Old and new models for the study of human ageing

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Human ageing is associated with high susceptibility to disease. Some aspects of ageing can be studied directly in humans and have revealed that ageing is influenced by many factors, including genetics, lifestyle, sex and socio-economic status. But identifying the factors that cause and modulate the ageing process often requires experimental interventions and modelling that can only be performed in model systems.

Classical non-vertebrate models

Classical non-vertebrate models — yeast, worm and fly — have been instrumental to identify conserved genes and pathways that delay ageing and extend lifespan[1,2]. Because non-vertebrate species tend to be short-lived (1 month for *Caenorhabditis elegans*; 2 months for *Drosophila melanogaster*) and are easily genetically tractable, they can be used for rapid and iterative experiments as well as high-throughput screenings. Studies in these organisms have uncovered chromatin regulators as well as genetic pathways that are involved in the regulation of longevity, including nutrient sensing (for example, the insulin and mTOR pathways) and protein homeostasis. Remarkably, many of these genes and pathways have been shown to modulate lifespan in mammals. This conservation has also helped to identify drugs or compounds that act on these pathways and that can modulate lifespan across species. A case study is provided by rapamycin, a compound that inhibits the mTOR pathway. Rapamycin extends lifespan in yeast, worms and mice, and it has been found to have beneficial effects in clinical trials in elderly humans[3]. However, non-vertebrate models, although instrumental to understanding the mechanisms that regulate ageing, lack organs and systems that are crucial in human ageing, including blood, a closed circulation system, somatic stem cells in multiple organs and an adaptive immune system.

Traditional vertebrate models

Traditional vertebrate models — mice and zebrafish — have organs and systems that are similar to humans. Mice have a lifespan of approximately 2.5–3 years and have been heavily used to study ageing[4], notably for the validation of genes and pathways identified in non-vertebrates (such as the insulin/IGF1 pathway). Mouse studies were also the first to uncover new genes in the growth hormone axis that are involved in vertebrate lifespan regulation. Several 'anti-ageing' or 'rejuvenating' interventions and drugs have been successfully tested in mice, notably dietary regimens, parabiosis (the sharing of young blood circulation) and senolytics (compounds that kill senescent cells). The most commonly used mouse strain for the study of ageing is the inbred line C57BL6, but other strains have been informative. For example, shorter-lived mouse models with mutations that mimic progeria have been leveraged to accelerate the discovery of anti-ageing compounds. Crosses between different inbred lines can mimic aspects of genetic diversity present in humans and reduce confounds resulting from inbreeding. These crosses can also help to identify genetic regions associated with differences in lifespan between strains (including in response to anti-ageing interventions). However, the ~3-year lifespan and high husbandry costs have rendered studies on ageing (especially genetic ones) difficult and low throughput. Finally, although cheaper than mice, zebrafish have not been extensively used for ageing research because these fish are relatively long-lived (5–6 years).

A new vertebrate model: the killifish

The African turquoise killifish *Nothobranchius furzeri* is a powerful new model to bridge the gap between short-lived invertebrates and longer-lived vertebrate models[5,6]. The killifish lives in Mozambique and Zimbabwe in ephemeral ponds that are only present for 4 months per year during the brief rainy season. This species has developed two key features for the adaptation to this exceptionally transient habitat. First, it exhibits explosive growth, rapid sexual maturation and progeny production (and therefore rapid ageing). Second, the embryos can enter a state of 'suspended life' called diapause, which allows the killifish species to survive through the long annual drought. In the laboratory, with constant water availability, the killifish lives ~4–6 months, making this species the shortest-lived experimental vertebrate model system thus far. The killifish reproduces readily in captivity and is easy to grow and maintain. During its short life, this fish displays ageing characteristics, including muscle loss,

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fertility decline and cognitive deterioration. Importantly, it is responsive to interventions that can delay ageing, such as intermittent fasting. Tools for genetic and genomic studies in the killifish have been developed (for example, genome assembly, transcriptomic datasets, CRISPR–Cas9 genome editing and transgenesis). This toolkit has transformed this species into a vertebrate model for ageing research that is amenable to rapid genetic and longitudinal studies. However, the killifish is different from mammals on several aspects, including reproductive strategies, respiratory system and haematopoiesis. Finally, the killifish — like traditional vertebrate models such as mice and zebrafish — exhibits evident differences with humans in terms of overall longevity, behaviour, disease susceptibility and drug responses.

