News Release

Title

Lansoprazole upregulates polyubiquitination of the TNF receptor-associated factor 6 and facilitates Runx2-mediated osteoblastogenesis

Key Points

- The drug repositioning strategy targeting on upregulation of Runx2, a master transcription factor of osteoblastogenesis, reveals that lansoprazole enhances osteoblastogenesis and fracture healing in rats.
- Lansoprazole activates the TAK1 (transforming growth factor beta-activated kinase 1)-p38 MAPK axis of the BMP (bone morphogenetic protein) signaling pathway *in cellulo*.
- Lansoprazole inhibits the enzymatic activity of CYLD, a deubiquitination enzyme, *in vitro* by tightly fitting in its pocket, and facilitates autopolyubiquitination of TRAF6 (TNF receptor-associated factor 6), an upstream of TAK1 and a ubiquitin ligase, *in cellulo*.

Summary

Professor Naoki Ishiguro, Associate Professor Hiroshi Kitoh, and Assistant Professor Kenichi Mishima (first author) at Orthopedics and Professor Kinji Ohno (corresponding author) at Neurogenetics of the Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, MD, PhD) demonstrated in collaboration with their colleagues that lansoprazole, which is commonly prescribed for gastroduodenal ulcers and reflux esophagitis, facilitated osteogenesis *in cellulo* and *in vivo*. They used the drug repositioning strategy, in which a novel application is identified for a drug that is already prescribed for another disease in clinical settings. In a mouse mesenchymal stem cell line and a human osteoblast-like cell line, lansoprazole increased the expression of *Runx2* gene, and upregulated the intranuclear accumulation and the transcriptional activity of Runx2. Lansoprazole also elevated osteoblastic differentiation markers. Lansoprazole enhanced matrix calcium deposition, indicating terminal differentiation of the osteoblast lineage, in primary human bone marrow cells isolated from young patients. Systemic administration of lansoprazole to a rat femoral fracture model increased calcified bone formation in gaps of the fracture sites and accelerated fracture healing. Lansoprazole activated the TAK1 (transforming growth factor beta-activated kinase 1)-p38 MAPK axis of the BMP (bone morphogenetic protein) signaling pathway and facilitated autopolyubiquitination of TRAF6 (TNF receptor-associated factor 6), which is an upstream adaptor molecule of TAK1, *in cellulo*. Moreover, lansoprazole inhibited the enzymatic activity of CYLD, a deubiquitination enzyme. CYLD specifically catalyzes disassembly of Lys63-linked polyubiquitination that participates in intracellular signaling transduction. An *in silico* search for ligand-binding sites of CYLD disclosed a unique binding pocket. The pocket was located

across which the C-terminal tail of ubiquitin was predicted to lie. A specific binding of lansoprazole to CYLD was predicted to inhibit its enzymatic activity by preventing the C-terminal tail from reaching the active site. The validity of the docking model was established by introducing artificial mutations in the binding pocket of CYLD. Lansoprazole is expected to be efficacious as an agent for osteogenesis promotion. This work will be published in *EbioMedicine* on Nov.24,2015.

Research Background

The advent of a “super-aged” society has witnessed increasing incidence of osteoporotic fractures that contributes to increased number of elderly patients who require nursing care. To overcome the situations for prolongation of health expectancy, an agent to promote osteogenesis is anticipated to emerge in addition to the development of superior anti-osteoporosis drugs. As of 2015, only a parathyroid hormone analogue is prescribed as an agent to promote osteogenesis in Japan. Because of the requirement of repeated injection and higher cost, however, chemical compounds that promote osteogenesis are eagerly anticipated to develop. The drug repositioning strategy is for the development of a novel application of previously approved medicine. Its advantage is that optimal doses, adverse effects, and contraindications are already established and can accelerate cost-effective development of a novel drug without safety concerns. In Japan, lansoprazole was released in 1992 as a potent acid suppressant for the treatment of gastroduodenal ulcer and reflux esophagitis.

