

mTORC1 and p53

Clash of the gods?

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A balance must be struck between cell growth and stress responses to ensure that cells proliferate without accumulating damaged DNA. This balance means that optimal cell proliferation requires the integration of pro-growth and stress-response pathways. mTOR (mechanistic target of rapamycin) is a pleiotropic kinase found in complex 1 (mTORC1). The mTORC1 pathway governs a response to mitogenic signals with high energy levels to promote protein synthesis and cell growth. In contrast, the p53 DNA damage response pathway is the arbiter of cell proliferation, restraining mTORC1 under conditions of genotoxic stress. Recent studies suggest a complicated integration of these pathways to ensure successful cell growth and proliferation without compromising genome maintenance. Deciphering this integration could be key to understanding the potential clinical usefulness of mTORC1 inhibitors like rapamycin. Here we discuss how these p53-mTORC1 interactions might play a role in the suppression of cancer and perhaps the development of cellular senescence and organismal aging.

The mTORC1 Growth Response: Dionysus and Silenus at the Banquet

mTOR (mechanistic target of rapamycin) is a serine/threonine kinase in the phosphatidylinositol 3-kinase (PI3K)-related family. It is found in the mTOR complex 1 (mTORC1) and is conserved across species

from yeast to mammals. mTORC1 regulates cell growth (mass) and proliferation (cell division) in response to the environment, thereby ensuring that proliferation occurs only when sufficient nutrients are available without destructive stresses such as DNA damage. We describe a simplified version of this pathway, but much greater detail can be found in several excellent reviews.¹⁻⁴

There are both upstream and downstream components to the mTORC1 pathway. Upstream of mTORC1 are growth factors that bind to receptor tyrosine kinases in the cell membrane. Receptor occupancy induces activation of PI3K, leading to phosphorylation of PIP3 (phosphatidylinositol-3,4,5-triphosphate), a second messenger in the cell membrane. The tumor suppressor PTEN (phosphatase and tensin homolog deleted on chromosome 10) attenuates this pathway through PIP3 dephosphorylation. Otherwise, PIP3 activates AKT, which, in turn, inhibits the TSC1 and TSC2 (tuberous sclerosis complex), a negative regulator of mTORC1. TSC1/2 are GTPase-activating proteins that inhibit the small GTPase RHEB (RAS homolog enriched in brain), thereby prohibiting mTORC1 activation.^{5,6}

Downstream of mTORC1 are components of the mRNA translation machinery. Activated mTORC1 regulates mRNA translation to enhance protein synthesis. mTORC1, in part, by phosphorylating S6K1 (S6 kinase 1), induces ribosome biogenesis and translation.^{2,3} In addition, mTORC1 phosphorylates 4E-BPs (eIF4E-binding proteins) to terminate binding to

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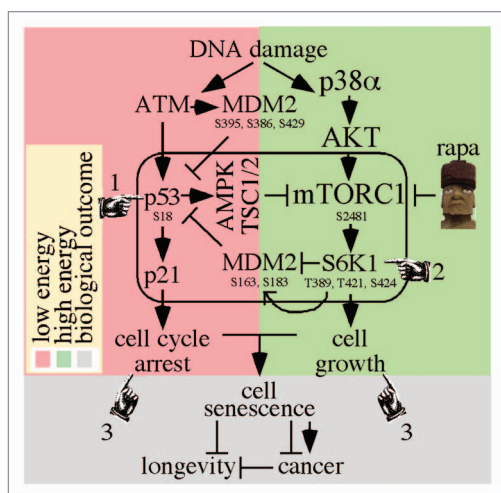


Figure 1. The integration of DNA damages response with cell growth. (1) The ATM response (red box) activates p53 to inhibit mTORC1 through AMPK/TSC1/2. (2) The p38 α /AKT/mTORC1/S6K1 response (green box) inhibits MDM2 suppression of p53 which inhibits mTORC1. (3) The combination of p53/p21 cell cycle arrest and mTORC1-induced cell growth may result in cellular senescence, which, in turn, may impact cancer and longevity.

eIF4E and relieve the block on translation. Thus, mTORC1 enables cell growth in response to mitogenic signaling in the presence of high nutrient/energy levels and low stress.

