Validation of anti-aging drugs by treating age-related diseases

Mikhail V. Blagosklonny

Cancer Center, Ordway Research Institute, Albany, NY 12208, USA

Running title: Anti-aging drugs and diseases
Key words: Anti-aging drugs, diseases, cancer, atherosclerosis, resveratrol, rapamycin, metformin
Correspondence: Mikhail V. Blagosklonny, MD, PhD, Roswell Park Cancer Institute, Elm St., Buffalo, NY 14203, USA
Received: 09/20/08; accepted: 03/28/09; published on line: 03/28/09
E-mail: Blagosklonny@oncotarget.com
Copyright: © 2009 Blagosklonny. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Abstract: Humans die from age-related diseases, which are deadly manifestations of the aging process. In order to extend life span, an anti-aging drug must delay age-related diseases. All together age-related diseases are the best biomarker of aging. Once a drug is used for treatment of any one chronic disease, its effect against other diseases (atherosclerosis, cancer, prostate enlargement, osteoporosis, insulin resistance, Alzheimer’s and Parkinson's diseases, age-related macular degeneration) may be evaluated in the same group of patients. If the group is large, then the anti-aging effect could be validated in a couple of years. Startlingly, retrospective analysis of clinical and preclinical data reveals four potential anti-aging modalities.

Problem

The discovery of anti-aging drugs is no longer a fantasy. Numerous genes for aging and longevity have been identified in diverse organisms, revealing potential targets for potential anti-aging drugs. But how could potential anti-aging drug be introduced to humans? There are two problems. First, the effect of anti-aging agents on human aging may require almost a lifetime to determine [1]. Second, it is seemingly desirable to test anti-aging drugs in healthy individuals. However, all drugs have side effects. And, in healthy individuals, side effects would preclude clinical trials. How might these problems be solved? How could we validate anti-aging drugs in humans without life-long trials in healthy individuals?

Solution

The solution includes two steps. First, we must find an indication for a drug to treat at least one chronic disease. Then this drug could be tested in humans, not as an anti-aging drug, but as therapy for a particular disease. In fact this approach has been suggested for introduction of activators of sirtuins to the clinic [2, 3]. Second, we must find a biomarker of aging that absolutely predicts longevity. Then using this biomarker, the anti-aging effect could be evaluated in the same patients.

Aging and age-related diseases

Aging can be defined as an increase in the probability of death. This is how the rate of aging can be measured. Humans die not from ‘healthy’ aging but from age-related diseases. Healthy aging (a late onset of disease) is associated with longevity. For example, centenarians show significant delay in the onset of age-related diseases, including cardiovascular disease, type 2 diabetes, cancer and Alzheimer’s disease. In other words, those who live longer are healthier and vice versa [4, 5]. Since, by definition, all age-dependent diseases are connected with aging, these diseases are connected to each other. In fact, aging humans often suffer from many diseases simultaneously: diabetes, atherosclerosis, hypertension, macular degeneration, prostate enlargement and prostate cancer (in men) or breast cancer (in women), Alzheimer’s disease and osteoarthritis. This is why elimination of one disease...
Metformin is used to induce ovulation in patients with polycystic ovary syndrome (PCOS). Six months of 1700 mg/d metformin treatment improved fertility in anovulatory PCOS women [11, 12]. Given such effects on infertility, type II diabetes, cancer and atherosclerosis, it is plausible that metformin slows aging. In fact, it extends life span in rodents [13-15].

Calorie restriction
Calorie restriction (CR) extends life span from yeast and worms to rodents and perhaps humans [16-18]. If we did not already know that CR slows aging, how might we figure that out based solely on clinical data? Unrestricted food consumption leads to obesity associated with diabetes, atherosclerosis, thrombosis, hypertension, cancer (especially breast, prostate and colon cancer), coronary heart disease, stroke, osteoporosis and Alzheimer’s disease [19-25]. In other words, unrestricted eating in humans (ad libitum in rodents) accelerates most, if not all, diseases of aging. So we can conclude that CR delays all diseases of aging. This suggests that CR is an anti-aging modality. And it is known that CR extends life span in almost all organisms from yeast to mammals.

From metformin and calorie restriction to rapamycin
Numerous factors including insulin, glucose and amino acids activate the nutrient-sensing TOR (target of rapamycin) pathway. When the TOR pathway is activated, it acts via S6K to deplete the insulin-receptor-substrate (IRS1/2), causing insulin resistance (Figure 1). As shown in Figure 1, metformin indirectly (by activating AMPK) inhibits TOR and thereby restores insulin sensitivity [26].

