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Cellular reprogramming and the rise of rejuvenation biotech

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Abstract

Cells can be rejuvenated and biological clocks reset using cellular reprogramming. Based on this premise, a growing number of companies now aim to use cellular reprogramming to develop therapies that induce rejuvenation of human beings. But how solid is our understanding of cellular programming to develop therapies for aging? Can the "young" science of rejuvenation, so far mostly based on in vitro studies, drive a new biotech field ultimately leading to clinical applications?

Key words: aging, longevity, Yamanaka factors, gene therapy

The rise of rejuvenation biotech

In spite of significant advances in our understanding of genetic, dietary, and pharmacological manipulations of aging in animals [1], known interventions retard but do not stop or reverse aging. For those seeking eternal youth, life-extending interventions like caloric restriction (capable of extending rodent lifespan up to 50%) and rapamycin (>20% in mice) will not be sufficient, and therefore reversing aging may be essential.

Based on recent developments in cellular rejuvenation using reprogramming (Box 1), a number of companies are now aiming to apply cellular reprogramming methods for the treatment of age-associated diseases and potentially rejuvenation of tissues, organs, and even whole humans. These include Turn Biotechnologies, YouthBio Therapeutics, NewLimit, AgeX Therapeutics, Iduna Therapeutics (a Life Biosciences subsidiary) and Shift Bioscience (Table 1). More recently, a $3B effort to apply reprogramming to rejuvenation purposes has emerged as part of a new company called Altos Labs, reportedly supported, amongst others, by one of the world's richest man, Jeff Bezos (https://www.technologyreview.com/2021/09/04/1034364/altos-labs-silicon-valleys-jeff-bezos-milner-bet-living-forever/). The news are not surprising in light of the recent growth in companies and investment of the anti-aging biotech sector [1]. In 2013, Google announced Calico, a company aimed at targeting aging. With a reported $2.5B in investment, Calico has thus far arguably failed to live up to the expectations of many people including its own initial Google backers (https://www.businessinsider.com/calico-life-sciences-founder-disappointed-with-progress-2020-5). Interestingly, Calico is now starting to focus on cellular reprogramming and has recently reported that different combinations of Yamanaka factors can restore youthful gene expression, suggesting we should be able to design potentially less risky reprogramming strategies [2].

Prospects and pitfalls for rejuvenation therapies

The explosion in the field of reprogramming as a technology for rejuvenation is not surprising given its paradigm-shifting potential, but what are its future prospects? At present, multiple in vitro studies have shown that it is possible to reset features of cellular aging with partial

reprogramming in different mouse and human cell types [3, 4]. In addition to OSKM overexpression (Box 1), transient expression of mRNAs (OSKM plus LIN28 and NANOG) has also been used for partial reprogramming and cellular rejuvenation [4], with mRNA technology being the basis for Turn Biotechnologies. Importantly, one of the aging features that can be reset by reprogramming is epigenetic age. Epigenetic clocks, based on the changes in DNA methylation levels of an often relatively small number of CpG sites, have emerged in recent years as the most accurate biomarker of age, correlating with diseases and mortality [5]. Nevertheless, the biological mechanisms behind epigenetic clocks and whether they are causes or consequences of aging remains unknown. In this line, it is unclear whether the epigenetic reset is driving cellular rejuvenation or is a consequence of the rejuvenation. Regardless, reprogramming can reset epigenetic age to zero in iPS cells, and likewise transient expression of the Yamanaka factors can partially reverse the epigenetic clock [4, 6], supporting the idea that cellular reprogramming is, at least at some level, a rejuvenation procedure. Other aging phenotypes like telomere length and mitochondrial dysfunction are also reset by reprogramming [3, 4]. However, mutations are not thought to be corrected. Overall, it appears that most (but not all) known cellular aging changes are reset during reprogramming, even in cells from very old individuals, like supercentenarians [6].

Although there is a growing body of knowledge regarding the role of cellular reprogramming in rejuvenation in vitro, the situation is far less clear in vivo. Indeed, there has been a relatively small number of studies investigating the induction of Yamanaka factors in the context of aging in vivo. One groundbreaking study led by Ocampo and colleagues showed that transient expression of the Yamanaka factors can ameliorate aging phenotypes and extend lifespan in a short-lived mouse model of progeria, a disease resembling accelerated aging [3]. Importantly, there is very limited data in normal aged animals. In this regard, Ocampo and colleagues observed benefits of partial reprogramming in normal aged mice, such as increased resistance to metabolic disease and improved recovery from muscle injury [3], but effects on lifespan are unknown. Another landmark study recently showed that three Yamanaka factors (OSK) can be used to restore vision in aged mice, also restoring youthful DNA methylation patterns [7]. Recently, partial reprogramming of cardiomyocytes has also been shown to drive heart regeneration in mice by inducing a neonatal gene expression program in adult, normally post-

mitotic, cardiomyocytes that results in dedifferentiation and cell cycle reentry [8]. Therefore, it appears that reprogramming can have therapeutic benefits, like promoting repair and regeneration, in at least some mouse tissues and organs.

