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Review

Pharmacological lifespan extension of invertebrates

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ABSTRACT

There is considerable interest in identifying small, drug-like compounds that slow aging in multiple species, particularly in mammals. Such compounds may prove to be useful in treating and retarding age-related disease in humans. Just as invertebrate models have been essential in helping us understand the genetic pathways that control aging, these model organisms are also proving valuable in discovering chemical compounds that influence longevity. The nematode *Caenorhabditis elegans* has numerous advantages for such studies including its short lifespan and has been exploited by a number of investigators to find compounds that impact aging. Here, we summarize the progress being made in identifying compounds that extend the lifespan of invertebrates, and introduce the challenges we face in translating this research into human therapies.

1. Introduction

Aging is the single largest risk factor for chronic disease in developed countries and is consequently responsible for an enormous social and economic burden. The development of therapies or preventive measures aimed at reducing or delaying age-related disease must be a priority for the biomedical community. However, the traditional models of drug discovery are failing when it comes to the major chronic diseases of the elderly. The disappointing outcomes of dozens of phase III clinical trials in Alzheimer's disease and Parkinson's disease, among others, suggest a general failure in our understanding of the mechanisms at play (Sperling et al., 2011). This has led some commentators to ask whether targeting aging mechanisms might lead to better outcomes.

Novel compounds that slow aging are highly sought after due to their potential for treating age related diseases. Here, we argue that recent growth of a new subfield, the chemical biology of aging, will lead to the identification of candidate compounds and mechanistic insights that will ultimately propel forward treatments of age related diseases. While identifying compounds that slow the aging of mammals is undoubtedly more relevant for human drug development, the prohibitive cost of mouse aging studies, make it extremely unlikely that large scale chemical screens will be carried out in mice. Basic research in more cost effective model systems is therefore a critical starting point for identifying such compounds and elucidating their mechanism(s) of action. Cell culture and invertebrate model organisms provide opportunities to screen hundreds of thousands of chemical compounds in an efficient manner. Moreover, once candidate compounds are identified, the strengths of these model systems in molecular genetics, allows for rapid elucidation of the genetic pathways being targeted by these compounds.

In this review, we summarize the contribution of invertebrate models to our understanding of the pharmacology of aging, and speculate on the directions the field is headed in the imminent future. We will almost exclusively focus on *Caenorhabditis elegans* research, since most of the chemical biology of aging studies to date, have been conducted in the nematode. However, we will also discuss a limited number of pharmacological aging studies undertaken in the fruit fly *Drosophila melanogaster*.

2. General consideration when conducting experiments to identify lifespan extending compounds

2.1. High-throughput chemical screens vs. candidate based approaches

In screening compounds for biological activity, investigators are often drawn to the idea of examining a wide range of chemical structures using high-throughput screens. Due to the prohibitive cost, labor and (for aging studies) the relatively long lifespan of the current vertebrate models, they are impractical for large scale chemical screens, particularly mice. Therefore, researchers have turned to in vitro and invertebrate models to conduct such large

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scale screens. It is routine for in vitro chemical screens to test tens of thousands, or even hundreds of thousands of synthetic and structurally diverse compounds.

Instead of high-throughput screens of synthetic chemical structures, some researchers have taken an alternative, more focused, candidate approach by screening individual classes of compounds or small libraries of compounds that are predicted to modulate biological processes such as oxidative stress, intracellular signaling and protein aggregation among others. This approach has worked very well in the *C. elegans* model. To our knowledge the vast majority of the chemicals that have been extensively characterized to modulate lifespan have stemmed from these targeted studies.

2.2. Choosing an appropriate model to identify lifespan extending compounds

2.2.1. In vitro based assays vs. whole organisms

In vitro biochemical or cell based assays have been the mainstay for decades of chemical screening. Such research has led to the identification candidate compounds that are of great interest to the aging community (Howitz et al., 2003) and will certainly continue to be an effective method of chemical screening. Chemical screens in simple eukaryotic models such as the brewers yeast (*Saccharomyces cerevisiae*) are particularly promising due to the ease of its culture, the wealth of information on the endogenous genetic pathways that contribute to lifespan, and the molecular tools available for the organism. Other cell based, and particularly in vitro biochemical assays can be designed to maximize specificity for particular targets. A major advantage of these assays is the opportunity to use human cells and recombinant proteins which provide direct relevance for the development of drugs for humans. Studies of the molecular genetics of aging have provided hundreds of potential gene product targets. Therefore, it is likely that in vitro biochemical assays which aim to target human homologs of these proteins, known to influence lifespan in model organisms, will lead to the discovery of compounds that hold great promise for treatment of age related diseases in humans.