CR decreases levels of nutrients and insulin and thus de-activates TOR (Figure 1). It is possible that the anti-aging effects of CR and metformin are due to inhibition of the TOR pathway. Like CR, rapamycin decreases size of fat cells and animal weight. When rats (15 weeks old) were either treated 1 mg/kg rapamycin 3 times per week for 12 weeks, rapamycin decreased their weight. Mean adipocyte diameter was decreased from 36 µm to 25 µm. At the end of the study, mean body weight in the rapamycin-treated rats was 356 g instead of 507 g, in spite of comparable food intake [27]. So rapamycin imitated CR. CR may also extend life span by activating sirtuins. Probably, sirtuins, AMPK and mTOR are linked in the common network [28].

Genetic inhibition of the TOR pathway slows down aging in diverse organisms, including yeast, worms, flies and mice [29-33]. If genetic inhibition of the TOR pathway slows aging, then rapamycin, a drug that

---

Biomarker of organismal aging

Given that (a) an increase in the death rate is a measure of aging and (b) the death rate is determined diseases taken together, then we can conclude that the sum of all age-related diseases is the best biomarker of aging. Any one age-related disease is not a biomarker of aging because, in addition to aging, numerous factors contribute to the incidence of a particular disease. For example, smoking increases the risk for lung cancer but not for Parkinson’s disease. Yet, aging is a risk factor for both diseases. And, even for lung cancer, aging is a bigger risk factor than is smoking. Aging is the biggest risk factor for all age-related diseases. Whether aging and disease have a common mechanism or whether aging simply increases vulnerability to diseases, in any case, the inhibition of aging will delay diseases, thus extending life span.

Disease-specific drugs versus anti-aging agents

Slowing aging would delay all age-related diseases. If a drug is effective against one particular disease only, such a drug is not anti-aging. And current drugs are not anti-aging. For example, insulin compensates diabetes. Yet, insulin does not treat cancer. And vice versa chemotherapy may treat cancer but does not treat diabetes. So neither chemotherapy nor insulin is an anti-aging modality. Furthermore, both insulin and chemotherapy may accelerate aging.

Metformin

The underlying cause of age-related type II diabetes is insulin resistance. Insulin treatment does not ‘treat’ the cause, it just compensates for resistance. Unlike insulin, metformin, an oral anti-diabetic drug, restores insulin sensitivity in type diabetes type II. Remarkably, metformin decreases the incidence of breast cancer [7, 8]. Also, metformin is considered for cancer treatment [9] and inhibits atherosclerosis in diabetic mice [10]. Metformin is used to induce ovulation in patients with...
inhibits TOR, must slow aging too. Once used for any indication, even unrelated to age-related diseases (such as renal transplantation, for instance), an anti-aging drug should slow down age-related diseases such as cancer, osteoporosis and atherosclerosis. Rapamycin is already used in renal transplant patients.

**Retrospective analysis of the clinical use of rapamycin**

Rapamycin has been used in renal-transplant patients for several years. Since rapamycin was viewed as an immunosuppressive drug (not as an anti-aging drug) it was expected that it would cause cancer.

Unexpectedly, it turned out that rapamycin prevented cancer, and even cured pre-existing cancer and Kaposi’s sarcoma in renal transplant patients [34-44]. Furthermore, temsirolimus, an analog of rapamycin, has recently been approved for cancer therapy [45]. Also, everolimus, a TOR inhibitor, markedly delayed tumor development in transgenic mice that spontaneously develop ovarian carcinomas [46]. Would TOR inhibitors extend life span in transgenic mice? Since rapamycin delays cancer, it must prolong the life span of cancer-prone mice, who would otherwise die from cancer. Of course, humans die from a variety of age-related diseases, not from just one disease. To prolong life span dramatically, rapamycin must delay most of them.

In renal transplant patients, rapamycin increases blood lipoproteins [47]. This is considered to be a negative side effect. Yet, this results from mobilization of fat from the fat tissue (lipolysis) [48, 49]. This is exactly what happens during starvation or calorie restriction (CR). And CR extends life span. Furthermore, rapamycin reduces the accumulation of cholesterol within the arterial wall [50, 51]. Thus, lipolysis of fat tissue and decreased uptake of cholesterol by tissues both contribute to high levels of lipids in blood (Figure 2). Despite hypercholesterolemia, rapamycin prevents atherosclerosis in animals [52]. In animal models, systemic administration of rapamycin reduces neointimal thickening and slows the progression of atherosclerosis in apoE-deficient mice with elevated levels of cholesterol [53-55]. In patients with coronary atherosclerosis, oral rapamycin prevents re-stenosis after implantation of metal stents [56]. As a case report, it has been described that conversion to everolimus (an analog of rapamycin) resulted in decrease in blood pressure [57]. In kidney transplant patients, 2 years after transplantation, body-mass index was significantly lower in the rapamycin-based treatment arm compared to cyclosporine [27].

