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学位論文名	GATA-4 regulates cardiac morphogenesis through transactivation of the N-cadherin gene （GATA-4はN-Cadherin遺伝子の転写活性を調節することにより、心臓の形態形成を制御する）
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論文内容の要旨

[Aim]

Cardia bifida (separated heart tubes) is seen in mice with targeted disruption of GATA-4, a subfamily of the zinc finger regulatory proteins. The similar phenotype has been found in animal models lacking of N-cadherin, RhoA, Furin, BMP, and Mesp1. Moreover, GATA-4 is a potent transactivator for numerous cardiac promoters. In this study, we used the RNA interference (RNAi) to delete the GATA-4 mRNA in the cardiac tubes of chick embryos and tried to find the down-stream targets of GATA-4.

[Methods and Results]

Chick embryo cultures and in vitro RNAi. At HH stage 7, chick embryos were removed from the eggs, placed on filter paper. Small interfering RNAs (siRNAs) for control, chick GATA-4 were chemically synthesized, and then electroporated into precardiac mesoderm of HH stage 7 chick embryo and embryos were allowed to develop up to HH stage 12. Depletion of the GATA-4 mRNA led to the cardia bifida in chick embryo.

Immunohistochemistry. Whole-mount immunohistochemistry was performed in various stages of chick embryos. N-cadherin expression within cardiac mesoderm began at HH stage 8 and then gradually increased until HH stage 12.

Analysis of RNA by RT-PCR. RT-PCR using mRNAs extracted from cardiac tubes revealed that the GATA-4-specific siRNA selectively suppresses expression of N-cadherin mRNA, one of the genes essential for the single heart formation, without affecting other cardiac marker mRNAs.

Transfection and luciferase assays. Cultured rat cardiac myocytes or HEK 293 cells were transiently transfected with luciferase constructs containing various lengths of the 5' flanking region of N-cadherin gene. The luciferase assays of the N-cadherin promoter demonstrated that the region spanning -570 bp to -556 bp is essential for its transcription.

Electrophoretic mobility shift assays. The electrophoretic mobility shift using a double-stranded DNA probe

of the N-cadherin promoter (from -590 bp to -530 bp) was performed. Mobility of DNA probe was shifted by incubation with not only cardiac extracts but also GATA-4 protein, indicating that DNA probe associate with GATA-4 protein. The supershift of this band by incubation with anti-GATA-4 antibody supported association of DNA probe with GATA-4 protein. This complex was competitively blocked by addition of excess unlabeled wild-type oligonucleotide, but not by unlabeled mutant oligonucleotide lacking of GATA-4 binding motif. Thus, the region spanning -590 bp to -530 bp of N-cadherin gene associated with GATA-4.

[Conclusion]

The morphological changes in heart tube formation caused by GATA-4 specific siRNA revealed that N-cadherin expression is crucial for fusion of precardiac cells and formation of the single heart tube. Promoter analysis of the N-cadherin gene demonstrated that N-cadherin expression is transactivated by GATA-4. We therefore conclude that the cardia bifida induced by deletion of GATA-4 may be caused by the down-regulation of the N-cadherin expression.

論文審査の結果の要旨

GATA-4の欠損マウスではCardia bifida（separated heart tube）をおこすことは知られているが、その下流にある標的遺伝子については明らかとされていない。本研究ではニワトリ胚のcardiac mesodermにGATA-4のRNAiを導入することでCardia bifidaをおこすモデルを確立させ、これによって得られたcardiac tubeより抽出したmRNAを用いてRT-PCRを施行した。この結果single heartの形成に重要なN-cadherinのmRNAの発現が特異的に抑制されていた。一方、cardiogenic markerであるNkx 2.5やVMHCについては変化はみられなかった。このことからGATA-4に対するRNAiが選択的にN-cadheirin遺伝子の転写を抑制したことが示唆された。さらにluciferase assayとelectromobility shift assayを用いてN-cadherin遺伝子の転写調節について検討した結果、GATA-4がN-cadherin遺伝子のプロモーター領域に直接結合し、転写活性を上昇させることが明らかとなった。以上より、GATA-4がN-cadherinの発現調節を介して心臓の形態形成に関わることが明らかとなった。

これらの知見は心臓の形態形成の新しい制御機構を発見した上で学位に値するものと認める。