

# Challenging but essential targets for genuine anti-aging drugs

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## Abstract

Contrary to what one might conclude from the popular press, anti-aging drugs do not yet exist, in the sense in which the term “drug” is normally used. Since a drug is assumed to be effective against its target human pathology, and since the vast majority of deaths in the developed world are from aging-related causes, it is inappropriate to describe something as an anti-aging drug unless one has good reason to believe that it will appreciably extend the life expectancy of those in the developed world who receive it. A drug that rejuvenates aspects of the aged body but does not increase life expectancy is an anti-frailty drug, not an anti-aging one. This distinction is critical for decision-makers in the drug discovery sphere because, while the market for anti-frailty drugs (even unproven ones) is large, that for genuine anti-aging drugs—which, as I explain here, are now foreseeable—will certainly be far larger. In this article I survey the main aspects of age-related degeneration that are believed to be essential targets for genuine anti-aging drugs—that is, without whose amelioration human life expectancy probably cannot be greatly increased—and some promising strategies for the design of such drugs.

**Keywords:** aging, anti-aging drugs, atherosclerosis, mutations, mitochondria, glycation, cell senescence, cell therapy, gene therapy

## 1. Is aging as complex as it seems? Yes and no

The complexity of aging, in practical terms, depends on one’s objective: comprehension or intervention. From the point of view of the biogerontologist, whose goal is the ever more detailed understanding of aging, aging is indeed fearsomely complex; for the biomedical gerontologist, however, whose goal is to do something about aging, it is not [1-3]. Aging is, at its root, a side-effect of being alive, and being alive is very complex indeed; furthermore, the pathologies that we term “age-related diseases” are also exceedingly complex in their progression. However, we cannot conclude from these facts that intervention in aging must also be extremely complex. This is because there is a “causal bottleneck” in the tangled chains of events that lead from metabolism (being alive) to pathology (being dead). There seem to be only half a dozen or so types of “damage” (a term that we will need to define with care—see below) caused by metabolism that we have any reason to believe develop into pathology (Table 1).

For present purposes, “damage” will be taken to mean any originating stable alteration in the composition of the body at the molecular or cellular level that accumulates as a side-effect of essential metabolic processes. “Originating” means that the precursors of the alteration are not stable, but are potentially repairable before they develop into it. For example, mutations in our DNA are stable but their precursors, such as oxidatively damaged bases, are not—we have enzymatic repair mechanisms that mend such lesions. The reason why mutations accumulate with age is that oxidatively damaged bases are not always repaired quickly enough before maturing into mutations and that mutations themselves are not repairable. Thus, mutations count as a form of “damage” by the definition used here.

This paucity of types of damage is the main reason why they are the correct set of targets for anti-aging drugs. The chain of events that leads to aging and death is not so much a chain as a tangled rope of strands of causality, each event or process causing and being caused by many others in concert. To treat aging effectively, this rope must be cut—and that means cutting every strand. The thinnest part of the rope is, *a priori*, the most attractive target. This exposes the error of the biogerontologist who concludes

from the complexity of aging that its effective treatment must be similarly complex: we do not need to disentangle the whole rope in order to cut it at its thinnest point.

The remainder of this article will focus in turn on four of the types of damage listed in Table 1. The ones that I will not address are cell loss/atrophy, because that is the subject of many excellent reviews (e.g. [4-6]), and nuclear mutations, because in my view their only pathogenic consequence is cancer, against which a wide variety of well-known therapeutic strategies is already ranged.

## 2. Intra- and extracellular aggregates

Most of the macromolecules in a cell are ephemeral. They are created as needed, but then are destroyed, either because their task is done or because they have been inactivated (by, for example, free radicals). Some are easier for the cell to degrade than others, and a small residue of highly cross-linked material targeted for degradation is resistant to any of the cell's numerous catabolic mechanisms. This residue ends up in the lysosome, an acidified compartment containing many powerful hydrolytic enzymes. If the cell is of a type that divides regularly, such material does not accumulate over time because cell division dilutes it. In non-dividing cells, however, it gradually occupies more and more of the cell volume, sometimes to the detriment of cell and organ function.

The most clear-cut examples of this are arterial macrophages, which enter the artery wall to degrade damaged or trapped lipoproteins and are eventually overcome by the indigestible fraction of such material. At that point they become “foam cells”, the first stage of an atherosclerotic lesion [7]. The significance of this incomplete degradation is thus immense: if macrophages could break down everything they take up, atherosclerosis would simply not occur and cardiovascular disease (particularly heart attacks and strokes) would be a minor cause of death, rather than the most frequent one in the Western world.

Other examples with somewhat less categorical, but still very probable, roles in major aspects of age-related decline are neuronal aggregates such as hyperphosphorylated tau in Alzheimer's disease [8], the fluorescent compound A2E in the retina in macular degeneration [9], and more controversially the highly heterogeneous material lipofuscin (“age pigment”) in the heart, motor neurons and various other postmitotic cell types [10].

