REVIEW

Molecular inhibition of RAS signalling to target ageing and age-related health

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ABSTRACT

The RAS/MAPK pathway is a highly conserved signalling pathway with a well-established role in cancer. Mutations that hyperactivate this pathway are associated with unregulated cell proliferation. Evidence from a range of model organisms also links RAS/MAPK signalling to ageing. Genetic approaches that reduce RAS/MAPK signalling activity extend lifespan and also improve healthspan, delaying the onset and/or progression of age-related functional decline. Given its role in cancer, therapeutic interventions that target and inhibit this pathway’s key components are under intense investigation. The consequent availability of small molecule inhibitors raises the possibility of repurposing these compounds to ameliorate the deleterious effects of ageing. Here, we review evidence that RAS/MAPK signalling inhibitors already in clinical use, such as trametinib, acarbose, statins, metformin and dihydromyricetin, lead to lifespan extension and to improved healthspan in a range of model systems. These findings suggest that the repurposing of small molecule inhibitors of RAS/MAPK signalling might offer opportunities to improve health during ageing, and to delay or prevent the development of age-related disease. However, challenges to this approach, including poor tolerance to treatment in older adults or development of drug resistance, first need to be resolved before successful clinical implementation.

KEY WORDS: MEK inhibitor, RAS pathway, Ageing

Introduction

Globally, people are living longer. In developed nations, most people are expected to live well into their 70s and beyond (www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-life-expectancy-and-healthy-life-expectancy). These changes are primarily due to declining mortality led by advances in hygiene, healthcare and education (Brown, 2015). However, healthspan (see Glossary, Box 1) is not keeping pace with lifespan; ageing individuals therefore experience an extended period of time of disability and ill health at the end of life. Increased age is the major risk factor for multiple chronic and debilitating diseases, including diabetes, cancer, cardiovascular disease and neurodegeneration (Nicolli and Partridge, 2012; Smetana et al., 2016; Hou et al., 2019). The prevalence of these diseases is therefore increasing worldwide alongside increased lifespans. The World Health Organization (WHO) has defined healthy ageing as the process of maintaining functional ability to enable wellbeing in older age (Rudnicka et al., 2020). The maintenance of healthy ageing therefore represents a global challenge with important socioeconomic consequences (McMaughan et al., 2020; Steptoe and Zaninotto, 2020).

Ageing has long been assumed to be an intractably complex process, caused by the accumulation of damage and pathologies because of the failure to maintain cellular integrity (López-Otín et al., 2013). However, genetic studies in model organisms have identified multiple evolutionarily conserved pathways that can be manipulated to increase lifespan and to improve multiple parameters of health during ageing. The most robust of these evolutionarily conserved longevity-associated mechanisms involve inhibition of nutrient signalling networks. For instance, genetic downregulation of insulin/IGF signalling (IIS) can increase lifespan across evolutionarily distinct organisms, including mammals (Kim and Lee et al., 2019). Moreover, genome-wide association studies in long-lived human populations have implicated a key downstream molecular effector of IIS, the FOXO3 transcription factor (Wilcock et al., 2008; Deelen et al., 2019; Ji et al., 2022) (Box 2).

At the molecular level, there is extensive crosstalk between IIS and the RAS/MAPK signalling pathway, resulting in a complex nutrient signalling network (Fig. 1). RAS small GTPases are activated in response to activation of receptor tyrosine kinases (RTKs; Box 1), including the insulin receptor (IR). As GTPases, RAS proteins function as binary molecular switches, cycling between inactive GDP-bound and active GTP-bound states, coordinated by the competing activities of guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) (Rajalingam et al., 2007). Activated RTKs recruit growth factor receptor-bound protein 2 (GRB2) to the intracellular surface of the cell membrane either directly or via association with the Src homology and collagen (SHC) adaptor protein. GRB2 then binds to and localises the RAS-GEF, son of sevenless (SOS) and, by association, activated RAS to the activated RTK-bound complex. Active GTP-bound RAS then binds to and activates its effector molecules, including RAF, initiating a phosphorylation cascade via MEK (also known as MAP2K) and the extracellular signal-regulated kinase (ERK) [also known as mitogen-activated protein kinase (MAPK)]. As a key downstream effector of RAS/MAPK signalling, activated ERK phosphorylates multiple cytoplasmic and cytoskeletal proteins and can also translocate to the nucleus, where it phosphorylates and activates several transcription factors, including members of the E-twenty-six (ETS) transcription factor family (Waslylk et al., 1998).

