RESEARCH REPORTS
Biological

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Bisphosphonates Inhibit Expression of p63 by Oral Keratinocytes

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APPENDIX

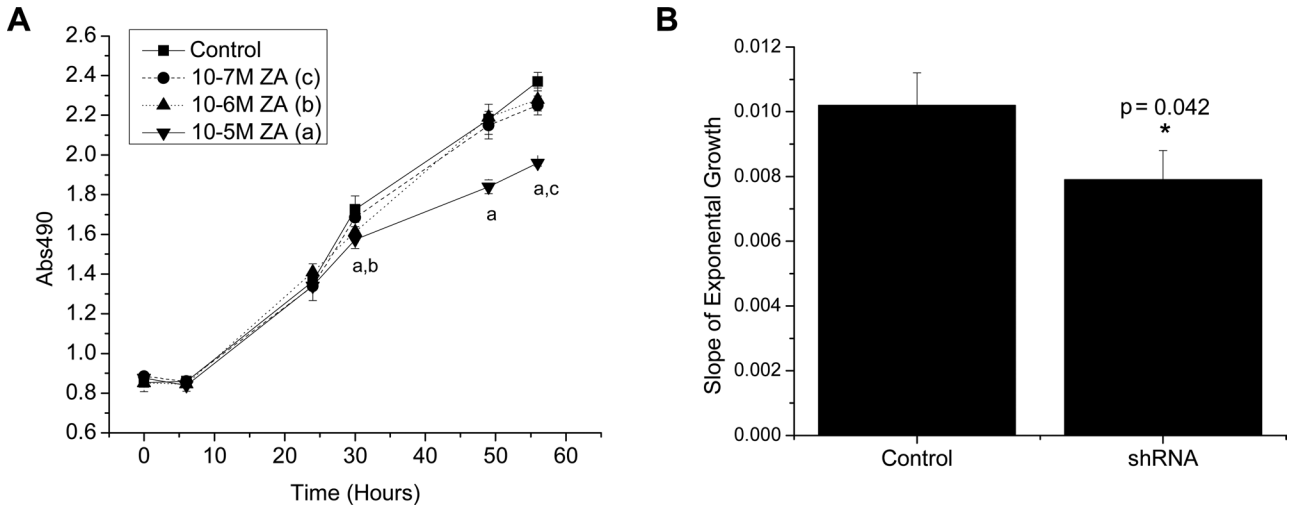
METHODS

Human Tissues

Approval was obtained from the University of Michigan Health Sciences IRB. Biopsies from the archives of the University of Michigan Oral Pathology Biopsy Service accessed between January 2006 and June 2008 were searched for the following key words: "necrotic bone", "exposed bone", "bisphosphonate", "aredia", "zometa", "fosamax", "osteonecrosis", "myeloma", "osteomyelitis", "pamidronate", "alendronate", "zoledronate", or "zoledronic". After clinical history review, cases were assigned to one of three groups: no BP history (control), ONJ with oral BP ("Oral-BP"), or ONJ with intravenous BP ("IV-BP"). We examined H&E-stained slides of these cases to ensure that each specimen contained epithelium and connective tissue. Histologic identification of non-viable bone with associated bacterial colonies was also required. Nine control and 10 bisphosphonate cases (5 Oral-BP, 5 IV-BP) were identified (Appendix Table 1).

NOK-SI Cell Proliferation

Cell proliferation was measured after overnight plating in 48-well plates (Time 0) by incubation with AQueous One Cell Proliferation Assay solution (Promega:G3580, Madison, WI, USA) for 1.5 hrs and measurement of absorbance at 490 nm.



Appendix Figure. (A) Proliferation defects at all time-points from 30 hrs on appeared only in the 10^{-5} M ZA group. However, proliferation decreases were also noted with 10^{-6} M ZA at 30 hrs and 10^{-7} M ZA at 56 hrs. **(B)** Proliferation of p63 knock-out cells was also impaired, as shown by a significant 22% decrease in the slope of their exponential growth phase when compared with that of scrambled controls. N = 3 *per* time-point, *per* treatment. (a) 10^{-5} M ZA significance, (b) 10^{-6} M ZA significance, (c) 10^{-7} M ZA significance. Results are reported as mean ± standard deviation.

Appendix Table 1. Summary of Known Patient Data from Analyzed Biopsy Specimens

Patient	Group	Age (yrs)	Gender	Specimen Diagnosis	Biopsy Site	Bisphosphonate	Known Systemic Conditions
1	Control	65	M	Bony sequestrum	Alveolar ridge		
2	Control	57	M	Non-specific ulcer	Mandibular gingiva		
3	Control	65	M	Traumatic ulcer	Hard palate		
4	Control	78	M	Traumatic ulcer	Alveolar ridge		
5	Control	54	F	Granulomatous reaction	Alveolar ridge		
6	Control	54	F	Ulcer and exposed bone	Retromolar pad		
7	Control	65	M	Bony sequestrum	Alveolar ridge		
8	Control	48	F	Bony sequestrum	Mandibular gingiva		
9	Control	84	F	Bony sequestrum	Alveolar ridge		
10	IV BP	82	M	ONJ	Alveolar ridge	Zometa®	Prostate cancer
11	IV BP	65	M	ONJ	Alveolar ridge	Zometa®	Multiple myeloma
12	IV BP	67	F	ONJ	Maxilla NOS	Zometa®	Multiple myeloma
13	IV BP	55	F	ONJ	Alveolar ridge	Zometa®	Breast cancer
14	IV BP	78	M	ONJ	Alveolar ridge	Zometa®	
15	Oral BP	70	F	ONJ	Alveolar ridge	Oral NOS	
16	Oral BP	81	F	ONJ	Alveolar ridge	Oral NOS	
17	Oral BP	65	F	ONJ	Alveolar ridge	Fosamax®	
18	Oral BP	67	F	ONJ	Alveolar ridge	Fosamax®	
19	Oral BP	88	F	ONJ	Mandible NOS	Fosamax®	

Known systemic conditions include those that were disclosed when the biopsy was submitted but do not represent a complete medical history. NOS: not otherwise specified.

Appendix Table 2. Patient Information Summary for Biopsies from Which Primary Oral Keratinocytes Were Derived

Patient	Age (yrs)	Gender	Race	Biopsy Site
1	38	F	Caucasian	Buccal mucosa
2	39	M	Caucasian	Alveolar ridge