Light-induced membrane potential regulates bone cell function; development of the light-responsive bone cells

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The PTH and VD₃ are not only osteogenic reagents but also major regulator for osteoclastogenesis. These molecules transiently increase bone resorption by increment of the expression level of RANKL, whereas their mechanism of RANKL-intracellular transportation (RANKL-iTP) remains unclear. The RANKL-iTP depends on lysosomal vesicles whose fusion is related to increment of intracellular Ca²⁺ concentration ([Ca²⁺]ᵢ) following depolarizing membrane potential (MP). Thus, MP could regulate osteoblastic function and RANKL-iTP. So far little is known about the role of MP on bone cells, because it is hard to control temporal patterns of MP. Here, by novel system of light-responsive osteoblastic MP, we show that the transiently increasing [Ca²⁺]ᵢ following depolarization regulates PTH- and VD₃-induced RANKL-iTP.

In membrane fraction of RANKL-GFP expressed MC3T3-E1 cell, PTH and VD₃ increase membrane-bound RANKL (mbRANKL) at 10 min (more than 300% increment compared to control), however those mRNA expression level were unchanged at same time. Confirming by time-lapse imaging, the addition of PTH and VD₃ translocated RANKL-GFP to cell membrane within 10 min. In previous reports, PTH and VD₃ transiently increase [Ca²⁺]ᵢ following depolarization. The [Ca²⁺]ᵢ spike was diminished by depolarization-activated calcium channel blocker (Diltiazem: Dil) or ER-dependent [Ca²⁺], blocker (Thapsigargin: Tg). The increment of mbRANKL by PTH and VD₃ was also decreased by Dil and Tg. These results showed that depolarization-dependent change of [Ca²⁺]ᵢ affected PTH- and VD₃-induced RANKL-iTP.

To address whether depolarization controls RANKL-TPi, we developed a novel system of light-responsive osteoblastic MP by channel-rhodopsin wide receiver (ChWR). MPs in ChWR stably expressed cell were depolarized immediately (<20 msec) after light-stimulus (ΔMP: 16.3 ± 1.1 mV) and were returned to the pre-stimulus potential after the off-stimulus. Light induced depolarization increase [Ca²⁺]ᵢ and mbRANKL at 10 min in similar to addition of PTH and VD₃. In co-culture of RAW267 and RANKL-GFP expressed MC3T3-E1 cell, TRAP activities were increased 4-fold by light stimulus and the development of mature osteoclasts was observed.

We showed that depolarizing MP is related to osteoblastic function. Our results indicates that RANKL-iTP is regulated by [Ca²⁺]ᵢ, following depolarization of MP. The mechanism of PTH- and VD₃-induced RANKL-iTP would have benefits for developing PTH- and VD₃-like medicine.