様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　４ 年　 ６ 月 １７ 日現在

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研究課題名（和文）The study of a novel Runx2 target, Tem8, in skeletal development and chondrocyte apoptosis

研究課題名（英文）The study of a novel Runx2 target, Tem8, in skeletal development and chondrocyte apoptosis

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：軟骨細胞でRunx2の標的遺伝子を探したところ,Runx2がTem8の発現を高めることがわかった。胚16.5日からTem8-/-マウスの四肢は軟骨細胞の増殖が減少し、短くなった。軟骨細胞特異性Tem8遺伝子組み換えマウスでは、軟骨内骨化が野生型マウスのように進行しているにもかかわらず、四肢が短くなった。軟骨細胞ではBrdUとアポトーシスの両方が増加し、アポトーシス高域で鉱化が起こる。これらの結果から、Tem8は軟骨細胞の増殖に重要な役割を果たし、その発現はRunx2によって制御されており、Tem8の過剰発現は軟骨細胞のアポトーシスと基質鉱化を伴うことが示唆された。

研究成果の学術的意義や社会的意義
Antxr1は、軟骨細胞の成熟や血管の軟骨侵入、骨形成細胞の分化に影響を及ぼさず、軟骨細胞の増殖を調節していることを明らかにした。さらに、軟骨細胞におけるAntxr1の2回の過発現は、明らかな軟骨細胞のアポトーシスを誘導するのに十分であり、これはカルシウムまたはリン酸塩の代謝に関連するメカニズムによって引き起こされる可能性がある。人間の遺伝疾患の研究では、Tem8の突然変異がGAPO症候群を引き起こすことがわかっている。そのため、生理的な骨の発達だけでなく、変形性関節炎やGAPO症候群などの骨の病気のメカニズム解明にも役立つ。

研究成果の概要（英文）：In the search of Runx2 target genes in chondrocytes, we found that Tem8 expression is upregulated by Runx2. Tem8 was highly expressed in cartilaginous tissues and was directly regulated by Runx2. In skeletal development, the process of endochondral ossification proceeded similarly in wild-type and Tem8-/- mice. However, the limbs of Tem8-/- mice were shorter than those of wild-type mice from embryonic day 16.5 due to the reduced chondrocyte proliferation. Chondrocyte-specific Tem8 transgenic mice exhibited shortened limbs, although the process of endochondral ossification proceeded as in wild-type mice. BrdU-uptake and apoptosis were both increased in chondrocytes, and the apoptosis-high regions were mineralized. These findings indicated that Tem8, of which the expression is regulated by Runx2, plays an important role in chondrocyte proliferation and that overexpression of Tem8 causes chondrocyte apoptosis accompanied by matrix mineralization.

研究分野：Bone biology

キーワード：Tem8　Runx2　chondrocyte　proliferation　apoptosis

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様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

To identify genes under the regulatory control of Runx2, we performed in vitro experiments using chondrocytes. We introduced *Runx2* by infection of adenovirus and screened molecular targets of Runx2 by microarray techniques. *Tem8* (gene symbol: *Antxr1*) was found as one of the candidate genes, and we confirmed that *Tem8* mRNA levels were up-regulated by Runx2 in chondrocytes by real-time RT-PCR (Fig.1).

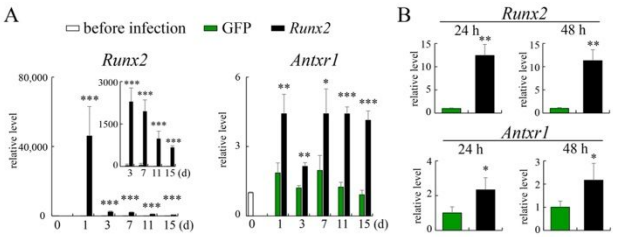

Fig.1 Induction of Antxr1 by Runx2.
(Int. J. Mol. Sci. 2020, 21, 2425)

Tem8 was first discovered as a gene highly expressed in tumor endothelial cells. It encodes a type I membrane protein that can act as a receptor for the anthrax toxin. However, there is no report of Tem8 in bone tissue. To examine gain of function phenotypes during skeletal development, we cloned *Tem8* full length cDNA and prepared transgenic mice overexpressing *Tem8* in chondrocytes by using the *Col2a1* promoter. Interestingly, Tg mice overexpressing *Tem8* displayed alkaline phosphatase (Alp)-independent ectopic calcification in epiphysis, and many chondrocytes in the mineralized regions were TUNEL-positive. Apoptosis occurred first and mineralization followed it (Fig.2). OA is the most common chronic disease of the joints. OA occurs when cartilage breaks down and wears away, causing the bones within the joint to rub together. The death of chondrocyte is implicated in the pathogenesis of OA. Runx2 is requisite for chondrocyte maturation and regulates the expressions of *Ihh*, *Col10a1*, *Spp1*, *Ibsp*, *Mmp13*, and *Vegfa*. Runx2 was reported to be involved in the pathogenesis of OA. Overexpression of *Runx2* induces the expressions of *MMP13* and *ADAMTS5*, which disrupt the matrix of articular cartilage. Since Tem8 induced apoptosis of chondrocytes, it may be involved in the pathogenesis of OA. The elucidation of the mechanism of apoptosis by Tem8 may explain a part of apoptosis in OA.