Thus, lysosomal aggregates are key examples of damage leading to aging and death. Moreover, the most significant *extracellular* aggregate in age-related pathology—amyloid plaques in Alzheimer's disease—is also potentially lysosomal: a promising approach to its removal is vaccination, which causes it to be taken up by microglia and thereby to be directed to microglial lysosomes [11]. The same strategy should be applicable to any other extracellular aggregate.

Though the above has mostly been well known for some time, this has not led to the development of drugs to dissolve such aggregates. The main reason is that they really are very hard to break down. There have been occasional reports of successful clearance of such material, but none has proved reproducible [12]. Thus, this is a prime target for the development of more sophisticated drugs whose design has not hitherto been possible, perhaps because it relies on advanced genomics. A plausible strategy is to identify microbial enzymes with the necessary function [13]; this is motivated by the simple observation that the target substances do not accumulate in the soil, even in graveyards. Delivery of such enzymes will be easier for some cell types than others; the macrophage is a particularly straightforward one, already being explored for therapy of lysosomal storage diseases much rarer than atherosclerosis [14], while delivery to (for example) neurons will require further improvements in gene therapy.

## 3. Protein-protein crosslinks

The overall structure of our tissues is maintained by the extracellular matrix, a network of structural proteins linked together in regular arrays to give both support and elasticity where it is needed. This

elasticity diminishes with age, largely as a result of the accumulation of additional cross-links (which, unlike the enzymatically-generated ones, are distributed randomly); the results include systolic hypertension, which contributes to numerous age-related pathological conditions. Most such random cross-links are thought to arise from chemical reaction with sugars present normally in the circulation; the process of their formation is termed glycooxidation, comprising a non-oxidative step (glycation) and an oxidative step (oxidation) [15]. The cross-links themselves are termed “advanced glycation endproducts” or AGEs.

There are many different AGEs with a wide variety of chemical structures. This complicates the search for agents that might remove or cleave them. Somewhat serendipitously, a family of small molecules was discovered several years ago that cleaves one class of AGE; the leading example is ALT-711, being pursued by Alton. ALT-711 has performed very well in several animal experiments and in Phase I and II clinical trials [16,17]; this indicates that the class of AGE that it cleaves is a major one in human tissue. However, it is certainly not the only major one. The opportunity therefore exists to identify drugs that can cleave other classes of AGE. These classes include chemically more stable ones, which may not be cleavable without coupling the reaction to an exergonic one such as ATP hydrolysis; in other words, the most effective agents may be ATP-consuming enzymes. Again, this is a field in which modern genomic and proteomic technology, combined with classical chemistry, can transform the quest for such agents from a nearly random search to a rational design process.

## 4. Mitochondrial mutations

Of the tens of thousands of proteins that our genes encode, a tiny minority—just 13—are encoded in the DNA of our mitochondria, not on our chromosomes. These proteins are essential components of the machinery of aerobic respiration, the process responsible for most of our ATP synthesis. Like nuclear DNA, the DNA in the mitochondrion (hereafter “mtDNA”) can suffer mutations. Each cell has at least a few hundred copies of the mtDNA, so initially a mutation has no phenotypic consequence. However, for reasons that are still debated [18], some mutant species enjoy a selective advantage—they expand clonally within the cell and eventually take it over entirely, thereby seriously compromising cell function.

The effect of this process on aging is controversial, largely because few cells succumb to this process [19]. However, the clear cross-species correlation between rate of aging and rate of mitochondrial oxidant production [20] has sustained it as a “prime suspect” within biogerontology. There are at least two ways in which a small number of mitochondrially mutant cells could exert a wide-ranging pathogenic effect on the body that could contribute to age-related decline. One is that mitochondrial mutations may cause sarcopenia (loss of muscle mass). Muscle fibres are extremely long syncytial structures that may be entirely lost even though just a small segment (~1mm) becomes mitochondrially mutant and thereby eventually atrophies [21]. This would amplify the effect of mtDNA mutations very greatly, especially given the critical homeostatic roles played by muscle and the consequent loss of resistance to challenge that results from loss of muscle mass with age [22]. The other plausible mechanism is that the metabolic alterations caused by loss of respiration may include a marked up-regulation of electron transfer through the cell membrane into the extracellular medium, with extracellular free radical production as a side-effect. This would potentially elicit a similar amplification of the problem, this time a chemical one—such free radicals could initiate lipid peroxidation chain reactions in circulating material such as low-density lipoprotein, for example. The result could be an increasingly oxidised circulating pool of such material (and indeed the redox state of the plasma becomes markedly more oxidised with age [23]), leading in turn to oxidative stress in mitochondrially non-mutant cells (the vast majority) that import such material [24,25].