Rapamycin and rapamycin derivatives (rapalogs) specifically inhibit mTOR and have been instrumental in studying the mTORC1 pathway. Rapamycin is a macrocyclic lactone discovered on the island of Rapa Nui (Easter Island).⁷ The bacterium *Streptomyces hygroscopicus* naturally produces rapamycin as an antifungal agent. Mechanistically, rapamycin binds to FKBP12, a protein folding chaperone required for mTORC1 activity, and, thus, inhibits mTORC1.⁸ There is a history of using rapamycin in the clinic as an adjuvant to the immunosuppressant cyclosporine for organ transplant patients, with few toxic side effects (even with chronic use). mTOR inhibitors are being examined for their ability to prevent post-transplant cancers.⁹ In addition to use in transplant patients, rapamycin also has potential value in ameliorating any disease whose etiology includes cell growth, the most obvious being cancer.¹⁰ Yet, exuberant cell growth may contribute to other maladies, especially those related to aging. Thus, rapamycin and other agents that target the mTORC1 pathway are candidates for a host of therapies.

The p53 DNA Damage Response: Nomos, Themis and Dike Set Divine Order

There must be an antagonist to balance these high energy and growth processes. For mTORC1, one such antagonist is p53. The p53 pathway is best known for suppressing tumors and is mutated in more than half of all human cancers.¹¹ p53, a transcription factor,¹² suppresses cellular proliferation in response to a variety of stimuli, including genotoxic stress and low energy levels.^{13–15} In response to genotoxic stress, ATM (ataxia telangiectasia mutated) phosphorylates p53 on serine 15/18 (pS15 in human, pS18 in mice) to stimulate p53 transcriptional activity.¹⁶

p53 pS15/18 induces transcription. The majority of p53's anticancer function derives from transactivation of a large number of genes, most of which suppress cell proliferation in response to various stresses, including damaged DNA.¹⁵ These p53 targets are generally classified into four groups: (1) proliferation arrest (p21, 14–3–3 α), (2) apoptosis (BAX, NOXA, PUMA, APAF1), (3) death receptor (KILLER/DR5, FAS and PIDD) and (4) mTORC1 inhibition (sestrins).¹⁷ In addition, p53 induces a negative autoregulatory loop by transactivating MDM2, which targets p53 for ubiquitination and subsequent degradation. ATM also phosphorylates

MDM2 on serines 395, 386 and 429 to induce p53 ubiquitination. This negative regulation is important because high p53 levels can be deleterious. In mice, for example, MDM2 deletion is early embryonic lethal, and lethality is rescued by p53 deletion.^{18,19} In addition to ubiquitination, other post-translational p53 modifications include phosphorylation and acetylation, neddylation, sumoylation and methylation, all of which are essential for modulating p53 activity. Thus, p53 is a highly regulated protein that inhibits cell proliferation and induces apoptosis in response to a variety of cellular stresses, including DNA damage. The p53 pathway is critical for regulating the pro-growth mTORC1 pathway.

The p53 DNA Damage Response Confronts mTORC1: Zeus Battles Cronus for His Day of Reckoning

p53 inhibits the mTORC1 pathway in response to cellular stresses, including DNA damage⁴ (Fig. 1). Activated p53 inhibits mTORC1 through AMPK (5' adenosine monophosphate-activated kinase) and REDD1 (regulated in development and DNA damage responses 1) via the TSC1/2 complexes.²⁰ This DNA damage response occurs in a low-energy environment, since AMPK responds to a high AMP/ATP ratio in the cell (Fig. 1, red box). REDD1 is a p53 transcriptional target under several kinds of stress conditions, including DNA damage.²¹ p53 also decreases S6K1 activity, 4E-BP1 dephosphorylation and protein synthesis, independent of its role in transcription regulation.²² Cytoplasmic p53 is covalently linked to 5.8S rRNA and is associated with ribosomes.^{23,24} Furthermore, p53 induces PTEN transcription that ultimately inhibits mTORC1, since PTEN inhibits PIP3/AKT.²⁵ Thus, p53 inhibits mTORC1 and protein synthesis through multiple mechanisms.