Species closer to humans: primates and dogs

Non-human primates (rhesus monkeys and marmosets) are evolutionarily closer to humans and have allowed testing of interventions and compounds[4]. Studies in rhesus monkeys, which live ~30 years, have confirmed that dietary restriction delays the onset and/or development of age-related traits and diseases in primates. The marmoset, which lives ~10 years, is currently being developed as a smaller and cheaper non-human primate to test the effects of drugs (for example, rapamycin) on lifespan and healthspan (the disease-free portion of life). However, non-human primate experiments remain long and costly, and environmental and genetic variables are difficult to control. A recent approach has been to study companion dogs because they share an environment with humans and their genetics is more tractable than that of primates[7]. Companion dogs are subjected to the same environmental factors as humans (pollution, noise and, to some degree, food and microbiome). The genetic characteristics of companion dogs, including pure pedigrees (with extreme and stereotypical phenotypes) or mixed pedigrees (similar to diverse human population), can also be leveraged. Of note, rapamycin is currently being tested in companion dogs with promising results. But studies in primates and dogs are more expensive and long term, thereby tending to validate rather than discover interventions that delay ageing.

Extremophiles with exceptional lifespans

Vertebrate species with exceptional longevity include naked mole rats (~30 years), beavers (~20 years), bats (~30 years), parrots (~80 years), rockfish (~120 years), Greenland sharks (400 years) and bowhead whales (200 years). Invertebrate species with exceptional longevity include Quahog clams (~400 years) and social insects such as bees and ants (~30 years). Some invertebrates, such as tardigrades, have dormant forms that make them exceptionally long-lived in their dormant state. Other species, including planarians, hydra and jellyfish, are 'immortal' (in their non-sexual form). Whereas some of these species are difficult to study in a laboratory setting (whales and sharks), others have been used and developed for experiments (naked mole rats, planaria and hydra)[6,8]. Regardless of their usability in the laboratory, species with exceptional lifespan characteristics can help to discover different strategies, pathways or gene variants that might be absent in humans but still promote health in humans.

Humans as a 'model organism'

Humans are long-lived (with a mean lifespan of ~80 years and a maximal lifespan of ~120 years). They are remarkably diverse in both their genetics and environment, having colonized nearly all habitats on the planet, even regions with extreme climates or altitudes. Humans are also responsible for social determinants that influence health, both positively (social support, antibiotics and sanitation) or negatively (pollution and climate change). Human genetic or biobank studies, such as those of UK biobanks, can provide association between genetic, environmental or societal factors and phenotype (for example, long lifespan). But how can causality be established? A possibility is to perform clinical trials for the study of age-related traits (because ageing per se is considered normal and as such is not classified as a disease by the Food and Drug Administration). Indeed, rapamycin has been tested in humans and has positive effects on the response to vaccination in elderly patients[3]. Other compounds (metformin, senolytics and young blood factors) have also been considered in strategies to delay age-related diseases in humans. Identifying ageing biomarkers or ageing clocks in humans (for example, DNA methylation, proteomics or multi-omics clocks)[9], coupled with longitudinal studies and imposed or self-reported interventions ('biohacking'), could contribute to increasing our understanding of human ageing.

Modelling human ageing in a dish

Culturing cells from old individuals (for example, old tissue stem cells or old fibroblasts) can recapitulate some, though not all, aspects of ageing. These in vitro systems can be helpful to characterize cell-intrinsic factors that change with age and analyse how they could be modulated by external interventions. 3D cultures or organoids can mimic a tissue/organ state better than a 2D culture. But a pre-requisite to generate most organoids is the reprogramming to an embryonic-like state, which has been shown to erase several hallmarks of ageing[10]. Direct transdifferentiation into specific cell types has been proposed to overcome this issue[10]. However, culture models, while promising for mechanistic studies, have limitations in understanding key aspects of organismal ageing, including drug responses, diseases, immune response, interaction between organs and tissues, and behaviour.

Conclusions and perspectives

The ensemble of model organisms already used for the study of ageing is reminiscent of Noah's ark. Traditional models will likely continue to provide a wealth of translatable information. Studying 'extremophiles' has the exciting potential of generating new concepts that could be implemented for the regulation of lifespan. The development of new experimental models uniquely tailored to ageing studies, such as the African killifish or others, will probably be essential in future years to have relatively high-throughput model systems that more closely recapitulate human physiology. The iteration

between such models and humans could be a particularly promising approach in delineating strategies to extend healthspan and promote healthy ageing.

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