The ETS transcription factors are conserved across animals. They all contain an ~85 amino acid ETS domain that folds into a winged helix-turn-helix DNA-binding motif that binds DNA at 5′-GGA(A/T)-3′ ETS-binding motifs (EBMs) (Dobson et al., 2019).
ETS transcription factors generally function as activators, but a few repress transcription, and different family members can be differentially regulated by activated ERK. For example, the ETS transcription factors Pointed (Pnt) and Anterior open (Aop) are key transcriptional effectors of RAS/MAPK signalling during Drosophila development (Boisclair Lachance et al., 2014). They regulate the expression of the same set of genes, but with opposing outcomes (Dobson et al., 2019). In Drosophila, Pnt is activated in response to RAS signalling and functions as a transcriptional activator, whereas Aop is activated by RAS inhibition and represses gene expression (Fig. 2).

Cross-regulation between RAS/MAPK signalling and IIS occurs at multiple nodes downstream of the activated IR. For example, activated RAS-GTP can bind directly to and allosterically activate the catalytic subunit of phosphoinositide-3 kinase (PI3K) (Castellano and Downward, 2011), and, during cell growth and proliferation in Drosophila, this interaction allows for maximal PI3K activation (Orme et al., 2006). Conversely, AKT can negatively regulate ERK activation by phosphorylating inhibitory sites in RAF, sequestering it within the cytosol away from RAS and MEK (Zimmerman and Moelling, 1999). Moreover, activated ERK directly binds to and phosphorylates FOXO transcription factors (Box 2) on conserved serine residues, targeting them for degradation via an MDM2-mediated ubiquitin-proteasome pathway (Yang et al., 2008).

Increasing evidence from across different model organisms has shown that, like the IIS pathway, inhibition of RAS/MAPK signalling can modulate both ageing and a range of age-associated phenotypes (Slack, 2017). Hyperactivating mutations within key RAS/MAPK signalling components are prevalent in human cancers, and this has led to the development of small molecule inhibitors of the pathway for use as cancer therapeutics (Yaeger and Corcoran, 2019; Degirmenci et al., 2020). The link between reduced RAS/MAPK signalling and longevity assurance mechanisms has raised the question as to whether these compounds could be repurposed as geroprotectors (Box 1) to improve multiple parameters of health during ageing. This link also highlights the possibility of using other compounds that do not interact directly with RAS/MAPK pathway components but nevertheless indirectly inhibit the pathway’s activity as geroprotectors.

Here, we review recent genetic evidence from model organisms that supports a role for RAS/MAPK signalling in ageing and discuss the impact of chemically inhibiting RAS/MAPK signalling, both directly and indirectly, on age-related health. We discuss the potential risks raised by this approach to improve healthy ageing and address key challenges for further clinical studies.

Single-gene mutations that extend lifespan

The first single-gene mutations reported to influence animal ageing were identified in the nematode worm Caenorhabditis elegans. Mutations in age-1, which encodes the C. elegans orthologue of PI3K, result in reduced function of this key effector of IIS and in a significant increase in lifespan (Friedman and Johnson et al., 1988). Subsequent mutations that decreased the activity of other core IIS molecules, including DAF-2, the worm orthologue of the insulin/IGF-1 receptor, also produced substantial increases in lifespan (Kenyon et al., 1993). The longevity effects of reducing IIS are conserved over large evolutionary distances, with similar effects on lifespan being well reported in the fruit fly, Drosophila melanogaster, and in mice (Tabizadeh, 2021). Moreover, genetic variants of FOXO3, which encodes a key downstream effector of IIS, are strongly associated with longevity in human population studies, particularly in centenarians and in individuals over 95 years of age (Deelen et al., 2019). However, the mechanisms that underlie longevity in humans have yet to be identified.

