The bone is the main storage site for Ca\(^{2+}\) and Mg\(^{2+}\) ions in the mammalian body. Although investigations into Ca\(^{2+}\) signaling have progressed rapidly and led to better understanding of bone biology, the Mg\(^{2+}\) signaling pathway and associated molecules remain to be elucidated. Here, we investigated the role of a potential Mg\(^{2+}\) signaling-related lysosomal molecule, two-pore channel subtype 2 (TPC2), in osteoclast differentiation and bone remodeling. Previously, we have found that under normal Mg\(^{2+}\) conditions, TPC2 promotes osteoclastogenesis via activating intracellular Ca\(^{2+}\) signaling. We observed that under low-Mg\(^{2+}\) conditions, TPC2 inhibited, rather than promoted, the osteoclast differentiation and that the phosphatidylinositol 3,5-bisphosphate [PI(3,5)P\(_2\)] signaling pathway played a role in the TPC2 activation under low-Mg\(^{2+}\) conditions.

Under normal-Mg\(^{2+}\) conditions, the deletion of TPC2 inhibited osteoclast differentiation in RAW267.4 and bone marrow cells, as determined by the TRAP activity and mRNA expression of osteoclast differentiation markers (Calcr, Itgb3, Ctsk). However, under low-Mg\(^{2+}\) conditions, it promoted osteoclast differentiation. To further confirm that TPC2 plays a role in osteoclastogenesis under Low-Mg\(^{2+}\) conditions, we focused on PI(3,5)P\(_2\), which is one of TPC2 ligands. The PI(3,5)P\(_2\) addition inhibited osteoclast differentiation only under low-Mg\(^{2+}\) conditions, and the effect was diminished in TPC2 deletion cells. These findings indicated that the TPC2 activity was mediated via the PI(3,5)P\(_2\) signaling pathway only under Low-Mg\(^{2+}\) conditions. Furthermore, PI(3,5)P\(_2\) depolarized the membrane potential by increasing the intracellular Na\(^{+}\) levels under low-Mg\(^{2+}\) conditions. To investigate how membrane depolarization affects osteoclast differentiation, we generated a light-sensitive cell line and developed a system for the light-stimulated depolarization of the membrane potential. The light-induced depolarization inhibited the osteoclast differentiation and TPC2 was involved in this membrane depolarization-induced inhibition. We then tested the effect of myo-inositol supplementation, which increased the PI(3,5)P\(_2\) levels in mice fed a low-Mg\(^{2+}\) diet. The myo-inositol supplementation rescued the low-Mg\(^{2+}\) diet-induced trabecular bone loss, which was accompanied by the inhibition of osteoclastogenesis. These results indicate that low-Mg\(^{2+}\)-induced osteoclastogenesis involves changes in the role of TPC2, which are mediated through the PI(3,5)P\(_2\) pathway. Our findings also suggest that myo-inositol consumption might provide beneficial effects in Mg\(^{2+}\) deficiency-induced skeletal diseases.