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Modulation of telomerase activity in fish muscle by biological and environmental factors☆

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ABSTRACT

Telomerase expression has long been linked to promotion of tumor growth and cell proliferation in mammals. Interestingly, telomerase activity (TA) has been detected in skeletal muscle for a variety of fish species. Despite this being a unique feature in fish, very few studies have investigated the potential role of TA in muscle. The present study was set to prove the concepts that muscle telomerase in fish is related to body growth, and more specifically, to muscle cell proliferation and apoptosis *in vivo*. Moreover, muscle TA can be influenced by biotic factors and modulated by environmental stress. Using three fish species, mangrove red snapper (*Lutjanus argentimaculatus*), orange-spotted grouper (*Epinephelus coioides*), and marine medaka (*Oryzias melastigma*), the present work reports for the first time that fish muscle TA was sensitive to the environmental stresses of starvation, foodborne exposure to benzo[a]pyrene, and hypoxia. In marine medaka, muscle TA was coupled with fish growth during early life stages. Upon sexual maturation, muscle TA was confounded by sex (female > male). Muscle TA was significantly correlated with telomerase reverse transcriptase (TERT) protein expression (Pearson correlation $r = 0.892$; $p \leq 0.05$), which was coupled with proliferating cell nuclear antigen (PCNA) cell proliferation, but not associated with apoptosis (*omBax/omBcl2* ratio) in muscle tissue. The results reported here have bridged the knowledge gap between the existence and function of telomerase in fish muscle. The underlying regulatory mechanisms of muscle TA in fish warrant further exploration for comparison with telomerase regulation in mammals.

1. Introduction

Telomerase is a ribonucleoprotein enzyme that was first found in the ciliate *Tetrahymena thermophila* for *de novo* telomere synthesis (Greider and Blackburn, 1987). Subsequently, telomerase has been detected in all eukaryotes, from single-celled organisms (e.g. yeast) to multi-cellular life forms (e.g. humans). In mammals, TA was found to be absent or dramatically down regulated in differentiated somatic tissues e.g. skeletal muscles (Kolquist et al., 1998; Hiyama et al., 2001; O'Connor et al., 2009). In contrast to mammals, telomerase has been detected in post-mitotic, somatic tissues for a wide variety of fish (Table 1). The most interesting observation is the presence of TA in fish muscles. Klapper et al. (1998) were the first to demonstrate high TA in muscles of rainbow trout (*Oncorhynchus mykiss*), and the levels were age-dependent (1-month-old > 30-month-old juvenile > 42-month-old adult) (Klapper et al., 1998). In zebrafish (*Danio rerio*), the levels of muscle TA in mature adults (from 5-month-old, 15-month-old to 24-month-old) were significantly higher than that of a 4-week-old mouse (Kishi et al., 2003). In medaka, muscle TA was constitutively expressed in both the marine *Oryzias melastigma* and the freshwater *Oryzias latipes* (Yu et al., 2006; Hatakeyama et al., 2008; Kong et al., 2008; Au et al., 2009).

Telomerase has long been linked to promotion of growth and cell proliferation in mammalian tumor cell systems. High levels of human telomerase reverse transcriptase (*hTERT*) mRNA expression and TA were restricted to proliferating tissues e.g. embryo, testis (Martin-Rivera et al., 1998; Greenberg et al., 1998; Hiyama and Hiyama, 2003; Park et al., 2004; Pannone et al., 2007; Herbert et al., 2007). *In vivo* studies using non-tumor tissues further revealed a good correlation between TA, cell proliferation and apoptosis (Inui et al., 2002; Oh et al., 2002; Lamy et al., 2013). The anti-apoptotic function of telomerase was first reported by Kondo et al. (1998), showing that inhibition of *hTERT* by anti-sense RNA significantly reduced TA in glioblastoma cells and, at the same time, increased cell susceptibility to cisplatin-induced apoptosis. The protective role of telomerase against apoptosis is not limited to mammalian cells and/or cancer cells (Fu et al., 1999; Mo et al., 2003; Djojosubroto et al., 2005). Plant cells (*Arabidopsis thaliana*) treated with the telomerase inhibitor telomestatin for two weeks also exhibited

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Table 1
A summary of telomerase activity and TERT expression (mRNA and protein) in muscle and other organs in different fish species.

Organism	TERT mRNA	TERT protein	Telomerase activity	Reference
Zebrafish (*Danio rerio*)	–	–	5-month, 15-month and 24-month-old adult: muscle	Kishi et al. (2003)
	–	–	Eggs, muscle, brain & eye	McChesney et al. (2005)
	Brain, retina, skin, gill, heart, kidney, ovary, liver & muscle	Ganglion cell layer, inner & outer nuclear layer	Brain, retina, skin, gill, heart, kidney, ovary & muscle	Lau et al. (2008)
Japanese medaka (*Oryzias latipes*)	7-month-old adult: Gonads, brain, liver, heart, spleen, kidney, gill, muscle & skin	-	Adult male & female: Testis, ovary, brain & heart	Pfennig et al. (2008)
	–	–	Embryo, 1 and 2-month, 1, 2, 3, 4-year-old Gonad, liver, kidney, brain, muscle, intestine, spleen and gill	Hatakeyama et al. (2008)
	–	–	1-year-old male and female: Gonad, intestines, heart, gill, liver, skin, kidney, eye, spleen, brain, muscle	Au et al. (2009)
			4, 8, 12, 15, 22-month-old Gill and Liver	Gopalakrishnan et al., 2013
Marine medaka (*Oryzias melastigma*)	4-month-old adult: Ovary, testis, kidney, intestine, gill, liver, muscle	4-month-old adult: Ovary, testis, kidney, intestine, gill, liver, muscle	4-month-old adult: Ovary, testis, kidney, intestine, gill, liver, muscle	Kong et al. (2008)
European Hake (*Merluccius merluccius*) Atlantic Cod (*Gadus morhua*)	Brain, ovary, testis, muscle, skin, gills, liver & kidney	–	–	López de Abechuco et al. (2014)
Rainbow trout (*Oncorhynchus mykiss*)	–	–	Kidney, liver, skin, heart, brain & muscle	Klapper et al. (1998a)
			eyed embryos	Yoda et al. (2002)