Conversely, mTORC1 activity increases p53 function in what appears to be a compensatory response to DNA damage with high energy levels (Fig. 1, green box). p38 α /AKT/mTORC1/S6K1 upregulated p53 function in tissue culture cells exposed to genotoxic agents, and rapamycin negated this effect (i.e., suppressed p53)

through S6K1.²⁶ More specifically, p38 α /AKT/mTORC1/S6K1 negatively regulated MDM2, which increased p53 levels in response to genotoxic stress, including DNA double-strand breaks.²⁶ In this case, activated mTORC1 phosphorylates S6K1 on T389, T421 and S424. Subsequently, activated S6K1 phosphorylates MDM2 on S163/183 (S166/186 in human). In addition, activated S6K1 binds to MDM2 pS163/183 to inhibit nuclear localization and p53 ubiquitination, thereby stabilizing p53. Rapamycin reverses this process.

Though it appears counterintuitive for a pro-growth pathway to enhance an anti-proliferation pathway, this makes biological sense if one considers it as a negative feedback loop that integrates metabolic and energy signals with the DNA damage response. To support this possibility, high serum levels enhance S6K1 activation and p53 induction for cells exposed to strong mitogenic signaling and genotoxic stress. In addition to this feedback loop, p53 activity is stimulated in response to activated mTORC1 caused by loss of TSC1/2.^{27,28} TSC1/2 loss causes tuberous sclerosis and is associated with multiple benign tumors that are maintained in a benign state, in part by the increase in p53 activity.²⁹ Therefore, p53 and mTORC1 are linked together to regulate protein translation with cell fate determination based on the combined levels of energy and cellular stress.

Given that both ATM and mTORC1/S6K1 induce a p53 response to DNA damage, our model shows how these two independent pathways could be integrated in an interdependent manner. First, in response to DNA damage, p53 inhibits mTORC1 through AMPK/TSC1/2 (Fig. 1, pointer #1). Second, in response to DNA damage, p38 α /AKT/mTORC1/S6K1 phosphorylates MDM2 and activated S6K1 binds to MDM2 thereby elevating p53 levels (Fig. 1, pointer #2) which, in turn, inhibits mTORC1. Thus, a circle of regulation (Fig. 1, rounded rectangle) would inhibit the growth of cells experiencing DNA damage. It is possible that high energy levels would favor the second pathway (Fig. 1, green box), perhaps in addition to the first (Fig. 1, red box) as an extra layer of regulation. This circle of regulation would integrate energy

levels with genotoxic stress to regulate cell cycle arrest and cell growth in a negative feedback loop and provide an extra measure of growth regulation and tumor suppression.

Arrested Cells Become Quiescent or Senescent: Nyx Bears Geras and Old Age Shrouds All

The integration of growth pathways like mTORC1 with cell cycle arrest pathways like p53/p21 can push cell fate toward either quiescence or senescence (Fig. 1 pointer #3).³⁰ Quiescence and senescence are both characterized by the absence of proliferation, but the former is reversible with low metabolism, while the latter is permanent with high metabolism.^{31,32} As mentioned above, p53 arrests the cell cycle in response to DNA damage. Most p53-arrested cells re-enter the cell cycle after the damage is repaired. However, if the damage is severe, then the arrested state could become permanent, or the cell could become senescent.