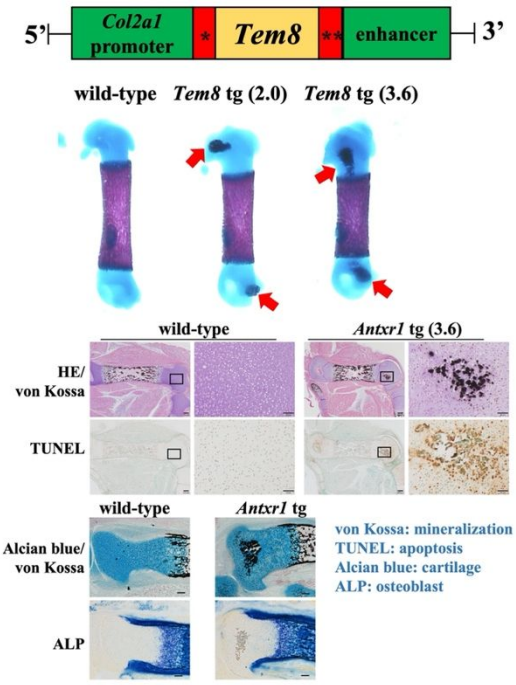

Fig.2 Ectopic Mineralization Was Caused by Apoptosis.
(Int. J. Mol. Sci. 2020, 21, 2425)

２．研究の目的

Runx2 is a transcription factor, which is essential for osteoblast differentiation and chondrocyte maturation. However, a small number of Runx2 targets in chondrocytes have been characterized. We screened Runx2 targets using microarray techniques and found that Tem8 (Tumor endothelial marker 8, also named as Antrx1) is one of the Runx2 targets in chondrocytes (unpublished data). We generated transgenic (Tg) mice overexpressing Tem8 in chondrocytes using Col2a1 promoter, which directs the expression to chondrocytes. Overexpression of Tem8 in chondrocytes induced chondrocyte apoptosis and disturbed the development of cartilaginous structures (unpublished data). The main purposes of this study are to elucidate the roles of Tem8 in skeletal development and the molecular pathway of the induction of apoptosis by Tem8, which may provide a novel insight into the pathogenesis of osteoarthritis (OA).

３．研究の方法

Runx2 is essential for osteoblast differentiation and chondrocyte maturation. A high-throughput screening of chondrocytes overexpressing Runx2 revealed a new target gene of Runx2 in chondrocytes, Tem8. Sequence analysis of Tem8 gene revealed that Runx2 protein directly binds to the 0.85K enhancer in the intron region of Tem8 gene to regulate Tem8 expression. The proliferation of chondrocytes in Tem8-/- knockout mice was significantly lower than that in the wild type, resulting in short limbs in embryonic and adult Tem8-/- mice. Chondrocytes matured and vascular invasion, and osteoblasts differentiated normally. The applicant used transgenic mice (Tem8-tg) that overexpressed Tem8 in chondrocytes, and further study found that Tem8-TG mice showed abnormal calcification in the bone secondary calcification center, which significantly induced chondrocyte apoptosis, leading to abnormal calcium and phosphorus metabolism in the body. Apoptosis is a risk factor for osteoarthritis, and Runx2 is one of the genes responsible for osteoarthritis. Human genetic disease studies have shown that Tem8 gene mutations cause GAPO syndrome, a rare autosomal recessive disorder characterized by growth retardation. Therefore, elucidating the regulation mechanism of Tem8 on chondrocyte proliferation and apoptosis is not only helpful to understand physiological bone development, but also helpful to elucidate the pathogenesis of bone diseases such as osteoarthritis and GAPO syndrome.

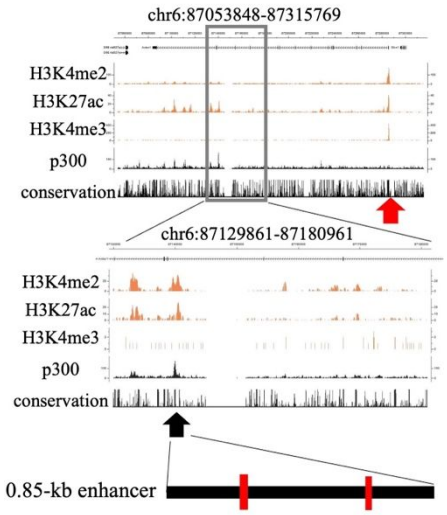

Fig.3 ChIP-seq using Runx2 antibody.
(Int. J. Mol. Sci. 2020, 21, 2425)