---

**Figure 1. The TOR intracellular signaling pathway.** Nutrients, GF (growth factors) and insulin activate the TOR pathway, which is involved in aging and age-related diseases. Other genetic factors and environmental factors (e.g., smoking) contribute to specific age-related diseases. Three potential anti-aging modalities (metformin, calorie restriction and rapamycin) all inhibit the TOR pathway.
Multiple indications for a single drug

If a drug is indicated to treat most age-related diseases, then this drug could be defined as an anti-aging drug. The probability that a non-anti-aging drug would have independent activities against all diseases is exceedingly low.

Rapamycin analogs are approved to treat certain cancers [45]. Based on preclinical data, rapamycin has been considered in such pathologies as obesity [58], atherosclerosis [53-55], cardiac hypertrophy [59-64], aortic aneurysm [65], osteoporosis [66-68], organ fibrosis (liver, renal, cardiac fibrosis) [64, 69, 70-75], neurodegeneration [76, 77], Alzheimer’s disease [78, 79], Parkinson’s disease [80-82], psoriasis [80], skin scars and keloids [83], multiple sclerosis [84], arthritis [85, 86], and renal hypertrophy in diabetes [87].

May rapamycin increase human life span?

In principle, life-extending effect of anti-aging drug might be limited by side effects. Although chronic administration of rapamycin is associated with some undesirable effects in transplant patients (see for references [88]), they might be avoided by administrating rapamycin in pulses (for example, once a week). For example, chronic administration of rapamycin impairs wound healing. In theory, a pulse treatment might rejuvenate wound-healing cells [88]. A single dose of rapamycin reverses insulin resistance, whereas chronic administration of rapamycin may precipitate diabetes in certain conditions. Clinical trials will be needed to determine benefits of pulse treatment with rapamycin. Alternatively, rapamycin can be combined with ‘complementary’ drugs. Thus, hyperlipidemia caused by rapamycin may deteriorate insulin-resistance. Yet, hyperlipidemia caused by rapamycin can be controlled by lipid-lowering drugs. A combination of rapamycin with resveratrol may be especially intriguing.

Resveratrol

Resveratrol, an activator of SIRT1 in mammals, extends life span in diverse species. [89, 90] Resveratrol was shown to prevent cancer, atherosclerosis, neurodegeneration and insulin-resistance (diabetes type II) [10, 91-100]. Resveratrol also indirectly inhibits PI-3K/mTOR/S6K pathway [101-105]. SIRT1 and mTOR could be members of the same sirtuin/TOR network. The link between TOR and sirtuins has been suggested [28]. It is likely that TOR (pro-aging pathway) and sirtuins (anti-aging pathway) antagonize each other [106]. However, inhibition of the TOR pathway by resveratrol occurs at near-toxic concentrations [107].

The ability of resveratrol to extend life span may be limited by its toxicity at high doses due to off-target effects. Therefore, more selective activators of SIRT1 undergo clinical trials [3]. Importantly, these drugs will be developed to treat age-related diseases such as type 2 diabetes [3]. This is the only possible strategy for a drug to enter the clinic. But here is an additional aspect: this is the only practical way of how anti-aging effect can be evaluated too. Once used for treatment of diabetes, sirtuin activators might delay heart diseases, cancer, neurodegeneration and other age-related diseases in the same patients. And delaying of all diseases must extend life span, thus validating a drug as anti-aging.

Conclusion

It was previously assumed that anti-aging drugs should be tested in healthy individuals. Ironically, the best biomarker of aging is the occurrence of age-related diseases. And this is how anti-aging drugs can be
validated in the clinic (by showing that a putative anti-
aging drug can prevent or delay the onset of all age-
related diseases). Then such drugs could be approved 
for prevention of any particular age-related disease in 
healthy individuals. Thus, potential anti-aging drugs 
should be introduced to the clinical trials for therapy of 
the particular disease but be ultimately approved for 
prevention of all age-related diseases in healthy 
individuals. And this is synonymous to the approval of 
a drug as anti-aging.

ACKNOWLEDGEMENTS

This work was not funded by any sources. The author is 
a founder of Oncotarget but is not employed by the 
company and declares no conflicts of interests.

