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Okayama University research: Fourth key molecule identified in bone development

(Okayama, 16 February) **Researchers at Okayama University have identified the role of an additional protein in controlling the signalling processes for bone repair and growth.**

Bone repair and remodelling is achieved by the resorption of bone tissue by cells called ‘osteoclasts’ and the generation of new bone material by ‘osteoblasts’. Previous research had suggested that the molecular signalling for the generation of osteoclasts relies on a trio of protein molecules: RANK, RANKL and OPG. Now results from researchers at Okayama University have identified the crucial contribution of a fourth protein in the process.

RANK is a membrane protein that is essential in the generation of osteoclasts. It is regulated by a RANK ligand (RANKL) and the protein OPG, which acts as a decoy receptor inhibiting RANK signalling.

Masaharu Takigawa and colleagues at Okayama University Dental School and Okayama University Graduate School of Medicine and Pharmaceutical Science focused their investigations on the secreted protein CCN2. Previous work had already suggested that CCN2 plays a multifunctional role on various skeletal cells. “However,” as the researchers explain in the report of their studies, “The precise mechanisms of CCN2 action in the RANK/RANKL/OPG system were not sufficiently elucidated, though the system is known as crucial in osteoclast differentiation.”

The researchers sought peptides that bind to CCN2 through bacteriophage display screening and identified RANK. They then used a range of techniques to pin down CCN2 activity in cells, such as binding behaviour and the translocation of factors in the cells.

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The researchers found that CCN2 binds to RANK and enhanced RANK signalling in the generation of osteoclast cells. They also found that CCN2 binds to OPG, an interaction that impinges on the binding of CCN2 to RANK, but also prevents OPG inhibiting RANK signalling.

In their report of the work Takigawa and his colleagues conclude, “Therefore we propose CCN2 as a candidate of the fourth key molecule working in RANK/RANKL/OPG.”

Background

Osteoclastogenesis

NF-kappa B (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA, and is integral in a range of processes. It is widely understood that NF-kappa B plays a crucial role in osteoclastogenesis – the generation of osteoclasts. Previous work has indicated that osteoclast cells mature by means of receptor activator of NF-kappa B (RANK) signalling. The factors JNK and p38 are also understood to mediate RANK signalling.

Prior studies had also highlighted two mechanisms that regulate RANK signalling. The RANK ligand (RANKL) binds to RANK, activating factors NF-kappa B and JNK to trigger differentiation of cells into specialised osteoclasts. The other regulator, osteoprotegerin (OPG) inhibits the signalling by binding to RANK instead.

CCN2

The acronym CCN describes a family of secreted proteins that are rich in the amino acid cysteine. Although the family now includes a total of six proteins, the acronym stems from the initials of the three founder members: cysteine-rich 61 (CCN1), connective tissue regrowth factor (CCN2 – the focus of the current research) and nephroblastoma overexpressed (CNN3).

The secreted protein CCN2 binds to a variety of cytokines, membrane proteins and extracellular matrix components, prompting the Takigawa and colleagues to explore its possible biological functions. They used ‘bacteriophage display screening’ to search for peptides binding to CCN2 and identified an amino acid sequence homologous to RANK.

Studies of CCN2 in cultured mouse cells revealed several functions that are important in osteoclastogenesis. It enhances the translocation of NF-kappa B from the cell cytoplasm to the nucleus. It also enhances the phosphorylation of p38 and JNK – a process that involves the addition of a phosphate group and is known to control protein activity. In addition it inhibits activity of the OPG regulator by binding to it.

Experiments

The investigation of CCN2 activity included assays to monitor the binding between proteins, as well as surface plasmon resonance measurements to analyse the strength of the binding. Staining NF-kappa B allowed the researchers to assess the rate of its translocation by comparing signal intensities in the cytosol and the nucleus of cells.

Mitogen-activated protein kinases (MAPK) are protein enzymes that catalyse the transfer of phosphates. The researchers used western blotting to monitor RANK signalling via MAPK in the presence and absence of CCN2. In addition they used polymerase chain reaction (PCR) analysis to monitor osteoclast differentiation markers when stimulated by RANKL with and without CCN2. These tests also confirmed that CCN2 amplifies RANK signalling.

Reference

Eriko Aoyama, Satoshi Kubota, Hany Mohamed Khattab, Takashi Nishida, Masaharu Takigawa, “CCN2 enhances RANKL-induced osteoclast differentiation via direct binding to RANK and OPG”, *Bone*, **72**, pp.242-248, (2015)

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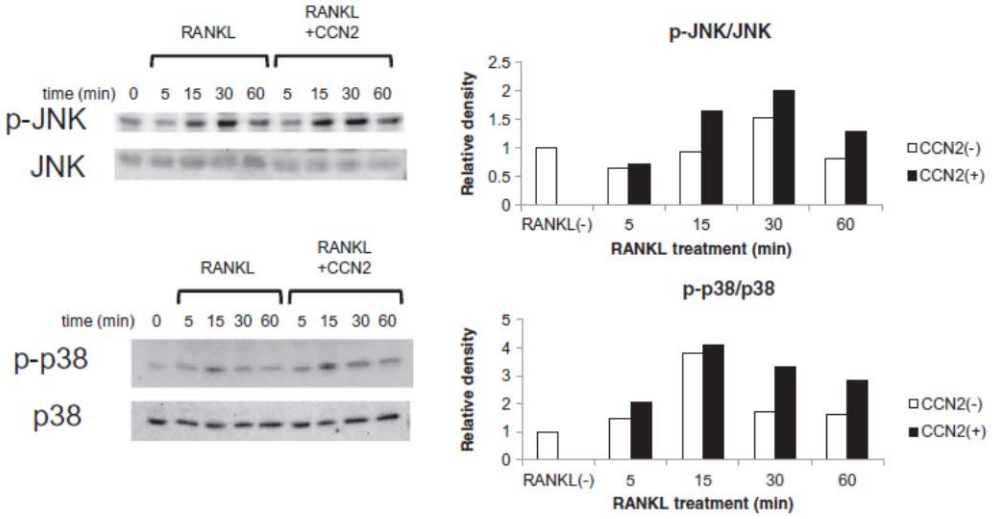

Figure caption

Amplification of p38 and JNK activation induced by RANKL stimulation of RAW264.7 (cultured mouse cells) by CCN2. RAW264.7 cells (6.6×10^4/well) were plated overnight, and the culture medium was changed to 1% FCS/αMEM. The next day, the cells were stimulated with RANKL-GST (25 ng/ml) in the presence or absence of CCN2 (100 ng/ml). After incubation for the indicated times, the cells were collected in RIPA buffer. The lysate was analyzed by Western blotting using anti-MAPK antibodies. Data are representative of those of 3 independent experiments.

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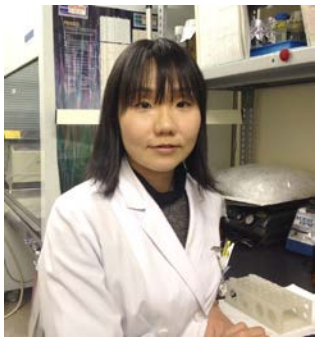

Further information

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About Okayama University

Okayama University is one of the largest comprehensive universities in Japan with roots going back to the Medical Training Place sponsored by the Lord of Okayama and established in 1870. Now with 1,300 faculty and 14,000 students, the University offers courses in specialties ranging from medicine and pharmacy to humanities and physical sciences. Okayama University is located in the heart of Japan approximately 3 hours west of Tokyo by Shinkansen.

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