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Recent progresses on anti-aging compounds and their targets in *Caenorhabditis elegans*

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ABSTRACT

Searching for drugs that extend healthy lifespan and the subsequent analysis of their mechanisms of action is a crucial aspect for aging research. However, identifying both longevity-enhancing drugs and their corresponding targets is challenging. The roundworm *Caenorhabditis elegans* is a suitable model for such research because of its short lifespan and genetic tractability. In this perspective, we discuss recent progresses on the identification of anti-aging drugs and characterization of their targets using *C. elegans* as a model organism. In particular, minocycline, JZL184, monorden, and paxilline increase *C. elegans* lifespan by inhibiting 18S rRNA/ribosome, fatty acid amide hydrolase-4, Hsp90, and the Ca^{2+}-activated K^+ (BK) channel SLO-1, respectively. Because many factors that regulate aging and lifespan in *C. elegans* are evolutionarily conserved, these newly identified lifespan-extending compounds may guide the development of anti-aging medicines for humans.

1. Introduction

In most living organisms, the process of aging is associated with physiological and molecular deterioration, including decreased cognitive, motor, and immune functions, as well as the accumulation of damaged DNA, RNA, and proteins. The identification of compounds that delay or reverse these age-related degenerative changes is important for the aging society as well as for scientific advances. However, the discovery of anti-aging drugs and their mechanisms of actions in mammals is time-consuming and costly, and has several complications.

The roundworm *Caenorhabditis elegans* is an outstanding model organism for aging research because of its short lifespan (approximately 3 weeks), simple physiology, and genetic tractability [1,2]. In addition, several fundamental genetic pathways that regulate aging in *C. elegans* are conserved in mammals [3]. In recent decades, numerous anti-aging chemical compounds have been identified in *C. elegans*. DrugAge (http://genomics.senescence.info/drugs/) is an excellent resource to obtain information regarding anti-aging drugs in various model organisms, including *C. elegans* [4]. The DrugAge database also enables functional enrichment analysis for the identification of direct and indirect targets of proposed life-extending drugs.

Herein, we briefly discuss the recent findings in *C. elegans* that have been published after the latest update of DrugAge database in 2017, focusing on four papers that identified anti-aging chemical compounds and their targets [5–8]. These papers show that minocycline, JZL184, monorden, and paxilline significantly increase the lifespan of *C. elegans* via targeting 18S rRNA/ribosome, fatty acid amide hydrolase-4 (FAAH-4), a cytosolic chaperone Hsp90, and a Ca^{2+}-activated K^+ (BK) channel SLO-1, respectively. Considering the evolutionary conservation of aging-regulating processes, these findings using *C. elegans* will provide valuable information for developing anti-aging drugs and evaluating their corresponding mechanisms in mammals.

1.1. Minocycline increases lifespan via targeting 18S rRNA/ribosome

Reducing mRNA translation and protein synthesis increases lifespan in many species, including *C. elegans* [9]. Several compounds, including rapamycin, which decreases mRNA translation via inhibiting mechanistic target of rapamycin (mTOR), extend lifespan and healthspan in many species, ranging from yeast to mammals [10]. Therefore, novel compounds that decrease protein synthesis are excellent candidates for anti-aging drugs.

Solis et al. reported that minocycline, a tetracycline analog used

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to treat acne vulgaris and sexually transmitted diseases [11], significantly increases lifespan by decreasing mRNA translation [5] (Fig. 1A). By screening the life-extending drugs that the authors had previously identified in *C. elegans* [12], initiating minocycline treatment on day 8 adults (middle-aged adult worms) is sufficient to increase longevity. In addition, minocycline treatment decreases age-dependent protein aggregation and increases survival under various stresses, such as heat and oxidative stresses. Unexpectedly, minocycline does not activate stress signaling pathways (SSPs) in adult worms, which are closely associated with longevity and aging [13]. Therefore, the authors conclude that minocycline confers protective mechanisms that bypass SSP activation. They then identified targets of minocycline, including proteins for ribosome assembly, via activity-based protein profiling using iodoacetamide as a probe and isotopic tandem orthogonal proteolysis methods. A previous report showed that prokaryotic 16S rRNA has a tetracycline-binding site [14], which is conserved in eukaryotic 18S rRNA. Therefore, it seems likely that minocycline binds 18S rRNA/ribosome, with subsequent effects on protein synthesis. Indeed, the administration of minocycline attenuates cytoplasmic mRNA translation in *C. elegans* as well as in cultured mammalian cells. Chemico-genetic epistasis further indicates that the life-extending effect of minocycline is dependent on reduced protein synthesis, which confers longevity in multiple species [9]. The study highlights minocycline as a promising anti-aging candidate drug that is effective, even in aged organisms, through direct targeting of 18S rRNA/ribosome and decreasing mRNA translation bypassing SSP activation.