Lifespan extension via the genetic inhibition of IIS in model organisms is often accompanied by improvements in physiological parameters of age-related health. In worms, age-related death has a multitude of causes: early death is associated with a swollen, infected pharynx and death later in life with pharyngeal atrophy. Loss-of-function daf-2 mutations decrease death caused by pharyngeal defects (Zhao et al., 2021) and increase the mutant worms’ resistance to colonisation by dietary bacteria (Podshivalova et al., 2017). Cardiac-specific overexpression of dFOXO in Drosophila protects against loss of cardiac function during ageing (Wessells et al., 2009). Genetic deletion of three of the Drosophila insulin-like peptides (dILPs) increases proteostasis (Box 1) in the gut, which maintains gut health and integrity in older flies (Tain et al., 2017). In mice, disruption of IIS activity by inhibition of growth hormone (GH; also known as GH1) release, or of GH action, reduces insulin levels, enhances insulin sensitivity, extends lifespan (Brown-Borg and Bartke, 2012), and promotes a shift in the balance between pro-inflammatory and anti-inflammatory cytokines, potentially enhancing insulin sensitivity and contributing to longevity (Salminen et al., 2011). Genetic knockout of IR substrate 1 (IRS1) in mice not only extends lifespan but is also associated with improvements in multiple markers of age-related physiological function, including motor performance, immune senescence and bone density (Selman et al., 2008).

Reducing the activity of IIS also protects against pathologies in mammalian models of age-related disease. Reduced neuronal...
expression of IRS2 in mice improves survival and delays the progression of motor deficits in a mouse model of Huntington’s disease (Sadagurski et al., 2011). Genetic deletion of IRS2 also suppresses amyloid deposition within the cerebral cortex in an amyloid precursor protein transgenic mouse model of Alzheimer’s disease (Wakabayashi et al., 2019). Disruption of IR function using a blocking peptide or by direct transcriptional knockdown using short hairpin RNA (shRNA) inhibits the growth of cultured endocrine-resistant breast cancer cells (Chan et al., 2016a).

Pharmacological interventions that diminish IIS could therefore offer wide-ranging treatments for multiple age-related pathologies. The oncogenic RAS small GTPase is a well-known mediator of IIS in mammals that is activated when the insulin/IGF-1 RTK is stimulated (Saltiel, 2021). RAS activation induces an intracellular signal transduction cascade via the phosphorylation and activation of ERK. Furthermore, regulatory feedback from RAS and ERK modulates the activity of several key molecules within the IIS pathway, including PI3K (Castellano and Downward, 2011; Singh and Smith, 2020). Targeting the activity of RAS itself or of components of its signal transduction cascade might therefore provide novel avenues for pharmacological inhibition of a highly conserved pro-longevity pathway. Indeed, there is now increasing evidence that genetic or pharmacological modulation of signalling through this pathway shows evolutionarily conserved effects on ageing across different species.

**RAS/MAPK signalling in ageing: genetic evidence from model organisms**

**Yeast**

Ageing in the budding yeast *Saccharomyces cerevisiae* is usually assessed by the measurement of replicative and/or chronological lifespan. Replicative lifespan corresponds to the number of daughter cells produced by an individual mother cell, whereas chronological lifespan measures the survival time of non-dividing cells in the stationary phase (Mirisola et al., 2014). *S. cerevisiae* possesses two RAS homologues, *RAS1* and *RAS2* (Fig. 3A). Deletion of *RAS1* extends replicative lifespan while that of *RAS2* extends chronological lifespan (Longo and Fabrizio, 2012). Similarly, genetic deletion of *CDC25*, encoding the yeast orthologue of mammalian SOS, which stimulates the nucleotide exchange of RAS proteins for their activation, results in ~50% increase in chronological lifespan (Lin et al., 2000).

