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

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The PTH and VD_3 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 Ca^{2+} concentration ($[Ca^{2+}]_i$) 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 $[Ca^{2+}]_i$ following depolarization regulates PTH- and VD_3- induced RANKL-iTP.

In membrane fraction of RANKL-GFP expressed MC3T3-E1 cell, PTH and VD_3 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 VD_3 translocated RANKL-GFP to cell membrane within 10 min. In previous reports, PTH and VD_3 transiently increase $[Ca^{2+}]_i$ following depolarization. The $[Ca^{2+}]_i$ spike was diminished by depolarization-activated calcium channel blocker (Diltiazem: Dil) or ER-dependent $[Ca^{2+}]_i$ blocker (Thapsigargin: Tg). The increment of mbRANKL by PTH and VD_3 was also decreased by Dil and Tg. These results showed that depolarization-dependent change of $[Ca^{2+}]_i$ affected PTH- and VD_3-induced RANKL-TPi.

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 $[Ca^{2+}]_i$ and mbRANKL at 10 min in similar to addition of PTH and VD_3. 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 $[Ca^{2+}]_i$ following depolarization of MP. The mechanism of PTH- and VD_3-induced RANKL-iTP would have benefits for developing PTH- and VD_3-like medicine.