REFERENCES

1. Hadley EC, lakatta EG, Morrison-Bogorad M, Warner HR, 
Hodes RJ. The future of aging therapies. Cell. 2005;120:557-567.
2. Sinclair DA, Gareau L. Unlocking the secrets of longevity 
genes. Sci Am. 2006;294:48-51.
3. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne 
DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, 
Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, 
Iffland A, Lavu S, Medvedik O, Sinclair DA, Olfesky JM, Jirousek 
MR, Elliott PJ, Westphal CH. Small molecule activators of SIRT1 as 
thearapeutics for the treatment of type 2 diabetes. Nature. 
2007;450:712-716.
4. Perlis T, Kunkel L, Puca A. The genetics of aging. Curr. 
Opin. Genet. Dev. 2002;12:362-369.
5. Curtis R, Geesaman BJ, DiStefano PS. Ageing and metabolism: 
drug discovery opportunities. Nat Rev Drug Discov. 2005; 
4:569-580.
6. Hayflick L. The future of ageing. Nature. 2000;408:267-269.
7. Evans JM et al. Metformin and reduced risk of cancer 
in diabetic patients. BMJ. 2005;330:1304-1305.
8. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased 
mortality associated with the use of metformin compared with 
sulfonylurea monotherapy in type 2 diabetes. Diabetes Care. 
2002;25:2244-2247.
9. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, 
Zhao F, Viollet B, Thompson CB. Systemic treatment with the 
antiabetic drug metformin selectively impairs p53-deficient 
tumor cell growth. Cancer Res. 2007;67:6745-6752.
10. Zhang M, Xu S, Maitland-Toolan KA, Zuccollo A, Hou X, Jiang B, 
Wierzbicki M, Verbeuren TJ, Cohen RA. Polyphenols stimulate 
AMP-activated protein kinase, lower lipids, and inhibit 
accelerated atherosclerosis in diabetic LDL receptor-deficient 
mice. Diabetes. 2006;55:2180-2191.
11. Cheang KJ, Sharma ST, Nestler JE. Is metformin a primary 
ovulatory agent in patients with polycystic ovary syndrome? 
Gynecol Endocrinol. 2006;22:595-604.
12. Palomba S, Orio FJ, Falbo A, Russo T, Tolino A, Zullo F. 
Clomiphene citrate versus metformin as first-line approach 
for the treatment of anovulation in infertile patients with 
polycystic ovary syndrome. J Clin Endocrinol Metab. 2007;92:3498-3503.
13. Dilman VM, Anisimov VN. Effect of treatment with 
phenformin, diphenylhydantoin or L-dopa on life span and 
tumour incidence in C3H/HeJ mice. Gerontology. 1980;26:241-
14. Anisimov VN, Egormin PA, Berstein LM, Zabezhinskii MA, 
Piskunova TS, Popovich IG, Semenchenko AV. Metformin 
decelerates aging and development of mammary tumors in HER-
2/neu transgenic mice. Bull Exp Biol Med. 2005;139:721-723.
15. Anisimov VN, Berstein LM, Egormin PA, Piskunova TS, 
Popovich IG, Zabezhinski MA, Tyndyk ML, Yurova MV, Kovalenko 
IG, Poroshina TE, Semenchenko AV. Metformin slows down 
aging and extends life span of female SHR mice. Cell Cycle. 
2008;7:2769-2773.
16. Heilbronn LK, Ravussin E. Calorie restriction and aging: 
review of the literature and implications for studies in humans. 
Am J Clin Nutr. 2003;78:361-369.
17. Kennedy BK, Steffen KK, Kaeberlein M. Ruminations on 
dietary restriction and aging. Cell Mol Life Sci. 2007;64:1323-
1328.
18. Everitt AV, Le Couteur DG. Life extension by calorie 
restriction in humans. Ann N Y Acad Sci. 2007;1114:428-433.
19. Anisimov VN. Premature ageing prevention: limitations and 
perspectives of pharmacological intervention. Curr Drug Targets. 
2006;7.
20. Ingram DK, Zhu M, Mamczarz J, Zou S, Lane MA, Roth GS, 
deCabo R. Calorie restriction mimetics: an emerging research 
field. Aging Cell. 2006;5:97-108.
21. Holloszy JO, Fontana L. Caloric restriction in humans. 
Exp Gerontol. 2007;42:709-12.
22. Wilson PW, Kannel WB. Obesity, diabetes, and risk of 
cardiovascular disease in the elderly. Am J Geriatr Cardiol. 
2002;11:119-123.
