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学 位 論 文 題 名

Role of SDF-1 signaling during gastrulation in *Xenopus laevis*

（アフリカツメガエル原腸胚期におけるSDF-1情報伝達の役割）

学位論文内容の要旨

Gastrulation is the process in which concerted cell movement leads to the arrangement of three germ layers to their proper locations. Chemokines known as chomotactic cytokines are small secreted proteins produced by number of hematopoietic and non hematopoietic stromal cells in adult tissues. Chemokines play crucial role in developmental processes and recent studies reveal their roles in a gastrulation movement. SDF-1, also known as CXCL12, is one of the chemokine which functions *via* chemokine receptors CXCR4 and CXCR7. Here, I revealed the expression pattern of xSDF-1α, xCXCR4, and xCXCR7, regulation of expression of xSDF-1α and xCXCR4, and the role of xCXCR4 signaling on the regulation of mesodermal gene.

Whole mount *in situ* hybridization (WISH) and quantitative real-time RT-PCR (qRT-PCR) analyses demonstrated that the transcript of xSDF-1α was increased in blastocoel roof during gastrulation, but not in involuted mesoderm. xCXCR4 was expressed in mesendoderm at the late blastula and retained throughout the gastrulation. xCXCR7 was found in dorsal lip around the blastopore at early gastrula stage and became localized in the presumptive notochord later.

CXCR4 is known as activin responsive gene and activin is a potent mesoderm inducing factor. Thus, I investigated the role of activin-like signaling in the regulation of xSDF-1α and xCXCR4 by WISH and qRT-PCR analyses. The embryos were injected with cer-s, a potent antagonist of Xnrs, to block the mesoderm induction. Increased xSDF-1α and

suppressed xCXCR4 expression in the mesoderm region were observed in cer-s injected embryos by WISH analysis. The efficacy of cer-s was confirmed by complete inhibition of a pan-mesodermal marker gene Xbra. For further confirmation, I examined the expression levels of xSDF-1α and its receptors in activin treated animal cap explants. The level of xSDF-1α expression was decreased in the explants treated with 5-500 ng/ml activin and that of xCXCR4 was increased at the 500 ng/ml activin. These results show that activin/nodal signaling can control the expressions of xCXCR4 and xSDF-1α reciprocally. Taken together, xSDF-1α and its receptors may contribute the arrangement of mesoderm cells and their expression patterns are partially regulated by activin/nodal signaling.

Furthermore, I examined the role of xSDF-1α and xCXCR4 in the regulation of mesodermal gene. WISH analysis revealed that the injection of CXCR4 resulted in the down regulation of Xbra but not other genes such as FGF, Mix.2, gsc, chd, and cer. At the time of gastrulation, dorsal mesoderm exhibits two different cell behaviors in two different regions, directional cell migration of prechordal mesoderm and convergent extension of chordamesoderm. Considering previous report that Xbra acts as a switch between cell migration and convergent extension, CXCR4 may control cell migration by regulating Xbra.

To confirm the role of SDF-1/CXCR4 signaling in the regulation of xSDF-1α and its receptor, I examined the expression levels of xSDF-1, xCXCR4, and xCXCR7 in the xSDF-1α and CXCR4 injected animal caps. However, I could not find any significant difference in the expression levels of xSDF-1α, xCXCR4, and xCXCR7 compared with control and injected animal caps. These results suggest the inability of SDF-1/CXCR4 signaling to regulate xSDF-1α and its receptor.

Chemokine receptor CXCR4 is up-regulated in many human cancers such as breast, lung, prostate, colon, and melanoma, and facilitates tumor at several key steps of metastasis. Recent investigations show exciting therapeutic applications of chemokines in inflammatory, infectious, and cancer-related diseases. The knowledge about the expression and regulation of chemokines and its receptors can be applied not only in developmental biology, but also in immunology and oncology for the development of therapies.

学位論文審査の要旨

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The dissertation describes the expression pattern of chemokine xSDF-1 and its receptors xCXCR4 and xCXCR7 and the regulation of xSDF-1/xCXCR4 signalling during gastrulation in *Xenopus laevis*. Role of chemokines in the regulation of gastrulation is still not fully understood but the detail study about expression patterns of xSDF-1, xCXCR4, and xCXCR7 may throw some lights on the role of these in the regulation of gastrulation. Furthermore the regulation of SDF-1/CXCR4 signalling is not clear yet, so the author tried to put some light on the some aspects which may be involved in the regulation of these signalling. Here, the author clarified the expression pattern of xCXCR7 during gastrulation, gene regulation of xSDF-1 by activin, and suppression of Xbra by xCXCR4 signaling.

The author analyzed the expression patterns of xSDF-1, xCXCR4, and xCXCR7 by Whole mount *in situ* hybridization analysis. The increase in the expression level of xSDF-1 was observed in the BCR with progression in the gastrulation stages. The expression of xCXCR4 was detected in the mesendoderm at the onset of gastrulation and remained in the mesendoderm throughout the gastrula stages. xCXCR7 was expressed in dorsal lip and later become localized in the presumptive notochord as depicted in the external and sagittal section of the embryo. The expression patterns were also confirmed by the q-RT-PCR analysis. xSDF-1 was expressed in the BCR explants but not in the mesendoderm. Furthermore xCXCR4 retained in the mesendoderm whereas xCXCR7 retained in the dorsal mesoderm explants but decreased in ectodermal explants. CXCR7 was found as second receptor for SDF-1 and can function via non G-protein signalling pathway or sequestrating SDF-1 signalling. These observations suggest that xCXCR7-expressing cells in axial mesoderm sequestrates xSDF-1 and forms a local xSDF-1 gradient, which supports xCXCR4-expressing mesendoderm cells migrate towards animal pale.

Regulation of expression of xSDF-1 and xCXCR4 is still not clear. xCXCR4 is reported as activin responsive gene and activin act as mesoderm inducers. Inhibition of activin/nodal signalling by cer-s resulted in the suppression of xCXCR4 and overexpression of xSDF-1 in the mesendoderm confirmed by Whole mount *in situ* hybridization and qRT-PCR analysis. Decrease in level of xSDF-1 with increase in concentration of activin was observed whereas level of xCXCR4 increased at high concentration of activin. Furthermore no such effect of activin on xCXCR7 was observed. These results show that Activin/Nodal signalling controls the expression pattern of xSDF-1 and xCXCR4.

As role of xCXCR4 in the regulation of mesodermal genes are poorly understood, the author examined the effect of xCXCR4 on mesodermal gene expression. Previous report showed that Xbra acts as a switch between cell migration and convergent extension. The author detected the suppression of Xbra, but not other genes Mix, gsc and FGF, by overexpression of xCXCR4 with Whole mount *in situ* hybridization analysis. These data suggest that CXCR4 supports directional migration in mesendodermal cells by suppressing Xbra.

In conclusion, the knowledge about the expression and regulation of chemokines and its receptors contributes not only in developmental biology, but also in immunology and oncology for the development of therapies. Therefore, we acknowledge that the author is qualified to be granted the doctorate of Science from Hokkaido University.