Importantly, in vitro studies have highlighted that reprogramming is currently quite inefficient with a small percentage of cells successfully reprogrammed to pluripotency. Therefore, it is possible that a selection process is occurring; in other words, only cells that have particular characteristics (e.g., lower level of damage or are younger) can be fully reprogrammed. As such, perhaps differences between tissues and organs will impact on the efficiency and therapeutic potential of reprogramming. Indeed, it may be necessary to identify specific combination of factors, or alternatives to the Yamanaka factors, for reprogramming specific tissues. As shown by the recent study from Calico [2], OSKM may be too blunt of an instrument and a fine tuning or maybe alternative factors might be necessary for effective and safe rejuvenation. In addition, even if reprogramming can be used therapeutically for rejuvenating human tissues, it will be necessary to dramatically increase its efficiency while at the same time avoiding the induction of cancer. Interestingly, ex vivo cell rejuvenation with reprogramming factors may be possible for some clinical applications. In this line, mice treated with skeletal muscle stem cells rejuvenated using transient reprogramming exhibited an improved regenerative ability upon transplantation [4]. While ex vivo reprogramming might thus be a possibility for some therapies, it is difficult to envision whole organismal rejuvenation with such therapies alone.

Even in cases where cellular reprogramming can be used therapeutically for rejuvenation without the induction of cancer or other serious side effects, another crucial hurdle is developing human therapies. While studies in mice can make use of an array of genetic approaches, in humans these are not possible. Our ability to modify human genes in vivo is still very basic, and it has long been argued that this inability is a major limitation to therapeutically target aging [9]. Given the narrow therapeutic window of cellular reprogramming [8], very tight dosage control and tissue, or even cell type specificity, will be necessary to induce rejuvenation in vivo without causing cancer. For this reason, developing a gene therapy, or alternatively a pharmacological approach, with such a control and specificity is likely to be technically challenging, even assuming that safer combinations of reprogramming factors can be discovered. Lastly, the technology to

change human genes is still at an early stage with current gene therapies mostly restricted to rare genetic diseases and cancer [10].

Concluding remarks

Overall, cellular reprogramming has emerged as the most promising approach for human rejuvenation, giving rise to the idea that aging may be reprogrammable. However, the field is still immature and there is limited in vivo data to infer safety and efficacy in a clinical setting. Though specific applications of reprogramming may emerge in regenerative medicine, developing broader rejuvenation therapies based on reprogramming is arguably built on shaky ground that may or may not work out. In our view, this endeavor cannot be compared to the Apollo or Manhattan projects, for which we had a strong theoretical understanding and became engineering problems. In the case of aging, our theoretical foundations may crumble at any time. In this line, we are not sure of the driving mechanisms of aging [11], or if the readouts used to infer rejuvenation are accurate or complete representations of biological age. Moreover, even if reprogramming can be used to rejuvenate mice using genetic manipulations, translating these discoveries into humans remains a major challenge as new technologies are necessary to make medicine (re)programmable. On the other hand, it is possible that if epigenetics is the major regulator of aging phenotypes and cellular reprogramming is rejuvenating cells at all relevant levels, we will be able to develop the technologies to apply reprogramming in human rejuvenation therapies. As such, in addition to considering potential drawbacks, it is equally important to point out that the potential medical benefits of rejuvenating human tissues are revolutionary. Given the current trajectory of rejuvenation biotech, the pace of new discoveries in this field, and the amount of capital coming into this sector, rejuvenation science and biotech will most likely explode in the coming years.

A little over 20 years ago scientists, companies and investors were excited about telomerase, the so-called “immortality enzyme”, as a way of reverting aging [12]. Based on this premise, companies like Sierra Sciences and Geron attempted to use telomerase to target aging. They failed, however, because although telomerase extends the lifespan of cells in a Petri dish, it does not work in the same way in organisms. In a Petri dish, cellular reprogramming is a very exciting

technology for rejuvenation, and it shows that at least some cells can be turned young again according to current readouts. Just like telomere shortening is only one facet of aging, we currently do not know whether reprogramming will reverse all key facets of aging in vivo. Importantly, figuring out whether cellular reprogramming can rejuvenate whole organisms and, if so, how we can harness this technology to rejuvenate human beings is one of the biggest open questions in biotechnology.

