TUMSAT-OACIS Repository - Tokyo

University of Marine Science and Technology

(東京海洋大学)

Establishment of germ cell transplantation system in bitterling

メタデータ	言語: eng 出版者: 公開日: 2019-06-24 キーワード (Ja): キーワード (En): 作成者: Anna Octavera メールアドレス: 所属:
URL	https://oacis.repo.nii.ac.jp/records/1757

［課程博士・論文博士共通］

博士学位論文内容要旨
Abstract

専　攻 Major	応用生命科学専攻	氏　名 Name	ANNA OCTAVERA
論文題目 Title	Establishment of germ cell transplantation system in bitterling （タナゴ類における生殖細胞移植技術の構築）		

Bitterling is small freshwater species belong to cyprinid family. Many bitterling species are facing extinction because of habitat destruction. Japan has 16 species or subspecies of bitterlings, which are mostly endemic and mostly endangered. There are eight species which are listed as critically endangered, five species are listed as endangered, and two species are listed as near-threatened in Red List Japan. Since cryopreservation of fish eggs is still impossible due to their large size and high yolk content, long-term and stable storage of bitterling genetic resources is currently not possible. Recent study was discovered the cryopreservation of immature germ cells is possible in several fish species and functional gametes derived from the frozen materials can be produced through their transplantation to embryonic recipients. The final goal of this study is to establish surrogate broodstock that can produce both eggs and sperm derived from cryopreserved testes of endangered bitterlings. However, bitterlings have uniquely shaped eggs and their embryos are extremely fragile, making it difficult to perform germ cell transplantation. Therefore, as a first step, we conducted intra-species spermatogonial transplantation using recessive albino Chinese rosy bitterling as donors and wild-type Chinese rosy bitterling as recipients to develop a system to convert freezable immature germ cells into functional gametes, particularly eggs.

Donor germ cells were dissociated from 3 months old albino testes (immature) and transplanted approximately 3,000 cells into the peritoneal cavity of 4 days old of triploid-treated recipients. To trace donor cells in the recipient gonads, donor cells were stained with the fluorescent membrane dye, PKH26. Sixteen days after the transplantation (dpt), incorporated donor-derived germ cells were observed by fluorescence microscopy. The remaining recipients were continuously reared until they reached to mature stage. Mature recipients were crossed with albino by using in vitro fertilization and donor-derived offspring were identified by retina pigmentation. Among the transplanted recipients, 58.33±3.33% of them carried donor cells in their gonads. In total, 14 (2n=6 fish; 3n=8 fish) out of 32 transplanted recipient larvae survived until maturity. In the crosses between diploid recipients and non-transplanted albino fish, two male and two female recipients produced some offspring without black pigmentation in their retina. The mean of germ line transmission rate of the donor-derived albino phenotype was 45.29±20.97% and 32.48±8.31% for male and female diploid recipients, respectively. Furthermore, all offspring produced by the triploid recipients showed albino phenotype. However, we found that triploid fish all become male. Therefore, this study was continuing by using germ cell-less recipient.

Germ cell-less recipients were produced by knocked down the *dead end* (*dnd*) gene, an essential gene for PGC migration and survival, using antisense morpholino oligonucleotides (MO). A total of 2 ng *dnd*-MO was microinjected into the cytoplasm of single-cell stage fertilized eggs. Observation of gonad morphology in 4-month-old knocked down fish revealed that dnd-AMO fish had smaller gonads compared with control fish. The germ cell depletion was further confirmed by histology and *in situ* hybridization (ISH) using *vasa* probe. Furthermore, expression of the germ cell marker *vasa* was not detected in *dnd*-AMO treated fish using reverse-transcription PCR. Expression of *gsdf*, as somatic cell marker was confirmed in gonadal RNA of both control and knocked down fish. This was also confirmed by ISH. Furthermore, we found two types of gonad in

germ cell-less fish (n=15): type I with male-type separated gonad pairs (n=9) and type II with female-type connected gonad pairs (n=6). Moreover, expression of the male marker *cyp11b* was confirmed in type I gonads and expression of the female marker *cyp19a1* was confirmed in type II gonads suggesting that germ cell-less fish was clearly both male and female.

Further, we performed intra-species transplantation by using *dnd*-MO treated fish as recipients. There is 84.44±13.88% recipients carrying donor-derived germ cells in their gonads. At 6 months, 19 out 23 (male=10 fish and female=9 fish) recipients produced gametes. Progeny tests of these recipients with non-transplanted albino fish revealed that six of the ten mature male and three of the nine mature female recipients produced only donor-derived albino gametes since there was no black pigmentation in the retinas of the resulting offspring. Further, three mature male and four mature female recipients produced a mixture of wild-type and albino offspring, with an average frequency of albino in the F1 generation of 80.94±4.93% and 75.98±3.96% for male and female recipients, respectively. The remaining one male and two female recipients produced only wild-type offspring. As a result, in nine of 19 recipients, endogenous germ cells were successfully eliminated by dnd-AMO microinjection and replaced with the transplanted donor-derived germ cells. Thus, we successfully established a germ cell transplantation system in an iconic endangered teleost, bitterling. The technology established in this study can be directly applied to produce functional gametes of endangered bitterlings using cryopreserved donor cells.