Review

Pharmaceutical and nutraceutical activation of FOXO3 for healthy longevity

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ABSTRACT

Life expectancy has increased substantially over the last 150 years. Yet this means that now most people also spend a greater length of time suffering from various age-associated diseases. As such, delaying age-related functional decline and extending healthspan, the period of active older years free from disease and disability, is an overarching objective of current aging research. Geroprotectors, compounds that target pathways that causally influence aging, are increasingly recognized as a means to extend healthspan in the aging population. Meanwhile, FOXO3 has emerged as a geroprotective gene intricately involved in aging and healthspan. FOXO3 genetic variants are linked to human longevity, reduced disease risks, and even self-reported health. Therefore, identification of FOXO3-activating compounds represents one of the most direct candidate approaches to extending healthspan or lifespan in a FOXO3-dependent manner. These compounds can be classified as pharmaceuticals, including PI3K/AKT inhibitors and AMPK activators, antidepressants and antipsychotics, muscle relaxants, and HDAC inhibitors, or as nutraceuticals, including primary metabolites involved in cell growth and sustenance, and secondary metabolites including extracts, polyphenols, terpenoids, and other purified natural compounds. The compounds documented here provide a basis and resource for further research and development, with the ultimate goal of promoting healthy longevity in humans.

1. Introduction

Advancing age is a predominant risk factor for developing frailty as well as a variety of diseases and degeneration, such as Alzheimer’s disease, cardiovascular disease, stroke, and cancer (López-Otin et al., 2013). Although life expectancy has nearly doubled over the past 150 years, increasing from 40 years in 1850 to an average life expectancy of ~80 years in 2019 for many countries, the age of onset of most health problems has not been significantly delayed in a commensurate manner (Kingston et al., 2018; Olshansky, 2018). This means that most people live to old age but spend greater time at older ages suffering from various age-associated diseases, frailty, disability, and functional loss (Crimmins, 2015). As such, delaying age-related functional decline and extending healthspan, the period free from disease and disability, should be an overarching objective of aging research.

Aging has long been mistakenly regarded as a passive and irreversible process. In recent years, studies in model organisms have discovered some of its root causes, including cellular senescence, epigenetic changes, chronic inflammation, mitochondrial dysfunction, deregulated proteostasis, stem cell exhaustion, and metabolic inflexibility (Kennedy et al., 2014; López-Otin et al., 2013). Intervening in these causes can not only prolong lifespan in animal models, including mammals, but also delay or reduce morbidity in most cases (Kennedy et al., 2014; Longo et al., 2015). Therefore, the findings from basic aging research provide...
proof-of-concept demonstrations that anti-aging treatment in humans might be a powerful and concrete solution to guarantee health and vitality in old age.

Indeed, promising strategies are emerging for combating age-related deterioration in humans. Those include recommendations for diet and lifestyle modifications such as reduced caloric intake and increased exercise. Intuitively, diet and lifestyle modification are believed to be safe strategies to combat aging, but these alone are not sufficient to fend off various diseases that occur at older ages. Soon, groups of agents known as geroprotectors, compounds that ‘protect’ the ‘gerontological’ phase of life, may likewise join the list of recommendations to further support healthy longevity. The development of geroprotectors is still in its infancy, though their potential availability represents an important healthy longevity solution (Moskalev et al., 2017, 2016). One example is metformin, which is currently in clinical trials as a geroprotector (Kulkarni et al., 2020).

Yet, drug discovery and development are never straightforward. To address the generally high rate of failure in drug discovery pipelines, the pharmaceutical company AstraZeneca described lessons learned from previous experience, establishing a framework that includes advice on selecting the right drug target. Notably, they observed that 73% of their drug development projects that began with a known genetic association with the disease were active or successful in Phase II trials, compared to only 43% successes for projects without such genetic associations (Cook et al., 2014). The findings of AstraZeneca suggest that genetic evidence for a link to longevity may be a prerequisite to develop a geroprotector with a high chance of success in humans.

While the GeneAge database of longevity interventions currently possesses over a hundred entries for genetic modifications that can influence lifespan in mice (Tacutu et al., 2018), human genetic studies have only identified two genes that consistently relate to human longevity: APOE and forkhead/winged helix box, group O, member 3 gene (FOXO3) (Brooks-Wilson, 2013; Morris et al., 2015). FOXO3 (the protein nomenclature for the gene FOXO3) is the only known transcription factor linked to longevity in yeast, hydra, worms (Caenorhabditis elegans), flies (Drosophila melanogaster), mice, and humans (Boehm et al., 2012; Hwangbo et al., 2004; Kwon et al., 2010; Lin et al., 1997; Martins et al., 2016; Morris et al., 2015; Postnikoff et al., 2012; Shimokawa et al., 2015). Drugs that activate FOXO3 may therefore represent the approach with the highest potential to increase healthy longevity in humans. This review will focus on the geroprotective potential of pharmacological activation of FOXO3, describing and discussing compounds and drug classes known to activate FOXO3 and its orthologs in other organisms.

2. FOXO3 and longevity

Studies investigating genealogical trees and lifespan variation in twins have estimated that heritability of longevity is approximately 12% (Kaplanis et al., 2018) to 25% (van den Berg et al., 2017). This is in line with observations that the greater the proportion of long-lived family members one has, the healthier their life should be while aging (Asl et al., 2015). Of note, long-lived individuals generally achieve long life despite having similar disease risks as everyone else in the population (Andersen et al., 2012), which suggests their genomes harbor protective genetic variants, rather than a lack of disease-causing genetic variants. While at least 57 genetic loci have been associated to longevity in humans (Morris et al., 2019), the gene FOXO3 is one of only two genes that have been replicated across multiple studies, labs, and ethnicities as being associated with human longevity (Brooks-Wilson, 2013; Morris et al., 2015). Indeed, after Wilcox, Donlon and colleagues established the association between FOXO3 and human longevity in 2008 (Wilcox et al., 2008), at least a dozen publications replicated this finding (Morris et al., 2015). This association has held true when scrutinized by genome-wide association studies and meta-analyses (Bao et al., 2014; Broer et al., 2015). Interestingly, the FOXO3 single nucleotide polymorphisms (SNPs) that have been associated with longevity in humans have been found in intronic and proximal 5′-flanking regions of the gene. While these SNPs have been linked to variation in FOXO3 expression (Banasik et al., 2011; Donlon et al., 2017; Flachsbart et al., 2017; Grossi et al., 2018), the full molecular consequences resulting from FOXO3 SNPs on human longevity have yet to be elucidated.

Nonetheless, not only are FOXO3 SNPs associated with human longevity and all-cause mortality, but also with reduced risk of cancer (Liu et al., 2018), stroke, and cardiovascular mortality (Kuningas et al., 2007). FOXO3 has, moreover, been associated with self-reported health in older individuals (Zettergren et al., 2018). Therefore, FOXO3 represents one of the most interesting candidate gene products to pharmacologically target for promotion of healthy aging, and potentially longevity in humans.

There may be multiple molecular mechanisms through which FOXO3 activation results in longevity. As a transcription factor, FOXO3 can be described as a central player in cellular stress resistance, contributing to redox regulation, autophagy, energy homeostasis, DNA repair, cell cycle arrest, telomere maintenance and stem cell homeostasis (Davy et al., 2018b, 2018a), the result of which is improved somatic maintenance and extended lifespan. Much of the knowledge on the upstream regulatory pathways of FOXO3 has been obtained from research in the nematode C. elegans (referred to as ‘worms’ in this review), where daf-16 is orthologous to the FoxO family of genes that includes FOXO3 (Sun et al., 2017) (FoxO3 in mice and rats). For the purposes of this review, we will interpret activation of C. elegans DAF-16 as equivalent to activation of mammalian FOXO3.

