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**Bisphosphonates inhibit Cyclin A2 expression
in normal human oral keratinocytes**

Objectives: Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is characterized by unhealed oral mucosa. Previously, we reported that bisphosphonates (BPs) inhibit proliferation and induce senescence in normal human oral keratinocytes (NHOK), but not in fibroblast (NHOF). Here, we examined the underlying mechanisms of BPs on NHOK.

Methods: Primary NHOK and NHOF were established from normal human oral epithelium and treated with BPs. Cell cycle analysis was performed using propidium iodide (PI) staining followed by the flow cytometry. Cell-cycle associated genes were screened using Western blotting and RT-PCR. Luciferase reporter vector containing cyclin A2 promoter (608bp from -516 to +92) was constructed and luciferase assay was performed in BP-treated NHOK and NHOF. Immunohistochemistry (IHC) staining was performed in BP-treated and untreated 3-dimensional (3D) oral mucosa tissue construct.

Results: Cell cycle analysis in NHOK treated with BPs revealed an increase in the S phase content. BP- treated NHOK drastically inhibited protein and mRNA expressions of Cyclin A2. No such changes were observed in NHOF. Luciferase reporter assay utilizing cyclin A2 promoter showed a reduced luciferase activity in BP-treated NHOK, indicating that BPs transcriptionally regulate Cyclin A2 expression. IHC staining in 3D raft culture model confirmed the similar effect at the tissue level.

Conclusion: Our study demonstrates that BPs inhibit the proliferation of NHOK by dysregulating the expression of Cyclin A2 at the transcriptional level, which may be associated with the underlying mechanisms of unhealed oral mucosa on the necrotic bone in BRONJ.