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Geroconversion: irreversible step to cellular senescence

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Cellular senescence happens in 2 steps: cell cycle arrest followed, or sometimes preceded, by gerogenic conversion (geroconversion). Geroconversion is a form of growth, a futile growth during cell cycle arrest. It converts reversible arrest to irreversible senescence. Geroconversion is driven by growth-promoting, mitogen-/nutrient-sensing pathways such as mTOR. Geroconversion leads to hyper-secretory, hypertrophic and pro-inflammatory cellular phenotypes, hyperfunctions and malfunctions. On organismal level, geroconversion leads to age-related diseases and death. Rapamycin, a gerosuppressant, extends life span in diverse species from yeast to mammals. Stress–and oncogene-induced accelerated senescence, replicative senescence in vitro and life-long cellular aging in vivo all can be described by 2-step model.

Keywords: aging, cell cycle arrest, gerogenic conversion, mTOR, oncogenic trsnformation

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Introduction

Defined as irreversible cell cycle arrest, cellular senescence is difficult to link to age-related diseases, which terminate our life span. If anything, cell cycle arrest per se should protect against atherosclerosis, hypertension, organ fibrosis, visceral adiposity, benign tumors and cancer. And why calorie restriction and rapamycin, which inhibit proliferation, extend life span. Something is missing. Indeed, aging is not just cell cycle arrest.[1-18] In analogy, although cell cycle progression is important in carcinogenesis, we do not define cancer as cell cycle progression. For one, intestinal and bone marrow progenitor cells proliferate faster than tumor cells. And the cancer cell cycle can be easily arrested by p21, which is not even a tumor suppressor. A cell can be proliferating but not cancerous. Similarly, a cell can be arrested but not senescent. Even permanently-arrested cells (such as neurons) are not necessarily senescent. Here we will define the essence of senescence, as it had been done for cancer.

The essence of cancer is oncogenic transformation, driven by oncogenes and antagonized by tumor suppressors.[19-31] Similarly, the essence of senescence is gerogenic conversion driven by gerogenes and antagonized by gerosuppressors. (There is an overlap between oncogenes and gerogenes[18,32]). But let us start from the beginning.

Two-types of Cell Cycle Arrest

Growth factors, hormones, cytokines and nutrients activate Ras/Raf/MEK/ERK and PI3K/Akt/mTOR signaling pathways.[33-36] In cancer cells, these pathways are constitutively activated. These pathways (MAPK/mTOR, for brevity) stimulate cellular mass growth coupled with cell cycle progression (**Fig. 1A**).

1. Quiescence: Without GF, the MAPK/mTOR network is deactivated (**Fig. 1B**). Cell cycle comes to a halt. The quiescent cell neither grows nor cycles. Yet the cell retains the proliferative potential: re-addition of GF causes activation of MAPK/mTOR, cell mass growth, cell cycle progression, mitosis and cell proliferation. In quiescent cells, mTOR is deactivated, levels of pS6, cyclin D1, p21 and p16 are all low.[37] "Everything is off." Contact inhibition also causes quiescence-like arrest. Contact inhibition in confluent culture inhibits mTOR and MAPK pathways.[38,39] Cells neither

