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Review

An aquatic organism as time machine: *Nothobranchius furzeri*

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Background. There is need for animal models to study ageing. Worms, flies and mice have been extensively explored with outstanding results.
Aims. Many studies have used *Nothobranchius furzeri* - the African turquoise killfish with a lifespan less than 1 year.
Results. Studies have shown that the ageing process of *N. furzeri* and humans share many features.
Discussion. Despite its relatively short lifespan for a vertebrate, *N. furzeri* shows many molecular, cellular and physiological ageing phenotypes, shared with many other organisms, including humans. We have shown a significant impairment of learning performance with age, when tested using an active avoidance task.
Conclusion. *N. furzeri* is an ideal model to explore – in short time – molecular mechanisms that control ageing in vertebrates, including humans.

Key words: Animal models for ageing, *Nothobranchius furzeri,* African turquoise kill fish, life span, Eastern Africa

INTRODUCTION

The usefulness of biological models in improving the understanding of disease mechanisms, diagnostics and treatment is undisputable. According to the definition of Wessler, an animal model is "*a living organism in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animal*" [1]. The three traditional multicellular model species for aging research (worms, flies, and mice) have been thoroughly studied. Breakdown in the maintenance of genomic stability, stochastic damage to DNA, and inadequate repair processes, as well as oxidative damage and impaired protein processing and folding have been widely implicated in their aging. Dietary restriction (DR) appears to prolong life span in all classical models. Similarly, single-gene mutations that alter life span have been identified in yeasts, worms, flies, and mice. Furthermore, a similar genetic mechanism and a concomitant biochemical pathway have been found across the phyla. However, there is an animal model, *Nothobranchius furzeri*, which is known as the vertebrate with the shortest lifespan ever described in captivity, representing a powerful and useful animal model to address key question on how organisms age and which are the mechanisms underlying the age-related diseases.

In the present manuscript, I intend to recapitulate all the main relevant biological characteristics which make the annual teleost fish *Nothobranchius furzeri* an excellent model for ageing studies in biomedicine. Research into ageing in vertebrates is hampered by the lifespan of available model systems and tractable laboratory species with a lifespan of less than 1 year are highly desirable. This fish has a naturally compressed life span and short generation time, and shows typical signs of ageing according to the worldwide accepted definition. Ageing is indeed described as *'a progressive, generalized impairment of function that results in a loss of adaptive response to stress and an increasing*

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probability of death' [2]. In the natural world, ageing is characterized by an increase in mortality and decrease in fertility. Ageing is a consequence of damage, through the gradual accumulation of faults in molecules, cells and organs, leading to loss of physical, cognitive and immune function, and increased frailty and vulnerability to age-related diseases.

LIFE CYCLE OF *N. FURZERI*

Nothobranchius furzeri, also known as African turquoise killifish, inhabits seasonal pools which are formed during the monsoon season in the Eastern Africa. Currently, two strains are available in laboratory conditions: GRZ and MZM. The GRZ strain has the shortest recorded lifespan with a median lifespan ranging from 9 to 16 weeks [3] depending on the culture conditions, whereas MZM are considered as longer-lived strains, with median lifespans ranging from 23 to 28 weeks [4].
The short life cycle is due to the adaption to the ephemeral and unpredictable conditions of the natural habitat, characterized by the alternance of dry and wet season. It has adapted to the routine drying of their environment by evolving desiccation resistant eggs that can remain dormant in the mud for one and maybe more years as embryos encased in the dry mud [5]. This delay in development is accomplished by the eggs entering into diapause where oxygen consumption is depressed. When it rains, the embryos hatch and reproduce in the few weeks before their habitat disappears. Therefore, fish rapidly hatch, grow rapidly and become sexually mature within three weeks, and reproduce before the dry season. This fish maintains its compressed life span and short generation time in the laboratory, when water is in constant supply. Accelerated maturation is observed in captivity as well; growth and maturation are accelerated even when compared with other, longer-living, species of the genus which originate from more humid climates with longer rain seasons.
Despite its relatively short lifespan for a vertebrate, *N. furzeri* shows many molecular, cellular and physiological ageing phenotypes, shared with many other organisms, including humans.

AGEING PHENOTYPES

Similarly to ageing mammals, who progressively lose hair and skin pigment with age, male *N. furzeri*, which are more colorful than females, progressively lose body and tail colour. Old age in this short-lived vertebrate is also associated with emaciation, abnormal spine curvature, defective vision, fin structure deterioration. At a behavioral level, there is a generalized reduction in spontaneous locomotor activity, with older *N. furzeri* individuals spending less time exploring compared to young ones; a significant impairment of learning performance with age, when tested using an active avoidance task [6].