3. Dietary restriction, PI3K, AKT, AMPK, mTOR and FOXO3

One of the main pathways regulating DAF-16/FoxO involves dietary restriction (Fontana et al., 2010). Dietary restriction (DR) can extend lifespan up to 50% in mice (McCay et al., 1989) while also extending healthy longevity (Fontana et al., 2010). In addition to rodents, extensive studies demonstrate benefits of DR for invertebrates, including yeast, worms, and flies (Kapahi et al., 2017), primates such as the grey mouse lemur (Pifferi et al., 2018), and even other primates, including humans (Longo and Mattson, 2014; Redman et al., 2018). Many DR variations have been demonstrated to extend lifespan in different organisms. These include reduction of calories (McCay et al., 1989), restriction of the period of feeding (Mitchell et al., 2019), carbohydrate restriction (Roberts et al., 2018), and restriction of protein components (Richardson et al., 2021).

While the universality of DR has been debated (Huberts et al., 2014; le Bourg, 2018), this is likely due to the wide variety of DR regimes that exist between studies (Kapahi et al., 2017; Vaughan et al., 2018). For example, while some studies in C. elegans have found that lifespan extension from DR is independent of the activity of DAF-16/FoxO (Houthof et al., 2003), others show that DR in worms is DAF-16/FoxO-dependent, and specific to the DR regime used (Greer and Brunet, 2009). In line with this, DR in C. elegans, implemented by diluting bacterial food, activates DAF-16/FoxO through AMP-activated protein kinase (AMPK) (Greer et al., 2009), while treating C. elegans with an intermittent fasting regime activates DAF-16/FoxO through a different mechanism involving the GTPase RHEB-1 and mechanistic target of rapamycin (mTOR) signaling (Honjo et al., 2008). Meanwhile, in mice, a DR study involving a 30% reduction in calories demonstrated that FOXO3 is required for lifespan extension (Shimokawa et al., 2015).

Despite the fact that not all DR protocols operate through the same molecular pathways (Kapahi et al., 2017), a picture has emerged of the potential mechanism mediating the effect of DR. DR interventions converge on the insulin-like growth factor-1 (IGF-1) signaling axis, a pathway that regulates DAF-16/FoxO activity in longevity (Kenyon, 2010). The proposed mechanisms is that DR firstly reduces IGF-1 signaling, in turn reducing the activity of the serine-threonine kinase AKT (also known as protein kinase B), which itself is a negative regulator...
of FOXO3 (Fig. 1) (Fontanata et al., 2010; Hay, 2011; Kenyon, 2010; Papadopoli et al., 2019). This signaling cascade ultimately results in activation of FOXO3 and extension of lifespan (Fig. 1) (Fontanata et al., 2010; Hay, 2011; Kenyon, 2010; Papadopoli et al., 2019). In parallel, the influence of DR on AKT may reduce activity of mTOR in the mTORC1 protein complex, both through AKT- and AMPK-related pathways, the net result of which also contributes to longevity (Fig. 1) (Fontanata et al., 2010; Hay, 2011; Kenyon, 2010; Papadopoli et al., 2019). Finally, AMPK itself can activate DAF-16/FoxO, as has been demonstrated during DR in C. elegans (Greer et al., 2007a).

Whereas the link between DR and AKT, AMPK, and FOXO3 signaling is well described, the link between DR and mTOR and FOXO3 signaling in longevity is more convoluted. mTOR consists of two complexes, namely, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Reduction of mTORC1 activity, which occurs during DR or by treatment with the mTORC1 inhibitor rapamycin, reduces phosphorylation levels of the ribosomal S6 kinase (S6K) and the eukaryotic translation initiation factor 4E (eIF4E) binding protein (4E-BP), which slows down the translation process, a signaling cascade that extends lifespan (Fontanata et al., 2010; Hay, 2011; Kenyon, 2010; Papadopoli et al., 2019). This is believed to be independent of DAF-16/FoxO (Velai et al., 2003). Importantly, a difference should be noted between acute and chronic inhibition of mTORC1, because chronic inhibition of mTORC1 also results in inhibition of mTORC2 (Sarbassov et al., 2006). Repression of mTORC2 can activate FOXO3 (Feehan and Shantz, 2016), but mTORC2 inhibition may ultimately prove detrimental, since mTORC2 plays an essential role in cytoskeletal operations, ion transport, and cell migration (Papadopoli et al., 2019). Indeed, selective inhibition of mTORC2 by depletion of the mTORC2 component Rictor, not found in mTORC1, reduces lifespan in both worms (Soukas et al., 2009) and mice (Lamming et al., 2014). This is likely due to the fact that Rictor is a target of FoxO factors and, as a component of the mTORC2 complex, is critical for AKT activation by S473 phosphorylation (Chen et al., 2010). Meanwhile, selective inhibition of mTORC1 by depletion of the mTORC1 component Raptor, which is not found in mTORC2, increases lifespan in worms (Jia et al., 2004). FOXO3 activation has also been shown to result in mTORC1 activation, and mTORC2 suppression, suggesting that FOXO3 activation additionally benefits longevity by acting upstream of these complexes as well (Chen et al., 2010) (Fig. 1).

To summarize, FOXO3 is intricately linked to the nutrient sensing longevity pathways (Houtkooper et al., 2010). While mTOR inhibition, such as by rapamycin or other mTORC1 inhibitors, may activate FOXO3 upon chronic treatment, FOXO3 activity during mTOR inhibition may likewise be a marker for reduced mTORC2 activity, an undesirable result when optimizing for longevity. Therefore, lessons learned from DR experiments suggest that targeting AMPK, which positively regulates the activity of FOXO3 (Greer et al., 2007b), or targeting PI3K and AKT, which negatively regulate activity of DAF-16/FoxO (Cahill et al., 2001), may be more desirable for achieving FOXO3 activation. As such, AMPK can be activated not only through DR but also by exercise (Richter and Ruderman, 2009) and metformin (Musi et al., 2002), each of which lead to activation of FOXO3 (Sato et al., 2012; Zeng et al., 2020). Additionally, inhibiting the PI3K/AKT axis with the PI3K inhibitor LY294002 enhances DAF-16/FoxO activity and increases lifespan and stress resistance in worms (Babar et al., 1999; Cahill et al., 2001) (Fig. 1).

4. Pharmaceuticals that activate FOXO3

FOXO3 activation can be achieved through a variety of cellular mechanisms. These include transcriptional activity, protein-protein interactions, regulation of protein stability, or through nuclear transport and/or retention (Calissi et al., 2021). Post-translational modifications of FOXO3 proteins that regulate these mechanisms can include phosphorylation, acetylation, ubiquitination and methylation (Calissi et al., 2021). Phosphorylation of FOXO3 can have an activating or inactivating role, depending on the amino acid involved (Fasano et al., 2019). In this review, we classify pharmaceuticals and nutraceuticals that result in at least one of the following: (1) FOXO3/DAF-16 nuclear accumulation, (2) upregulation of FOXO3/DAF-16 activity itself, (3) increased lifespan in a manner dependent on DAF-16/FoxO, (4) modulation of phosphorylation of FOXO3/DAF-16, and/or (5) production of an increase in expression of downstream FOXO3 target genes (Table 1, Supplemental Table 1, Fig. 2). Activation of FOXO3 indicates the activation of the protein, for example nuclear translocation or downstream transcriptional activity, while activation of FOXO3 indicates stimulation of transcription of the gene. Some studies reviewed here describe activation of ‘FOXO3A’. Because FOXO3B is now generally considered to be a pseudogene (Flachsbart et al., 2012), the nomenclature has transitioned to using the term ‘FOXO3’ to describe FOXO3A. While there is recent evidence that FOXO3B is actually protein-coding (Santo and Paik, 2018), the nomenclature for FOXO3A as simply FOXO3 has persisted, and we will use only FOXO3 in this review.