23. Zamboni M, Mazzali G, Zoico E, Harris TB, Meigs JB, Di 
Francesco V, Fantin F, Bissoli L, Bosello O. Health consequences 
of obesity in the elderly: a review of four unresolved questions. 
Int J Obes. 2005;29:1011-1029.
24. Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie 
restriction is highly effective in reducing the risk for 
atherosclerosis in humans. Proc Natl Acad Sci U S A. 
2004;101:6659-6663.
25. Bray GA. Medical consequences of obesity. J Clin Endocrinol 
Metab. 2004;89:2583-2594.
26. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho 
RA, Montminy M, Cantley LC. The kinase LKB1 mediates glucose 
homeostasis in liver and therapeutic effects of metformin. 
Science. 2005;310:1642-1646.
27. Rovira J, E MA, Burke JT, Brault Y, Moya-Rull D, Bañón-
Maneus E, Ramírez-Bajo MJ, Gutiérrez-Dalmay A, Revuelta I, 
Quintana LF, Campistol JM, Diekmann F. Effect of mTOR inhibitor 
on body weight: from an experimental rat model to human 
transplant patients. Transpl Int. 2008;21:992-998.
28. Medvedik O, Lammers DW, Kim KD, Sinclair DA. MSN2 and 
MSN4 Link Calorie Restriction and TOR to Sirtuin-Mediated 
Lifespan Extension in Saccharomyces cerevisiae. PLoS Biol. 
2007;5:e261.
29. Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller 
F. Genetics: influence of TOR kinase on lifespan in C. elegans. 
Nature. 2003;426:620.
30. Jia K, Chen D, Riddle DL. The TOR pathway interacts with 
the insulin signaling pathway to regulate C. elegans larval
development, metabolism and life span. Development. 2004;131:3897-3906.
31. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science. 2001;292:107-110.
32. Kapahi P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S. Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr Biol. 2004;14:885-890.
33. Bartke A. Long-lived klotho mice: new insights into the roles of IGF-1 and insulin in aging. Trends Endocrinol Metab. 2006;17:33-35.
34. Law BK. Rapamycin: an anti-cancer immunosuppressant? Crit Rev Oncol Hematol. 2005;56:47-60.
35. Nungaray N, Arriola M, Gutierrez MJ, Oliva E, Hernandez E, Gonzalez E, Andres A, Morales JM. Rapamycin at six years can exhibit normal renal function without proteinuria or neoplasia after renal transplantation. A single-center experience. Transplant Proc. 2005;37:3727-3728.
36. Kauffman HM, Cherikh WS, Cheng Y, Hanto DW, Kahan BD. Maintenance immunosuppression with target-of-rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. Transplantation. 2005;80:883-889.
37. Zmonar SK, Boratynska M, Rabczynski J, Kazimierczak K, Klinger M. Regression of Kaposi’s sarcoma in renal graft recipients after conversion to sirolimus treatment. Transplant Proc. 2005;37:964-966.
38. Rizell M, Cahlin C, Friman S, Hafstrom L, Lonn L, Olausson M, Lindner P. Impressive regression of primary liver cancer after treatment with sirolimus. Acta Oncol. 2005;44:496.
39. Stallone G, Schena A, Infante D, Di Paolo S, Loverre A, Maggio G, Ranieri E, Gesualdo L, Schena FP, Grandaliano G. Sirolimus for Kaposi’s sarcoma in renal-implant recipients. N Engl J Med. 2005;352:1317-1323.
40. Yakupoglu YK, Buell JF, Woodle S, Kahan BD. Individualization of Immunosuppressive Therapy. Ill. Sirolimus Associated With a Reduced Incidence of Malignancy. Transplant Proc. 2006;38:358-361.
41. Campistol JM, Eris J, Oberbauer R, Friend P, Hutchison B, Morales JM, Claesson K, Stallone G, Russ G, Rostaing L, Kreis H, Burke JT, Brault Y, Scarola JA, Neylan JF. Sirolimus Therapy after Early Cyclosporine Withdrawal Reduces the Risk for Cancer in Adult Renal Transplantation. J Am Soc Nephrol. 2006;17:581-589.
42. Mathew T, Kreis H, Friend P. Two-year incidence of malignancy in sirolimus-treated renal transplant recipients: results from five multicenter studies. Clin Transplant. 2004;18:446-449.