1.2. JZL184 promotes longevity by targeting FAAH-4

Screening the libraries of electrophilic compounds that irreversibly bind to proteins [15,16] is a suitable strategy for identifying chemical compounds and their corresponding endogenous targets. This method has been used in cellular systems to identify targets that interact with inhibitory compounds via covalent attachments. However, the use of such screening methods in whole-animal systems or longevity regulation remains limited.

Chen et al. first performed a targeted chemical screen for approximately 100 electrophiles that inhibit serine hydrolases, a large and diverse family of enzymes, and found that the aryloxy carbamate JZL184 results in a 45% increase in the lifespan of *C. elegans* [6] (Fig. 1B). JZL184 is an inhibitor of monoacylglycerol lipase (MAGL), which hydrolyzes 2-arachidonoylglycerol (2-AG), a prominent endocannabinoid ligand in mammals [17]. Interestingly, sequence homology search indicates that *C. elegans* lacks an ortholog of MAGL. The authors therefore employed unbiased quantitative mass spectrometry (MS) and competitive activity-based protein profiling, and identified FAAH-4 as a direct target of JZL184. FAAH-4 mediates the hydrolysis of 2-AG, a process inhibited

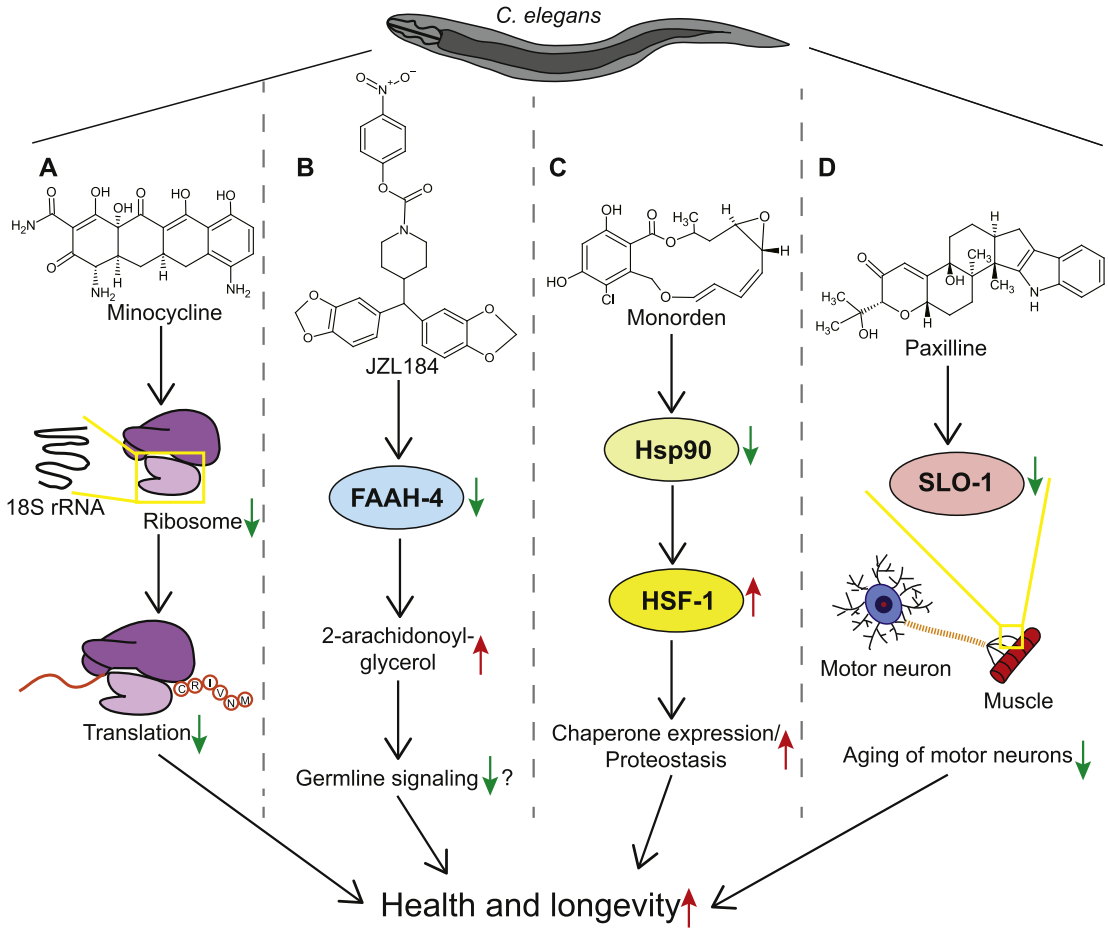

Fig. 1. The four anti-aging compounds and their mechanisms of actions in *Caenorhabditis elegans*. Illustration of the four recently discovered anti-aging drugs in *Caenorhabditis elegans* and their direct endogenous targets discussed in this perspective. **A**. Minocycline directly targets 18s rRNA/ribosome and inhibits translation for increasing lifespan. **B**. The drug JZL184 directly inhibits fatty acid amide hydrolase 4 (FAAH-4), thereby increasing the level of 2-arachidonoylglycerol that, in turn, significantly extends lifespan via possibly reducing germline signaling. **C**. Monorden targets Hsp90 and up-regulates heat shock transcription factor 1 (HSF-1); this increases chaperone expression and proteostasis that promote health and longevity. **D**. Paxilline inhibits the Ca^{2+}-activated K^+ channel SLO-1, and delays the aging of motor neurons to increase lifespan.

by JZL184. In addition, genetic mutations in *faah-4* is sufficient to increase lifespan. Thus, FAAH-4, a target of JZL184 in *C. elegans*, shares pharmacological and genetic profiles as well as metabolic functions with mammalian MAGL. Thus, this study demonstrates the utility of *C. elegans* in pharmacological screening for anti-aging compounds and its potential for identifying functionally analogous protein targets in mammals.