Compound classes that, by acting on the DR pathway, inhibit PI3K/
Table 1
Geroprotective FOXO3 activators as described in this review. More extensive information can be found in Supplemental Table 1.

| Compound       | Category                        | Extends lifespan | FOXO3/DAF-16 nuclear localization | FOXO3/DAF-16 mRNA or protein upregulation | FOXO3/DAF-16 phosphorylation | FOXO3/DAF-16 target genes modulation | FOXO3/DAF-16 activation | References                                                                 |
|----------------|---------------------------------|------------------|------------------------------------|------------------------------------------|-------------------------------|--------------------------------------|--------------------------|--------------------------------------------------------------------------|
| staurosporine  | protein kinase inhibitor        | x                | x                                  | x                                        | x                             |                                      |                          | (Zanella et al., 2008)                                                  |
| UCN01          | protein kinase inhibitor        | x                |                                    |                                          |                               |                                      |                          | (Zanella et al., 2008)                                                  |
| LY294002       | PI3K inhibitor                  | x                | x                                  |                                          |                               |                                      |                          | (Babar et al., 1999; Zanella et al., 2008)                                |
| wortmannin     | PI3K inhibitor                  | x                | x                                  |                                          |                               |                                      |                          | (Damluk et al., 2013; Zanella et al., 2008)                                |
| PIK-75         | PI3K inhibitor                  | x                |                                    |                                          |                               |                                      |                          | (Zanella et al., 2008)                                                  |
| PI-103         | PI3K inhibitor                  | x                |                                    |                                          |                               |                                      |                          | (Zanella et al., 2008)                                                  |
| ETP-45658      | PI3K inhibitor                  | x                | x                                  |                                          |                               |                                      |                          | (Link et al., 2009)                                                     |
| ETP-46321      | PI3K inhibitor                  | x                | x                                  |                                          |                               |                                      |                          | (Granda et al., 2013)                                                    |
| capivasertib   | AKT inhibitor                   | x                |                                    |                                          |                               |                                      |                          | (Davies et al., 2012)                                                    |
| withaferin-A   | AKT inhibitor                   | x                | x                                  |                                          |                               |                                      |                          | (Das et al., 2016; Koval et al., 2021)                                     |
| metformin      | AMPK activator                  | x                | x                                  | x                                        | x                             | x                                    |                          | (Anisimov et al., 2008; Cabreiro et al., 2013; Hartwig et al., 2021; Sato et al., 2012; Zhang et al., 2018) |
| quercetin      | flavonoid/AMPK activator        | x                | x                                  | x                                        |                               | x                                    |                          | (Kampfet al., 2008; Nguyen et al., 2017; Saul et al., 2008)               |
| resveratrol    | flavonoid/ stilbene/AMPK activator x | x                |                                    |                                          |                               |                                      |                          | (Bour et al., 2006; Franco et al., 2014; Wood et al., 2004)               |
| berberine      | ammonium salt/ AMPK activator   | x                |                                    |                                          |                               |                                      |                          | (Dang et al., 2020)                                                     |
| cilostazol     | PDE3 inhibitor/ AMPK activator  | x                |                                    |                                          |                               |                                      |                          | (Anestis et al., 2020)                                                   |
| AICAR          | AMP analog/AMPK activator       | x                | x                                  |                                          |                               |                                      |                          | (Nyström and Lang, 2008; Sanchez et al., 2018)                            |
| simvastatin    | statin/AMPK activator           | x                |                                    |                                          |                               |                                      |                          | (Spindler et al., 2012)                                                  |
| mianserin      | antipsychotic                   | x                | x                                  |                                          |                               |                                      |                          | (Zanella et al., 2008)                                                  |
| bepridil       | antipsychotic                   | x                | x                                  |                                          |                               |                                      |                          | (Park et al., 2016)                                                     |
| trifluoperazine| antipsychotic                   | x                | x                                  |                                          |                               |                                      |                          | (Park et al., 2016)                                                     |
| calmidazolium  | antipsychotic; calmodulin inhibitor | x                |                                    |                                          |                               |                                      |                          | (Park et al., 2016)                                                     |
| atracurium     | muscle relaxant                 | x                | x                                  | x                                        | x                             |                                      |                          | (McIntyre et al., 2021)                                                  |
| chlorzoxazone  | muscle relaxant                 | x                | x                                  | x                                        |                               |                                      |                          | (Deng et al., 2020)                                                     |
| trichostatin A | HDAC inhibitor                  | x                | x                                  |                                          |                               |                                      |                          | (Calvert et al., 2014; Guo et al., 2017; Tao et al., 2004; Zhang et al., 2015a) |
| TMP269         | HDAC inhibitor                  | x                |                                    |                                          |                               |                                      |                          | (Usami et al., 2020)                                                     |
| panobinostat   | HDAC inhibitor                  | x                |                                    |                                          |                               |                                      |                          | (Körlholz et al., 2021)                                                  |
| vorinostat     | HDAC inhibitor                  | x                |                                    |                                          |                               |                                      |                          | (Körlholz et al., 2021; McDonald et al., 2013)                            |
| MS-275         | HDAC inhibitor                  | x                |                                    |                                          |                               |                                      |                          | (Aune et al., 2014)                                                     |
| valproic acid  | HDAC inhibitor                  | x                | x                                  | x                                        |                               |                                      |                          | (Everson et al., 2008)                                                   |
| β-hydroxybutyrate | HDAC inhibitor               | x                | x                                  |                                          |                               |                                      |                          | (continued on next page)                                                |
Table 1 (continued)

| Compound               | Category                  | Extends lifespan | Evidence for FOXO3/DAF-16 activation | References                                         |
|------------------------|---------------------------|------------------|-------------------------------------|----------------------------------------------------|
|                        |                           |                  | FOXO3/DAF-16 nuclear localization | upregulation of FOXO3/DAF-16 mRNA or protein | lifespan extension dependent on FOXO3/DAF-16 | modulates FOXO3/DAF-16 phosphorylation | activation of FOXO3/DAF-16 target genes |
| lovastatin             | statin                    | x                | x                                   | x                                                  | x                                                  | x                                        | (Edwards et al., 2014; Shimazu et al., 2013) |
| pitavastatin           | statin                    | x                | x                                   | x                                                  | x                                                  | x                                        | (Jahn et al., 2020)                      |
| bortezomib             | other; proteosome inhibitor | x                |                                     | x                                                  | x                                                  |                                          | (Lee et al., 2020b)                      |
| BAPTA-AM               | other; calcium chelator   | x                |                                     | x                                                  | x                                                  |                                          | (Jagani et al., 2009)                    |
| zoledronate            | other; bisphosphate       | x                |                                     | x                                                  | x                                                  |                                          | (Zanella et al., 2008)                   |
| celecoxib              | other; anti-inflammatory   | x                |                                     | x                                                  | x                                                  |                                          | (Chen et al., 2021)                     |
| α-ketoglutarate        | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Shahmirzadi et al., 2020; Chin et al., 2014; Su et al., 2019) |
| malate                 | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Edwards et al., 2013)                  |
| fumarate               | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Edwards et al., 2013)                  |
| phosphatidylcholine    | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Kim et al., 2019a)                     |
| N-acetyl-l-cysteine    | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Jirack et al., 1997; Oh and Park, 2017) |
| NAD                    | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Hashimoto et al., 2010)                |
| PQQ blueberry extracts | primary metabolite        | x                |                                     | x                                                  | x                                                  |                                          | (Wu et al., 2016)                       |
| apple peel extracts    | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Wang et al., 2018a)                    |
| Trans-communic acid   | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Song et al., 2020)                     |
| Rhododendron rusea     | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Kim et al., 2019a)                     |
| pomegranate juice extract | secondary metabolite    | x                |                                     | x                                                  | x                                                  |                                          | (Zheng et al., 2017)                    |
| tart cherry extract    | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Jayarathe et al., 2020)                |
| Chondrus crispus       | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Han et al., 2013)                      |
| Anacardium occidentale extract | secondary metabolite    | x                |                                     | x                                                  | x                                                  |                                          | (Duangkan et al., 2019)                 |
| Culcasulina mimosoides extracts | secondary metabolite | x                |                                     | x                                                  | x                                                  |                                          | (Bansinith et al., 2019)                |
| Moringa oleifera       | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Im et al., 2016)                       |
| Holothuria scabra      | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Jattu et al., 2018)                    |
| Mora bombycis          | secondary metabolite      | x                |                                     | x                                                  | x                                                  |                                          | (Hoe et al., 2016)                      |
| piceatannol curcumin   | stilbene                  | x                |                                     | x                                                  | x                                                  |                                          | (Shen et al., 2017)                     |
| epigallocatechin-3-gallate (EGCG) | phenol                | x                |                                     | x                                                  | x                                                  |                                          | (Xiong et al., 2018)                    |
| flavone                | flavone                   | x                |                                     | x                                                  | x                                                  |                                          | (Lin et al., 2015)                      |
| apigenin               | flavone                   | x                |                                     | x                                                  | x                                                  |                                          | (Kawasaki et al., 2010; Lin et al., 2013) |
| luteolin               | flavone                   | x                |                                     | x                                                  | x                                                  |                                          | (Lesmanova et al., 2017; Lin et al., 2015) |
| syringaresinol         | lignin                    | x                |                                     | x                                                  | x                                                  |                                          | (Kim et al., 2020)                      |
| fisetin                | flavonoid                 | x                |                                     | x                                                  | x                                                  |                                          | (Ramphatker et al., 2007; Wood et al., 2004; Yousefzadeh et al., 2018) |
| kaempferol             | flavonoid                 | x                |                                     | x                                                  | x                                                  |                                          |                                           |

