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研究課題名（和文）Understanding flagellum formation in mouse spermatozoa

研究課題名（英文）Understanding flagellum formation in mouse spermatozoa

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研究成果の概要（和文）：N-DRCは鞭毛と繊毛の運動やその形成の調節に関わる11の構成因子から成るタンパク質複合体です(DRC1-11)。11の構成因子のうち8個についてはノックアウト（KO）マウスが作製されており、臓器発達の異常（DRC1)、精子無力症（Drc5 / Tcte)などの表現型を示します。本研究では、未解析の3因子(DRC2、10、および11)をCRISPR/Cas9によりKOし、その解析を行いました。その結果、Drc2のKOマウスは水頭症が原因で生後致死を示しました。Drc11のKOマウスは繁殖能力も正常で明らかな異常は見つかりませんでした。Drc10の解析は進行中です。

研究成果の学術的意義や社会的意義
The N-DRC is an important macromolecular complex that help regulate flagella and cilia formation and function. My research examined the last remaining components of the N-DRC that have yet to be studied. This research completes the analysis of all components of the N-DRC.

研究成果の概要（英文）：My proposal set out the examine cilia and flagella formation by studying 3 components of the Nexin-Dynein Regulatory Complex (N-DRC). The N-DRC is a macromolecular protein complex that helps regulate the beating motion of flagella and cilia as well as their formation. Flagella and cilia are important for cell and organ function. The N-DRC is composed of 11 subunits (DRC1-11). Eight N-DRC components have been studied in the mouse that lead to various phenotypes such as abnormal organ development (DRC1), short sperm tails (Drc7), immotile sperm (Drc5/Tctel), or no phenotype at all (Drc6/Fbxl13). The remaining genes yet to be studied include DRC2, 10 (Iqcd), and 11 (Iqca). Using CRISPR/Cas9, I managed to knockout all 3 genes. I found that Drc2 knockout mice are perinatal lethal and exhibit hydrocephalus. Drc11/Iqca mice are fertile with no apparent abnormalities, while Drc10/Iqcd analysis is still ongoing.

研究分野：Developmental Biology

キーワード：spermatogenesis　sperm　N-DRC　sperm motility　male fertility　hydrocephalus

様　式　C－１９、F－１９－１、Z－１９（共通）

１．研究開始当初の背景

Cilia are critical cellular organelles that extend from the cell and that function in locomotion and signaling. Proper cilia formation and regulation is essential as defects in cilia formation lead to many syndromic diseases termed ciliopathies (e.g. Polycystic Kidney Disease). Ciliopathies affect many organs such as the kidneys, lungs, and reproductive organs, and have severe consequences for patients. Using the flagellum of mouse spermatozoa, which is a modified cilium, I examined components that regulate cilia formation and function.

２．研究の目的

The purpose of this research was to understand genetic mechanisms of cilia formation by using flagellum formation in mouse spermatozoa as a model. Cilia are critical cellular organelles that function in locomotion and signaling. Proper cilia formation and regulation is essential as defects in cilia lead to many syndromic diseases termed ciliopathies (e.g. Polycystic Kidney Disease). Ciliopathies affect many organs such as the kidneys, lungs, and reproductive organs, and have severe consequences for patients. Using genetically modified mouse models, I examined the mechanisms of flagellum (a modified cilium) formation, which will give insights into both male infertility and ciliopathies.

３．研究の方法

Using the flagellum of mouse spermatozoa, which is a modified cilium, I examined components that regulate cilia formation and function. The key scientific question this research attempted to answer is understanding the mechanisms that govern cilia formation and function. The research plan included two main aims:
Specific Aim 1: Determine whether uncharacterized components of the flagellum are essential for flagellum formation and function.
The axoneme is essential for providing structural support to the flagellum and generating motility through the motor activity of dynein. While many genes that comprise the axoneme have been characterized, there remains several genes that have yet to be examined in mouse. I knocked out three genes (*Drc2*, *Drc10*, and *Drc11*) in mouse zygotes (with CRISPR/Cas9) and characterize the phenotype using cell biological techniques and by examining sperm function through mating tests and Computer Assisted Sperm Analysis (CASA).
Specific Aim2: Describe the molecular function of the three DRC proteins.
By inserting epitope tags into the three DRC proteins from Aim 1, I wanted to perform pulldown experiments and identify the proteome of the three genes with mass spectrometry. However, one gene (*Drc11*) displayed no phenotype so I decided not to pursue that gene. Another gene (*Drc2*) showed hydrocephalus and premature death in mice and therefore prohibited analysis of the gene in sperm flagella. Analysis of the last gene (*Drc10*) is still on going.

４．研究成果

Using the CRISPR/Cas9 system, I managed to knockout (KO) three components (*Drc2*, *Drc10*, and *Drc11*) of the Nexin-Dyenin Regulatory Complex (N-DRC, Figure 1) that had not been studied. The N-DRC is important for flagella and cilia form and function, and knockouts of various components of this complex lead to various phenotypes from primary ciliary dyskinesia,

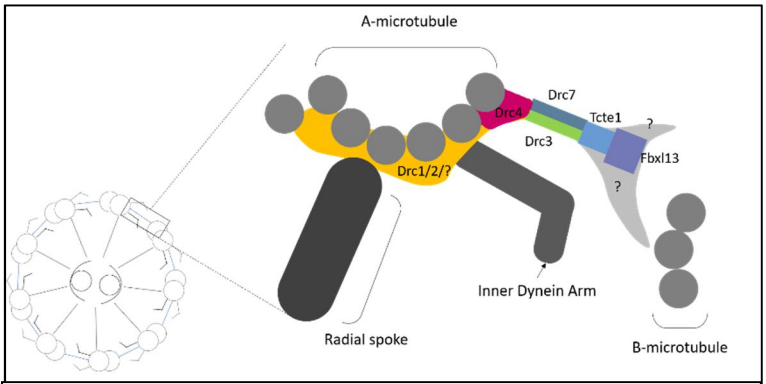

Figure 1. The N-DRC of the axoneme. The components of the N-DRC are highlighted in color.

hydrocephalus, and male infertility (Summarized in Table 1).