The precise mechanism that drives arrested cells into senescence is not understood. It is presumed that p53 is at least partly culpable, perhaps by inducing prolonged p21 expression. However, p53 might not induce cellular senescence beyond stimulating p21 transcription, so the role of p53 in inducing senescence is unclear. It is also possible that p53 actually inhibits cellular senescence (a process coined gero-suppression).³⁰ For example, prolonged p21 overexpression induces senescence, but simultaneous p53 overexpression prohibited senescence in favor of quiescence.³³ Furthermore, mTORC1 could induce senescence in these p21-expressing cells (a process coined geroconversion).³⁰ mTOR activation converts quiescent cells into senescent cells when they were growth arrested by nutlin-3a (nutlin-3a activates p53-induced quiescence but inhibits p53-induced senescence).³⁴ Rapamycin reverses this process. Furthermore, TSC1-dependent mTORC1 suppression was crucial for maintaining naive T cells in a quiescent state.³⁵ Finally, p53-mediated DNA damage responses could be inconsequential for tumor suppression. Mice that express a mutant p53 defective for acetylation of three lysines (3KR: K117R,

K161R and K162R) are unable to mount a DNA damage response but are protected from cancer.³⁶ Even though the DNA damage response is negated, p53^{3KR/3KR} mutant mice can still regulate glycolysis and reactive oxidation species production, activities that also influence growth pathways like mTORC1.^{37,38} Thus, p53 and mTORC1 (and their integration) can influence the choice between cellular quiescence and senescence (or apoptosis).

p53 Inhibits mTORC1 to Suppress Cancer: Nemesis Enacts Divine Retribution against Hubris

mTORC1 inhibitors like rapamycin show promise as anticancer therapies.¹⁰ Based on p53/mTORC1 interactions that ultimately elevate p53, it is of interest to know whether rapamycin can suppress tumors that lack p53. This knowledge is important because p53 is mutated in about half of all human tumors.¹¹ Some reports suggest p53 is important for rapamycin-mediated tumor suppression because p53 reduces cellular proliferation after mTORC1 inhibition. For example, RAD001 (a rapalog) suppressed hepatocyte proliferation during chronic liver injury that was dependent on p53.³⁹ Further, RAD001 combined with cisplatin suppressed the growth of hepatocellular carcinoma cells, which was enhanced by p53 expression.⁴⁰ Another genotoxin, doxorubicin, caused acute cardiac dysfunction and reduced cardiac mass through p53-dependent mTOR inhibition.⁴⁰ Furthermore, a p53 activator (chloroquine) in combination with RAD001 reduced mammary tumors in a mouse model.⁴¹ Finally, consistent with rapamycin's ability to block bladder cancer development, deletion of p53 and PTEN led to invasive bladder cancer with upregulated mTOR.⁴² These data support the possibility that p53 is important for rapamycin-mediated tumor activity.

There is also evidence that does not support the notion that rapamycin is dependent on p53 to fight cancer. For example, in a cancer treatment model, RAD001 synergized with ionizing radiation to increase cell death in pre-B acute lymphocytic leukemia cells independent of p53.⁴³ In addition, rapamycin prevented early onset of oral mucosal papillomas

deleted for p53 and expressing mutant K-Ras,⁴⁴ and rapamycin delayed the onset or progression of tumors in *p53^{-/-}* and *p53^{+/-}* mice to extend their lifespan.^{45,46} Thus, rapamycin's dependence on p53 for suppressing tumor growth could be influenced by context and might not be absolute.

DNA Damage Responses Vs. Cell Growth and Aging: Achilles' Death Spawns Immortality

Data suggests that DNA damage responses influence longevity. The cyclin-dependent kinase inhibitors p21 and p16 are considered aging biomarkers, and their overexpression induces cellular senescence.⁴⁷ In addition, removal of p16-expressing cells improves survival and delays aging for the BubR1-deficient progeroid mouse model.⁴⁸ Furthermore, at the cellular level, p53 overexpression induces replicative senescence,⁴⁹ while p53-deletion induces immortality.⁴⁹ Naturally, cellular immortality does not equate to organismal longevity, but instead to cancer predisposition (the arrow that found Achilles' heel).

