日本財団 The Nippon Foundation 助成事業

財団法人　日中医学協会

2010年度共同研究等助成金報告書－在留中国人研究者－

2011年　3　月　8　日

財団法人　日中医学協会　御中

貴財団より助成金を受領して行った研究テーマについて報告いたします。

添付資料：**研究報告書**

中国人研究者氏：王　霜　　印

指導責任者名：小方　頼昌

所属部署名：日本大学松戸歯学部　　職名：歯周治療講座

所　在　地：千葉県松戸栄町西2-870-1

電　　話：047-360-9365　　内線：365

1．助成金額：　600.000　円

2．研究テーマ

インターロイキン１１にする骨シアロタンパク質の転写調節

3．成果の概要

IL-11は骨芽細胞の分化を誘導し　石灰化結合組織特異的に発現する骨シアロタンパク質（BSP）の遺伝子発現を増加させた。この転写調節にBSP遺伝子プロモーター中のCRE，FREおよびHOX配列が重要な役割をしている。

4．研究業績

（1）学会における発表　　無・有（学会名・演題）

第96回アメリカ歯周病学会　共催日本歯周病学会　2010年大会

Effect of Interleukin-11 on Bone Sialoprotein Gene Transcription

第53回春季日本歯周病学会

Calcium Hydroxide Regulates Bone Sialoprotein Gene Transcription

（2）発表した論文　　無・有（雑誌名・題名）

「Gene」掲載　Transcriptional regulation of bone sialoprotein gene by Interleukin-11

「日大口腔科学」掲載　Regulation of Bone Sialoprotein Gene Transcription by Keampferol and Calcium Hydroxide

－日中医学協会助成事業－

インターロイキン－11による骨シアロタンパク質の転写の調節

研究者　氏名　　王　霜
中国所属機関　　中国天津医科大学口腔医院歯内科
日本研究機関　　日本大学松戸歯学部歯周治療講座
指導　責任者　　教授　小方　頼昌
共同研究者名　　佐々木庸子　周黎明　松村浩禎
荒木正大　目澤優　高井英樹　陈阵

要旨

Interleukin-11 (IL-11) is a stromal cell-derived cytokine. IL-11 has many biological activities and has roles in hematopoiesis, immune responses, the nervous system and bone metabolism. Bone sialoprotein (BSP) is a mineralized tissue-specific protein expressed in differentiated osteoblasts that appears to function in the initial mineralization of bone. IL-11 (20 ng/ml) increased BSP mRNA levels at 12 h in osteoblast-like ROS 17/2.8 cells. In a transient transfection assay, IL-11 (20 ng/ml) increased luciferase activity of the construct (-116 to +60) in ROS 17/2.8 cells and rat bone marrow stromal cells. Introduction of 2 bp mutations to the luciferase constructs showed that the effects of IL-11 were mediated by a CRE, a FRE and a HOX. Luciferase activities induced by IL-11 were blocked by protein kinase A inhibitor, tyrosine kinase inhibitor and ERK1/2 inhibitor. Gel shift analyses showed that IL-11 (20 ng/ml) increased nuclear protein binding to CRE, FRE and HOX. CREB1, phospho-CREB1, c-Jun, JunD and Fra2 antibodies disrupted the formation of CRE-protein complexes. Dlx5, Msx2, Runx2 and Smad1 antibodies disrupted FRE- and HOX-protein complex formations. These studies demonstrate that IL-11 stimulates BSP transcription by targeting CRE, FRE and HOX sites in the proximal promoter of the rat BSP gene. Moreover, CREB1, c-Jun, JunD, Fra2, Dlx5, Msx2, Runx2 and Smadl transcription factors appear to be key regulators of IL-11 effects on BSP transcription.

Key Words　骨シアロタンパク質，　インターロイキン－11，骨芽細胞，　転写

緒言

Interleukin-11 (IL-11) is a stromal cell-derived cytokine that belongs to the interleukin-6 family of cytokines (1, 2). IL-11 has many biologic activities and has roles in hematopoiesis, immune responses, the nervous system and bone metabolism (2-6). IL-11 increased alkaline phosphatase (ALP) activities, which are a marker of osteoblasts (7); therefore, it is possible that IL-11 may have an important role in osteogenesis. however, little is known about the role of IL-11 in osteogenesis, osteoblast differentiation and bone formation.