1.3. Monorden lengthens lifespan by inhibiting Hsp90

In addition to pharmacological screening using model organisms, such as *C. elegans*, screening involving human cell culture systems with computational interpretation of the results may enable the identification of anti-aging drugs. However, the reports of combinatorial approaches using both model organisms and human cells in the context of anti-aging drug screening are rare.

Janssens et al. identified promising candidate anti-aging compounds by employing transcriptomic analysis of human cell data and subsequent experimental validation using *C. elegans* [7]. First, they identified eight candidates using Connectivity Map (CMap), an extensive transcriptome resource obtained from the results of the studies on cultured human cells exposed to various compounds [18], and machine-learning models that predict the anti-aging effects on the transcriptome. The topmost lifespan-extending candidate was monorden, also known as radicicol, an antitumor antibiotic that directly inhibits Hsp90 [19]. Monorden robustly increases lifespan and improves the general health parameters of *C. elegans* [7] (Fig. 1C). RNAi knockdown targeting *daf-21*, which encodes *C. elegans* Hsp90, significantly increases lifespan, similar to the observations of treatment with monorden. Inhibition of Hsp90 by monorden leads to up-regulation of heat shock transcription factor 1 (HSF-1), a master regulator of cytosolic chaperones, and improves protein homeostasis and healthspan. Thus, this paper presents an elegant combinatorial approach using both human cell line transcriptomics and *C. elegans* physiology, demonstrating the potential of monorden as an anti-aging compound.

1.4. Paxilline delays motor-neuron aging and promotes longevity by targeting BK-channel, SLO-1

One of the most prominent age-dependent physiological changes is a decrease in motor function, which is exemplified by the fact that aged worms are considerably less active than young adult ones [20]. At the cellular level, neuromuscular junctions (NMJs), which connect motor neurons to muscles, deteriorate with increased age [21].

Paxilline is a tremorgenic indole alkaloid and a potent BK-channel blocker [22]. Li et al. reported that the administration of paxilline in the middle age (day 5 or 7 of adulthood) in *C. elegans* substantially delays age-dependent declines in motor functions and extends lifespan [8] (Fig. 1D). The *C. elegans* ortholog of the BK channel SLO-1 is expressed in the neurons as well as the muscles [23]. The expression of SLO-1 decreases synaptic transmission at NMJs via the inhibition of motor-neuron excitability and blockage of synaptic release from the motor neurons [23]. Importantly, the lifespan-extending and anti-motor aging effects of paxilline are dependent on *slo-1*, suggesting that paxilline increases lifespan by inhibiting SLO-1. They further demonstrated that the beneficial effects of SLO-1 inhibition on longevity and delayed motor aging act via FOXO/DAF-16, a key longevity transcription factor located in insulin/IGF-1 signaling. In conclusion, the temporal blockade of BK channels may represent a novel pharmacological approach for delaying motor aging and increasing longevity.

2. Conclusions

Key objectives of aging research include the identification and analysis of compounds that delay degenerative and deleterious changes associated with aging. However, complex organisms present challenges because the analysis of physiological aging and mapping of direct target proteins are time-consuming and costly. Combinatorial drug and gene/protein screening performed in *C. elegans* overcomes some of these challenges because of the advantages this model organism offers, including rapid aging, genetic tractability, and evolutionarily conserved lifespan-regulating processes. Minocycline, JZL184, monorden, and paxilline are recently identified drugs that significantly increase lifespan in *C. elegans*. These drugs directly target 18S rRNA/ribosome, FAAH-4, Hsp90, and the SLO-1 BK channel, respectively. Moreover, the mechanisms of actions have been suggested as the reduction of translational rate, inhibition of 2-AG hydrolysis, up-regulation of HSF-1 with consequent improvements in protein homeostasis, and decrease in motor neuron aging via the activation of FOXO, respectively.

The studies discussed in this perspective provide up-to-date information regarding potential strategies for the discovery of novel anti-aging drugs as well as identification of their endogenous targets using *C. elegans*. In addition, several compounds described in this perspective are currently used as therapeutic medications for the treatment of various diseases. Therefore, the findings using *C. elegans* regarding these drugs will pave the way for future research into anti-aging drugs for humans with minimal safety concerns.

Conflict of interest

The authors declare there is no conflict of interest.

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