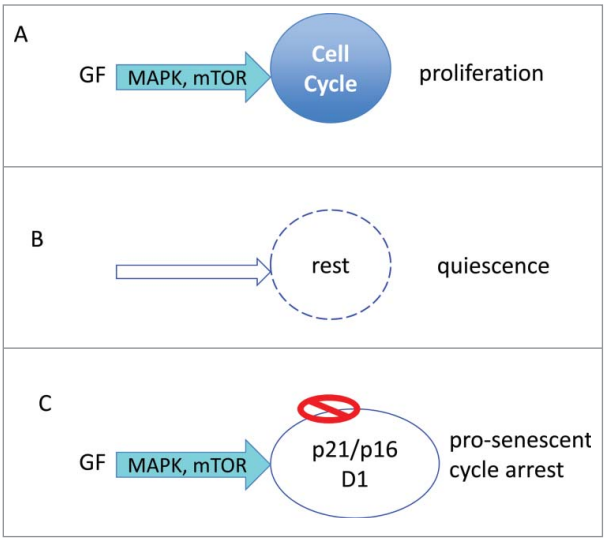

Figure 1. Proliferation versus arrest. (**A**) Proliferation: Growth factors (GF) activate MAPK and mTOR pathways, driving cell growth (in size) and cell cycle. Cellular growth is balanced by cell division. (**B**) Quiescence: In the absence of GF, cell growth and cycle are at rest. (**C**) Pro-senescent cycle arrest: When the cycle cycle is blocked by p21 or p16, the growth promoting pathway (MAPK/mTOR) causes growth in size (hypertrophy).

grow in size, nor cycle and can restart proliferation after splitting.

2. Hyper-mitogenic type of arrest (**Fig. 1C**). Cell cycle can be arrested by CDK inhibitors such as p21 and p16. In this case, the cell cycle is blocked but mTOR and MAPK are still active (**Fig. 1C**). In futile attempt to overcome the block, growth-promoting pathways push a cell to become hypertrophic, hyper-active, hyper-functional and secondary signal-resistant and β-Gal-positive.[40] Cyclin D1 goes above the roof. It is like pushing the brakes and the gas simultaneously. (In contrast, in quiescence the motor is off: normal parking).[40] Activation of mTOR, when the cell cycle is blocked, leads to senescent morphology, including loss of the ability to re-start proliferation.[41] This mTOR-driven process is gerogenic conversion.[18]

Depending on whether a cell is proliferating or arrested, mTOR drives either growth or geroconversion. Geroconversion is a form of growth, when actual growth is restricted.[42] It leads to cellular hyperfunctions, hypertrophy and compensatory signal-resistance and lysosomal hyperfunction (β-Gal-positivity). Rapamycin slows down geroconversion.[43]

p21- and p16 -Induced Senescence

To test 2-step hypothesis experimentally, we first employed the simplest model of senescence: p21- or p16-induced senescence. In HT-p21 and HT-p16 cells, expressing IPTG-inducible p21 and p16, respectively, cell cycle and mTOR can be manipulated separately.[41,43,44]. Arrest can be caused by p21 or p16. During 2 days, cells are arrested but still can resume proliferation, when p21 (or p16) is switched off. After 3–4 days, p21/p16-arrested cells lose proliferative potential. If (after 3–4 days) p21 is switched off, cells cannot divide.[45] The proliferative/mitotic potential is lost. What drives this conversion from initial arrest to irreversible state?

In proliferating HT-p21/p16 cells, mTOR is activated. When p21 (or p16) is induced, the cell cycle is arrested but mTOR is still active (**Fig. 2A**). It drives cellular mass growth (growth in size) leading to a large and flattened morphology. This hypertrophy is counterbalanced by increased lysosomal activity (lysosomal hyperfunction), manifested by β-Gal-staining. Loss of mitotic competence is one of manifestations of senescent phenotype.[45]

Rapamycin suppresses geroconversion (**Fig. 2B**), so the arrested cells retain the proliferative potential. They can re-start proliferation, if cell-cycle arrest is abrogated. Importantly, rapamycin does not abrogate p21-or p16-induced arrest. Cells are still arrested but when p21 or p16 is switched off, they can restart proliferation.[43-45]

Rapamycin by itself slows down cell cycle. It slows growth (in proliferating cells) and geroconversion (in arrested cells). Literally, rapamycin slows down time. Geroconversion takes 3 times longer.[43]

Not only rapamycin but also any condition that deactivates mTOR in turn suppresses geroconversion.[38,46-48] In particular, hypoxia, starvation and contact inhibition all suppress geroconversion.[38,47-49] As we will discuss, this can explain how quiescent cells remain quiescent in the organism for so long.