Research Results

The research team screened a panel of preapproved drugs for dose-dependent increase of human *RUNX2* P1 promoter activity, and identified lansoprazole. Lansoprazole increased expressions of *Runx2* gene in a mouse mesenchymal stem cell line, a human osteoblast-like cell line, human mesenchymal stem cells, and rat primary bone marrow cells in a dose-dependent manner. Lansoprazole also upregulated the intranuclear accumulation and the transcriptional activity of Runx2, and elevated expression of its target gene, *Spp1*, and an osteoblastic differentiation marker, ALP activity, in a dose-dependent manner. Moreover, sustained culture of human bone marrow cells, which were extracted from young patients, with osteogenic medium supplemented with lansoprazole facilitated matrix calcium deposition indicating terminal differentiation of the osteoblast lineage. Systemic administration of lansoprazole to a rat femoral fracture model increased calcified bone formation within mesenchyme of the fracture gaps, and accelerated fracture healing. Dissection of signaling pathways revealed that lansoprazole activated the TAK1 [transforming growth factor beta (TGF-β)-activated kinase 1]-p38 MAPK axis of the BMP (bone morphogenetic protein) signaling pathway in human mesenchymal stem cells. The TAK1-p38 MAPK pathway is critical for the activation of Runx2. TAK1, a MAPKKK, is autophosphorylated and activated by binding to a lys63-linked polyubiquitin chain formed on TRAF6. TRAF6, an adaptor molecule linking between TGF-β/BMP receptor and TAK1, possesses ubiquitin ligase activity and is

autopolyubiquitinated on intracellular signal transduction. *In cellulo* ubiquitination assay exhibited that lansoprazole facilitated autopolyubiquitination of TRAF6. Moreover, lansoprazole inhibited the enzymatic activity of CYLD, a deubiquitination enzyme that specifically catalyzes disassembly of Lys63-linked polyubiquitination. An *in silico* search for ligand-binding sites of CYLD disclosed a unique pocket. The pocket was located across which the C-terminal tail of ubiquitin was predicted to lie. A specific binding of lansoprazole to CYLD was predicted to inhibit its enzymatic activity by preventing the C-terminal tail from reaching the active site. Introduction of artificial mutations in the binding pocket of CYLD retained the deubiquitination activity of CYLD, but abolished its responsiveness to lansoprazole, which directly proved that the lansoprazole tightly fits in the binding pocket of CYLD and inhibits its enzymatic activity.

Research Summary and Future Perspective

Currently there are no chemical compounds that were permitted to apply for promoting osteogenesis and fracture repair in humans. Lansoprazole is a safe drug that has been used as a proton pump inhibitor in Japan from 23 years ago, in USA from 20 years ago, in EU from 24 years ago. Feasibility for local administration of lansoprazole is currently under investigation using an animal model of bone defect. In addition, clinical application of lansoprazole to fracture repair will be explored in the future.

Article

Mishima K, Kitoh H, Ohkawara B, Okuno T, Ito M, Masuda A, Ishiguro N, Ohno K. Lansoprazole upregulates polyubiquitination of the TNF receptor associated factor 6 and facilitates Runx2-mediated osteoblastogenesis. *EBioMedicine*; Nov. 24, 2015.

Japanese ver.

http://www.med.nagoya-u.ac.jp/medical/dbps_data/_material_/nu_medical/_res/topix/2015/Lansoprazole_20151127jp.pdf

