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BIOMARKERS OF AGING REMAIN ELUSIVE AS RESEARCHERS TRY TO SLOW THE BIOLOGICAL CLOCK

Billions of dollars are pouring into longevity biotechs, but measuring success is challenging, given how little is known about the biology of aging. **By Mike May**

All of us age, and so we probably believe that we understand aging. If nothing else, all of us learn over time what aging feels like, from the strength and vigor of youth to increasing infirmity and death. Experiencing the feeling of aging, though, is far from explaining the underlying processes. Many human cultures have wondered what causes aging and how to slow it, but for those researchers who study aging, learning more about the science raises even more questions.

"At the biological level, ageing results from the impact of the accumulation of a wide variety of molecular and cellular damage over time," according to the World Health Organization[1]. "This leads to a gradual decrease in physical and mental capacity, a growing risk of disease and ultimately death."

That sounds straightforward enough, but the details of how cells, organs, and organisms age remain largely unknown. "There's no consensus in the field on what aging is," says Alan Cohen, associate professor of environmental health sciences at Columbia University. "I think you can actually make a strong argument that there is no biological process of aging." Instead, he says, the aggregate of lots of individual processes increase a person's mortality risk over time.

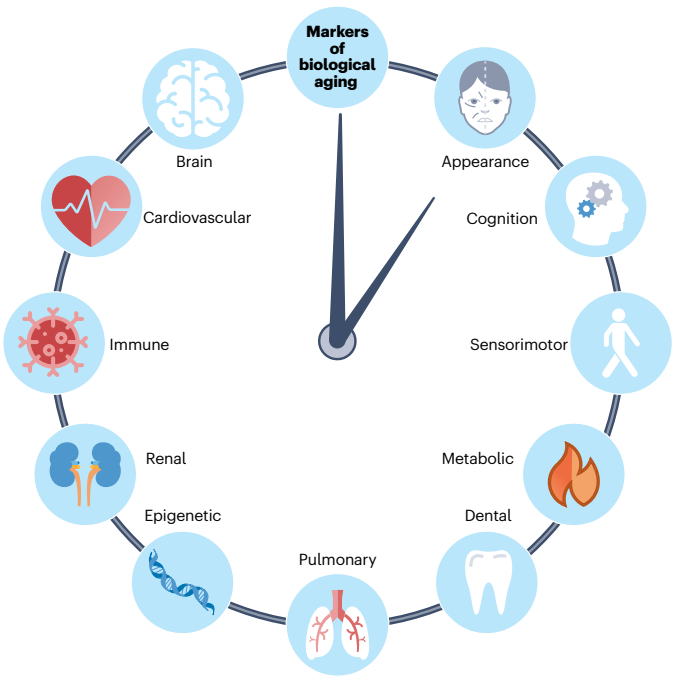

Fig. 1 | The markers of biological aging. Biomarkers in multiple organ systems can be used to predict a person's biological age, which may be older or younger than their chronological age. Adapted from M. L. Elliott et al. *Nat. Aging* **1**, 295–308 (2021), Springer Nature.

Biological curiosity drives aging research, but so does the centuries-old search for a fountain of youth. Rather than a natural spring purported to be a life-giving elixir (which Spanish explorer Ponce de Leon erroneously claimed to have found in Florida in the sixteenth century), many scientists search for indicators or markers of aging, and other scientists look for ways to slow or stop it, or prevent age-related diseases.

Biomarkers lack predictive power

In the search to better understand aging, many scientists look for molecular or functional markers (Fig. 1). "I guess you need to have biomarkers," says Christoph Handschin, professor of pharmacology at the University of Basel. "Otherwise, you have to follow people over decades."

Finding those biomarkers of aging, however, creates a daunting challenge. "The best biomarkers we have are still functional biomarkers, like cardiovascular fitness, maximal oxygen consumption, even grip strength and muscle mass," says Handschin. "Those are really good predictors of morbidity and mortality, and they still surpass – by far – the predictive power of molecular biomarkers"[2].

According to Handschin, functional biomarkers can be causally related to the likelihood of death. "We lack that for the molecular markers, where it's all correlation," he says. Still, Handschin hopes that scientists find ways to turn biomolecular correlations into causal explanations of aging processes.

Part of the challenge in understanding aging arises from how scientists look at it. Chronological and biological age, for example, are two different things because of the heterogeneity in how people age. A calendar can be used to measure the former, and scientists build clocks of biological aging for the latter.