Gerogenes and Gerosuppressors

Conditions that activate mTOR accelerate geroconversion. Therefore, we can predict genes that are gerogenes and gerosuppressors. Gerogenes activate the growth-promoting mTOR pathway.[32,50,51] They include growth factor receptors (IGF-1, insulin, EGF, ErbB), Ras, Raf, Mek, PI-3K, Akt and other growth-promoting oncogenes. Gerosuppressors antagonize mTOR pathway and include PTEN, TSC1/2, AMPK, and other tumor-suppressors. This was discussed in detail.[32,50-52] Gerogenes are oncogenes that promote cellular mass growth in cancer and senescence. One of surprising predictions is that p53, a tumor-suppressor that inhibits mTOR, will suppress geroconversion.[50]

p53-Induced Senescence

Like many tumor-suppressors such as PTEN, p53 can suppress cancer in part by inhibiting mTOR.[50,53,54] p53 induces senescence because p53 is an extremely potent inducer of cell cycle arrest.[55,56] When the cell cycle is arrested by p53, mTOR drives geroconversion,[47,57,58] (**Fig. 3**, green arrow). But in some cell

types, at high levels p53 also inhibits mTOR and cellular mass growth (**Fig. 3**, yellow arrow). When p53 is capable to inhibit mTOR, it induces reversible arrest (quiescence), instead of senescence.[47,57-60] For example, when cell cycle is arrested by ectopic p21, then induction of p53 can inhibit geroconversion in p21-arrested cells.[47] In some types, while p21 and p16 caused senescence, their combination with p53 caused quiescence.[47] In MEFs, p53 does not inhibit mTOR,[57] so it does not prevent geroconversion during replicative senescence. As a powerful inducer of arrest, p53 is involved in stress-induced, oncogene-induced and replicative senescence.[6,9,55,56,61-63] p53 "induces" senescence by causing cell cycle arrest, while failing to inhibit mTOR.[57,58] Noteworthy, rapamycin may partially substitute for p53 in mice.[64-67]

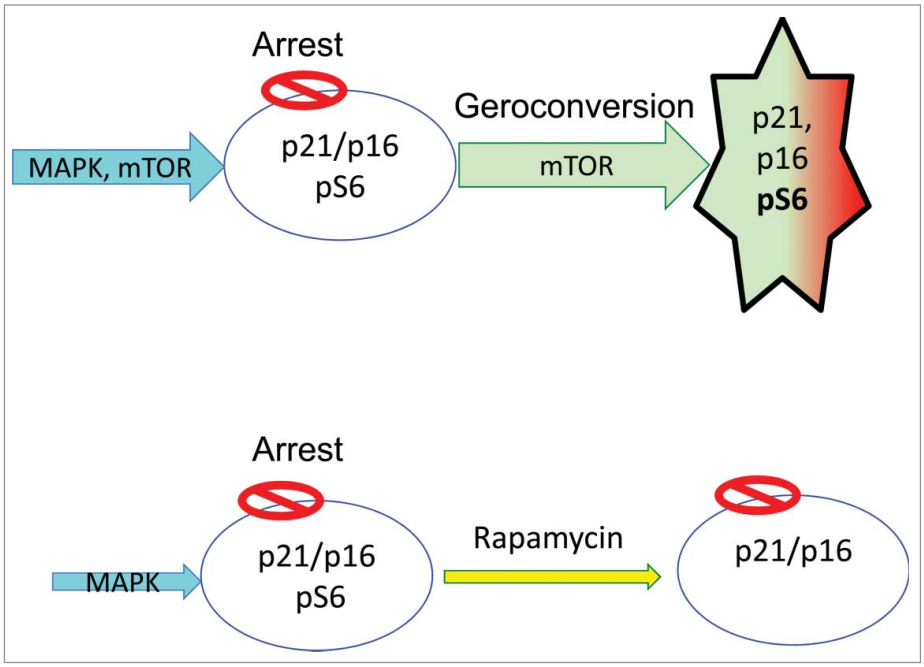

Figure 2. Senescence vs. quiescence.