What are the implications of this for anti-aging drugs? The requirement is either to stop mutant mtDNA from clonally expanding, or to introduce complementing functionality into the nuclear DNA so that such

mutations do not block respiration. A third option, eliminating cells that have become mitochondrially mutant, is more questionable given the sarcopenia possibility mentioned above.

Clonal expansion seems to be rather rapid once it begins, so most cells may not be rescuable from a mutant state by a reversal of the selective advantage that amplified the mutation—there will probably be little non-mutant DNA to amplify. Luckily, however, the cell type most likely to matter in aging (according to either of the models described above) is skeletal muscle. As noted above, only short segments of fibres are taken over by mutant mtDNA; hence, in theory a reversal of the selective pressure would cause such segments to be repopulated from either end by functional mitochondria [26]. Certain rhodamine compounds, particularly rhodamine 6G, cause rapid degradation of all mitochondria in cultured cells; analogues may not be hard to find that are selective for dysfunctional mitochondria, since there exist quite overt signatures of such dysfunction, such as an abnormally low membrane potential. A genetic approach with the same motivation is to inhibit the replication of mutant mtDNA with agents that bind specifically to mutant sequences [27]; this seems inapplicable to aging, however, because different cells accumulate different mutations.

A more comprehensive strategy would be to rescue the genetic defect without destroying the mutant mitochondria. Approaches involving the direct introduction of replacement DNA into mitochondria have been proposed [28], but seem unlikely to be effective while the selective pressure for their elimination remains in place. This can be avoided by introducing the replacement DNA not into the mitochondria but into the nucleus, with certain modifications causing its encoded proteins to be imported into mitochondria (which is the natural pathway for around 1000 other proteins) [29]. There are complications in this strategy, but after many years of frustration it has seen three recent successes [30-32]; its time has definitely come.

## 5. Senescent cells

The spontaneous and apparently irreversible conversion of a mitotically competent cell into a state where it cannot divide, termed “replicative senescence”, is a phenomenon well known in cell culture. *In vivo*, however, such cells seem to be about as common as mitochondrially mutant cells—that is, not very common at all in otherwise healthy tissue [33]. However, as with mitochondrially mutant cells, there are reasons to believe that these cells may be pathogenic despite their low abundance. The mechanism currently attracting most interest [34] derives from the discovery that senescent cells secrete a number of proteins that potentially promote cancer. Two key classes are growth factors that promote cell division and proteases that degrade the extracellular matrix. Clearly the senescent cell itself cannot develop into a cancer—it cannot divide at all, let alone uncontrollably—but a nearby cell, which may have undergone some of the mutational events needed to initiate a tumour, may be helped on its way by these extracellular factors.

A highly promising approach to averting this chain of events is the ablation of senescent cells. This lacks the drawback noted above of killing mitochondrially mutant cells, because the cell types most affected do not include syncytia that might be entirely lost as a result of deleting one small segment. Two main strategies are evident. The first is a “suicide gene” approach, in which gene therapy is used to introduce into cells a gene that will be expressed only if and when the cell becomes senescent, and whose protein product is highly toxic. This may be challenging to implement, both because of its reliance on gene therapy and because of the risk that the gene will be adventitiously activated in healthy cells, thus damaging the tissue more than it protects it.

An alternative is to kill senescent cells from the outside: to target cytotoxic agents on the basis of cell surface markers. This could be done by exploiting the immune system, within which a class of cells (cytotoxic T lymphocytes) kills and engulfs cells that are presenting foreign peptides on their surface. As yet there is no report of a sufficiently senescence-specific antigen, but the wide range of proteins up-

regulated in senescent cells strongly suggests that some may exist whose basal expression is low enough for this to be a practical approach.

## 6. Conclusion

Aging is dauntingly complex from the basic science perspective, but less so from the biomedical one. We are on the threshold of the development of genuine anti-aging drugs—pharmaceutical and genetic therapies that, jointly, can rationally be expected to postpone and even reverse age-related degeneration in humans and thereby markedly extend healthy lifespan. The market for such treatments will without doubt dwarf anything that medicine has ever seen; those with the vision and determination to spearhead this advance will be richly rewarded.

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Category of damage	Feasibly repairable (or effects avoidable) by
Cell loss and atrophy	Cell and growth factor therapy
Oncogenic nuclear mutations	Anti-cancer therapies
Mitochondrial mutations	Allotopic expression; destruction of mutant mitochondria
Intra- and extracellular aggregates	Lysosomal enhancement with microbial hydrolases
Protein-protein crosslinks	Small-molecule and enzymatic crosslink-breakers
Senescent cells	Selective killing of senescent cells

**Table 1. Categories of age-related molecular and cellular damage and promising ways to repair them.**