Although Steve Horvath, a principal investigator at the Altos Labs San Diego Institute of Science, appreciates the utility of the term biological clock, he prefers something more specific, like 'epigenetic clock', which relies on DNA methylation as a marker of aging.

Epigenetic clocks

Over the years, Horvath and his colleagues have developed various approaches to correlating collections of biomarkers with chronological and biological age. In 2019, for example, Horvath and his colleagues described GrimAge, which combines information from plasma proteins and biomarkers that serve as indicators of DNA methylation[3]. With GrimAge, Horvath says, "we understand the methylation signal in terms of plasma proteins, and it works really well at predicting mortality risk." Horvath and his colleagues showed that GrimAge outperformed several other epigenetic clocks in connecting mortality with indicators of functional health, like grip strength[4].

Tools like GrimAge can also be used in clinical trials. As one example, Greg Fahy, chief scientific officer and co-founder of Intervene Immune, and his colleagues – including Horvath – ran what they reported "may be the first human clinical trial designed to reverse aspects of human aging"[5]. Patients in the study, who were aged 51 to 65, received recombinant human growth hormone, which has been shown to trigger growth of the thymus gland and reverse aging of the immune system in animals. From that work, the authors wrote: "The GrimAge predictor of human morbidity and mortality showed a 2-year decrease in epigenetic vs. chronological age that persisted six months after discontinuing treatment."

Perhaps most surprising of all, epigenetic clocks reveal something that appears to be universal about aging. Horvath and a huge team of collaborators applied DNA-methylation analysis to 128 mammalian species, and the epigenetic clock accurately estimated the chronological age of all of them[6]. "This shows that epigenetic aging is highly conserved, so it cannot be entirely stochastic," Horvath explains. Instead, Horvath suggests that this result supports the idea that an as-yet unknown biological program controls aging in most animals. "Maybe you can target that program," he says.

A systems biology approach

One biological system thought to be relevant for aging is homeostasis – the idea of biological systems staying in balance, which may become impaired during aging. "Most of what's happening in our bodies is more sophisticated than that," says Cohen. The term homeostasis implies a static (stasis) aspect of the process, but Cohen says that "is kind of misleading." Instead, Cohen sees the body in a form of dynamic equilibrium[7], which he describes as "being in the state that you should be at given your external and internal conditions."

Although no one knows exactly how a person's dynamic equilibrium changes with aging, Cohen points out a few general examples. "There are adaptive processes where, for example, you probably need a different way to have your immune system functioning later in life because you're not meeting as many new pathogens as when you were young,"

***Caenorhabditis elegans* worms are a model organism for aging research.**

he says. "There are also compensatory processes, adjustments to something else that is going wrong."

Those adjustment processes, though, vary between people. It's even difficult to distinguish between a good or bad change because the processes interact. "We've evolved to regulate macro-level processes, like a person's level of inflammation, through large numbers of lower-level molecules that coordinate those responses," Cohen says. Consequently, the level of any particular molecule can only be interpreted relative to many other molecules, in a systems biology approach. "Only then can you get some idea of what the system is trying to do," he says.

This complexity of aging has led Cohen and his colleagues to analyze groups of biomarkers — occasionally as few as 3 to 5, but more often dozens or even hundreds. "The overall idea is that if we combine a bunch of markers, we're going to get a better sense of what the whole system is doing," he says. Understanding dynamic equilibrium over a person's life, though, will require much more work.

Decoding dysregulation

One fact that is agreed upon by aging researchers is that biological systems will fail over time. "There are almost an infinite number of ways that a system can go wrong," says Morgan Levine, a principal investigator at the Altos Labs San Diego Institute of Science. "By decoding how the system works, you can see how various perturbations might cause it to fail." This is known as system dysregulation.

To get at that dysregulation, scientists in Levine's lab simultaneously measure as many as hundreds of thousands of variables in people's genomes, such as DNA methylation and RNA expression. Then they use artificial intelligence (AI) to look for patterns in the data, such as variables that appear to be connected to age or health.

As one example, Levine and her colleagues applied a broad combination of variables and computational techniques to develop PCBrainAge, an AI-driven tool that they trained on cortical samples. The scientists concluded that PCBrainAge could be a useful tool for studying the various changes in the brain over time that predict the impact of Alzheimer's disease[8].

Similar approaches can be applied to other organs or biological systems to start building a better understanding of how dysregulation develops.