Stress-induced Senescence

In most cases, stress-induced senescence is p53-dependent (**Fig. 3**). Stresses such as acute DNA damage induce p53-dependent arrest, while mTOR drives geroconversion from arrest to senescence (**Fig. 4A**). Rapamycin and serum withdrawal decelerate geroconversion, (**Fig. 4B**) during stress-induced senescence.[68]

Oncogene-induced Senescence

Oncogene-induced senescence is caused by oncogenes (Ras, Raf, MEK, AKT) that activate MAPK/mTOR pathways and also induce p53, p21 or p16[69-74] These oncogenes are gerogenes, which cause 2 processes: cell cycle arrest and geroconversion. For example, Ras promotes growth, leading to hypertrophy (pro-gerogenic conversion,**Fig. 4C**). This pro-gerogenic conversion induces p53-dependent arrest.[69,70,71,75] During cell cycle arrest, Ras-activated mTOR pathway completes geroconversion to senescence (**Fig. 4C**). Rapamycin suppresses Ras-induced growth and geroconverion.[76] In contrast, loss of p53 prevents cell cycle arrest, without affecting geroconversion. Geroconversion without cell cycle arrest is cancer.

Replicative Senescence in Human Cells

In replicative senescence, telomere shortening culminates in DNA damage response in human cells. Noteworthy, there is a correlation with longevity in vivo[77-79] Replicative senescence of human cells is a variant of stress-induced senescence (**Fig. 4D**). The main peculiarity is that stress (telomere crisis) occurs after many rounds of cell divisions. After a definite number of divisions (Hayflick limit), the process can be described by

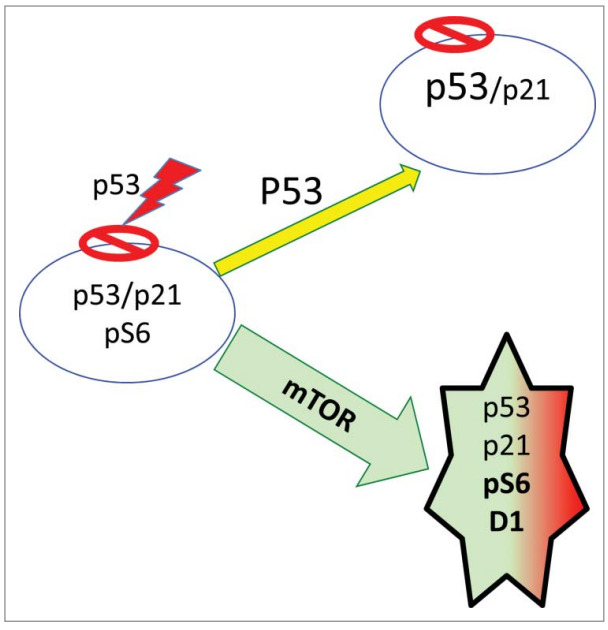

Figure 3. The dual role of p53 in senescence. P53 causes Arrest that is followed by geroconversion (green arrow). Yet, at very high levels, p53 can inhibit mTOR, suppressing geroconversion (yellow arrow) and leading to quiescence.

