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Bisphosphonates Induce Senescence in Normal Human Oral Keratinocytes

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APPENDIX

MATERIALS & METHODS

Proliferation Curve

NHOK and NHOF (2 x 10^4 cells) were plated onto the 6-well plate. After 24 hrs, cells were treated with or without 10 μM PAM. Every two days, cells were either collected for cell counting or the medium was exchanged with fresh medium containing 10 μM PAM.

MTT Assay

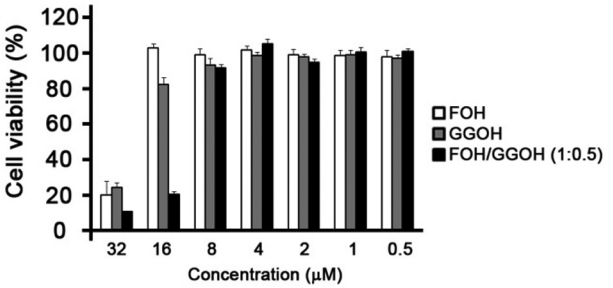
Cell viability in response to PAM treatment was accessed with the use of an MTT Cell Proliferation Assay (ATCC, Manassas, VA, USA), according to the manufacturer's protocol. For NHOK and NHOF, PAM was treated for 4 days in the range of 500 μM to 0.5 μM or 200 μM to 0.2 μM, respectively. The plates were read in 570 nm with an ELx800 Absorbance Microplate Reader (BioTek, Winooski, VT, USA).

Laser-captured Microdissection (LCM)

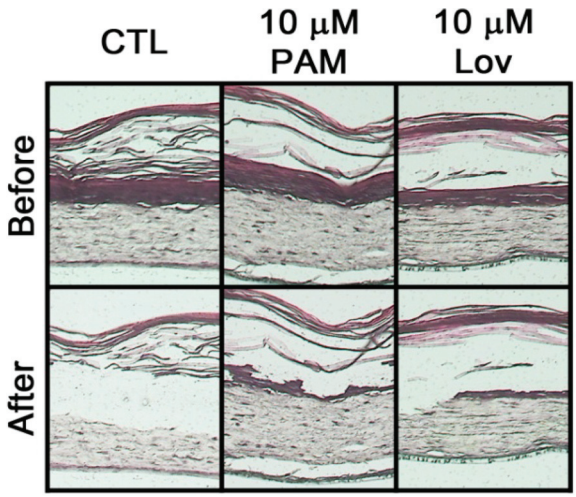
The organotypic raft culture samples were formalin-fixed, paraffin-embedded, and sectioned at 5 μm onto PEN-Membrane slides (Leica Microsystems, Inc., Bannockburn, IL, USA). Samples were H&E-stained and kept in a desiccator for at least 1 hr. Epithelial cells were laser-captured by means of an Arcturus Pixcell IIE Microscope (Arturus Engineering Inc., Mountain View, CA, USA) onto LCM caps (Arturus Engineering Inc.). Total RNAs were extracted with the use of a High Pure RNA Paraffin Kit (Roche, Indianapolis, IN, USA), and a 200-ng quantity of total RNAs was used to make cDNA.

Quantitative Real-time PCR (qRT-PCR)

Total RNAs were isolated and cDNAs were made as described previously (Kim *et al.,* 2010). qRT-PCR was performed in triplicate for each sample with LC480 SYBR Green I master (Roche) and universal cycling conditions on a LightCycler 480 (Roche). In total, 45 cycles were executed, and the second derivative Cq value determination method was used to compare fold-differences. The following primers were used for qRT-PCR: IL-6 primers, 5′-CAGTACCCCCAGGAGAAGATTCCAA-3′ (forward) and 5′-GTCTCCTTTCTCAGGGCTGAGATGC-3′ (reverse); IL-8 primers, 5′-CTCTCTTGGCAGCCTTCCTGATTTC-3′ (forward) and 5′-AATTTGGGGTGGAAAGGTTTGGAGT-3′ (reverse); and MMP-3 primers, 5′-GTGAGGACACCAGCATG AACCTTGT-3′ (forward) and 5′-CCTCCAATCCAAGGAAC TTCTGCAT-3′ (reverse).



Appendix Figure 1. Cytotoxic effects of FOH, GGOH, and FOH/GGOH. NHOK were seeded onto a 96-well plate and treated with the indicated amounts of FOH, GGOH, or FOH/GGOH (1:0.5 ratio) for 4 days. Cells were subjected to MTT assay. Experiments were performed in quadruplicate, and bars indicate standard errors.



Appendix Figure 2. Oral mucosal tissue constructs before and after LCM. The epithelia of the oral mucosal tissue constructs were dissected by LCM. The photographs were taken before and after the dissection at 10X magnification.