Ancient infections

In some cases, dysregulation might arise from past infection. Recently, Guang-Hui Liu, an expert in regenerative medicine at the Chinese Academy of Sciences, and his colleagues described the impact of retroviral infection on aging. The best-known retrovirus is human immunodeficiency virus (HIV), but Liu's team studied the impact of endogenous retroviruses (ERVs), which infiltrated the human genome tens of millions of years ago and so are sometimes known as viral fossils.

ERVs, Liu says, "are increasingly expressed in senescent cells, aged tissues and organs, as well as the serum of elderly individuals, thus serving as novel biomarkers of aging"[9,10]. For example, he points out that the reverse transcription products of ERVs in senescent cells trigger chronic inflammation by activating innate immune pathways. "Furthermore, ERV viral particles released from senescent cells can effectively transmit and amplify aging signals among cells and organs via paracrine (cell signaling) or fluid-mediated manners," Liu says.

As Liu tries to better understand the biology of ERVs in aging, he hopes to turn the knowledge into treatments. "By analyzing the life cycle of ERVs, including latency, reactivation and intercellular transmission, we have identified potential targets for intervention," he says.

In a study of the brain's frontal lobe in primates, for instance, Liu and his colleagues found that B-type lamins, proteins making up part of a scaffold in the nucleus that influences its function, decrease over time, and that triggers ERV expression, which leads to neuronal senescence and inflammation. Working with physiologically aged mice or aged human neurons in culture, Liu's team showed that abacavir, a nucleoside reverse transcriptase inhibitor used to treat HIV, inhibits the neuronal aging[11].

Uncoupling biomarkers from aging

Worms have proven to be a useful model to understand aging. At the Centre for Genomic Regulation in Barcelona, Spain, Nicholas Stroustrup, group leader of the systems biology research program, studies aging in the nematode *Caenorhabditis elegans*, a well-known model organism for biological studies.

The lifespan distribution of humans resembles that of *C. elegans*, even though the worms only live a couple of weeks. "If you overlay survival curves for humans and these worms and erase the units, you'd really have to be an

expert to be able to tell the difference," Stroustrup says.

Worms have helped determine if a biomarker is causally related to aging or if it just a correlation. To explain this distinction, Stroustrup discusses a human example of skin wrinkles. "Consider an intervention like cosmetic plastic surgery – you're not actually changing aging," he says. "You can eliminate wrinkles, but doing so merely decouples skin appearance as a biomarker for aging, and the causal structure of aging isn't affected." As he adds: "Looking better doesn't make you physiologically younger." The same could be true of many molecular biomarkers and the interventions that modify them. Such interventions might act downstream of the causal process – the one that could really impact aging.

In 2022, Stroustrup and his colleagues reported that age-associated changes in behavior are causally distinct from lifespan in *C. elegans*[12]. Vigorous movement is a powerful predictor of lifespan in worms, but this relationship is decoupled by many interventions. "The idea is that you can speed up aging studies by identifying markers that correlate with staying healthy and then use these markers as study endpoints is very much true for humans and biological aging," Stroustrup explains. "Yet, when you do an intervention on an aging process, like giving somebody a drug or changing their diet, you might see biomarkers looking better – the people younger in terms of some molecular question – but you might unknowingly be altering the relationship between the biomarker and the aging process, not directly modifying the aging process itself."

AI-driven drug discovery

In many cases, the biological processes of aging trigger specific diseases. Alex Zhavoronkov, CEO of Insilico Medicine, focuses on "dual-purpose aging and disease drug discovery at scale with robotics." As one example, Zhavoronkov and his colleagues combined data from 29 studies of glioblastoma multiforme, an aggressive brain cancer, in an AI-driven computational tool called PandaOmics, which combines information from genomics, transcriptomics, epigenomics, proteomics and more[13]. From this work, the scientists found several genes that might serve as targets for treating both the cancer and aging.

To combine computational power with the analysis of biological samples, Insilico Medicine built a fully automated drug-discovery lab in Jiangsu, China. "It automatically discovers targets using real-time data acquisition from human or animal samples," Zhavoronkov says.

Such automation and drug discovery should expose more about the biological processes of aging, and maybe how to delay them. "Only when we fully understand the biomarkers of human aging can we truly understand how old we really are," says Liu. "We need a ruler that accurately measures the biological age of humans, which is an important prerequisite for aging intervention." The science of aging, however, is not there yet.

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