43. Mohsin N, Budruddin M, Pakkarya A, Darweesh A, Nayer M, Amitalbah J, Daar AS. Complete regression of visceral Kaposi’s sarcoma after conversion to sirolimus. Exp Clin Transplant. 2005;3:366-369.
44. Cullis D, D’Souza R, McCullagh P, Harries S, Nicholls A, Lee R, Bingham C. Sirolimus-induced remission of posttransplantation lymphoproliferative disorder. Am J Kidney Dis. 2006;47:e67-72.
45. Hudes G, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med. 2007;356:2271-81.
46. Mabuchi S, Altomare DA, Connolly DC, Klein-Szanto A, Litwin S, Hoelzle MK, Hensley HH, Hamilton TC, Testa JR. RAD001 (Everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. Cancer Res. 2007;67:2408-2413.
47. Legendre C, Campistol JM, Squifft JP, Burke JT, Group. SERTS. Cardiovascular risk factors of sirolimus compared with cyclosporine: early experience from two randomized trials in renal transplantation. Transplant Proc. 2003;35:1515-1535.
48. Morrisset JD, Abdel-Fattah G, Hoogeveen R, Mitchell E, Ballantyne CM, Pownall HJ, Opekun AR, Jaffe JS, Oppermann S, Kahan BD. Effects of sirolimus on plasma lipids, lipoprotein levels, and fatty acid metabolism in renal transplant patients. J Lipid Res. 2002;43:1170-1180.
49. Morrisset JD, Abdel-Fattah G, Kahan BD. Sirolimus changes lipid concentrations and lipoprotein metabolism in kidney transplant recipients. Transplant Proc. 2003;35:1435-1505.
50. Basso MD, Namibi P, Adelman SJ. Effect of sirolimus on the cholesterol content in ApoE knockout mice. Transplant Proc. 2003;35:3136-3138.
51. Ma KL, Ruan XZ, Powis SH, Moorhead JF, Varghese Z. Antiatherosclerotic effects of sirolimus on human vascular smooth muscle cells. Am J Physiol Heart Circ Physiol. 2007;292:H2721-2728.
52. Mueller MA, Beutner F, Teupser d, Ceglae139, Thierry J. Prevention of atherosclerosis by the mTOR inhibitor everolimus in LDLR/(-) mice despite hypercholesterolemia. Atherosclerosis. 2008;198:39-48.
53. Pakala R, Stabile E, Jang GJ, Clavijo L, Waksman R. Rapamycin attenuates atherosclerotic plaque progression in apolipoprotein E knockout mice: inhibitory effect on monocyte chemotaxis. J Cardiovasc Pharmacol. 2005;46:481-486.
54. Elloso MM, Azrolan N, Seahal SN, Hsu PL, Phiel KL, Kopec CA, Basso MD, Adelman SJ. Protective effect of the immuno-suppressant sirolimus against aortic atherosclerosis in apoE deficient mice. Am J Transplant. 2003;3:562-569.
55. Waksman R, Pakala R, M.S. B, et al. Oral rapamycin inhibits growth of atherosclerotic plaque in apoE knockout mice. Cardiovasc Radiat Med. 2003;4:34-38.
56. Rodriguez AE, Granada JF, Rodriguez-Alemparte M, Vigo CF, Delgado J, Fernandez-Pereira C, Pocovi A, Rodriguez-Granillo AR, Schulz D, Raizenner AE, Palacios I, O’Neill W, Kaluza GL, Stone G, Investigators OI. Oral Rapamycin After Coronary Bare-Metal Stent Implantation to Prevent Restenosis The Prospective, Randomized Oral Rapamycin in Argentina (ORAR II) Study. J Am Coll Cardiol. 2006;47:1522-1529.
57. Pascal J, Fernandez AM, Marcén R, Ortuño J. Conversion to everolimus in a patient with arterial hypertension and recurrent cutaneous neoplasia—a case report. Nephrol Dial Transplant. 2006 Jul;21 Suppl 3:i38-41. 2006;3:38-41.
58. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G. Absence of SK61 protects against age- and diet-induced obesity while enhancing insulin sensitivity. Nature. 2004;431:200-205.
59. Oudit GY, Sun H, Kerfant BG, Crackower MA, Penninger JM, Backx PH. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. J Mol Cell Cardiol. 2004;37:449-471.
60. Tu VC, Bahl JJ, Chen QM. Signals of oxidant-induced cardiomyocyte hypertrophy: key activation of p70 S6 kinase-1 and phosphoinositide-3-kinase. J Pharmacol Exp Ther.
Activation Haddad Wu 2002;300:1101-1110.