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Conflict of interest

JPM is an advisor/consultant for the Longevity Vision Fund, Centaura, NOVOS, YouthBio Therapeutics and the founder of Magellan Science Ltd, a company providing consulting services in longevity science. AO is founder of Longevity Consultancy Group, a company providing consulting services in longevity science.

Box 1: Seeking rejuvenation: From ancient China to partial reprogramming

The quest for eternal youth is as old as humankind. From the Chinese emperor Qin Shi Huang, who over 2,000 years ago reportedly died from taking mercury pills believing it was the elixir of youth, to Russian physician Alexander Bogdanov, who in early 20th century died from blood transfusions he was hoping would rejuvenate him, many have sought immortality. At the cellular level, we have known since John Gurdon's famous frog cloning experiments that we can artificially revert time and reset biological age. Later nuclear transfer experiments, including the generation of the famous sheep Dolly, also demonstrated that it is possible to take an adult aged, potentially damaged cell, and restart the course of life. In this line of research, the discovery of induced pluripotency (iPS) by expression of four transcription factors (OCT4, SOX2, KLF4, and MYC, also known as Yamanaka factors or OSKM) further revolutionized our view of biological age and time, demonstrating that the cell's biological clocks can be reset to an embryonic state [6]. These series of observations raise very important questions that challenge our current unidirectional view of the aging process. If cells can be rejuvenated, could this open the door for rejuvenating organisms? Initial studies activating reprogramming factors in vivo showed that reprogramming can induce cancer and teratomas in mice [13], perhaps not surprisingly as MYC is a well-known proto-oncogene. Nonetheless, more recent approaches using a short-term induction of the reprogramming factors, termed partial or transient reprogramming, show that it is possible to partially reset biological age in the absence of pluripotency - i.e., separating dedifferentiation from epigenetic rejuvenation and therefore maintaining cell identity [3, 4, 14]. In theory, if we could reset biological age while maintaining cell identity at the organismal level, we could induce rejuvenation of an organism and minimize cancer risk. Such approaches, if successful, would represent a paradigm shift in medicine, because instead of slowing down the pace of aging and the onset of age-related diseases, like neurodegenerative diseases, sarcopenia, osteoarthritis or cardiovascular diseases, we would be able to rejuvenate the affected tissues and prevent or even revert the manifestation of age-related diseases and degenerative changes. As aging is the major risk factor for most human diseases, a therapy capable of preventing many of these diseases altogether would represent a miracle of medicine not seen since the development of antibiotics.

Table 1: Rejuvenation biotech companies using cellular reprogramming

Name	Aim	Approach	URL	Reference
AgeX Therapeutics	Unlock cellular immortality and regenerative capacity to reverse age-related changes in the body	Using pluripotent stem cells plus partial reprogramming to induce tissue regeneration (iTR)	https://www.agexinc.com/	[15]
Altos Labs	Reversing disease to transform medicine	Cellular rejuvenation programming to restore cell health and resilience	https://altoslabs.com/	
Calico	Understand the biology of aging and age-related diseases	Various, including the use of transient reprogramming	https://www.calicolabs.com/	[2]
Gameto	Solve the problem of accelerated ovarian aging	Applying cellular reprogramming to create human reprogrammed cells of the ovary	https://gametogen.com/	
Iduna Therapeutics (Life Biosciences)	Develop epigenetic reprogramming therapies that allow the rejuvenation and replacement of tissues	Proprietary gene therapy that expresses OSK to reprogram the epigenome back to a younger state	https://www.lifebiosciences.com/	[7]
NewLimit	Radically extending human healthspan	Using epigenetic reprogramming therapies	https://www.newlimit.com/	
Retro Bio	Develop therapies for diseases driven by the biology of aging	Pursuing multiple programs, including cellular reprogramming approaches	https://retro.bio/	
Shift Bioscience	Develop drugs that safely reset cells and tissues to a youthful state	Employing machine learning to better understand the causes of cellular rejuvenation	https://www.shiftbioscience.com/	
Turn Biotechnologies	Develop mRNA medicines, focused on reprogramming the	Using mRNA cocktails for the delivery of reprogramming factors	https://www.turn.bio/	[4]

	epigenome to restore capabilities that are often lost with age			
YouthBio Therapeutics	Develop gene therapies aimed at restoring more youthful epigenetic profiles	Employing partial reprogramming	https://youthbiotx.com/	

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