

Tetracycline analogs inhibit osteoclast differentiation by suppressing MMP-9-mediated histone H3 cleavage

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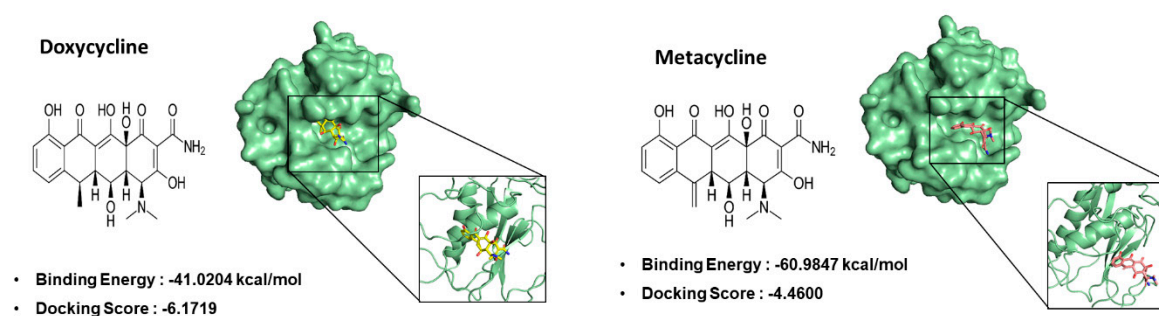


Figure S1. Molecular docking analysis of MMP-9 and tetracycline derivatives.

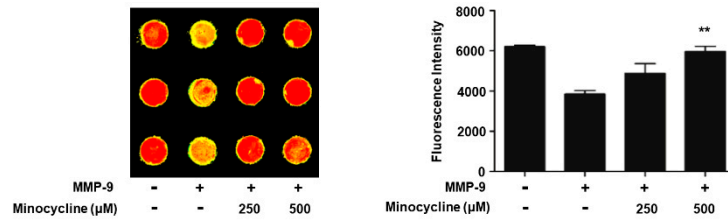


Figure S2. Peptide chip assay. Peptide arrays were fabricated by immobilizing TAMRA-Histone H3 peptide onto well-type amine array. Histone cleavage assays were performed as in Material and methods. Results are obtained from three independent experiments. Bars, the mean result \pm S.D. ** $p < 0.001$ relative to H3 peptide treated with only MMP-9.

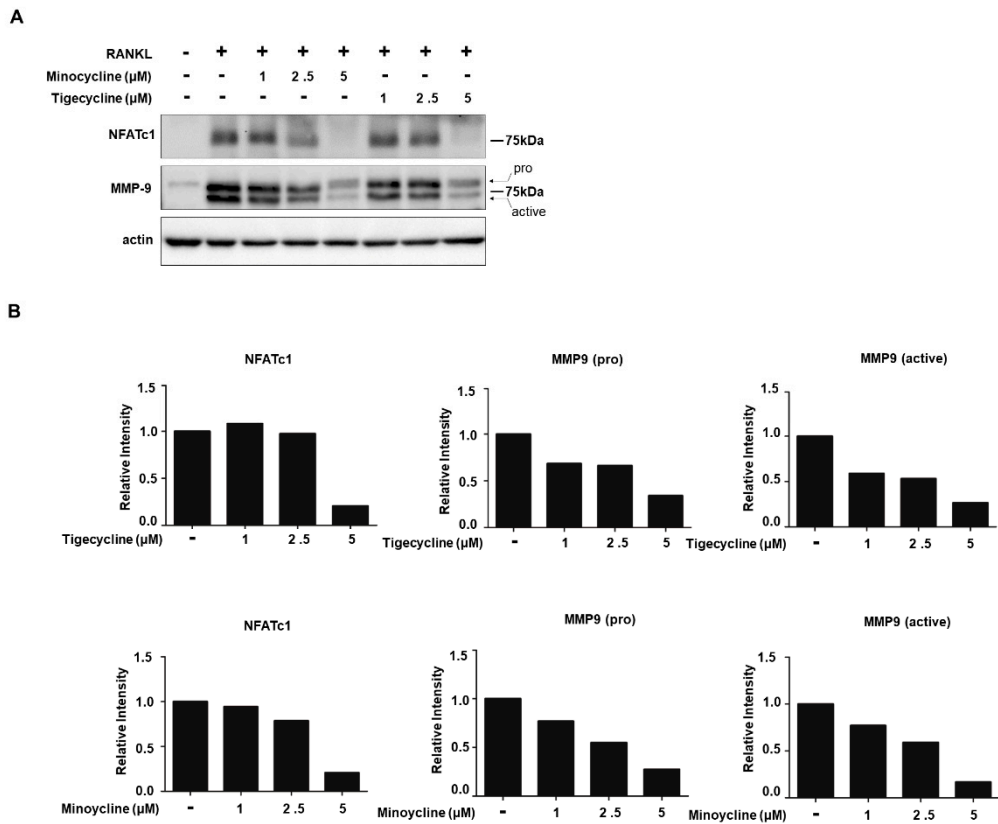


Figure S3. Western blot analyses of NFATc1 and MMP-9 after treating with OCP cells with tigecycline and minocycline. (A) BMM cells were pretreated with tigecycline or minocycline and further with RANKL for 3 days. Whole cell lysates were prepared and analyzed by Western blotting with the indicated antibodies. β -actin was used as a loading control. (B) The band intensities of the Western blots were quantified by densitometry.

Supplementary Materials and Methods

Peptide chip analysis

Well-type amine arrays were prepared by attaching Teflon tape on to silane-coated glass slides (Muto Pure Chemicals, Tokyo, Japan). To make well-type amine arrays containing TAMRA-labeled Histone H3 (aa 14-28), 4.9 mM sulfo-GMBS (ThermoFisher Scientific, Waltham, MA, USA) were dropped into 1.5mm tape hole. After 2h incubation, the arrays were washed with 50 mM sodium bicarbonate buffer (pH 7.0). Then, 1 μ M of H3 peptide were applied to array wells and further incubated at 37 °C for 1h. To investigate the effect of minocycline on MMP-9-mediated H3 proteolysis, the mixture of MMP-9 with or without minocycline was applied to TAMRA-Histone H3 peptide array and incubate at 37 °C for 1h. Following washing with 0.1% Tween 20 in phosphate buffered saline and with milli-Q-purified water, the arrays were dried and scanned on a GenePix 4000B (Molecular Devices, San Jose, CA, USA) scanner using 532 nm lasers.