(continued on next page)
AKT or activate AMPK often result in FOXO3 activation. Additionally, a variety of other pharmaceutical classes have emerged that can activate FOXO3. These other classes can be grouped into categories, including (a) antidepressants and antipsychotics, (b) muscle relaxants, (c) histone deacetylase (HDAC) inhibitors, and (d) statins. Individual compounds outside of these categories have also been identified.

4.1. PI3K/AKT inhibitors and AMPK activators

The DR pathway is relevant not only for aging, but also for cancer research and many other age-related disease fields. With interest coming from multiple fields, identifying and developing compounds that modulate the DR pathway has been pursued extensively, resulting in multiple drugs and other chemical compounds that act either directly or indirectly to modify PI3K/AKT and AMPK signaling. For example, in a study aimed at finding tumor suppressors, nuclear accumulation of FOXO3 was described in response to treatment with generalized protein kinase inhibitors, such as staurosporine and UCN01 (Zanella et al., 2008), as well as the PI3K inhibitors LY294002, wortmannin, PIK-75, and PI-103 (Zanella et al., 2008). FOXO3 nuclear accumulation was measured using a stably transfected green fluorescent protein (GFP)-tagged FOXO3 plasmid in U2OS human osteosarcoma cells, and was termed U2foxRELOC (Zanella et al., 2008). Of relevance to aging research and in line with their role as FOXO3 activators, several of these compounds increase lifespan in model organisms (Babar et al., 1999; Danilov et al., 2013). Soon after that, the same U2foxRELOC system was used to identify FOXO3-activators specifically (Link et al., 2009). After identifying the capacity of pyrazolopyrimidine derivatives to promote FOXO3 activation. Additionally, a variety of other pharmaceutical classes have emerged that can activate FOXO3. These other classes can be grouped into categories, including (a) antidepressants and antipsychotics, (b) muscle relaxants, (c) histone deacetylase (HDAC) inhibitors, and (d) statins. Individual compounds outside of these categories have also been identified.

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4.1. PI3K/AKT inhibitors and AMPK activators

The DR pathway is relevant not only for aging, but also for cancer research and many other age-related disease fields. With interest coming from multiple fields, identifying and developing compounds that modulate the DR pathway has been pursued extensively, resulting in multiple drugs and other chemical compounds that act either directly or indirectly to modify PI3K/AKT and AMPK signaling. For example, in a study aimed at finding tumor suppressors, nuclear accumulation of FOXO3 was described in response to treatment with generalized protein kinase inhibitors, such as staurosporine and UCN01 (Zanella et al., 2008), as well as the PI3K inhibitors LY294002, wortmannin, PIK-75, and PI-103 (Zanella et al., 2008). FOXO3 nuclear accumulation was measured using a stably transfected green fluorescent protein (GFP)-tagged FOXO3 plasmid in U2OS human osteosarcoma cells, and was termed U2foxRELOC (Zanella et al., 2008). Of relevance to aging research and in line with their role as FOXO3 activators, several of these compounds increase lifespan in model organisms (Babar et al., 1999; Danilov et al., 2013). Soon after that, the same U2foxRELOC system was used to identify FOXO3-activators specifically (Link et al., 2009). After identifying the capacity of pyrazolopyrimidine derivatives to promote FOXO3 activation. Additionally, a variety of other pharmaceutical classes have emerged that can activate FOXO3. These other classes can be grouped into categories, including (a) antidepressants and antipsychotics, (b) muscle relaxants, (c) histone deacetylase (HDAC) inhibitors, and (d) statins. Individual compounds outside of these categories have also been identified.

Fig. 2. FOXO3 activators. Pharmaceutical and nutraceutical categories described in this review as eliciting FOXO3 activation.
FOXO3 nuclear localization, these researchers developed ETP-45658, which also stimulates FOXO3 nuclear localization, and modulates FOXO3 phosphorylation (Link et al., 2009). The same group later showed that ETP-46321, another PI3K inhibitor, had the same capabilities (Granda et al., 2013). A study aimed at finding Akt inhibitors for FOXO3-dependent apoptosis of prostate cancer cells showed that withaferin-A, a small molecule derived from various nightshades, caused nuclear translocation of FOXO3 (Das et al., 2016), and extended lifespan in flies (Koval et al., 2021). The Akt inhibitor capivasertib also stimulates FOXO3 nuclear localization (Davies et al., 2012). These compounds represent a small fraction of the molecules targeting the PI3K/Akt pathway. For a comprehensive understanding of the compounds resulting from the enormous efforts dedicated to targeting PI3K (Yang et al., 2019) and AKT (Song et al., 2019) the reader is referred to dedicated reviews cataloging these compounds (Song et al., 2019; Yang et al., 2019).

Similar to the processes by which PI3K and AKT inhibitors were developed because of their relevance to cancer treatment and research, many compounds have been developed and studied for their role in AMPK signaling for a variety of other diseases. For example, metformin, a drug originally used to lower the blood sugar in people with type 2 diabetes, may have become the most well described and studied AMPK activator in the context of aging (Barzilai et al., 2016; Musi et al., 2002). Metformin extends lifespan in a number of model organisms (Anisimov et al., 2008; Cabreiro et al., 2013). Metformin also activates FOXO3 in a variety of human cell types, stimulating FOXO3 phosphorylation, nuclear localization and activation of downstream targets (Hartwig et al., 2021; Sato et al., 2012; Zhang et al., 2018). Of note, the broad range of chemical structures capable of causing AMPK activation has made classifying compounds solely on their ability to activate AMPK a challenge. For example, flavonoids, including quercetin and resveratrol, the quaternary ammonium salt berberine, the phosphodiesterase type 3 (PDE3) inhibitor cilostazol, the analog of adenosine monophosphate (AMP), 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR), and the lipid-lowering statin, simvastatin, have all been studied specifically for their therapeutic potential as AMPK activators, although most exhibit a diverse range of targets (Assefa et al., 2020). In line with these compounds activating AMPK, AICAR increases Foxo3 expression in muscle of mice (Nystrom and Lang, 2008) and stabilizes FOXO3 in primary myotubes (Sanchez et al., 2018), while simvastatin and berberine extend lifespan in flies and mice, respectively (Dang et al., 2020; Spindler et al., 2012). Several of these compounds will be discussed in other sections of this review, and for a recent overview of pharmaceutical developments regarding AMPK activators, the reader is referred to a dedicated review (Steinberg and Carling, 2019).

