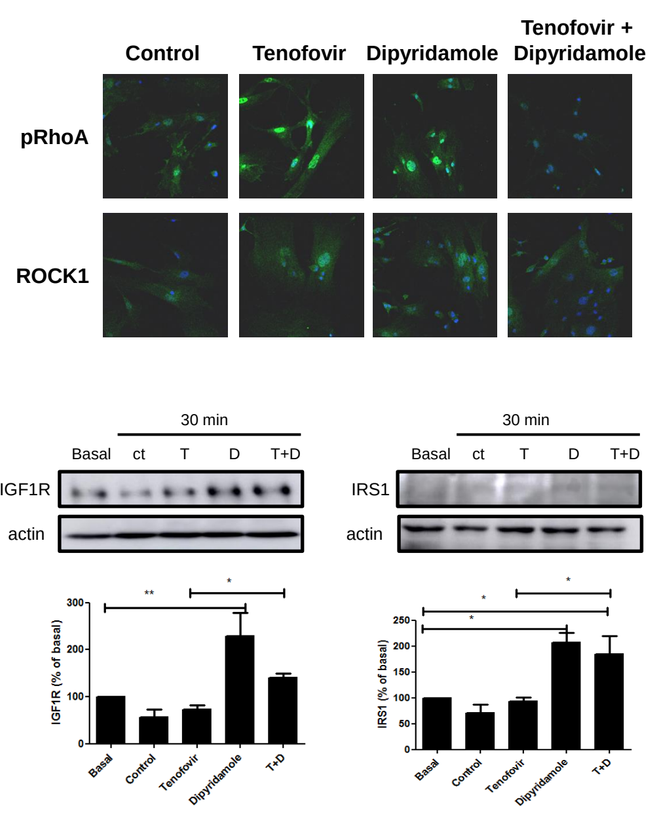


Tenofovir at 1μM increased Sema4D expression (119±32% increased vs. basal, p<0.05) and secretion (51±20% increased vs. basal, p<0.05) 24 hours after stimulation in osteoclast precursors, and pre-treatment with dipyridamole at 1μM reverted this effect.

No changes were observed at the protein level 24 hours after osteoblast precursors in Plexin B1 expression.

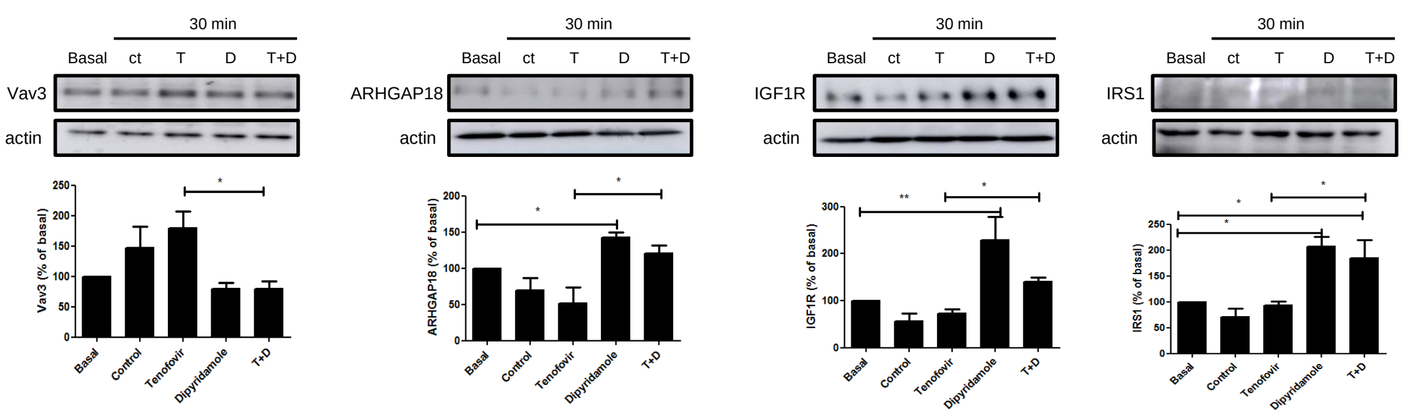
Phosphorylation of RhoA and ROCK1 activation were increased by tenofovir in osteoblasts





Tenofovir at 1μM increased RhoA phosphorylation (32±11% increase vs. basal, p<0.05) and ROCK1 activation (83±35% increase vs. basal, p<0.05) at 10 and 15 minutes, respectively, after osteoblast precursor stimulation, and pre-treatment with dipyridamole at 1μM reverted this effect.