RAS signalling in yeast is responsible for relaying nutrient inputs into cell growth and division (Fontana et al., 2010). In the presence of nutrients, activation of RAS-cAMP-PKA signalling mediates the activation of a pro-growth program, which partially
depends on the inhibition of the stress-responsive transcription factors MSN2/4 (Conrad et al., 2014). Conversely, downregulation of the RAS–cAMP–PKA pathway activates MSN2/4 to enhance cellular protection by activating stress responsive genes such as heat-shock proteins, superoxide dismutase, catalase and autophagy (Box 1)-related factors (Martínez-Pastor et al., 1996; Fabrizio et al., 2004; Mizuno and Irie, 2021). Activation of MSN2/4 is required for the effects of reduced RAS–cAMP–PKA signalling on chronological lifespan (Fabrizio et al., 2004) and may also be required for replicative lifespan extension in response to RAS–cAMP–PKA inhibition (Medvedik et al., 2007).

The transcriptional activity of MSN2/4 is regulated through direct phosphorylation by the serine/threonine kinase effector of PKA, RIM15 (Lee et al., 2013). RIM15 is activated in response to low RAS–cAMP–PKA signalling and is required for the chronological lifespan extension of Ras2 deletion mutants (Wei et al., 2008). RIM15 activity is also regulated by SCh9, the yeast homologue of AKT/S6K. RIM15 translocation to the nucleus is required for RIM15-dependent activation of MSN2/4 and SCh9 directly inhibits the nuclear localisation of RIM15 through an unidentified mechanism (Pedruzi et al., 2003). Moreover, genetic deletion of SCh9 extends replicative lifespan in yeast, which requires both RIM15 and MSN2/4 (Kaeberlein et al., 2005). RAS proteins in yeast also directly activate the adenylyl cyclase CYR1, genetic deletion of which is associated with extension of chronological lifespan (Fabrizio et al., 2001).

**Drosophila**

In *Drosophila*, RAS signalling functions downstream of reduced IIS in modulating lifespan. IRS proteins couple insulin/IGF-1 receptor stimulation to the activation of downstream molecules (Fig. 3B) and recruit Sos to the activated receptor through associations with the GRB2 adaptor protein [Dreadlocks (Drk)]. *Drosophila* express a single IRS protein, Chico. Mutation of Chico within the proposed Drk binding site disrupts Chico–Drk interactions *in vitro* (Slack et al., 2015). The same mutation within a transgenic genomic rescue construct failed to rescue the lifespan extension associated with *chico* null mutation (Slack et al., 2015).

Mimicking the effects of reduced RAS signalling by manipulating the expression of its transcriptional effectors, either by expression of an activated form of Aop or by RNA interference (RNAi)-mediated knockdown of Pnt within the adult *Drosophila* fat body (Box 1), is sufficient to extend lifespan (Alic et al., 2014). Aop activation is also required for the extension of lifespan induced by loss-of-function mutations in *chico* (Slack et al., 2015). Moreover, direct inhibition of RAS activity itself within the adult fly, either by RNAi or by overexpressing a dominant-negative isoform, extends lifespan in *Drosophila* (Slack et al., 2015). Inhibition of RAS signalling also alleviates the pathophysiological effects of neuronal mitochondrial dysfunction in a *Drosophila* model of familial Parkinson’s disease, in which loss-of-function mutations in Ras85D rescued the locomotory defects of *park* homozygous mutant flies (Duncan et al., 2018).