4.2. Antidepressants and antipsychotics

Antidepressants and antipsychotics (psychotropics) are another general class of pharmaceutical compounds that have emerged as FOXO3 activators. A high throughput screen involving 88,000 chemicals in C. elegans, identified the antidepressant mianserin as a lifespan-extending drug (Petrascheck et al., 2007). The study found that lifespan extension was abrogated in a model mimicking DR, and, in line with this, was partially dependent on DAF-16/FoxO (Petrascheck et al., 2007). The lifespan effects were dependent on the presence of serotonin and two neurotransmitter receptors (SER-3 and SER-4). It has been speculated that serotonin and the neurotransmitter may, in response to the inhibitory effects of mianserin, cause the worms to ‘perceive’ themselves as undergoing DR, despite ad libidum feeding (Petrascheck et al., 2007). Another study used a cell-based enzymatic (ELISA) assay and a commercially available small-molecule library of 640 FDA-approved drugs to screen for compounds able to modify FOXO3 phosphorylation (Park et al., 2016). The study identified two antipsychotics, bepridil and trifluoperazine, that were able to inhibit Akt and promote FOXO3 nuclear localization (Park et al., 2016). Both bepridil and trifluoperazine target calmodulin, a calcium-binding messenger protein. Another inhibitor of calmodulin, calmidazolium, also causes FOXO3 nuclear localization, as demonstrated in a screen using U2foxl/RELOC cells to screen for FOXO3 activators (Zanella et al., 2008).

4.3. Muscle relaxants

Very recently, muscle relaxants have been identified as powerful FOXO3 activators. Members of our team performed a computational screen for compounds that transcriptionally mimic the RNA expression signature of FOXO3 overexpression, in order to identify FOXO3 activators (McIntyre et al., 2021). Out of 2837 compounds, we identified the muscle relaxant atracurium as a FOXO3 activator. Atracurium causes DAF-16/FoxO nuclear translocation, extends C. elegans lifespan in a manner dependent on DAF-16/FoxO and upregulates DAF-16/FoxO gene targets in worms and in mammalian cell culture (McIntyre et al., 2021). The effects of atracurium occurred via the ortholog of the same acetylcholine receptor subunit in C. elegans that it canonically targets in humans, resulting in a novel signaling cascade for activation of DAF-16/FoxO involving the neuromuscular acetylcholine receptor (McIntyre et al., 2021). While in humans atracurium causes muscle relaxation and is used in surgery for intubation, in C. elegans it resulted in larger, healthier worms having greater muscular activity, as measured by pharyngeal pumping and body movement (McIntyre et al., 2021). The discrepancy between these two phenotypes requires a deeper investigation. Interestingly, another study performed a computational screen for FDA approved drugs able to modulate immune function in mesenchymal stem cells (Deng et al., 2020). That study identified chlorzoxazone, a muscle relaxant that is used to treat muscle spasms, although the exact mode of its action is unknown (Deng et al., 2020). Chlorzoxazone modulated phosphorylation of FOXO3 in a manner independent of AKT (Deng et al., 2020).

4.4. Histone deacetylase inhibitors

HDAC inhibitors were originally developed by cancer biology researchers to induce cell cycle arrest and apoptosis. They have since then been explored for the additional effects they elicit in cells, which includes the induction of pro-longevity autophagy genes (Rikishi, 2011). As such, the potential for HDAC inhibitors to increase healthy longevity has been extensively reviewed by some of the authors of this review (McIntyre et al., 2019). Interestingly, our recent computational drug screen for compounds that mimic the transcriptional signature of FOXO3 overexpression (McIntyre et al., 2021) found that HDAC inhibitors were a top enriched drug class amongst the highest ranked compounds from the screen. This is in line with the observation that a number of HDAC inhibitors cause FOXO3 nuclear localization and activation of downstream effectors. For example, treatment of rat H9c2 cardiac cells with the organic compound trichostatin A, a class II HDAC inhibitor, led to increased H4 acetylation of the FOX30 promotor region, enhancing its expression (Guo et al., 2017). Trichostatin A also increases FOXO3 (and FoxO1) expression levels and downstream targets in human colon and liver cancer cell lines (Zhang et al., 2015c). Likewise, treatment of pancreatic cancer cells with TMP269, a potent, selective class Ia HDAC inhibitor, upregulated FOXO3 expression and FOXO protein levels in a dose-dependent manner (Usami et al., 2020). Furthermore, treatment of neuroblastoma cells with the non-selective HDAC inhibitors panobinostat and vorinostat led to FOXO3 nuclear accumulation and induction of FOXO3-target autophagy genes (Korholz et al., 2021). This potentially explains the finding that vorinostat extends lifespan in flies (McDonald et al., 2019).

Despite these straightforward findings, discrepancies exist in the field. For example, although trichostatin A extends lifespan in flies (Tao et al., 2004) and C. elegans (Calvert et al., 2016), it does so in worms in a DAF-16/FoxO-independent manner (Calvert et al., 2016). Furthermore, HDAC1 may also activate FOXO3 in muscles, and treatment with the
HDAC inhibitor MS-275 is sufficient to reduce FOXO3 activity and autophagy, resulting in less muscle wasting that would normally occur during muscle disuse and sarcopenia (Beharry et al., 2014). This too has been contested, however, and multiple other research groups have suggested that the autophagy induced by FOXO3 activity is not sufficient to cause the muscle atrophy observed in sarcopenia (Sandri et al., 2013; Stefanetti et al., 2018). Despite these open questions, there is significant evidence suggesting the geroprotective effects of HDAC inhibitors may involve FOXO activation. In worms, the class I HDAC inhibitor valproic acid promotes DAF-16 nuclear localization and extends lifespan via DAF-16 activation (Evason et al., 2008). In mice with induced heart ischemia, MS-275 increases FOXO3 nuclear localization in heart tissue, as well as upregulating FOXO3-downstream targets (Aune et al., 2014). Lastly, FOXO3 activation has been demonstrated with an HDAC inhibitor, β-hydroxybutyrate, that has multiple other metabolic and cell signaling roles (Shimazu et al., 2013). β-hydroxybutyrate is a specific inhibitor of class I HDACs, and administration increased global histone acetylation in mouse tissues, increased histone acetylation at FoxO3 promoters, and upregulated FOXO3 (Shimazu et al., 2013). β-hydroxybutyrate also extends lifespan in C. elegans, and this effect was dependent on DAF-16/FoxO (Edwards et al., 2014). Taken together, HDAC inhibitors illicit a broad range of transcriptional changes, which can include FOXO3 activation and ultimately can promote healthy aging and lifespan (McIntyre et al., 2019).

4.5. Statins

Statins reduce circulating cholesterol levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Jahn et al., 2020). As mentioned above, simvastatin is known to activate AMPK (Assefa et al., 2020), thereby activating FOXO3. In C. elegans, simvastatin and lovastatin extend lifespan, and the lifespan extension conferred by lovastatin is dependent on DAF-16/FoxO, and by causing DAF-16/FoxO nuclear localization (Jahn et al., 2020). In a human oral squamous cell carcinoma cell line, another statin, pitavastatin, stimulated FOXO3 nuclear translocation and modulated FOXO3 phosphorylation (Lee et al., 2020b). The effect of pitavastatin was regulated by AKT and AMPK, suggesting AMPK as a common regulator of statin-controlled FOXO3 activation. Other statins also extend lifespan in C. elegans (Andreux et al., 2014), suggesting that additional drugs in the statins class could function through this same pathway.