an increase of apoptosis-like cell death (Zhang et al., 2006). These results suggest that the anti-apoptotic role of telomerase is a general phenomenon regardless of cell type. The role of telomerase plays in cell proliferation and apoptosis, however, have not been explored in fish muscle.

Fish growth and development are highly dynamic processes, which revolve around a delicate balance of cell proliferation and apoptosis in muscle. Fish skeletal muscle constitutes approximately 30–80% of body weight (Weatherley & Gill, 1987), and is the primary site for fish growth. Muscle growth in fish is accomplished by (i) hyperplastic growth, which refers to an increase in muscle fiber number resulting from the formation of new fibers and (ii) hypertrophic growth, an increase in the size of individual muscle fibers, mainly achieved by the fusion of newly formed myofibrils to the existing ones. Both types of muscle growth have been well studied in the common carp *Cyprinus carpio* (Koumans et al., 1993), white seabass *Atractoscion nobilis* (Zimmerman and Lowery, 1999), herring *Clupea harengus* L. (Johnston et al., 1998), rainbow trout (Suresh and Sheehan, 1998), zebrafish and giant danio *Danio aequipinnatus* (Biga and Goetz, 2006). In general, the relative importance of hyperplastic muscle growth is maximal in young fish and decreases with age (Rowlerson et al., 1995). Moreover, the degree of hyperplastic muscle growth is larger in fast-growing fish than in their slow-growing counterparts (Alami-Durante et al., 1997). Muscle stem cells, called myosatellite cells, are involved in both hyperplastic and hypertrophic muscle growth in fish. Histologically, pluripotent myosatellite cells are characterized by (i) small size (less than 5 μm), (ii) the presence of a heterochromatic nucleus, reduced cytoplasm with few organelles, (iii) a positive reactivity with the cell proliferation marker proliferating cell nuclear antigen (PCNA), indicating their cell proliferative properties, and (iv) being situated between the sarcolemma (plasma membrane of muscle cell) and at the basal region of fully differentiated skeletal muscle fibers. The *in vivo* relationship between muscle telomerase expression and proliferation of myosatellite cells can be investigated using quantitative immunohistochemistry.

The expression of telomerase in skeletal muscle is unique in fish. Surprisingly, very limited knowledge is available in literature on the *in vivo* relationship between muscle TA and body growth in fish. It is not known whether the biological factors that are known to affect fish growth (Zaboukas et al., 2006) can also affect muscle TA. Anthropogenic associated environmental stresses have been shown to retard fish growth (Johnston et al., 1998; Dubé et al., 2005; Kruitwagen et al., 2006). It is not known whether muscle TA may be suppressed in these fish. The relationship between suppression of fish muscle telomerase and growth impairment remains unclear. The present study aims to prove the concepts that muscle telomerase in fish can be modulated by biotic factors and is related to fish growth, and more specifically to muscle cell proliferation and apoptosis *in vivo*. A number of experiments were conducted, using different fish species, to investigate the impact of selected environmental stresses and the influence of biotic factors on telomerase, cell proliferation and apoptosis in muscle.

2. Methods

2.1. Studies of growth and muscle telomerase activity in fish under stress

To prove the concept that muscle TA could be modulated in fish under stress, three preliminary experiments were conducted using two juvenile fish species, the mangrove red snapper (*Lutjanus argentimaculatus*) and orange-spotted grouper (*Epinephelus coioides*), subjected to: (i) chronic food deprivation, (ii) re-feeding after chronic starvation, and (iii) prolonged exposure to benzo[a]pyrene (B[a]P), a well-known carcinogenic polycyclic aromatic hydrocarbons (PAHs).

2.1.1. Experiment 1: chronic food deprivation

Juvenile mangrove red snapper were obtained from a South China commercial hatchery. After seven days of acclimation, fish were divided into the control and the un-fed groups and maintained in separate rafts (1.5 m × 1.5 m × 1.5 m) at a fish farm at the Kat O Fisheries Substation of the Agriculture, Fisheries and Conservation Department (seawater temperature 29.7 ± 0.5 °C, pH 7.8 ± 0.02), in the northeastern New Territories of Hong Kong. Control fish were fed once daily with moist pellets (trash fish mixed with fish meal, vitamins and binders, provided by Kat O Fisheries Research Station) until satiation. Fish in the un-fed