Bone sialoprotein (BSP) is a highly sulfated, phosphorylated, and glycosylated protein that is expressed almost exclusively in mineralizing tissues (8, 9). Regulation of the BSP gene appears to be important in the differentiation of osteoblasts, in bone matrix mineralization and in tumor metastasis.

To elucidate the molecular mechanism of IL-11 regulation of the BSP gene, we analyzed the effects of IL-11 on the expression of the BSP gene in osteoblast-like cells.

対象と方法

対象は、Rat osteoblast-like ROS 17/2.8 cells and rat stromal bone marrow cells (RBMC)

方法は、Northern Hybridization, Transient Transfection Assays and Gel Mobility Shift Assays.

結果

1. Effects of IL-11 on BSP mRNA

To study the regulation of BSP transcription by IL-11, we performed Northern hybridization analysis of total RNA extracted from osteoblastic ROS 17/2.8 cells. First, In dose-response IL-11 increased BSP mRNA levels at 1, 5, 20 and 100 ng/ml and had a maximal effect at 20 ng/ml (Fig. 1A). Thus, 20 ng/ml IL-11 was used to determine the time courses of BSP mRNA expression. IL-11 (20 ng/ml) induced BSP mRNA levels at 3 h and reached maximal at 12 h (Fig. 1B).

2. Transient transcription analyses of rat BSP promoter constructs

To determine the site of IL-11-regulated transcription in the 5'-flanking region of the BSP gene, we did transient transcription analyses. The transcriptional activity of pLUC3, pLUC4, pLUC5 and pLUC6 was increased after 12 h treatment with 20 ng/ml IL-11 in ROS 17/2.8 and RBMC cells (Fig. 2A, B). Since protein kinases mediate IL-11 signaling activities, we investigated the effects of the PKC inhibitor H7, the PKA inhibitors H89 and KT5720, the tyrosine kinase inhibitor HA and the ERK1/2 inhibitor inhibitor U0126 on IL-11-mediated transcription. Whereas IL-11-induced pLUC3 promoter activation was inhibited by U0126, HA, KT5720 and H89, no effect was observed for H7 (Fig. 3). After introducing 2 bp mutations into the putative response elements within pLUC3 and pLUC4, transcriptional induction by IL-11 (20 ng/ml) was partially inhibited in the M-CRE (pLUC3), M-FRE and M-HOX (pLUC3 and pLUC4) constructs (Fig. 4).

3. Gel mobility shift assays

To identify nuclear proteins that bind to the CCAAT, CRE, FRE, Pit-1 and HOX elements and mediate IL-11 effects on transcription, we did gel mobility shift assays. Inverted CCAAT and Pit-1-protein complexes did not change after stimulation by IL-11 (Fig. 5, lanes 1-4, 13-16). After stimulation by 20 ng/ml IL-11 (3–12 h), CRE- and FRE-protein complexes were increased at 12 h (Fig. 5, lanes 5-8, 9-12), and HOX-protein complexes were increased at 3 h and reached maximal at 12 h (Fig. 5, lanes 17-20). To further characterize the proteins in the complexes formed with CRE, FRE and HOX, we used antibodies to several transcription factors. The addition of phospho-CREB1 antibody induced supershift (Fig. 6A, lane 5), and CREB1, c-Jun, JunD and Fra2 antibodies partially disrupted CRE-protein complex formation (Fig. 6A, lanes 4, 7-9). Dlx5, Msx2, Runx2 and Smad1 antibodies partially disrupted FRE- and HOX-protein complex formation (Fig. 6B and C, lanes 4-7).

