Fishing functional analogs in distantly related organisms with active-site-directed irreversible inhibitors to reveal a lipid pathway in lifespan regulation

Preview for "Chen *et al*. Pharmacological convergence reveals a lipid pathway that regulates *C. elegans* lifespan. Nat Chem Biol. 2019, 15(5): 453-462"

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Introduction

Finding small molecules with lifespan extending capability obtains self-explanatory impact. Since the complexity of aging, the search for such molecules requires reliable and efficient methodology, which is also a big technical challenge. One widely adopted strategy is screening. By running in parallel with many replicates of animals interacting with different small molecules, one can pick the molecules that induce substantial phenotypic changes. *C. elegans* makes good model animals for this purpose because of its short lifespan and easiness to maintain and manipulate. In addition, the selection of molecule starting list for screening is another challenge. It often requires prior knowledge such as the pathways or targeted enzyme classes. The interaction between small molecules and targeted enzymes can influence the screening effectively as well. Commonly used molecules undergoing reversible interaction with enzymes can be less sensitive when the potency is low. The irreversible binding compounds, on the other hand, can provide much better efficiency at low potency regime. The authors used such a compound library with activity-based protein profiling (ABPP) to systematically investigate how molecules targeting serine hydrolases can have effects on *C. elegans*' longevity and identified JZL184 as a lifespan promoting molecule. JZL184 is an inhibitor of the mammalian endocannabinoid (eCB) hydrolase monoacylglycerol lipase (MAGL) [1]. In the endocannabinoid system, the MAGL can degrade 2-Arachidonoylglycerol (2-AG) and terminate endocannabinoid signaling. JZL184 can inhibit the degradation of 2-AG by MAGL and keep the cannabinoid receptors in activation since accumulation of endocannabinoids, which can lead to behavioral effect in animals. *C. elegans*, however, does not obtain an ortholog of MAGL, but can respond to JZL184. The authors found the monoglyceride lipase fatty acid amide hydrolase-4 (FAAH-4) to be the target of JZL184. In *C. elegans*, FAAH-4 can hydrolyze the 2-AG, which is the same as MAGL in mammalian cells.

Results

The authors first generated a functional map of serine hydrolases (SHs) with ABPP coupled with quantitative mass spectrometry (MS). The probe for ABPP is a biotinylated fluorophosphonate (FP) that binds to the conserved serine nucleophile in SH active sites. This FP probe enriched 92 SHs from the *C. elegan*s proteome, including SHs with both high and low mRNA levels, indicating the specificity of the FP probe and the activity-directed binding of the FP probe to

SHs. To further demonstrate the specificity of the FP probe and the activity of an SH-directed small-molecule library, the authors performed the competitive gel-based ABPP. Competitive ABPP shows that some molecules from the library irreversibly bind to the same target proteins as the FP probe and compete the FP probe off the binding sites in SHs, leading to lower signaling in a gel-based assay. A subset of the SH-directed small-molecule library containing ~100 compounds was analyzed in regard to their effect on the lifespan of *C. elegans*. This mini screen identified a lifespan extending compound from the library, namely JZL184. This carbamate compound can extend the lifespan of *C. elegans* by roughly 45% at a concentration from 25 uM to 50 uM.

Sequence alignment with the JZL184 mammalian target MAGL failed to identify a homolog in the *C. elegans* genome. Aiming to determine the targets of JZL184 and unravel the mechanism of action in lifespan regulation, the authors utilized JZL184 mediated competitive ABPP with FP probe, coupled with quantitative MS to identify target proteins. In theory, the binding of the FP probe to the target proteins and thus the MS signal would be decreased by the addition of JZL184 to the worm lysate. By correlating target protein profiling and differential lifespan effects between JZL184 and structurally related analogs, the authors narrowed the protein target of JZL184 down to FAAH-4. Along the same line that JZL184 was able to extend the lifespan of *C. elegans*, CRISPR/Cas9 mediated deletion of faah-4 substantially increased the lifespan of these animals. Interestingly, JZL184 feeding further extended the lifespan observed in the long-lived Δfaah-4, indicating additional targets of JZL184 besides FAAH-4 in lifespan regulation. Both Δfaah-4 animals and wild-type animals treated with JZL184 showed greater resistance to paraquat-induced death compared to control non-treated animals, indicating improved oxidative stress management with FAAH-4 compromised either genetically or pharmacologically.

The authors finally set out to test the biochemical functions of FAAH-4 as an analog of the mammalian MAGL. Not surprisingly, FAAH-4 can robustly hydrolyze 2-AG and this enzymatic activity is blocked by JZL184, which is similar to MAGL. The lipid hydrolysis function was also evaluated *in vivo* with the Δfaah-4 mutant. These mutant animals displayed increased mono- and polyunsaturated MAGs (including 2-AG) and the elevated MAG levels were similar to that in the JZL184-treated worms.

By coupling active-site-directed irreversible inhibitor binding with ABPP, this study identified the functional analog of MAGL in worms, solving the perplexing mystery that *C. elegans* does not possess an MAGL ortholog. Taking advantage of the powerful genetics this model organism can provide, the authors further linked this lipid hydrolysis pathway to the regulation of lifespan possibly by modulating the oxidative stress response pathway.

Significance and Future Directions

This paper revealed that small-molecule phenotypic screening coupled with proteomics techniques is a powerful strategy to identify a biological pathway in distantly related organisms. Different from the traditional genetic screenings to unveil mechanisms of lifespan regulation, this study applied the pharmacologically irreversible drugs or small-molecule compounds to inhibit target protein binding to the FP probe to find the pathways that regulate lifespan in *C. elegans*. This strategy can be used to avoid the ignorance of evolutionarily unrelated proteins in lifespan studies of *C. elegans* to demonstrate the corresponding features of lifespan in the mammal. For this study, it is worth testing the selectivity of JZL184 and studying the role of 2-AG to get a better understanding of the mechanism that contributes to extending lifespan in the future. Many other questions still remain to be explored. For example, the enzymatic kinetics study *in vitro* should be performed and the possibility of a negative feedback loop after blocking FAAH-4 by JZL184 should be investigated. On the other hand, the maintenance of specificity, selectivity, and effectiveness *in vivo* of the small molecules to a specific enzyme is still challenging. Generally, this small-molecule phenotypic screening strategy will not be limited to the SHs enzymes but any enzymes that have irreversible binding compounds. The biotinylated fluorophosphate probe ensures a quick finding in both the compound that has a desired biological effect and the target of the compound. Overall, this study highlights the potential of combining phenotypic screening with ABPP for revealing lipid pathways in *C. elegans* which is not gene-conserved but relevant to the one in mammals.

References

[1] Long, Jonathan Z., et al. "Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects." Nature chemical biology 5.1 (2009): 37.