How Do Osteoclast Precursors Escape Bisphosphonate-induced Cell Death?

Osteoclast Precursors Develop Resistance Against Zoledronic Acid by Activation of p38 MAPK Signaling.

Shang-Lin Tsai, TA-WEI TAI, I-MING JOU.
National Cheng Kung University (NCKU) Hospital, Tainan, Taiwan.

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Introduction: Osteoporosis is characterized by low bone mineral density (BMD) and abnormal bone quality. It leads to decreased bone strength and increased susceptibility to fractures. There are some successful pharmacotherapeutic strategies to reverse this situation. Bisphosphonates are the most popular drug categories used for the treatment of osteoporosis. The percentage of patients who experience a decrease in BMD after treatment with oral bisphosphonates vary from 8% to 41% among the different anatomical parts and different drugs. The non-responsive situation is similar in some patients who receiving annual IV zoledronic acid. A small case series showed 15% (3/20) patients had a declined BMD on spine and 40% (8/20) had a declined BMD on hip after 1-year treatment of zoledronic acid. A large-scaled long-term cohort study demonstrated that the zoledronic acid non-responders had higher risk of osteoporotic fracture than those who responded to treatment. Furthermore, the long-term results of zoledronic acid treatment were reported not superior to the short-term results. The 3-year HORIZON-PFT study was extended to 6 years to investigate long-term effects of zoledronic acid on BMD and fracture risk. The report revealed small differences in bone density and markers in those who continued treatment to 6 years versus those who stopped treatment after 3 years. This phenomenon suggests the anti-resorptive effect is not proportional to the duration of treatment. After a period of treatment, osteoclasts may start to develop resistance to zoledronic acid and find more ways to escape drug-induced cell death. The molecular mechanism of non-response or developing resistance to zoledronic acid for osteoporosis treatment is not clear. The purpose of this study is to investigate the mechanism of resistance to zoledronic acid in the treatment of osteoporosis.

Methods: This in vitro study was conducted with standard molecular technology. Cell proliferation assay, western blotting, flow cytometry, real-time PCR, and immunochemistry staining were used to identify the signal pathway.

Results: Here we showed that part of murine osteoclast precursors, RAW264.7 cells developed resistance against ZA after treatment. These resistant cells showed increased expression of Bcl-xL, a protein for cell survival. Further investigation revealed the expression of p38 MAPK in the resistant cells. Inhibiting p38 MAPK decreased Bcl-xL mRNA and protein expression and increased the apoptosis of cells under ZA treatment (Figure 1). The resistant cell also showed nuclear import of β-catenin mediated by p38 MAPK. Inhibiting β-catenin declined Bcl-xL mRNA expression and increased the apoptosis (Figure 2). Inhibitory phosphorylation of glycogen synthase kinase (GSK)-3β was also found. The phenomenon diminished by inhibiting p38 MAPK. Furthermore, p38 MAPK inhibition and ZA worked synergically to suppress Tartrate-resistant acid phosphatase (TRAP) activity and osteoclast differentiation induced by Receptor activator of nuclear factor kappa-B ligand (RANKL).

Discussion: This in vitro study demonstrated that osteoclasts or its precursors developed resistance under the continuous exposure of zoledronic acid. The resistance was mediated by strong activation of the p38 mitogen-activated protein kinases (p38 MAPK)-dependent pathway. We also showed that inhibiting this pathway reversed the resistance and promoted zoledronic acid-induced cell death in osteoclast.

Significance: We demonstrate that the p38 MAPK-mediated Bcl-xL overexpression under exposure to ZA in osteoclast precursors has a critical role in the induction of resistance (Figure 3), which may result in treatment failure for osteoporosis or complications caused by bone metastases. Inhibiting p38 MAPK, β-catenin or Bcl-xL can reverse the resistance.

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