61. Sadoshima J, Izumo S. Rapamycin selectively inhibits angiotsin II-induced increase in protein synthesis in cardiac myocytes in vitro. Potential role of 70-kd kinase in angiotsin II-induced cardiac hypertrophy. Circ Res. 1995;77:1040-1052.

62. Shiou T, McMullen JR, Kang PM, Douglas PS, Obata T, Franke TF, Cantley LC, Izumo S. Akt/protein kinase B promotes organ growth in transgenic mice. Mol Cell Biol. 2002;22:2799-2809.

63. Ha T, Li Y, Gao X, McMullen JR ST, Izumo S, Kelley JL, Zhao A, Haddad GE, Williams DL, Browder IW, Kao RL, Li C. Attenuation of cardiac hypertrophy by inhibiting both mTOR and NFkappaB activation in vivo. Free Radic Biol Med. 2005;39:1570-1580.

64. McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shiou T, Izumo S. Inhibition of mTOR signaling with rapamycin represses established cardiac hypertrophy induced by pressure overload. Circulation. 2004;109:3050-3055.

65. Lawrence DM, Singh RS, Franklin DP, Carey DJ, Elmore JR. Rapamycin suppresses experimental aortic aneurysm growth. J Vasc Surg. 2004;40:334-338.

66. Kneissel M, Luong-Nguyen NH, Baptist M, Cortesi R, Zumstein-Mecker S, Kossida S, O'Reilly T, Lane H, Susa M. Everolimus suppresses candaoss cell loss, cell resorption, and cathepsin K expression by osteoclasts. Bone. 2004;35:1144-1156.

67. Glantschnig H, Fisher JE, Wesołowski G, Rodan GA, Reszka AA. M-CSF, TNFalpha and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. Cell Death Differ. 2003;10:1165-1177.

68. Sugatani T, Hruska KA. Akt1/Akt2 and mammalian target of rapamycin/Bim play critical roles in osteoclast differentiation and survival, respectively, whereas Akt is dispensable for cell survival in isolated osteoclast precursors. J Biol Chem. 2005;280:3583-3589.

69. Shegogue D, Trojanowska M. Mammalian target of rapamycin positively regulates collagen type I production via a phosphatidylinositol 3-kinase-independent pathway. J Biol Chem. 2004;279:23166-23175.

70. Gabele E, Reif S, Tsukada S, Bataller R, Yata Y, Morris T, Schrum LW, Brenner DA, Rippe RA. The role of p70S6K in hepatic stellate cell collagen gene expression and cell proliferation. J Biol Chem. 2005;280:13374-13382.

71. Bonegio RF, Fuhr O, Wang Z, Valeri CR, Andry C, Salant DJ, Lieberthal W. Rapamycin ameliorates proteinuria-associated tubulointerstitial inflammation and fibrosis in experimental membranous nephropathy. J Am Soc Nephrol. 2005;16:2063-2072.

72. Poulahlon N, Farge D, Roos N, Tacheau C, Neuzillet C, Michel L, Mauvel A, Verrecchia F. Modulation of collagen and MMP-1 gene expression in fibroblasts by the immunosuppressive drug rapamycin: A direct role as an anti-fibrotic agent? J Biol Chem. 2006;281:33045-52.

73. Gao XM, Gong G, Wang B, Kiriazis H, Moore XL, Su YD, Dart A, Du XJ. Inhibition of mTOR reduces chronic pressure-overload cardiac hypertrophy and fibrosis. J Hypertens. 2006;24:1663-1670.

74. Shiou T, McMullen JR, Tarnavski O, Converso K, Sherwood MC, Manning WJ, Izumo S. Rapamycin attenuates load-induced cardiac hypertrophy in mice. Circulation. 2003;107:1664-1670.

75. Wu MJ, Wen MC, Chiou YT, Chiou YY, Shu KH, Tang MJ. Rapamycin attenuates unilateral ureteral obstruction-induces renal fibrosis. Kidney Int. 2006;69:2029-2036.

76. Khurana V, Ly Y, Steinhibl ML, Oldham S, Shulman JM, Feany MB. mTOR-mediated cell-cycle activation causes neurodegeneration in a Drosophila tauopathy model. Curr Biol. 2006;16:230-241.

77. Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, Schmitt I, Wullner U, Evert BO, O'Kane CJ, Rubinsztein DC. Rapamycin alleviates toxicity of different aggregate-proteins. Hum Mol Genet. 2006;15:433-442.

78. Li X, Alafuzoff I, Soininen H, Winblad B, Peij J. Levels of mTOR and its downstream targets 4E-BP1, eEF2, and eEF2 kinase in relationships with tau in Alzheimer's disease brain. FEBS J. 2005;272:4211-4220.

79. An WL, Cowburn RF, Li L, Braak H, Alafuzoff I, Iqbal K, Iqbal IG, Winblad B, Peij J. Up-regulation of phosphorylated/activated p70 S6 kinase and its relationship to neurofibrillary pathology in Alzheimer's disease. Am J Pathol. 2003;163:591-607.

80. Marsland AM, Griffiths CE. The macrolide immunosuppressants in dermatology: mechanisms of action. Eur J Dermatol. 2002;12:618-622.

81. Wullschleger S, Loewith R, Hall MN. mTOR signaling in growth and metabolism. Cell. 2006;124:471-484.

82. Pannu J, Trojanowska M. Recent advances in fibroblast signaling and biology in scleroderma. Curr Opin Rheumatol. 2004;16:739-745.

83. Ong CT, Khoo YT, Mukhopadhyay A, Do DV, Lim JJ, Aalami O, Phan TT. mTOR as a potential therapeutic target for treatment of keloids and excessive scars. Exp Dermatol. 2007;16:394-404.

84. Farrell R, Heaney D, Giovannoni G. Emerging therapies in multiple sclerosis. Expert Opin Emerg Drugs. 2005;10:797-816.

