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NOTUM and RSPO3 in Bone

WNT signaling modulators regulating the skeleton

Akademisk avhandling
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av **Karin Nilsson**

Fakultetsopponent:
Professor Subburaman Mohan
Loma Linda University, USA

Avhandlingen baseras på följande delarbeten

I. Movérare-Skrtic S*, Nilsson KH*, Henning P, Funck-Brentano T, Nethander M, Rivadeneira F, Coletto Nunes G, Koskela A, Tuukkanen J, Tuckermann J, Perret C, Souza PPC, Lerner UH, Ohlsson C. **Osteoblast-derived NOTUM reduces cortical bone mass in mice and the NOTUM locus is associated with bone mineral density in humans.** *FASEB J.* 2019 Oct;33(10):11163-11179. *Contributed equally

II. Nilsson KH, Henning P, El Shahawy M, Wu J, Koskela A, Tuukkanen J, Perret C, Lerner UH, Ohlsson C, Movérare-Skrtic S. **Osteocyte- and late osteoblast-derived NOTUM reduces cortical bone mass in mice.** *Am J Physiol Endocrinol Metab.* 2021 May 1;320(5):E967-E975.

III. Nilsson KH*, Henning P*, El Shahawy M, Nethander M, Andersen TL, Ejersted C, Wu J, Gustafsson KL, Koskela A, Tuukkanen J, Souza PPC, Tuckermann J, Lorentzon M, Ruud LE, Lehtimäki T, Tobias JH, Zhou S, Lerner UH, Richards JB, Movérare-Skrtic S, Ohlsson C. **RSPO3 is important for trabecular bone and fracture risk in mice and humans.** *Nat Commun*. 2021 Aug 13;12(1):4923. *Contributed equally

IV. Nilsson KH, Wu J, Gustafsson KL, El Shahawy M, Koskela A, Tuukkanen J, Tuckermann J, Henning P, Lerner UH, Ohlsson C, Movérare-Skrtic S. **Estradiol and RSPO3 regulate vertebral trabecular bone mass independent of each other.** *Am J Physiol Endocrinol Metab.* 2022 Mar 1;322(3):E211-E218

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Abstract

Osteoporosis is a skeletal disease affecting millions of people worldwide, often leading to fragility fractures and a decreased quality of life. There is an unmet medical need for new anabolic, bone building, treatments. WNT proteins, signaling via the WNT canonical or non-canonical pathway, are known to regulate cortical bone, trabecular bone, or both. There is currently a knowledge gap on the mechanisms behind this regulation, and it would be of great interest to find targets to specifically manipulate the different compartments. The aim of this thesis is to study the effect that WNT signaling modulators NOTUM and RSPO3 have on bone, using both *in vivo* and *in vitro* methods. In mouse studies, we inactivated NOTUM and RSPO3 globally and in specific cells using the Cre-*loxP* system, enabling us to, in detail, study these modulators mechanistically. First, we found that endogenous NOTUM, a WNT inhibiting secreted lipase, is a specific regulator of cortical bone, and that inactivation of NOTUM in all osteoblast-lineage cells increased cortical bone quality and thickness by increasing periosteal bone formation. Cell culture experiment showed that this effect is via enhanced osteoblast differentiation, and further studies concluded that it is the NOTUM expressed by late osteoblasts/osteocytes that is the main source of NOTUM. RSPO3, part of the R-spondin family, enhances WNT signaling and inactivation of RSPO3 in osteoblast-lineage cells specifically decreases trabecular bone in the vertebral column, with no effect on cortical bone. Osteoblast cultures revealed that inhibition of RSPO3 decreased proliferation and differentiation of osteoblasts through inhibition of WNT canonical signaling. In ovariectomized mice, a model for postmenopausal osteoporosis, RSPO3 was nonessential for the beneficial effects of estrogens on trabecular bone, but it was, surprisingly, needed for a full effect of estrogen on cortical bone. Together, the results presented in this thesis provide important new knowledge about the WNT signaling modulators NOTUM and RSPO3 and reveals them to be new possible targets for anabolic drugs against osteoporosis.

Keywords: NOTUM, RSPO3, WNT signaling, bone

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