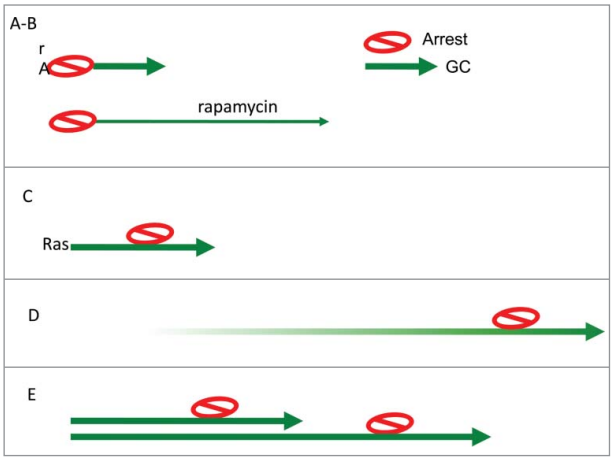

Figure 4. Arrest-Geroconversion model. Schematic representation of types of senescence (Arrest-red stop sign. Geroconversion – green arrow). (**A**) Typical arrest-induced senescence. DNA damage-induced senescence. CDK (p21 and 16)–induced senescence. (**B**) In the presence of rapamycin: geroconversion is slowed down and extended. (**C**) Oncogene-induced senescence. Oncogenes such as Ras empower growth, cause arrest and then empower geroconversion. (**D**) Replicative senescence of human cells in culture. Telomere shortening during cell proliferation eventually causes Arrest. Then geroconversion ensures senescence. (**E**) Replicative senescence of rodent cells in culture. Ovestumulation of mTOR by mitogen/nutrient/oxygen rich medium causes cellular hypertrophy.

2-step model: arrest followed by geroconvesion (**Fig. 4D**). Rapamycin suppress replicative senescence in human fibroblasts.[76]

Replicative Senescence in Rodent Cells

This type of senescence reminds protracted "oncogene-induced" senescence (**Fig. 4E**), because it is telomere-independent.[80] Primary rodent cells are overstimulated by serum, high glucose (DMEM contains 5 fold excess of glucose) and other nutrients and non-physiological oxygen levels. Under such overstimulation of mTOR, cells gradually undergo geroconversion. Pro-geroconversion triggers p53-dependent arrest. Continous geroconversion makes the process irreversible. Rapamycin prevents replicative senescence.[81,82] Similarly, low mitogenic-conditions delay replicatiove senescence.[83] In hypoxia, MEFs do not undergo senescence[84] Not co-incidentally, hypoxia inhibits mTOR.

Yeast Replicative Senescence

Yeast and rodent replicative aging are comparable (**Fig. 5E**). Yeast mother cell becomes progressively hypertrophic before ceasing proliferation. Geroconversion triggers the arrest. "CR" and genetic TOR inhibition extend replicative lifespan.[85]

Chronologic yeast aging is a different phenomenon.[86,87] It has no analogy to traditional senescence in mammals. Instead, it is identical to metabolic self-destruction of cancer cells[88,89] Both yeast and mammalian "chronological aging" is inhibited by rapamycin.[90] The reason is that the same pathways that drive geroconversion and organismal aging also increase glycolisis production.[88,91]

Emerging Summary

1. In cell culture, a senescent program includes 2 events: cell cycle arrest and geroconversion. Cell cycle arrest is ultimately caused by CDK inhibitors such as p21 and p16. Geroconversion is driven by growth-signaling pathways such as mTOR.
2. When the cell cycle is blocked, growth-promoting pathways drive geroconversion. Geroconversion can be viewed as a continuation of growth, a quasi-program of growth. Like cellular growth, gerconversion is slowed down by rapamycin. Geroconversion leads to hypertrophy, hyper-differentiation and hyperfunctions such as SASP.
3. The action of growth-promoting pathways causes the opposite reaction. For example, a cell cannot grow in size infinitively. Re-activation of lysosomes and autophagy counteracts protein synthesis and growth. Signal resistance counteracts cellular overstimulation. Hyperfunctions may eventually lead to malfunctions, hypertrophy to atrophy.

From Cell Culture to the Organism

Arrest and geroconversion are obligatory steps of cellular senescence, including stress-induced, oncogene-induced and replicative senescence (**Fig. 4**). In cell culture, geroconversion is a rapid step because in cell culture mTOR is overactivated, especially in cancer cells. When arrested for several days, cells become senescent due to rapid geroconversion. If mTOR is not inhibited by contact inhibition or by starvation, then geroconversion is automatic in arrested cells. Therefore, arrest is a key event in most models of senescence in vitro (if arrest occurs, then geroconversion follows). Not surprisingly, it is arrest that attracted all attention, so that senescence was defined as "permanent arrest."