85. Carlson RP, Hartman DA, Tomchek LA, Walter TL, Lugay JR, Calhoun W, Sehgul SN, Chang JY. Rapamycin, a potential disease-modifying antiarthritic drug. J Pharmacol Exp Ther. 1993;266:1125-1138.

86. Foronciewicz B, Mucha K, Paczek L, Chmura A, Rowinski W. Efficacy of rapamycin in patient with juvenile rheumatoid arthritis. Transpl Int. 2005;18:366-368.

87. Sakaguchi M, Isono M, Iishiki K, Sugimoto T, Koya D, Kashiwagi A. Inhibition of mTOR signaling with rapamycin attenuates renal hypertrophy in the early diabetic mice. Biochem Biophys Res Commun. 2006;340:296-301.

88. Blagosklonny MV. Aging, stem cells, and mammalian target of rapamycin: a prospect of pharmacologic rejuvenation of aging stem cells. Rejuvenation Res. 2008;11:801-808.

89. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisielewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature. 2003;425:191-196.

90. Bauer JH, Goupil S, Garber GB, Helfand SL. An accelerated assay for the identification of lifespan-extending interventions in Drosophila melanogaster. Proc Natl Acad Sci U S A. 2004;101:12980-12985.

91. Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science. 1997;275:218-220.

92. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov. 2006;5:493-506.
93. Westphal CH, Dipp MA, Guarente L. A therapeutic role for sirtuins in diseases of aging? Trends Biochem Sci. 2007;555-60.
94. Lavu S, Boss O, Elliott PJ, Lambert PD. Sirtuins--novel therapeutic targets to treat age-associated diseases. Nat Rev Drug Discov. 2008;10:841-853.
95. Wang RH, Sengupta K, Li C, Kim HS, Cao L, Xiao C, Kim S, Xu X, Zheng Y, Chilton B, Jia R, Zheng ZM, Appella E, Wang XW, Ried T, Deng CX. Impaired DNA damage response, genome instability, and tumorigenesis in SIRT1 mutant mice. Cancer Cell. 2008;14:312-323.
96. Guarente L. Sirtuins in aging and disease. Cold Spring Harb Symp Quant Biol. 2007;72:483-488.
97. Kim D, Nguyen MD, Dobbin MM, Fischer A, Sananbenesi F, Rodgers JT, Delalle I, Baur JA, Sui G, Armour SM, Puigserver P, Sinclair DA, Tsai LH. SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. EMBO J. 2007;26:3169-3179.
98. Harikumar KB, Aggarwal BB. Resveratrol: a multitargeted agent for age-associated chronic diseases. Cell Cycle. 2008;7:1020-1035.
99. Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin, C., Daussin F, Messadeg N, Milne J, Lambert P, Elliott P, Geny B, Laakso M, Puigserver P, Auwerx J. Resveratrol Improves Mitochondrial Function and Protects against Metabolic Disease by Activating SIRT1 and PGC-1alpha. Cell. 2006;127:1109-1122.
100. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. Nature. 2006;444:337-342.
101. Chan AY, Dolinsky VW, Soltys CL, Viollet B, Baksh S, Light PE, Dyck JR. Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and Akt. J Biol Chem. 2008;283:24194-24201.
102. Cao Z, Fang J, Xia C, Shi X, Jiang BH. trans-3,4,5'-Trihydroxystibene inhibits hypoxia-inducible factor 1alpha and vascular endothelial growth factor expression in human ovarian cancer cells. Clin Cancer Res. 2004;10:5253-5263.
103. Brito PM, Devillard R, Nègre-Salvayre A, Almeida LM, Dinis TC, Salvayre R, Augé N. Resveratrol inhibits the mTOR mitogenic signaling evoked by oxidized LDL in smooth muscle cells. Atherosclerosis. 2008; in press.
104. Fröjdö S, Cozzone D, Vidal H, Pirola L. Resveratrol is a class IA phosphoinositide 3-kinase inhibitor. Biochem J. 2007;406:511-518.
105. Haider UG, Sorescu D, Griendling KK, Vollmar AM, Dirsch VM. Resveratrol suppresses angiotensin II-induced Akt/protein kinase B and p70 S6 kinase phosphorylation and subsequent hypertrophy in rat aortic smooth muscle cells. Mol Pharmacol. 2002;62:772-777.
106. Blagosklonny MV. An anti-aging drug today: from senescence-promoting genes to anti-aging pill. Drug Disc Today. 2007;12:218-224.
107. Demidenko ZN, Blagosklonny MV. At concentrations that inhibit mTOR, resveratrol suppresses cellular senescence. Cell Cycle. 2009;8, in press.