4.6. Other pharmaceuticals

There are additional pharmaceuticals outside of the categories we have discussed that also activate FOXO3. Bortezomib is a proteasome inhibitor often used in cancer treatment. In a leukemia cell line, bortezomib increased the level of FOXO3 protein, and modulated FOXO3 phosphorylation (Jagani et al., 2009). Additionally, the calcium chelator BAPTA-AM increases FOXO3 nuclear localization (Zanella et al., 2008).

Two other compounds extend lifespan in model organisms coinciding with FOXO3 activation. Celecoxib is an anti-inflammatory pharmaceutical that extends lifespan in worms, causes DAF-16/FoxO nuclear localization and activation of target genes (Ching et al., 2011). The lifespan extension is dependent on DAF-16/FoxO, yet independent of the drug’s canonical target, COX-2 (Ching et al., 2011). Zoledronate is a nitrogen-containing bisphosphonate used for the treatment of skeletal bone disorders, including osteoporosis (Chen et al., 2021). In flies, zoledronate extends lifespan, as well as increasing resistance to oxidative stress (Chen et al., 2021). The oxidative stress resistance effect is dependent on dFOXO (Chen et al., 2021), the fly ortholog of FOXO3. Zoledronate also inhibits protein prenylation, affecting the cholesterol synthesis pathway (Green and Clezardin, 2002). It is therefore possible that zoledronate activates FOXO in a similar manner as statins.

5. Nutraceuticals that activate FOXO3

In addition to pharmaceuticals, a large body of evidence has implicated nutraceuticals as being potent FOXO3 activators (Table 1, Supplemental Table 1, Fig. 2). They include natural extracts, phenols, terpenoids, and other purified natural compounds, as well as either primary or secondary metabolites, with more specific subgroupings of the secondary metabolites possible.

5.1. Primary metabolites

β-hydroxybutyrate, described above as an HDAC inhibitor, is a ketone body and involved in many metabolic processes in the cell. Its levels increase in response to calorie restriction, in line with its role as a FOXO3 activator (Han et al., 2020). This serves to highlight the multiple roles compounds may have in an organism. A number of primary metabolites directly involved in growth and sustaining the cell have been shown to activate FOXO3. For example, the tricarboxylic acid (TCA) cycle intermediate α-ketoglutarate, that accumulates in tissues during dietary restriction, extends worm lifespan in part (though not fully) in a DAF-16/FoxO-dependent manner (Chin et al., 2014). α-Ketoglutarate also extends lifespan in flies, while increasing AMPK and FoxO expression (Su et al., 2019), and extends lifespan of female mice (Asadi Shahmirzadi et al., 2020). Interestingly, supplementation with other TCA cycle constituents, including malate and fumarate, also increase lifespan in worms, doing so by activating nuclear translocation of DAF-16/FoxO (Edwards et al., 2013). Not all TCA cycle components possess the ability to activate DAF-16/FoxO however, as supplementation with isocitrate, citrate, and succinate failed to reliably produce lifespan extension (Chin et al., 2014; Edwards et al., 2013). Similarly, when considering primary lipids, treatment of worms with phosphatidylcholine, one of the most abundant phospholipids in the cell, resulted in DAF-16/FoxO nuclear accumulation and lifespan extension (Kim et al., 2019b). The amino acid N-acetyl-L-cysteine (NAC), which is found in allium plants such as garlic and onion (yet is classified as a drug rather than a supplement), has been shown to mimic calorie restriction in worms and extend lifespan in a DAF-16/FoxO-dependent manner (Oh and Park, 2017). NAC also extends lifespan in flies (Brack et al., 1997). When considering coenzymes linked to fundamental metabolic processes, nicotinamide adenine dinucleotide (NAD) is perhaps the best-known example. NAD supplementation extends lifespan in worms in a manner dependent on DAF-16/FoxO and activates the DAF-16/FoxO downstream target gene sod-3 (Hashimoto et al., 2010; Mouchiroud et al., 2013). Finally, the cofactor pyrroloquinoline quinone (PQQ) enhances resistance to oxidative stress and extends the lifespan of worms in a DAF-16/FoxO dependent manner (Wu et al., 2016).

5.2. Secondary metabolites

Many extracts from natural sources such as plants and fungi, as well as purified or synthesized natural compounds, have been investigated for their role in healthspan and lifespan regulation. These compounds are often considered ‘secondary’ metabolites, fulfilling specialized roles in the cell. They may be specific to certain species, as opposed to being ‘primary’ metabolites central to cell growth and sustenance. Many of these secondary metabolites, which often derive from plant and fungal extracts, exert benefits in a manner dependent on FOXO3. The compounds can be non-exclusively grouped as (a) natural extracts, (b) phenols and polyphenols, (c) terpenoids, and (d) other purified natural compounds, as further outlined below.

5.2.1. Natural extracts

Natural extracts, such as those derived from plants and fungi, comprise a wide mixture of chemicals and have long been used in traditional medicine in attempts to positively influence human physiology and health. Studies have been performed to evaluate how such
extracts may influence stress resistance and longevity in *C. elegans*. Many of these studies have found that treatment with natural extracts results in lifespan extension and improved stress resistance, which is often dependent on, or coincides with, upregulation of DAF-16/FoxO. Interestingly, these natural extracts are often rich sources of antioxidants that aid in stress resistance, and while both antioxidative and oxidative treatments are able to increase lifespan in *C. elegans* in a manner dependent on DAF-16/FoxO, they seem to operate through different upstream pathways (Kim et al., 2014).

Blueberry extracts increase lifespan, motility, and stress resistance in worms, and upregulate DAF-16/FoxO as well as the antioxidant transcriptional system (Wang et al., 2018a). Blueberry extracts additionally extend lifespan in flies, while activating FoxO target genes (Peng et al., 2012). Apple peel extracts have shown similar effects, and apple peel and blueberry extracts synergistically increase lifespan and stress resistance in worms as well as causing DAF-16/FoxO nuclear localization (Song et al., 2020). Trans-communic acid, extracted from Korean red pine (*Pinus densiflora*), directly binds to FoxO3, as demonstrated through surface plasmon resonance assays and in worms, and extends lifespan of *C. elegans*, dependent on DAF-16/FoxO (Kim et al., 2019a).

Extract from *Rhodiola rosea*, a perennial flowering plant in the family Crassulaceae, increases lifespan in worms on a manner dependent on DAF-16/FoxO, and improves stress resistance against heat shock, ultraviolet radiation and parquat (Jiang et al., 2021). Pomegranate juice extract also extends lifespan in worms, in a manner partially dependent on DAF-16/FoxO (Zheng et al., 2017). Furthermore, tart cherry extract extends lifespan in worms, and this too is dependent on DAF-16/FoxO (Jayaratnhe et al., 2020). A study using a C. elegans model of innate immunity demonstrated that supplementation with *Chondrus crispus*, a species of red algae, improves immune function in worms in a manner dependent on DAF-16/FoxO (Liu et al., 2013). *Anacardium occidentale* promotes DAF-16/FoxO nuclear localization (Duanjing et al., 2019), and extracts from *Caesalpinia mimosoides* (Rangsinth et al., 2019), a vegetable consumed in Thailand, leaf extracts of *Moringa oleifera* (Im et al., 2016) and extracts from *Holothuria scabra* (*Jattuwan et al., 2018*), a sea cucumber, were all shown to improve aspects of healthspan and lifespan in worms in a DAF-16/FoxO-dependent manner. Extracts of *Morus bombycis*, used in multiple Asian countries as a traditional medicine, activates FOXO3 in human embryonic fibroblasts (Heo et al., 2016). Undoubtedly, many more extracts exist that show benefits on lifespan and healthspan that are associated with DAF-16/FoxO.