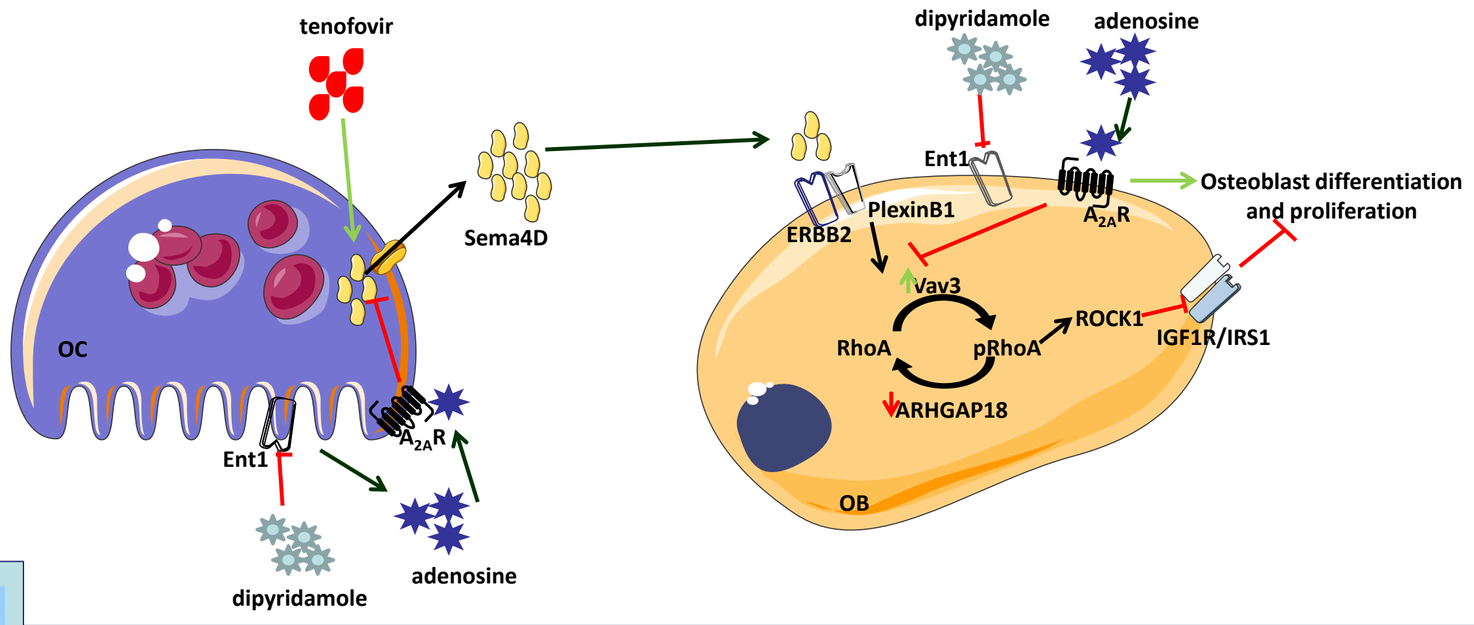
To confirm the Western blot results, immunofluorescence was carried out for pRhoA and ROCK1. Tenofovir at 1μM increased pRhoA and ROCK1 staining 15 minutes after treatment, and this was reverted in the presence of dipyridamole at 1μM.



Treatment with tenofovir at 1μM increased the expression of GEF protein Vav3 30 minutes after treatment (79±27% increase vs. basal, p=ns), with a consequent decrease in GAP protein ARHGAP18 (49±21% increase vs. basal, p=ns), which were both significantly reverted by dipyridamole (p<0.05), inhibited IGF1R activation (27±9% decrease vs. basal, p=ns) and did not change IRS1 (7±7% decrease vs. basal p=ns).

CONCLUSIONS

These data suggest that tenofovir increases bone loss by activation of Sema4D/PlexinB1 signaling that inhibits osteoblast differentiation. Agents that increase local adenosine concentrations, like dipyridamole, might prevent this bone loss following inhibition of the pathway.



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