Cytoplasmic dynein 2 is required to the regulation of intraflagellar transport system in sperm flagellum formation.

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Sperm flagellum formation is one of the major events in spermiogenesis. In sperm flagellum formation, many substances necessary for flagellar formation are transported along microtubules, and intraflagellar transport (IFT) system is responsible for this process. During the cilium formation, which are motile similar to sperm flagella, kinesin 2 and cytoplasmic dynein 2 have motility activity and play a fundamental role in the function of the IFT system. Kinesin 2 is known to important for sperm flagellum formation, but involvement of cytoplasmic dynein 2 is unclear. It is generally believed that IFT system is common to both cilia and sperm flagella. However, since numerous testis-specific genes are related to sperm flagellar formation, it is significant to study IFT system with a focus on the sperm flagella. Therefore, the purpose of this study is to elucidate the molecular mechanism of IFT system focusing on cytoplasmic dynein 2. Our study leads to understand the largely unknown mechanism of IFT system of sperm flagellum formation. To explore the function of cytoplasmic dynein 2, we generated knockout mice (KO mice) in which one of the cytoplasmic dynein 2 subunits was delated. We then evaluated the fertility of the generated male KO mice. Next, we conducted phenotypic analysis of the male KO mice using tissue staining and immunofluorescence, mainly for the following three. 1): Histological analysis of seminiferous tubules and epididymal spermatozoa. 2): Dynamic analysis of cytoplasmic dynein 2 subunit. 3): Dynamic analysis of IFT protein (IFT88,140) and kinesin 2 (KIF3A), which are a component protein of the IFT system.The male KO mice showed complete infertility. Periodic Acid Schiff - Hematoxylin staining in the seminiferous tubules showed that flagella defects were observed in KO mice. In epididymal cauda, most of spermatozoa flagella were severely shortened. In addition, disruption of the cytoplasmic dynein 2 subunit expression was detected in the sperm flagella of KO mice, such as loss of localization and ectopic localization, which were not seen in WT. Moreover, IFT protein and kinesin 2 also showed the same abnormal localization in KO mice. These results indicate that the entire structure of IFT system is impaired in KO mice. Thus, in KO mice, failing to normal function of IFT system should cause the disruption of transport of substances necessary for flagellar formation, and lead to the severe shortened flagella. In this study, we revealed for the first time that cytoplasmic dynein 2 is important for male fertility and sperm flagellum formation. Furthermore, we showed that cytoplasmic dynein 2 regulates the structural maintenance and function of the entire IFT system.