### 5.2.2. Phenols and polyphenols

The benefits from plant extracts that increase healthspan and lifespan in a DAF-16/FoxO-dependent manner may, at least in part, come from the phenols and polyphenols that they contain. Polyphenols represent a large class of compounds that have been explored extensively in aging research. Thousands of polyphenols have been identified in nature. They are classified relative to their phenol ring number or how the rings are structurally connected. Flavanoids, phenolic acids, phenolic alcohols, stilbenes, and lignans are the most common, and flavonoids are further subdivided into commonly studied classes including flavones, isoflavones, flavonones, flavonols, chalcones, catechins, and anthocyanidins (D’Archivio et al., 2007; Panche et al., 2016; Pandey and Rizvi, 2009).

Perhaps the most famous example of a polyphenol in aging research is the stilbene resveratrol (Markus and Morris, 2008), known to extend lifespan in both worm and mouse models (Sass et al., 2007; Wood et al., 2004) and mice (Baur et al., 2006). Resveratrol increases lifespan in worms through SIR-2.1 (Viswanathan et al., 2005), which itself has interactions with DAF-16/FoxO, although DAF-16/FoxO may not be necessary for the lifespan extending effects of resveratrol in worms (Viswanathan et al., 2005). Nonetheless, in mammalian cells, data exists indicating that resveratrol inhibits the PI3K/Akt pathway, resulting in FOXO3 activation (Franco et al., 2014). Piceatannol, another stilbene, increased lifespan in worms in a manner dependent on DAF-16/FoxO (Shen et al., 2017). The well-known compound curcumin is a FOXO3 activating polyphenol, as the curcumin metabolite tetrahydrocurcumin increased lifespan in flies in a manner dependent on dFOXO (Xiang et al., 2011). Furthermore, the flavonoids quercetin, found in many fruits, vegetables, leaves, seeds, and grains, and epigallocatechin-3-gallate (EGCG), found in green tea, activate FOXO3, as demonstrated in breast cancer cell lines (Belguise et al., 2007; Nguyen et al., 2017). These compounds both extend lifespan in model organisms. Quercetin extends lifespan in worms (Kampkötter et al., 2008), yet one study showed this effect was not entirely dependent on DAF-16/FoxO (Saül et al., 2008). EGCG extends fly lifespan (Wagner et al., 2015) and worm lifespan, dependent on DAF-16/FoxO (Xiong et al., 2018). EGCG also extends lifespan in rats coinciding with an increase in FOXO3 levels (Niu et al., 2013).

Flavonoids, including flavone (the simplest of the flavone class), apigenin and luteolin, induce FOXO3 expression in human cells by inhibiting PI3K signaling (Lin et al., 2015). In line with this, apigenin stimulates DAF-16/FoxO nuclear localization in worms (Kawasaki et al., 2010). Luteolin extends lifespan in worms and flies (Lashmanova et al., 2015). The lignin syringaresinol was shown to bind to FOXO3, offering an interesting hypothesis for how it may directly activate FOXO3 (Kim et al., 2020).

The flavonoid fisetin extends lifespan in flies and mice and has been implicated as a senolytic (Wood et al., 2004; Yousefzadeh et al., 2018). Fisetin and another flavonoid, kaempferol, improved thermotolerance, oxidative stress resistance in worms, and elicited translocation of DAF-16/FoxO to the nucleus (Kampkötter et al., 2007). Similarly, the flavonoid chrysin extends worm lifespan in a DAF-16/FoxO-dependent manner (Guerrero-Rubio et al., 2021). In line with this, 6,8-diprenylool, a flavonoid derived from *Glycyrrhiza uralensis* roots, when administered to several human hepatocellular carcinoma cell lines, upregulated FOXO3, among other proteins, as measured by western blotting (Lee et al., 2020a). Genistein, derived from soy, and also used as an antineoplastic agent, causes FOXO3 nuclear localization (Zanella et al., 2008). Genistein extends lifespan and activates DAF-16/FoxO target genes in worms (Lee et al., 2015). Additionally, similar to other polyphenols, echinacoside, a natural polyphenolic compound isolated from the echinacea plant, increases stress tolerance and lifespan in worms, and increases the transcript level and nuclear localization of DAF-16/FoxO (Wang et al., 2015). These many polyphenol examples suggest that there are even more compounds in this category with FOXO3-activating properties.

### 5.2.3. Terpenoids

Terpenoids are the largest group of plant secondary metabolites (Yazaki et al., 2017). They are beginning to gain more attention in the aging field for their geroprotective properties (Proshkina et al., 2020). Terpenoids are divided into groups based on the number of five-carbon units comprising their skeletons. These include five (hept-), ten (mono-), fifteen (sesqui-), twenty (di-), twentyfive (sester-), thirty (tri-) and forty (tetra-) carbons (Yazaki et al., 2017). One study found that the sesquiterpene β-caryophyllene extended lifespan in worms, and while β-caryophyllene was predicted to have a molecular docking interaction with DAF-16/FoxO, it nonetheless could elicit its lifespan extending effects independently of DAF-16/FoxO (Pant et al., 2014). In another study in worms, the triterpene oleanolic acid increased nuclear localization of DAF-16/FoxO, extended lifespan dependent on DAF-16/FoxO, and increased DAF-16/FoxO target genes (Zhang et al., 2015b). Interestingly, perhaps the most renown of the terpenes are the tetraterpenes, also known as carotenoids. From this class, astaxanthin, a terpenoid found in many red-colored aquatic organisms, was shown in worms to initiate DAF-16/FoxO nuclear translocation, and lifespan extension that was dependent on DAF-16/FoxO (Yazaki et al., 2011). Astaxanthin is implicated specifically as a geroneuroprotector as it can increase brain-neurotropic factor levels, therefore slowing down brain aging (Sorrenti et al., 2020). This supports the potential to translate use of
these FOXO activators to treat individual age-related diseases.

5.2.4. Other purified natural compounds

Other purified components of natural extracts, which may not necessarily fall under the categories described above, have been studied for their influence on health and lifespan. Indeed, a number of these compounds exert their benefits in a manner dependent on DAF-16/FOXO. For example, catalpol, a natural product in the class of iridoid glycosides that can be isolated from the rhizmannia root, extends worm lifespan, and increases tolerance to heat and oxidative stress via DAF-16/FOXO (See et al., 2015). Ergosterol peroxide, a sterol purified from the fungus Ganoderma lucidum, increases the expression of FOXO3 mRNA and protein in HepG2 cells (Li et al., 2016). Interestingly, after treatment, immunostaining showed that FOXO3 remained primarily in the cytosol (Li et al., 2016). Acetylshikonin, a napthoquinone pigment compound derived from roots of Lithospermum erythrorhizon, induced nuclear translocation of FOXO3 in human colorectal cancer cells (Lim et al., 2021). In addition, identified in a screen for FOXO3-activating compounds, is piperlongumine, found in the fruit of the long pepper, and harmine, a plant-derived β-carboline, each caused FOXO3 nuclear translocation in a reporter cell line, and also caused FOXO3-dependent transcription (Jimenez et al., 2021). Lastly, the popular supplement melatonin, sometimes utilized as an antidepressant, increases FOXO3 nuclear localization, activates downstream targets, and modulates FOXO3 phosphorylation in HepG2 cells (Carbajo-Pescador et al., 2012). In the brains of mice, melatonin increases the level of FOXO3 and modulates FOXO3 phosphorylation, as measured by western blotting (Ali et al., 2020). Melatonin also extends lifespan of flies (Bonilla et al., 2002).

