学 位 論 文 の 要 約

三 重 大 学

所 属	三重大学大学院医学系研究科 甲 生命医科学専攻 ゲノム再生医学講座 薬理ゲノミクス分野	氏 名	梅 本 紀 子

主論文の題名

Fluorescent-Based Methods for Gene Knockdown and Functional Cardiac Imaging in Zebrafish
（蛍光を基盤としたゼブラフィッシュの遺伝子ノックダウン法および心機能イメージング法）

Noriko Umemoto, Yuhei Nishimura, Yasuhito Shimada, YukikoYamanaka, Seiya Kishi, Saki Ito, Kana Okamori, Yuuki Nakamura, Junya Kuroyanagi,
Zi Zhang, Liqing Zang, Zhipeng Wang, Norihiro Nishimura, Toshio Tanaka

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主論文の要約

Introduction（導入）

Gene targeting technology, such as gene knockout (KO) and gene knockdown, is used inthe targeted analysis of specific gene function in human disease. When downregulation, or reduced activity, of a gene product is inferred to be associated with the cause and progress of a disease, a KO animal for that gene may be developed to evaluate phenotypic similarity to the human disease. However, a particular difficulty arises when the homozygous KO animal results in embryonic lethality and the heterozygous KO animal with 50 % reduction in the expression of a targeted gene rarely results in a detectable phenotype. Therefore, a method is required to generate animal models exhibiting intermediate phenotypes in which expression of the gene of interest is precisely reduced.

Background（背景）

Zebrafish is an attractive model organism for investigation of human disease because of its genetic tractability, external fertilization, early optical transparency. In particular, t

he two major advantages of zebrafish embryos are simple gene manipulation using mor pholino antisense oligonucleotides (MOs). Knockdown of any target protein is readily ach ieved with an MO. The expression of the targeted protein is reduced by MOs in a dose -dependent manner. However, zebrafish morphants injected with MO for a target protei n often show heterogeneous phenotypes, despite controlling the injection volume of the MO solution in all embryos.

Objectives（目的）

The aim in this study to establish both novel and useful method for quantitative gene knockdown by using MO and fluorescent cardiac imaging without expressed fluorescent protein in heart.

Methods（方法）

To manipulate a targeted gene dosage effect, we developed a method for estimati ng the quantity of MO injected into each living morphant, based on the co-injection of a control MO labeled with the fluorophore lissamine. In the current study, we determin ed the fluorescence intensity (FI) from Lis-MO in each morphant injected with a mixtur e of the targeted MO and Lis-MO, and determined the relative expression levels of mR NA for the target gene in each morphant. Although previous reports have shown that t nnt2a morphants with no cardiac contraction completely inhibit translation of tnnt2a pr otein by injecting a high concentration of tnnt2a-MO we were able to develop tnnt2a m orphants with a homogeneous intermediate phenotype that is in between homozygous an d heterozygous Tnnt2 KO models by applying our method. In addition, to characterize v entricular impairment in the tnnt2a morphants with impaired cardiac function, we also developed cardiac assessment of zebrafish to measure the performance of the inner-ventr icular wall using a commercially available fluorescent dye, Bodipy-ceramide.

Results（結果）

By applying this method for knockdown of cardiac troponin T (tnnt2a) in zebrafish, we co uld efficiently select to develop tnnt2a morphants. The morphant showed decreased heart rate and impairment of cardiac contraction. To investigate cardiac impairment of the tnnt2 a morphant, we performed fluorescent cardiac imaging using Bodipy-ceramide.The functio nal cardiac imaging of zebrafish stained with Bodipy-ceramide was able to detect both 109 systolic and diastolic dysfunction in the heart, albeit a fluorescent protein-labeled heart. T herefore, we could found that moderate reduction of tnnt2a impaired diastolic distensibility and decreased contraction and relaxation velocities.

Consideration（考察）

By using the two methods developed in this study, we could efficiently select th e partial tnnt2a-depleted zebrafish with a decreased heart rate and an

alyze cardiac function of tnnt2a morphants impairment of cardiac contraction. They showed a homogeneous intermediate phenotype that is in between homozygous an d heterozygous Tnnt2 KO models without cardiac construction. To the best of our knowl edge, this is the first report to analyze the role of tnnt2a in cardiac function in tnnt2a-depleted living animals.

Conclusion（結論）

Our combinatorial approach developed in this study can be applied for analyzing molecul ar function of proteins associated with human cardiac diseases, especially when the homo zygous KO is lethal and the heterozygous KO animal shows no significant abnormality.