At the organismal level, the impact that p53 has on longevity is complex. Mice with extra copies of intact p53 have an enhanced DNA damage response and less cancer, but a normal lifespan.^{50,51} Likewise, mice with extra copies of the INK4a/Arf locus, which encodes both p16^{INK4a}, a positive regulator of retinoblastoma tumor suppressor protein, and Arf, a positive regulator of p53, are cancer-resistant but have a normal lifespan.⁵² However, mice with extra copies of both the p53 locus and the INK4a/Arf locus are both cancer-resistant and long-lived. These studies suggest that enhanced—but regulated—p53 activity might promote longevity in conjunction with other cell cycle regulators such as p16^{INK4a}, independent of its ability to suppress cancer. On the other hand, mice that carry one wild-type p53 gene and one p53 gene that produces an N-terminally truncated protein show early aging and a shortened lifespan, despite a low cancer incidence.^{53,54} Because p53 normally acts as a tetramer, the truncated p53 interacts with full-length p53 to increase the stability, nuclear localization and activity of the tetramer.⁵⁵ Thus, enhanced but

unregulated (or differently regulated) p53 promotes aging. N-terminally truncated p53 occurs naturally, so these products may influence lifespan.⁵⁶ Taken together, the current evidence suggests that balanced p53 activity is essential for optimal longevity. This idea is consistent with the data discussed above showing a dynamic tension between the pro-growth activities of mTORC1 and the growth-suppressive activities of p53.

Likewise, several lines of evidence suggest that the mTORC1 pathway shortens lifespan—that is, mTORC1 activity enhances organismal senescence, not just cellular senescence. In support of this possibility, S6K1 deletion increases lifespan in mice.⁵⁷ Furthermore, the NIH Intervention Testing Program showed that microencapsulated and enterically released rapamycin (eRapa) increased lifespan when fed to genetically heterogeneous and inbred mice.⁵⁸⁻⁶⁰ It is likely that tumor suppression contributes to rapamycin-mediated lifespan extension, since several forms of tumors presented at a lower rate or a delayed onset. In addition, rapamycin reduced cancer by 46% for human kidney transplant patients.⁹ However, rapamycin likely ameliorates multiple aspects of aging beyond tumors because it increases lifespan for a variety of evolutionarily diverse species that do not suffer from cancer including yeast, worms and flies.⁶¹ In addition, eRapa enhances learning and memory in young mice and maintains memory in old mice, and it acts as an anti-depressant.⁶² Rapamycin also attenuated many age-related changes in mice, independent of cancer.⁶³ Furthermore, rapamycin improved survival and cardiac/skeletal muscle function for lamin A/C-deficient mice.⁶⁴ Lamin A deficiencies lead to multiple diseases in humans, one being Hutchinson-Gilford progeria syndrome that is due to a truncated form of prelamin A called progerin. Of interest, small amounts of progerin also occur naturally during aging and may contribute to the normal aging process. Progerin is aberrantly farnesylated lamin and farnesyltransferase inhibitors ameliorate Hutchinson-Gilford progeria syndrome in children⁶⁵ and in mouse models.⁶⁶ Therefore, it is noteworthy that rapamycin also ameliorates a lamin A defect,

which must occur by indirect mechanisms because there is no evidence that rapamycin directly alters lamin A expression or processing, so rapamycin does not have any means for direct intervention. Thus, rapamycin can ameliorate aging for both wild-type and lamin A-deficient mice, suggesting that mTORC1 contributes to aging in both cases.

Conclusion: Orpheus Takes a Look Back

Cell growth is essential for development and survival. Likewise, the mTORC1 pathway is essential across species yet can curtail longevity if left unrestrained. Like Dionysus and Silenus at a banquet, intemperate mTORC1 causes problems. Without some restraint, excessive growth can result in a variety of afflictions, including cancer and age-related pathologies (and perhaps a waistline that would confound Atlas). Cell cycle responses answer the call to mete out justice or at least maintain the feast at an acceptable level of ecstasy. The relationship between the pro-growth pathways and the stress response pathways are likely to be extremely complicated and heavily influenced by environment and cell type. Nonetheless, this integration is key to understand growth and aging. With an understanding of these pathways and their integration, therapeutics could be developed and used to prevent, delay or treat a good number of pathologies like cancer and cardiovascular disease, sarcopenia and possibly other age-related afflictions.

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