In long-lived organisms, it is geroconversion that is the limiting event. A cell can be quiescent for years without becoming senescent. In the organism, the mTOR activity is low and a cell can be arrested (quiescent and contact inhibited) without undergoing geroconversion. Geroconversion can either follow or precede arrest (or both). For example, geroconversion of stem cells may initially increase their proliferation followed by delayed arrest (stem cell exhaustion).[15,92-97] Similar geroconversion exhausts oocytes,[98,99] leading to menopause.[100]

Permanent cell cycle arrest is not a crucial marker of senescence in the organism. Senescent cell can re-enter cell cycle: in some situations with devastating consequences for the organism. (One example is death of neurons upon S-phase re-entry in Alzheimer's disease. Another example is cancer.)

Cell cycle arrest per se cannot be linked to age-related diseases. For one, organismal aging is associated with tumors, atherosclerosis, fibrosis and other hyperplastic and hypertrophic (obesity) conditions, rather than with cessation of cellular proliferation. It is geroconversion that leads to diseases.[101] Overstimulation of mTOR in post-development makes cells hyperfunctional and signal resistant (e.g., insulin resistant). Examples of hyperfunctions include oxidative burst by neutrophils or contraction by SMC. Macrophages are converted to foam cells, smooth muscle cells (SMC) become hypertrophic and calcificated, hepatocytes produce lipoproteins and pro-inflammatory factors, adipocytes are hyperactive. mTOR dependent geroconversion renders cells " pathogenic, thus leading to atherosclerosis, for instance. Atherosclerosis, hypertension, hyper-aggregation of blood cells and hyper-coagulation can culminate in stroke and infarction (for instance) and organismal death.

Geroconversion creates pathogenic or gerogenic cells, leading to diseases and organismal aging. Gerogenic and pathogenic abilities are tightly linked. In fact, organismal aging is an increase of the probability of death. It is the sum of age-related diseases and disease-like conditions[102-105] Inhibition of mTOR by calorie restriction or rapamycin decelerates geroconversion, and increases health–and life span. In other words deceleration of cellular geroconversion slows down organismal aging. The link between geroconversion and organismal aging (via diseases) I will discuss in forthcoming paper "Geroconversion: irresistible path to organismal aging"

From Gerontology to Medicine

Geroconversion renders cells gerogenic (the ability to cause organismal aging) and pathogenic (the ability to cause diseases) in the organism. In rare cases, (stress-induced and oncogene-induced senescence), geroconversion renders cells "typically senescent," resembling senescence in vitro.[5,106-111] A striking example is oncogene-induced senescent nevi.[112] Gerontologists are focused on these "typically" senescent cells.[113]Yet, these senescent cells are only a tip of the iceberg. Characterized by increased cellular functions (hyperfunctions), gerogenic cells are not necessarily resemble "in vitro senescent fibroblasts."

By feedback loops, cells from different organs/tissues over-stimulate each other.[100,114] Paracrine geroconversion also takes place.[115,116] Gradually geroconversion involves most cells in diverse tissues. The extend of geroconversion can range from slightly gerogenic to typically senescent cells. In general, heterogeneity is one of hallmarks of aging.[117] We know the contribution of all gerogenic cells together. Gerogenic cells are pathogenic by causing hyperlipedemia, pro-inflammation, hypertension, cardiac and so on. Foam cells (in atherosclerotic plaques), tumor cells, hypertrophic adipocytes and calcificated SMC are pathogenic. In medicine, the effect of all gerogenic cells together is determined by alterations in blood biochemistry, cardiac and renal functions and other laboratory and functional tests. Yet, medical science misses geroconversion as the universal process, which initiates age-related pathology and alters laboratory tests. Medicine concerns with thousands genetic and external factors of human diseases such as genetic predisposition, smoking and wrong diet. Although smoking and fat food can accelerate pathology, this pathology is developed anyway without any hazards (such as smoking or fat food) because. There is an universal process – geroconversion. And geroconversion is a continuation of developmental growth.

Senescence in cell culture does not model all the complexity of organismal aging. Still as we discussed here, these in vitro models reveal the process of mTOR-dependent geroconversion. Geroconversion is applicable to the organism, explaining why we develop age-related pathology (and cosmetic problems) and eventually die.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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