An acid-activatable fluorescent probe for imaging osteocytic bone resorption activity in deep bone cavities

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Abstract

A pH-activatable fluorescent probe (pHocas-RIS) with moderate bone-binding affinity was developed by conjugating a BODIPY fluorophore to bisphosphonate-targeting risedronate ligands. The probe enabled the imaging of acidic osteocytic lacunae that contain bone resorbing osteocytes in deep bone tissues in living animals.

Background & Results

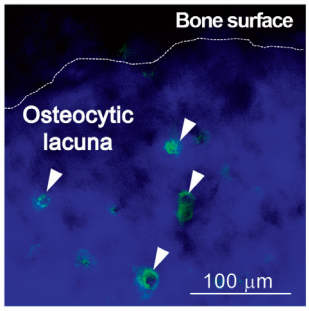
Long lived osteocytes constitute around 90–95% of all bone cells, which exist within disk-shaped cavities called osteocytic lacunae that are present throughout the mineralized bone matrix. Osteocytes are known to directly remodel the bone walls by lacunae bone wall mineralisation producing larger cavities in a process known as osteocytic osteolysis. However, the physiological role of osteocytes in direct bone mineralisation is still open to debate, so the availability of high-resolution real time imaging method would be extremely useful.

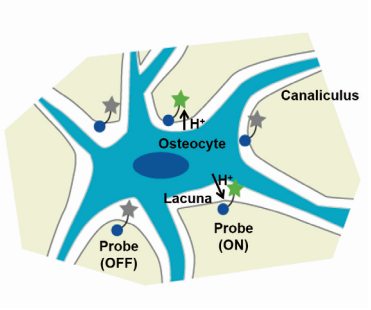
Thus, we developed a new probe (pHocas-RIS) that could be used to visualize the low pH environments of osteocytic lacunae that contained acid secreting osteocytes. Conjugation of the moderate bone binding drug risedronate to a pH-activatable BODIPY fluorophore afforded pHocas-RIS in order to penetrate osteocytic lacunae cavities embedded deep within the bone matrix. The fluorescence intensity of the pHocas-RIS probe increased approximately 15-fold as the pH decreased from 8.0 to 4.0, with changes in its quantum yield at different pH levels used to calculate the pK_a value of its aniline moiety as 6.8.

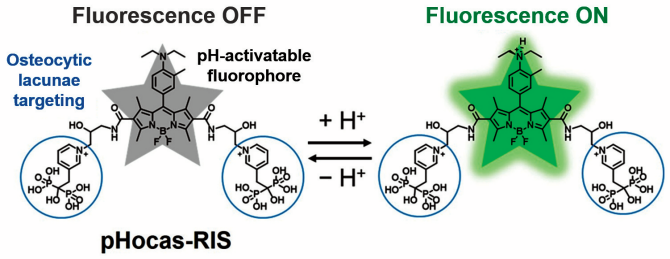
After subcutaneous administration of the probe to mice over a 3 day period followed by aesthetisation, we performed two-photon imaging of the medullary cavities of their thin calvaria parietal bones. Imaging studies revealed the presence of small numbers of ring-shaped green fluorescence signals of the protonated pHocas-RIS that were present in bone obtained from deep cortical regions. It is proposed that these fluorescent signals originate from probes bound to the walls of osteocytic lacunae that contained bone resorptive osteocytes that were actively secreting acid to create a low pH environment. The pH responsiveness of the pHocas-RIS probe within the lacunae of the bone matrix was confirmed by carrying out ex vivo fluorescent imaging of cryogenically prepared deep bone slices at different pH levels.

Significance of the research and Future perspective

These results clearly demonstrated that the intensity of the pH-activatable fluorescent response of the pHocas-RIS probe can be used to effectively image the pH of osteocytic lacunae in living bone. Our pH responsive probe can be used to visualize the bone mineralizing activities of acid producing osteocytes in real time, thus providing a valuable imaging method to explore their central role in remodeling bone-matrix in health and disease.







Patent

Treatise

Hashimoto, Ryu; Minoshima, Masafumi; Kikuta, Junichi et al. An Acid Activatable Fluorescence Probe for Imaging Osteocytic Bone Resorption Activity in Deep Bone Cavities. Angew. Chem. Int. Ed. 2020; 59, 20996-21000. doi: 10.1002/anie.202006388.

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URL

Keyword fluorescent probe, osteocytes, intravital imaging, bone resorption, pH