AGE-RELATED MARKERS IDENTIFIED IN *N. FURZERI*

Several ageing biomarkers have been identified to characterize the physiological age of this teleost fish. Lipofuscin, a yellow-brown autofluorescent pigment whose concentration increases with age in several species, including humans, accumulates in the brain and liver of old *N. furzeri.* Senescence-associated β-galactosidase (SA-β-gal) staining, a marker for cellular senescence and stress response in human cells, significantly increases in the skin of aged fish. Neurodegeneration – measured by Fluoro-Jade B, which stains cell bodies, dendrites and axons of degenerating neurons – increases in fish brains from as early as 2 months of age, strongly suggesting a spontaneous age-dependent increase in neurodegeneration. Spontaneous neoplastic lesions have been observed and measured in *N. furzeri* strains using several tumor-associated proteins, including Bcl-2, cytokeratin-8, carcinoembryonic antigen and mutated p53.
Histopathological examinations have revealed the nature of age-related organic decay: kidneys undergo tubule dilatation and crystal deposition; cardiac lesions ascribable to hypertrophy of the cardiomyocytes; age-dependent liver lipid accumulation.
At the molecular levels, the major changes described are: age-dependent telomere attrition; reduced mitochondrial numbers (as assessed by mtDNA copy numbers) and function, especially in liver, muscle and brain. Age-related capacity to regenerate the caudal fin after amputation in the long-lived MZM - 0703 strain has been documented [5].

EXPERIMENTAL MODULATION OF LIFESPAN

N. furzeri lifespan and ageing have been experimentally modulated through external intervention such as diet, temperature and chemicals supports, indicating that this organism can be used as an experimental platform for large-scale screens of age-modulating genes and chemicals.
The effect of dietary restriction (DR) has been tested in both strains of *N. furzeri*, which were fed every other day instead of daily. The results were strain-dependent: DR resulted in prolonged lifespan in the short-lived GRZ

strain but not in a wild-derived, long-lived MZM-0410 strain. Under the DR regimen, the short-lived strain showed reduced neurodegeneration, slower accumulation of lipofuscin, improved learning performance and decreased occurrence of tumours [7].

Lower water temperature extends both the median (1 week) and maximum (1.5 weeks) lifespan of *N. furzeri*. Furthermore, it leads to a 40% decrease in adult size compared to control animals grown under regular culturing temperature, suggesting a dramatic influence of temperature on metabolism. Several age-associated phenotypes, such as lipofuscin accumulation, spontaneous locomotor activity and learning performance, are also significantly improved in fish cultured at a lower temperature [8].

The supplementation of the natural polyphenol resveratrol, known to increase lifespan and delay ageing in several invertebrate and vertebrate organisms, can increase median and maximum lifespan in a dose-dependent manner in both male and female *N. furzeri*. In addition, compared to control-fed fish, resveratrol-fed fish are physically active for a longer time, and display late onset of typical ageing phenotypes [9].

GENOMIC AND TRANSCRIPTOMIC RESOURCES

The sequencing of the genome and a large number of transcriptome data (i.e. which genes are switched on or off during the process of aging) are now publicly available to the research community. By comparing the ageing process at different tissue levels (i.e. brain, liver and skin), it has been found out that genes responsible for ageing in *N. furzeri* are not spread randomly, but rather, are concentrated into specific small regions of the genome [10]. The discovery of these ageing hot spots in the genome let assume that the genes within these regions are somehow coordinated in their activation or de-activation. In addition, knowing how ageing-relevant genes are organized and how they can be (in)activated can help to identify key mechanisms regulating human aging as well.

Analysis of whole transcriptomes of the two strains, GRZ and MZM-0403, supported the difference in ageing pace between these strains. When comparing time-normalized fold changes of identified differentially expressed genes, age-related transcript level changes are stronger in GRZ than in MZM-0403, confirming previous findings, based on the analysis of age-related biomarkers, showing that ageing is accelerated in the short-lived strain GRZ compared to the longer-lived strain MZM-0403 [11].

GENETIC MANIPULATIONS

Efficient and reliable ways to generate precise changes to the genome of model organisms have been a long-standing goal of discovery-based and translational research. Protocols providing powerful genetic tools for studying vertebrate ageing and aging-related diseases in *N. furzeri* are available. By taking advantage of the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 nuclease (CRISPR/Cas9) system and the *N. furzeri* genome, it is possible to enable the generation of knockout alleles via non-homologous end joining (NHEJ) and knock-in alleles via homology-directed repair (HDR) [12]. There are also available strategies for Tol2-based transgenesis, to generate transgenic *N. furzeri* that express green fluorescent protein with germline transmission of the integrated transgene [13].

Both these methods are rapid and highly efficient, permitting generation of stable transgenic lines more rapidly than in any other available vertebrate model.

CONCLUSIONS

In conclusion, the ageing process of *N. furzeri* and humans share many common features, both being vertebrates. More importantly, for those researchers who use *N. furzeri* as a model system, the sequencing of its genome and a large number of transcriptome data (i.e. which genes are switched on or off during the process of aging) are now publicly available to the research community. Several studies over the last decade have shown that, in many aspects, ageing in *N. furzeri* resembles mammalian ageing. In addition, the existence of several natural strains with different lifespans and the possibility of engineering the genome as well as the recent availability of its genome sequence have helped *N. furzeri* become an accepted model for ageing research. It is indeed an ideal model to explore – in short time – molecular mechanisms that control ageing in vertebrates, including humans.

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