DRC10

Drc10 is located on mouse chromosome 5 and encodes for a protein that is 458 amino acids long. On the reserve strand is another gene (*Rita*) that is implicated in Notch signaling. Due to the presence of *Rita* on the reverse strand, only exon 2 (out of 5 exons) was deleted. Exon 2 contains the start codon and codes for half of *Drc10*. KO males have been produced and analysis of this gene is ongoing.

DRC11/IQCA

Drc11 (official name: *Iqca*) encodes a protein up to 857 amino acids long. The location of this protein within the N-DRC has not been determined. I successfully removed the majority of *Drc11* (107 kB, 17 out of 19 exons) and generated KO mice by intercrossing *Drc11* heterozygotes. Two *Drc11* KO males and 3 *Drc11* heterozygous males were paired with wild-type females for 8 weeks to examine fertility. *Drc11* KO males sired pups at comparable levels to heterozygous control animals (8.7±2.1 versus 7.0±2.7 pups per litter, respectively). This data suggests that *Drc11* is not required for male fertility. Analysis of spermatogenesis through histology did not reveal differences between wild type and *Drc11* KOs (Figure 2). CASA analysis also did not reveal differences between KO and control males. This work was submitted and accepted in the upcoming special issue on contraception in the Biology of Reproduction journal (Sun et al, 2020).

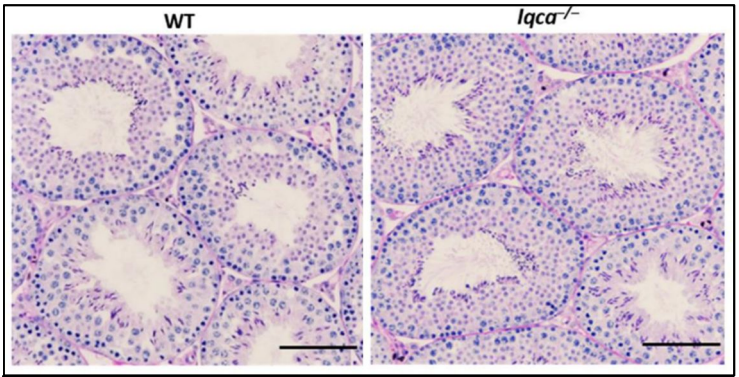

Figure 2. PAS-stained cross-sections of testis from control and *Drc11* KO (*Iqca*).

DRC2

Drc2 encodes a protein that is 493 amino acids long. Based on studies from Chlamydomonas, DRC2 is localized near DRC1 and contacting the A-microtubule of the outer doublet microtubules of the axoneme (Figure 1). *Drc1* mouse KOs display primary ciliary dyskinesia with defects in the heart, lungs, and infertility. Of the *Drc2* heterozygous intercrosses I performed for this study, I obtained 169 pups total, of which 26 were *Drc2* KO. Of the 26 *Drc2* KOs, only 3 survived into adults while the remaining died near weaning age (21 days after birth). All *Drc2* KO mice that died early displayed hydrocephalus (Figure 2). Hydrocephalus is characterized by excessive cerebral spinal fluid in the ventricles of the brain. Hydrocephalus was seen in another N-DRC component (*Drc3*), so the result of the *Drc2* KO is not surprising. Only one male survived into adulthood and was paired with three wild-type females to determine the fertility status. After 3 months of pairing the *Drc2* KO male with females, no pups were produced despite the presence of cervical plugs. This suggests that *Drc2* KO males that survive into adulthood are infertile; however, as only one mouse was examined, no definitive conclusions can be reached at this moment.

Figure 3. *Drc2* KO mouse displaying hydrocephalus (white arrowhead).

Summary

Below is a table summarizing the phenotypes of the eleven components of the N-DRC in mouse. The most severe phenotypes occur in Drc1-4 which affect multiple organs. Based on work in Chlamydomonas, it is proposed that Drc1-4 is in close contact with the microtubules. Expression analysis of Drc1-4 in mouse shows expression in a broader range of tissues, while Drc5, 6, 7, 9, and 11 is more restricted to the testis. This might explain the less severe phenotype of these genes.

	Mouse Orthologue	KO phenotype
DRC1	*Drc1/Ccdc164*	Primary ciliary dyskinesia
DRC2	*Ccdc65*	hydrocephalus
DRC3	*Drc3/Lrrc48*	mild hydrocephalus, male infertile
DRC4	*Gas8*	Primary ciliary dyskinesia
DRC5	*Tcte1*	Immotile sperm (Castaneda PNAS)
DRC6	*Fbxl13*	Not essential
DRC7	*Ccdc135*	Short sperm tails
DRC8	*Efcab2*	unpublished
DRC9	*Iqcg*	Short sperm tail
DRC10	*Iqcd*	Ongoing analysis
DRC11	*Iqca*	Not essential

Table 1. Components of the DRC. Genes in grey are the subject of this proposal.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Julio Castaneda
２．発表標題 Spar1 is a novel transmembrane gene required for acrosome formation in mouse
３．学会等名 Society for the Study of Reproduction
４．発表年 2018年

１．発表者名 Julio Castaneda
２．発表標題 Spar1 is a novel transmembrane gene required for acrosome formation in mouse
３．学会等名 Germ Cells Meeting, Cold Spring Harbor
４．発表年 2018年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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