6. DNA damaging agents

DNA damaging agents and other agents that disrupt cellular function as part of commonly used cancer treatments can elicit FOXO3 nuclear localization. These are, however, most likely to be unsuitable for geroprotection. For example, doxorubicin, a DNA damaging agent that intercalates with DNA, activates FOXO3 in leukemia cells (Hui et al., 2008). Mitoxanthrone, another chemotherapeutic that disrupts DNA synthesis and DNA repair, induces apoptosis in osteosarcoma cells through the AKT/FOXO3 pathway (Park et al., 2018). Paclitaxel and SN38 stimulate FOXO3 nuclear localization (Hu et al., 2014; Sunters et al., 2006) and paclitaxel modulates FOXO3 phosphorylation (Sunters et al., 2006). In line with this, the chemotherapeutic vinblastine was identified as a top hit in a screening study aimed at identifying FOXO3 activators (Zanella et al., 2008). Interestingly, at least in worms, these chemotherapeutics may nonetheless provide hormesis-based healthspan benefits at the appropriate dose, since it was demonstrated that an organoruthenium(II) complex, which is traditionally used in metal-based cancer chemotherapy, ameliorates oxidative stress and extends lifespan in worms in part through DAF-16/FOXO signaling (Devagi et al., 2018).

7. Limitations and future outlook

This review includes a number of assumptions and limitations. These should be considered when moving forward towards human therapy. One major limitation of targeting FOXO3 for healthy aging is the fact that FOXO3 activation is likely not universally beneficial but instead context- and time-dependent. Indeed, FOXO proteins have diverse roles and can also be detrimental to clinical outcomes (Maiese et al., 2009). As noted in the above section on HDAC inhibitors, there is debate about the possible negative impact of activating FOXO3, and therefore autophagy, in conditions of muscle atrophy, such as sarcopenia (Beharry et al., 2014; Sandri et al., 2013; Stefanetti et al., 2018). Additionally, FOXO3 (and FOXO1) activation may contribute to cardiac laminopathies (Auguste et al., 2018). These laminopathies are due to mutations in the gene LMNA, which encodes the nuclear inner membrane protein lamin A/C. LMNA mutation activates FOXOs, in addition to causing heart failure, conduction defects and arrhythmias (Auguste et al., 2018). Furthermore, in cancer, the benefit of FOXO activation is highly time- and context-dependent. FOXO proteins are generally classified as tumor suppressors, but can also promote tumorigenesis if activated once a tumor is already present (Maiese et al., 2009). In line with this, single deletion of FoxO3 in mice inhibits hematopoietic stem cell repopulation and results in apoptosis in these stem cell populations (Miyamoto et al., 2007; Tothova et al., 2007). This suggests that FOXO3 is a key player in maintaining stem cell populations. Therefore, continuous FOXO3 activation may prove to be detrimental due to increased cancer risk in long-lived organisms such as humans (Maiese et al., 2009). Taken together, the beneficial effects of FOXO3 activation in aging humans may be time dependent and assumes no underlying chronic diseases, a factor that requires further investigation.

A second major limitation concerns the translatability of the compounds listed in this review to human use. As discussed in the introduction to Section 2, FOXO3 SNPs have been consistently associated with longevity (Bao et al., 2014; Broer et al., 2015; Morris et al., 2015; Wilcox et al., 2008), and these variants are associated with increased FOXO3 mRNA expression (Banask et al., 2011; Donlon et al., 2017; Flachsbart et al., 2017; Grossi et al., 2018). However, increased activation of FOXO3 and downstream effectors has yet to be demonstrated in humans having the longevity-associated alleles of FOXO3 SNPs. The work described in this review is mostly based on experiments in cells or model organisms, in which FOXO3 activation consistently extends lifespan. However, for geroprotective treatment with FOXO3 activators to be clinically relevant, the connection must also be made in humans. In line with this, many of the compounds identified were studied in the worm C. elegans. While worms are a useful model organism in aging research, they have significant limitations, including the fact that they are post-mitotic and do not naturally develop cancer. Moreover, in translating these findings from model systems to humans it is of utmost importance to understand dosing requirements and explore the pharmacokinetics and pharmacodynamics for each of these treatments. Taken together, while these compounds extend lifespan in simple model organisms, some may require unfeasibly large doses to elicit FOXO3 activation, and/or additionally possess toxic profiles or produce side effects in more complex animals such as humans.

Another limitation involves the certainty or robustness of some of the studies described. While there is evidence to suggest that all of these compounds cause FOXO3 activation in the models tested, this does not always include direct evidence that FOXO3 is actually modulating transcription of downstream effectors. Furthermore, for the natural extracts described (Table 1), the specific compound within each extract that activates FOXO3/DAF-16 has not been identified. Additionally, while many compounds described here have been robustly replicated, there are a number of compounds or extracts listed that have been identified by single studies. For a compound to be assessed further as a geroprotector, the active component must be reproducibly shown to activate FOXO3, and preferably also demonstrate activation of downstream FOXO3 targets.

Looking to the future, we compiled an inventory of FOXO3 activators based on the criteria that they: (1) caused FOXO3/DAF-16 nuclear accumulation, (2) upregulated FOXO3/DAF-16 activity itself, (3) increased lifespan in a manner dependent on DAF-16/FOXO, (4) modulated phosphorylation of DAF-16, and (5) produced an increase in expression of downstream FOXO3 target genes. Almost all compounds we identified fulfilled more than one of these criteria. It should be noted, however, that studies that demonstrated activation of FOXO3 protein and downstream effectors provide the most clear and decisive evidence. These include metformin and melatonin (Table 1). In light of this, and should there be support for the benefit of FOXO3 activation in humans despite uncertainties, the health benefits of both metformin and melatonin make them excellent candidates for additional...
Nutraceutical, that directly or indirectly activate FOXO3 is of great interest. Therefore, identification of small molecules, either pharmaceutical or other, to activate FOXO3 will be discovered in the years to come. Computational drug screening, as employed by some of the authors of this review and others (Calvert et al., 2016; Hagenbuchner et al., 2019; Janssens et al., 2019; Link et al., 2009), and high-throughput screens in C. elegans based on motility and viability (Carretero et al., 2016; Petrascheck et al., 2007; Ye et al., 2014), are all means by which these novel FOXO3 activators have been, and will continue to be, identified. Additionally, the development of type-specific modulators of FOXO family members may be facilitated by more precise determination of differences in the structures of the DNA binding domains between FOXO family members (Psenakova et al., 2019). This may allow more precise pharmacological intervention with potentially lower risks of side effect. Irrespective, efforts are already underway to translate certain FOXO3 activators into human use (Barzilai et al., 2016). It is therefore a matter of ‘when’, not ‘if’, the right FOXO3 activator will reach the general population for prophylactic use to lower the prevalence of age-related diseases.

Competing interests
The authors declare no competing interests.

Acknowledgements
MH is supported by a Chinese Science Council scholarship. The work of BJM, TAD and BJW is supported by the US National Institutes of Health (contract N01-AG-4–2149, Grants 5 U01 AG019349-05, 5R01AG027060 [Kuakini Hawaii Lifespan Study], 5R01AG038707 [Kuakini Hawaii Healthspan Study], and contract N01-HC-05102 from the National Heart Lung and Blood Institute. GEJ is supported by a VENI grant from ZonMW, Talent Development and Innovation grants from the AGEM institute, and a Longevity Impetus Grant from Norn Group.

Author contributions
All authors have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Appendix A. Supporting information
Supplementary data associated with this article can be found in the online version at doi:10.1016/j.arr.2022.101621.

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