考察

These studies show that IL-11 increases BSP transcription in osteoblast-like cells by targeting CRE, FRE and HOX elements in the proximal promoter of the BSP gene. IL-11 (20 ng/ml) induced BSP mRNA expression in ROS17/2.8 cells (Fig. 1). When we used rat stromal bone marrow cells (RBMC), IL-11 also increased BSP transcription (Fig. 2B); therefore, IL-11 increases BSP transcription not only in transformed ROS 17/2.8 cells but also in normal osteoprogenitors (RBMC). Transcriptional regulation by IL-11 was abrogated by M-CRE, M-FRE and M-HOX in pLUC3 or pLUC4 (Fig. 4). The involvement of CRE, FRE and HOX elements is further supported by gel shift assays in which nuclear proteins that formed complexes with CRE, FRE and HOX elements were

increased by IL-11 (20 ng/ml) in ROS 17/2.8 cells (Fig. 5). Results of gel shift assays using antibodies (Fig. 6) suggest that IL-11 induced BSP transcription through CREB1, phospho-CREB1, c-Jun, JunD and Fra2 targeting CRE, and through Dlx5, Msx2, Runx2 and Smad1 targeting FRE and HOX in the rat BSP gene promoter. The ERK1/2 inhibitor U0126, the tyrosine kinase inhibitor HA and the PKA inhibitors H89 and KT5720 inhibited the effects of IL-11 on BSP transcription, suggesting that ERK1/2, tyrosine phosphorylation and PKA signaling pathways are crucial for IL-11 effects on BSP transcription.

In conclusion, we have characterized a region of the rat BSP gene promoter that is required for IL-11 mediated transcription. This region contains CRE, FRE, and HOX, which are required for the IL-11 response. Further, IL-11-induced transcription was inhibited by tyrosine kinase inhibitor HA and protein kinase A inhibitor. Moreover, CREB1, C-Jun, JunD, Fra2, Dlx5, Msx2, Runx2 and Smadl transcription factors appear to be key regulators of IL-11 effects on BSP transcription and bone formation.

参考文献

1. Paul, S.R., Bennett, F., Calvetti, J.A., Kelleher, K., Wood, C.R., O'Hare, R.M., Leary, A.C., Sibley, B., Clark, S.C., Williams, D.A., Yang, Y.C., 1990. Molecular cloning of a cDNA encoding interleukin-11, a stromal cell derived lymphopoietic cytokine. Proc. Natl. Acad. Sci. USA 87,7512–7516.
2. Yin, T.G., Schendel, P., and Yang, Y.C. 1992. Enhancement of in vitro and in vivo antigen-specific antibody responses by interleukin 11. J. Exp. Med. 175. 211-216.
3. Du, X.X., Williams, D.A., 1994. Interleukin-11; a multifunctional growth factor derived from the hematopoietic microenvironment. Blood 83, 2023-2030.
4. Du, X.X., Williams, D.A., 1997. Interleukin-11; review of molecular, cell biology, and clinical use. Blood 89, 3897-3908.
5. Mehler, M,F., Rozental, R., Dougherty, M., Spray, D.C., and Kessler, J.A., 1993. Cytokine regulation of neuronal differentiation of hippocampal progenitor cells. Nature 362, 62-65.
6. Musashi, M., Yang, Y.C., Paul, S.R., Clark, S.C., Sudo, T., and Ogawa, M. 1991. Direct and synergistic effects of interleukin 11 on murine hemopoiesis in culture. Proc. Natl. Acad. Sci. USA 88, 765-769.
7. Gimble, J,M., Wanker, F., Wang, C.S., BAS, H., Wu, X., Kelly. K., Yancopoulos, G.D., Hill, M.R., 1994. Regulation of bone marrow stromal cell differentiation by cytokines whose receptors share the gp130 protein. J. Cell. Biochem. 54, 122-133.
8. Oldberg, Å., Franzen, A., and Heinegård, D., 1988. The primary structure of a cell-binding bone sialoprotein. J. Biol. Chem. 263,19430–19432.
9. Fisher, L.W., McBride, O.W., Termine, J.D., Young, M.F., 1990. Human bone sialoprotein. J. Biol. Chem. 265, 2347-2351.

注: 本研究は、2010年11月1日「第96回アメリカ歯周病学会」にてポスター発表、「Gene」(2011Jan 26)に掲載、「日大口腔科学」（2011）に掲載。

作成日：2011 年 3 月 1 日