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Production of reproductively sterile fish: A mini-review of germ cell elimination technologies

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ABSTRACT

As seafood consumption shifts from fisheries harvests to artificially propagated aquatic species, the increase of aquaculture activities poses a biological threat to our environment. Selectively bred, non-native and (eventually) genetically engineered farmed fish may escape from aquaculture operations, propagate and/or interbreed with wild stocks and subsequently alter the genetic makeup of populations in the environment. Thus, an effective strategy for bio-containment of farmed fish is critically needed. Farming reproductively sterile fish is the most environmentally sustainable approach to ensure complete bio-containment in large-scale aquaculture operations. Chromosome set manipulations to produce sterile fish, including polyploidy and hybridization, are currently the most common practices in the aquaculture industry. However, they do not always result in 100% sterility of the treated fish. Moreover, triploid fish typically do not perform as well as the non-manipulated diploids under commercial culture conditions. In the last half decade, several genetic engineering methods have been developed to produce sterile fish. In this review, we will address the latest technologies that use transgenic approaches to eliminate germ cells, resulting in the production of sterile fish. These latest advances also led us to the development of egg/embryo immersion methodologies to deliver and screen compounds that can be used to eliminate primordial germ cells and produce sterile fish. This emerging non-transgenic strategy for the production of reproductively sterile fish in aquaculture will also be discussed.

1. Introduction

Technologies to produce reproductively sterile fish are becoming increasingly important to resolve the current and projected spread of genetic contamination caused by aquaculture escapees and the invasion of non-native species. As the shift in dependence from fisheries harvests to artificially propagated aquatic species continues, optimization of aquaculture methods will be necessary not only to maximize food production, but also to minimize ecological impacts. In conjunction with rapidly growing aquaculture activities, an effective bio-containment strategy for large-scale commercial aquaculture operations is imperatively needed to achieve the long-term environmental sustainability of our seafood supplies. The most proficient genetic containment strategy possible is the use of reproductively sterile farmed fish. Sterility carries environmental significance, as the escape of fertile cultured fish poses the threat of ecological imbalance and genetic contamination of wild populations (Muir and Howard, 1999; Stigebrandt et al., 2004). Sterile fish are also a prominent tool for biological control of non-native fish. The sterile-male-release technique (Bergstedt et al., 2003; Twohey et al., 2003), similar to the sterile-insect-technique (Krafsur, 1998), can be used to control the populations of non-native species by competitively interrupting reproduction. Furthermore, sterility is also desirable for other reasons in aquaculture operations. Many farmed fish attain sexual maturity before reaching market size. Sexual maturation is associated with intensive gonadal growth, resulting in a diversion of energy toward development of the gonads, deterioration of flesh quality, and an increase in susceptibility to stress and disease (Zohar, 1989). Sterilization minimizes energy input toward gonadal growth while enhancing muscle (flesh) development and promoting fish health (Manzoor Ali and Satyanarayana Rao, 1989). Moreover, sterility is a useful means for protecting valuable strains from unauthorized propagation.

The most common and practical methods currently used to induce sterility in the aquaculture industry are chromosome manipulations to cause triploidization or interspecies hybridization (Arai, 2001; Donaldson and Benfey, 1987). Disrupting the normal pairing of homologous chromosomes during meiosis, a key process in early gametogenesis, results in sterility. Many excellent reviews and publications are available on chromosome set manipulation

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that describe methodologies, efficiency of sterility and its effects on fish physiology, behavior and performance (Arai, 2001; Benfey, 1999; Donaldson and Benfey, 1987; Laing and Utting, 1994; Piferrer et al., 2009; Rottmann et al., 1991; Weber et al., 2014). Therefore this approach will not be further discussed in this mini-review. Other approaches such as radiation, hormone, chemical, antisense technology and surgical sterilization have also been reported (Hu et al., 2007; Twohey et al., 2003; Uzbekova et al., 2000; Yamazaki, 1976). However, the practice of these respective technologies has been limited to a small scale or laboratory setting and, as such, they will not be addressed in this mini-review. Instead, this paper reviews several of the latest technologies aimed at eliminating germ cells in order to produce sterile fish using genetic engineering approaches (Hsu et al., 2010; Hu et al., 2010; Lauth and Buchanan, 2012; Wong and Collodi, 2013b). These latest advances led us to develop an immersion-mediated delivery methodology to screen for compounds that can be used to eliminate primordial germ cells (PGCs) and produce sterile fish. This emerging non-transgenic strategy for the production of reproductively sterile fish in aquaculture will be discussed in detail.

2. Germ cell elimination by nitroreductase/prodrugs system

Nitroreductases are a family of flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) dependent enzymes that catalyze the reduction of nitro-substituted compounds using nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as reducing substrates. Nitroreductases can be used to convert (nitroreduction) non-toxic prodrugs such as CB1954 or metronidazole into cytotoxic metabolites that cause DNA crosslinks, which results in cell death through apoptosis. Therefore, the nitroreductase/prodrugs system has been used for cell ablation with a focus toward cancer therapy (Connors, 1995; Patterson et al., 2003; Searle et al., 2004). Together with transgenic approaches, nitroreductase was expressed specifically in certain tissues and cells when a tissue- or cell-specific promoter is employed. Consequently, the prodrugs were converted into their cytotoxic metabolites only in nitroreductase-expressing cells, which leads to targeted cell ablation under an inducible, spatial, temporal and tissue-specific control (Gusterson et al., 2003; Patterson et al., 2003). With fusion protein engineering and fluorescence microscopy, the nitroreductase-GFP fusion protein allows the progress of cell ablation to be monitored and visualized in a real-time manner, which makes it easier to optimize the treatment conditions (Curado et al., 2008).