Whole organism screens have some distinct advantages over cell-based assays, since they allow for the pharmacological investigation of complex phenotypes. Whole organism screens can be designed to inform on various discrete aspects of biology simultaneously, such as: growth rate, behavior, fertility, and specific pathological features. However, whole organism screens can present difficulty for the very reason that they are deemed attractive; while novel proteins or pathways can be identified that alter a complex phenotype of the whole organism, characterizing the mechanism of the action of the compound can be difficult due to the complexity of the very phenotype being assessed and the corresponding increase in off target opportunities.

2.2.2. Using C. elegans in chemical screens

Due to its ease of culture and short lifespan *C. elegans* is rapidly becoming the invertebrate model of choice for chemical tests on aging and age-related phenotypes. Indeed, *C. elegans* not only represents a model for assessing the biological effects of a large number of compounds, but the great number of genetic tools available in the nematode also make it a powerful system for determining the mechanism of action of known pharmacological treatments (Fitzgerald et al., 2006). The organism's relative simplicity and the wealth of knowledge of its biology, along with the large number of genetic tools available, make it an attractive organism for pharmacological research. The nematode has proved useful to assay both the compound's bioactivity and for determining its mechanism of action. Additionally, its rapid growth and high fecundity make *C. elegans* well suited for high-throughput chemical screens. Its utility as a pharmacological tool has been highlighted by its recent use to identify small molecules that; influence development, act as antifungals, inhibit neurotransmission, facilitate neuro-regeneration and act as toxic agents (Breger et al., 2007; Cao et al., 2010; Kokel et al., 2006; Kokel and Xue, 2006; Kwok et al., 2006; Moy et al., 2006; Samara et al., 2010). These diverse studies have demonstrated the versatility of the model for chemical testing and screening. This being said, worm idiosyncrasies should be minimized when testing the effects of chemicals on lifespan. This topic has previously been covered and we will refer to their arguments for consistency in the lifespan assays (Gruber et al., 2009). However, it is important to highlight here a particularly important feature of the worm that can confound chemical assays; the worm is unusual in animal models in its diet. *C. elegans* feeds on live bacterial cultures. This means there is an inherent possibility when observing a response to a chemical, that this is due in some way to the compounds interaction with the bacteria. This possibility should always be addressed. This can be done by growing and maintaining the worms on killed bacteria or by using bacteria free culture conditions during the chemical response assay.

While large-scale screens have been performed with *C. elegans* on other phenotypes, few researchers have reported large scale small molecule screens for compounds that can extend the lifespan of *C. elegans*. One of the first, and certainly the largest, was conducted by Petrascheck et al. who screened 88,000 chemical structures for the ability to extend *C. elegans* lifespan (Petrascheck et al., 2009). They identified numerous compounds with lifespan extending properties and presented a structure and mechanistic study for one of their hits from this screen. Interestingly, the authors focused on a hit that was structurally related to known human drugs and we will discuss their results in detail in the section below that relates to human drugs used to modulate invertebrate lifespan. To our knowledge there has yet to be a reported detailed characterization of an entirely novel structure to come out of a high throughput screen for longevity in *C. elegans*, likely because these high-throughput chemical lifespan screens have only recently been developed and implemented. Due to the vastness of possible chemical structures it seems certain that a large number that exhibit a positive influence on longevity remain to be identified. We expect that in the near future many novel chemical structures and descriptions of their mechanisms of action will emerge from these high-throughput screens that target aging.

2.2.3. Using Drosophila in chemical screens

Screening large numbers of compounds in *D. melanogaster*, is more difficult than in *C. elegans*, but the existence of complex behavioral phenotypes and several good models of human age-related diseases in *Drosophila*, make such challenging endeavors worthwhile. *Drosophila* has been successfully used to identify chemical inhibitors of functional protein domains involved in cell signaling (Chen et al., 2007) as well as compounds with therapeutic potential against fragile X syndrome (Chang et al., 2008) among others.

There is a long history of *Drosophila* being used to test for chemical effects on aging. *Drosophila* can be cultured as adults in large population cages which allows for the testing of bio-demographic effects. As early as 1948, the fruit fly was used to determine the biological effects of discrete chemical components of royal jelly, and to this end pantothenic acid was suggested to extend longevity of the fly (Gardner, 1948a, b). A recent review outlines the current strengths of the model and in particular sets out specific guidelines for evaluating the significance of chemical hits (Jafari, 2010). With the emergence of an increasing number of compounds shown to slow aging in *C. elegans*, *D. melanogaster* presents the opportunity to test whether the action of these compound on aging is species specific or is conserved.