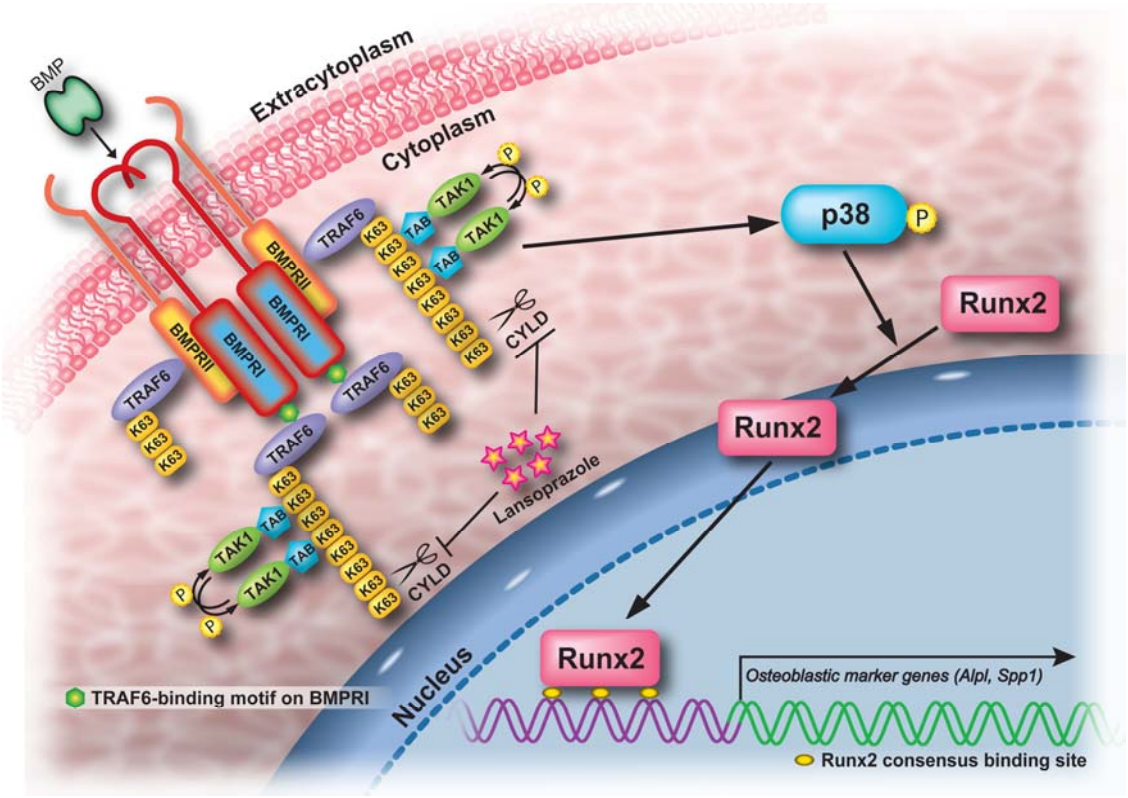

Figure 1. Proposed model of lansoprazole-induced activation of Runx2. Lansoprazole activates TRAF6-catalyzed Lys63-linked autopolyubiquitination of TRAF6 by inhibiting a deubiquitination enzyme, CYLD. Retained Lys63-linked polyubiquitinated chains of TRAF6 subsequently trigger autophosphorylation of TAK1. The TAK1-p38 MAPK pathway then activates transcriptional activity of Runx2. The binding of BMP ligand to BMPRI and BMPRII markedly potentiates lansoprazole-induced activation of Runx2.

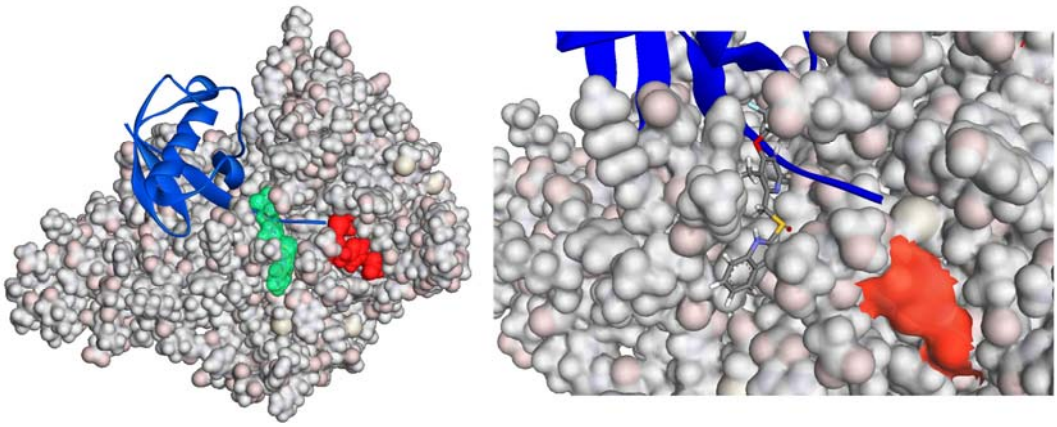

Figure 2. (Left) Simulated crystal structure of CYLD (gray) bound to ubiquitin (blue). The C-terminal tail of ubiquitin extends to the active site of CYLD (red). Lansoprazole (green) tightly fits into a pocket to cross the C-terminal tail of ubiquitin. **(Right)** An enlarged image of the pocket where lansoprazole (sticks) fits. Artificial mutations of arginine at codon 758 to alanine (not shown) and phenylalanine at codon 766 to alanine (not shown), both in the lansoprazole pocket, retained the deubiquitination activity of CYLD, but abrogated the effect of lansoprazole.