To generate transgenic fish that possess germ cell-specific expression of nitroreductase, promoters of the testis-specific genes, A-kinase anchoring protein-associated protein (*asp*), outer dense fibers (*odf*), and sperm acrosomal membrane-associated protein (*sam*), as well as the oocyte-specific gene, zona pellucida (*zpc*), were employed to drive nitroreductase-GFP fusion protein expression (Hsu et al., 2010; Hu et al., 2010). The germ cell-specific nitroreductase expression was confirmed by fluorescence microscopy to detect GFP-expressing germ cells. When transgenic fish were treated with metronidazole, gonads of treated fish displayed various germ cell depletion. In some cases, 100% sterility was achieved. Although these technologies demonstrate that a combination of genetic and pharmacological approaches can be used to achieve inducible infertility in fish populations, the overall sterility achieved in the population was not 100% despite prodrug exposure for two weeks in the culture system, and each transgenic line can only be sterilized in one sex but not the other, which is a detriment for its large scale application. A flow chart of a potential nitroreductase/prodrugs system for germ cell elimination is illustrated in Fig. 1.

3. Germ cell elimination by maternal sterility technology

Maternal sterility technology (MST) targets the elimination of PGCs, a specific population of embryonic cells that give rise to the eggs and sperm of the adult, using a maternal deposit mRNA that encodes a pro-apoptotic protein. Expression of pro-apoptotic protein subsequently activates caspases and DNAses that lead to apoptosis and the elimination of PGCs (Lauth and Buchanan, 2012). The mRNA also carries a specific 3′UTR of germ-cell gene to direct its expression solely in PGCs. In order to achieve spatial- and temporal-specific delivery of a transgene product in PGCs, the MST approach employs a maternal sterility construct (MSC) to generate transgenic fish. The MSC comprises (1) a promoter of maternal gene, such as *zpc*; (2) a polynucleotide that encodes a pro-apoptotic protein such as B-cell lymphoma 2 (Bcl2) associated X protein (Bax); and (3) a germ cell specific cis-acting element such as 3′UTR of dead end (*dnd*) or *nanos3* that directs (Koprunner et al., 2001; Weidinger et al., 2003) and prolong (Wong and Collodi, 2013a) PGC-specific expression. Females carrying the MSC transgene are lineage-ending females. They give rise to the embryos that express pro-apoptotic factor specifically in PGCs. Thus, PGCs are eliminated through the programmed cell death activated by a pro-apoptotic factor. Without PGCs, the embryos develop into sterile adults. Males carrying the MSC transgene are not able to pass pro-apoptosis mRNA to the embryos because the maternal-specific promoter *zpc* is female-specific. As a result, they give rise to fertile progeny when crossed with wild-type females. Hence, MSC transgenic males are used to propagate the transgenic line.

The MST has been successfully established in zebrafish. Its feasibility to aquaculture species is currently under evaluation. The challenges of the MST approach are (1) unwanted apoptosis may occur in oocytes or somatic cells of early embryos. The maternal mRNA encoded pro-apoptotic protein is deposited into the developing oocytes, which may drive oocytes into apoptosis if pro-apoptotic protein mRNA is translated in oocytes (and thus affect female fecundity). The same risk may also occur later in the early embryos if a sufficient amount of pro-apoptotic protein is expressed in somatic cells, which may eventually kill embryos. (2) The production of MSC lineage-ending females and hemizygous MSC male broodstock is relatively time and labor consuming. Since MSC females are lineage-ending females, the fertile MSC females would need to be generated from wild-type females crossed with hemizygous MSC males. Only one half of the offspring would carry MSC and need to be screened to identify lineage-ending females and MSC hemizygous male broodstock. A flow chart of MST is illustrated in Fig. 2.

4. Germ cell elimination by disrupting PGC migration – a transgenic approach

In fish, PGCs are specified by the inclusion of maternally-derived germ plasm during early development (Braat et al., 1999; Herpin et al., 2007). The PGCs then migrate to the developing gonad with guidance from a gradient of the chemokine, stromal-derived growth factor (Sdf1a) (Doitsidou et al., 2002; Herpin et al., 2008). Disruption of the Sdf1a signaling pathway prevents normal PGC migration in the fish embryo (Doitsidou et al., 2002; Knaut et al., 2003). Therefore, an inducible over-expression of Sdf1a in the zebrafish embryo has been designed to disrupt the formation of the Sdf1a gradient that guides PGC migration and to saturate the Sdf1a receptor, Cxcr4b, on PGCs, which prevents PGCs from responding to the endogenous Sdf1a signal. As a result, PGCs mis-migrate to an ectopic region without reaching the developing gonads. Consequently, the treated fish developed into sterile individuals with severely under-developed gonads that lacked of germ