４．研究成果

Antxr1/Tem8 is highly expressed in tumor endothelial cells and is a receptor for anthrax toxin. Mutation of Tem8 causes GAPO syndrome, which is characterized by growth retardation, alopecia, pseudo-anodontia, and optic atrophy. However, the mechanism underlying the growth retardation remains to be clarified. Runx2 is essential for osteoblast differentiation and chondrocyte maturation and regulates chondrocyte proliferation through *Ihh* induction. In the search of Runx2 target genes in chondrocytes, we found that Tem8 expression is upregulated by Runx2. Tem8 was highly expressed in cartilaginous tissues and was directly regulated by Runx2. In skeletal development, the process of endochondral ossification proceeded similarly in wild-type and Tem8-/- mice. However, the limbs of Tem8-/- mice were shorter than those of wild-type mice from embryonic day 16.5 due to the reduced chondrocyte proliferation. Chondrocyte-specific Tem8 transgenic mice exhibited shortened limbs, although the process of endochondral ossification proceeded as in wild-type mice. BrdU-uptake and apoptosis were both increased in chondrocytes, and the apoptosis-high regions were mineralized. These findings indicated that Tem8, of which the expression is regulated by Runx2, plays an important role in chondrocyte proliferation and that overexpression of Tem8 causes chondrocyte apoptosis accompanied by matrix mineralization.

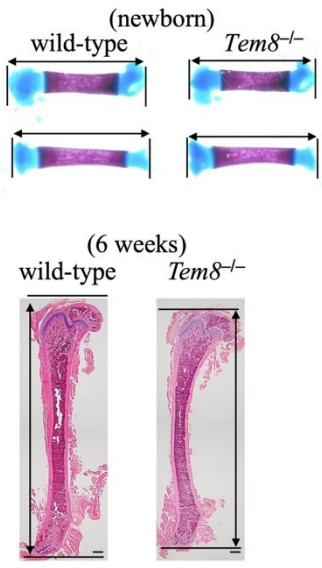

Fig.4 Shorter Femurs and Tibiae in Antxr1–/– Mice.
(Int. J. Mol. Sci. 2020, 21, 2425)

５．主な発表論文等

〔雑誌論文〕　計4件（うち査読付論文　4件／うち国際共著　4件／うちオープンアクセス　4件）

１．著者名 Jiang Qing、Qin Xin、Yoshida Carolina Andrea、Komori Hisato、Yamana Kei、Ohba Shinsuke、Hojo Hironori、Croix Brad St.、Kawata-Matsuura Viviane K. S.、Komori Toshihisa	４．巻 21
２．論文標題 Antxr1, Which is a Target of Runx2, Regulates Chondrocyte Proliferation and Apoptosis	５．発行年 2020年
３．雑誌名 International Journal of Molecular Sciences	６．最初と最後の頁 2425～2425
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijms21072425	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Qin Xin、Jiang Qing、Nagano Kenichi、Moriishi Takeshi、Miyazaki Toshihiro、Komori Hisato、Ito Kosei、Mark Klaus von der、Sakane Chiharu、Kaneko Hitomi、Komori Toshihisa	４．巻 16
２．論文標題 Runx2 is essential for the transdifferentiation of chondrocytes into osteoblasts	５．発行年 2020年
３．雑誌名 PLOS Genetics	６．最初と最後の頁 1009169～1009169
掲載論文のDOI（デジタルオブジェクト識別子） 10.1371/journal.pgen.1009169	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Matsuura Viviane K. S. Kawata、Yoshida Carolina Andrea、Komori Hisato、Sakane Chiharu、Yamana Kei、Jiang Qing、Komori Toshihisa	４．巻 21
２．論文標題 Expression of a Constitutively Active Form of Hck in Chondrocytes Activates Wnt and Hedgehog Signaling Pathways, and Induces Chondrocyte Proliferation in Mice	５．発行年 2020年
３．雑誌名 International Journal of Molecular Sciences	６．最初と最後の頁 2682～2682
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/ijms21082682	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Qin Xin、Jiang Qing、Komori Hisato、Sakane Chiharu、Fukuyama Ryo、Matsuo Yuki、Ito Kosei、Miyazaki Toshihiro、Komori Toshihisa	４．巻 36
２．論文標題 Runt‐related transcription factor‐2 (Runx2) is required for bone matrix protein gene expression in committed osteoblasts in mice	５．発行年 2021年
３．雑誌名 Journal of Bone and Mineral Research	６．最初と最後の頁 2081～2095
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/jbmr.4386	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計1件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Qing Jiang
２．発表標題 Runx2はマウスの骨芽細胞分化後の骨基質蛋白質遺伝子発現に必要である
３．学会等名 第 39 回日本骨代謝学会学術集会
４．発表年 2021年～2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関