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A New Protocol to Discover Novel Anti-Aging Compounds

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Abstract

Several natural and chemical compounds have been suggested to have effects as anti-aging drugs. For example, caloric restriction (CR) mimetics *trans*-resveratrol (Rsv) and 2-deoxy-D-glucose (2DG) are candidate compounds that can elongate life span of organisms, and they might also have roles in the regulation of telomere maintenance and mitochondrial functions. Recently, pharmaceutical medicaments rapamycin, an immunosuppressant and metformin, a medicine for diabetes, have been shown to act on the insulin/IGF1 signaling pathway. Therefore, they are also expected to have anti-aging effects. Here we propose a new protocol to discover novel compounds that can be used as a remedy to slow senescence and control aging in the aspect of promoter activities of telomere and energy metabolism-regulating factor encoding genes.

Keywords: Caloric restriction; Cellular senescence; 2-deoxy-D-glucose; Lignin carbohydrate complex; Metformin; Oxidative stress; Rapamycin; Resveratrol

Introduction

All living things are exposed to various stresses throughout their life span, including sun light, radiation, natural and chemical compounds, viruses, and other stimuli that cause damage to cellular DNA. Protection of chromosomes from various damages is critical requirement for organisms, because aging is thought to be caused by accumulation of damage at the chromosomes [1].

It has been shown that telomere-shortening accelerates cellular senescence, and that protection of telomeres from DNA replication process and/or DNA-damage is necessary to keep chromosomal integrity [2,3]. Therefore, induction of telomere-maintenance mechanisms should be applied for anti-aging therapy. One such target for anti-aging therapy might be *LMNA*, in which a loss of function mutation is known to cause Hutchison-Gilford progeria syndrome (HGPS) [4]. The *LMNA* gene encodes lamin A protein, an essential structural component of the nuclear membrane [5]. Telomeres are thought to be heterochromatic and associate with nuclear matrix membrane *via* heterochromatin protein 1 (HP1) [6]. Therefore, HGPS could be partly explained by disruption of telomere maintenance.

Another aspect of aging is that life span of organisms is regulated by the reactive oxygen species (ROS) and energy stresses that are associated with mitochondrial functions [7,8]. Recently, it was shown that telomere dysfunction affects mitochondria, where ROS are mainly generated, *via* p53 mediated suppression of peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α) [9]. It should also be noted that the tumor suppressor protein p53 regulates mitochondrial functions including respiration and glycolysis [10,11].

Recent studies have shown that the sirtuin (SIRT) protein family, comprising of SIRT1-SIRT7, plays important roles in controlling metabolism and health span [12]. Among these, SIRT1, an NAD^+ dependent deacetylating enzyme, has been widely known to act on glucose metabolism by modulating functions of various targets including PGC-1α, FOXO1, p53, HIF1α, UCP2, and other proteins [12]. Growth hormone (GH) resistance and deficiency of GH increase longevity of mice [13], suggesting that the signals induced by the action of GH on IGF-1, which belongs to the insulin signaling system, affects aging of organisms [14]. Moreover, GH does not only regulate IGF-1, but also stimulates secretion of insulin [14]. Genetic studies in *C. elegans*, *Drosophila* and mice showed that the insulin signaling system plays important roles in determining life span of the organisms [15]. Considering that mTOR and AMPK are key proteins in the insulin signaling pathways, these lines of evidences strongly suggest that these gene products could be attractive targets for anti-aging therapies [16].

Taken together, the biological factors involved in determination of life span may be categorized into several groups, telomere metabolism, ROS and mitochondrial functions, and insulin signaling system.

Analytical Methods

Our previous studies demonstrated that caloric restriction (CR) mimetic compounds, such as 2-deoxy-D-glucose (2DG) and *trans*-resveratrol (Rsv) moderately enhance telomerase activity along with induction of *WRN* gene expression in HeLa S3 cells [17,18]. Luciferase-reporter transfection experiments showed that these CR mimetic compounds up-regulate relative promoter activities of the 5'-upstream regions of human telomere-associated proteins and shelterin-encoding genes when compared with that of the *PIF1* gene [19]. The *PIF1* encodes a protein containing homology with a Rec D type DNA helicase that negatively regulate telomere length [20,21]. Furthermore, by using these relative values, we observed that β-thujaplicin (hinokitiol) has similar effects on promoter activities of these genes [22]. We also found that not only Rsv, but also pine cone lignin carbohydrate complex (LLC)

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Compound	Relative TMAE	Reference
Rsv (10 μM)	1.399	[19]
2DG (4 mM)	4.115	[19]
2DG (8 mM)	2.804	[19]
β-thujaplicin (10 μM)	2.078	[22]
Rapamycin (1 μM)	1.256	unpublished

Relative TME = $TME_{(CR\ mimetics)}/TME_{control}$
Here, each constant was set as $k_1=k_2=k_3=\ldots=k_N=1$.

Table 1: TME value in HeLa S3 cells.

and β-thujaplicin up-regulate human *SIRT1* promoter activity, these have already been reported to have favorable effects on mammalian cells [22-25]. Thus, we propose that the anti-aging effects of natural and synthetic compounds could be easily screened by the promoter activity ratios of the *SIRT1* and telomere-maintenance factor encoding genes normalized with that of the *PIF1*.

Based on these observations, the formula that indicates telomere-maintenance associated anti-aging effect (TME) of a specific compound A will be given as follows:

$$TME_A=(k_1[\text{gene } x_1]_p+k_2[\text{gene } x_2]_p+k_3[\text{gene } x_3]_p\ldots+k_N[\text{gene } x_N]_p)/([PIF_1]_p\ xN)$$

$$k_1+k_2+k_3+\ldots+k_N=N$$

In this formula, genes x_1 to x_N encode telomere maintenance factors, and $[\text{gene } x_1]_p$ and k_1 represent promoter activities of gene x_1, and contribution constant of $[\text{gene } x_1]_p$.

Results

At present, constant k_1 to k_N is unknown. Therefore, tentatively all constants were set equally at 1. Then TME values of Rsv (10 μM), 2-DG (4 mM and 8 mM), and β-thujaplicin (10 μM) were estimated from the previous results [19,22]. Table 1 shows the TME values of these CR mimetic compounds are over 1.00 in HeLa S3 cells. The result suggests that this estimation could be useful to predict the efficacy of anti-aging drugs, when applied to the analysis of promoter activities for telomere maintenance factor encoding genes. It is widely known that p53-mediated cellular responses are generated from its cellular levels and phosphorylation, which are mainly post-transcriptionally regulated. However, we propose here that evaluation of the *TP53* gene expression could be also included in the above formula. Because increased p53 protein level may affect cell proliferation, which is also a feature of cellular senescence, inducing not only cell cycle check point-associated genes but also suppressing expression of mitochondrial function-regulatory gene *PGC-1α* [9].

Discussion

Rapamycin and metformin, which are known to inhibit mTOR and activate AMPK, respectively, could be lead compounds that might retard aging [26]. Although these two drugs are regarded as CR mimetics, the molecular mechanism, through which they extend life span, is thought to be different from that of Rsv, 2DG and LLC. That is, rapamycin negatively regulates telomere length in yeast [27] and telomerase in human cells [28]. To present, there is no report that shows the effect of metformin on telomeres. These observations imply that extension of life span is not defined solely by telomere and telomerase regulation. Although we are proposing that the balance between telomere elongation/maintenance and its shortening-inducing signals will be useful to find anti-aging compounds, there should be other mechanisms that determine senescence and aging in a telomere/telomerase-independent manner.

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