

課題番号 (36)

## Roles of vasohibins - CCN2/CTGF interactions in angiogenesis and cartilage/bone-related tumors

### [ 1 ] 組織 (Research group)

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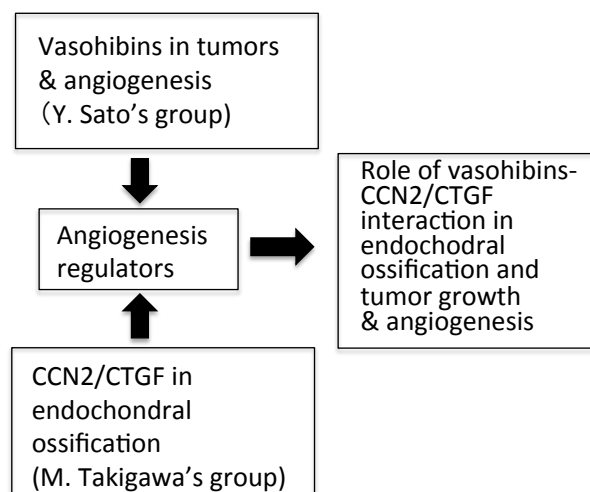
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### [ 2 ] 研究経過 (Research setup)

Angiogenesis is involved in various biological processes such as endochondral ossification and solid tumor growth. It is regulated by the local balance between angiogenesis stimulators and inhibitors. Recently, Prof. Y. Sato isolated vasohibin-1 (VASH1) as a negative feedback regulator of angiogenesis produced by endothelial cells (ECs) and subsequently vasohibin-2 (VASH2) as a homologue of VASH1. VASH1 is expressed in ECs to terminate angiogenesis, whereas VASH2 is expressed in cells other than ECs to promote angiogenesis in the mouse model of angiogenesis. Thus, much attempt to clarify roles of VASHs in tumor growth has been made so far but there has

been no report on role of VASHs in endochondral ossification.

In the process of endochondral ossification, chondrocytes proliferate, differentiate, mature and become hypertrophic chondrocytes and then blood vessels penetrate into cartilage, which is an avascular tissue, from bone rich in ECs. Prof. M. Takigawa et al. isolated a gene named *hcs24* (hypertrophic chondrocyte specific gene 24), which is predominantly expressed in hypertrophic chondrocytes and also in actively proliferating and migrating ECs. Its gene products Hcs24, recently re-named CCN2 (also known as connective tissue growth factor:CTGF) promotes proliferation and differentiation of chondrocytes, osteoblasts, and ECs and induce angiogenesis in vivo, resulting promotion of endochondral ossification. However, the role of CCN2 in a variety of malignancies is highly variable and controversial. This might be due to unique molecular characteristics of CCN2. Because CCN2 binds to various growth factors, extracellular matrix and cell surface receptors through 4 modules in the molecule, CCN2 may orchestrates extracellular signaling by interaction with the binding partners of which combinations (microenvironment) are different among cell types, and differentiated states of the same cells, and



disorders of this orchestration may lead to malignancies.

Therefore, in this study, we aimed to reveal the role of VASHs in cartilage/bone formation, especially endochondral ossification, as well as their malignant changes. Specifically, we investigated the involvement of interaction with CCN2 and VASHs, possible mutual regulation between VASHs and CCN2 and its significance in endochondral ossification, co-expression of VASH2 and CCN2 in growth plate chondrocytes.

To perform this project, we have discussed so often by e-mails. Examples include on May 13 and 14, June 12, 26 and 29, July 24, November 11, 24 and 27, December 3, 2015 and January 14, 25 and 28, February 2 and 5. Moreover, we discussed our results in detail at the 11<sup>th</sup> Vasohibin Meeting at LA FORET ZAO, Zao on January 23-24, 2016.

### [ 3 ] 成果 (Research outcomes)

#### ( 3 - 1 ) 研究成果 (Results)

During this fiscal year, we have obtained the following results.

- 1) Mouse chondroprogenitor ATDC5 cells were cultured in the chondrogenic differentiation medium for 4 weeks and gene expression of VASH1, 2, CCN2, VEGF, MMP13, Col2a1, aggrecan, ColXal, and alkaline phosphatase (ALP) were analyzed by RT-PCR. As a result, gene expression of CCN2 and VASH1 increased as gene expression of ColXal, ALP, MMP13, which are makers of hypertrophic chondrocytes, increased. Gene expression of VEGF, another angiogenesis factor, increased slightly later than that of CCN2 and VASH2.
- 2) Expression of VASH2 was high in the early stage of differentiation of ATDC5 and decreased gradually as the cells differentiated.
- 3) Human chondrocytic cell line HCS-2/8 express collagen type II and aggrecan which are two major makers of chondrocytes but does not differentiate to hypertrophic chondrocytes. Expression level of VASH1 in HCS-2/8 cells was low. Taken together with the finding in 1), the result suggests that VASH1 is expressed only in hypertrophic chondrocytes during chondrocyte differentiation.

- 4) When ATDC5 cells at early hypertrophic stage were treated with recombinant CCN2, mRNA level of VASH1 was decreased after 12 h.
- 5) Before investigating interaction between CCN2 and vasohibins, we investigated interaction between various angiogenic factors and CCN2, and found that FGF-1, FGF-2 and VEGF bound to CCN2 with high affinities.

#### ( 3 - 2 ) 波及効果と発展性など (Future perspectives)

There had been no report on relation between vasohibins and cartilage metabolism or on relation between vasohibins and CCN proteins. This study showed at the first time that VASH1 is involved in chondrocyte hypertrophy and the involvement has something to do with expression of CCN2. These novel findings are fruits from a collaboration between two different research fields/groups, producing a chance of development of new research fields, e.g., "Vasohibins in cartilage metabolism" and "Interaction between vasohibins and CCN proteins".

### [ 4 ] 成果資料 (List of Papers)

There has been no paper presenting the data obtained from this collaboration yet. The followings are a related book and papers.

- (1) Takigawa M.: CCN Proteins: Methods and Protocols, Springer, in press.
- (2) Aoyama E, Kubota S, Khattab HM, Nishida T, Takigawa M.: CCN2 enhances RANKL-induced osteoclast differentiation via direct binding to RANK and OPG. *Bone*, 73:242-8, 2015.
- (3) Khattab HM, Aoyama E, Kubota S, Takigawa M.: Physical interaction of CCN2 with diverse growth factors involved in chondrocyte differentiation during endochondral ossification. *J. Cell Commun. Signal.* 9(3):247-54, 2015.

Presentation at a meeting.

Murase Y, Aoyama E, Kattab H, Abd Elkader T, Suzuki Y, Sasaki A, Kubota S, Sato Y, Takigawa M: Role of vasohibins-CCN2/CTGF interaction in endochondral ossification. The 11<sup>th</sup> Vasohibin Meeting, LA FORET ZAO, Zao, January 23-24, 2016