Surprisingly, the effects of RAS/ MAPK suppression on neuronal mitochondrial dysfunction phenotypes were recapitulated with RNAi-mediated knockdown of either Aop or Pnt, which are generally considered act antagonistically in the canonical model of RAS/MAPK signalling. However, in *Drosophila* motor neurons, Aop and Pnt appear to both act as positive effectors (Duncan et al., 2018). Thus, although direct inhibition of RAS/MAPK signalling offers beneficial outcomes in models of neuronal mitochondrial dysfunction and during normal ageing, Aop may help.
have different functions in these two scenarios. Indeed, RNAi-mediated knockdown of Aop specifically in adult neurons can be beneficial in models of neuronal mitochondrial dysfunction but significantly shortens lifespan in normal flies (Dobson et al., 2019). This could reflect differential regulation of Aop across different tissues. For example, Aop stability in neurons is differentially regulated by RAS/MAPK signalling depending on their differentiation state (Peláez et al., 2015). Of course, RAS/MAPK signalling has additional upstream inputs from other RTKs as well as IR activation, and these may contribute to the differential regulation of RAS/MAPK transcriptional effectors such as Aop. For example, expression of a dominant-negative isoform of Egrf, but not of IR, in the intestinal enterocytes of adult flies extends lifespan, similarly to the expression of activated Aop in the same cells (Dobson et al., 2019). Furthermore, signalling via RAS/MAPK has additional transcriptional outputs, and tissue-restricted expression or regulation of these other RAS-responsive transcription factors in these two model systems might regulate the transcriptional targets with the worm FOXO orthologue, DAF-16 (Thyagarajan et al., 2010). Therefore, despite RAS signalling having different roles during ageing in C. elegans and flies, ETS transcription factors in these two model systems may therefore account for the differential effects of RAS inhibition on lifespan.

Despite the absence of a direct orthologue of Aop in C. elegans, the transcription factor ETS-4 may represent a functional homologue. Epistasis analysis using loss-of-function alleles for ets-4 suggests that it acts in a parallel pathway to DAF-2 during ageing (Thyagarajan et al., 2010). ETS-4 activity is limiting for worm lifespan and restoring ETS-4 expression specifically within the intestine in ets-4 mutants is sufficient to rescue lifespan defects (Thyagarajan et al., 2010). Similar to Aop and dFOXO in Drosophila, ETS-4 also shares transcriptional targets with the worm FOXO orthologue, DAF-16 (Thyagarajan et al., 2010). Therefore, despite RAS signalling having different roles during ageing in C. elegans and flies, ETS transcription factors in these two model systems might regulate the expression of common target genes that affect lifespan.

The importance of LET-60 signalling for vulval integrity might also affect the ability of let-60 loss of function to increase lifespan in C. elegans. Loss of vulval integrity leads to decreased lifespan, whereas preventing vulval defects correlates with longevity (Leiser et al., 2016). The ETS transcription factor LIN-31 is a vulval-specific effector of LET-60 signalling in worms.

**Fig. 2. Regulation of the ETS transcription factors Aop and Pnt in response to RAS/MAPK signalling in Drosophila.** (A) Activation of RAS/MAPK signalling leads to the phosphorylation and activation of ERK, which translocates to the nucleus and interacts with the ETS transcription factors Aop and Pnt. Phosphorylation by ERK activates Pnt, which then induces the expression of RAS-responsive genes. Conversely, phosphorylation of Aop inactivates it, preventing its association with the regulatory regions of its target genes and promoting its nuclear exclusion. In parallel, activation of PI3K/AKT signalling leads to phosphorylation of dFOXO and promotes its nuclear exclusion. (B) When RAS/MAPK signalling is inactive, ERK is not phosphorylated and therefore does not enter the nucleus, which leads to inactivation of Pnt and activation of Aop. Aop functions as a transcriptional repressor of RAS-responsive genes. In parallel, inactivation of PI3K/AKT leads to loss of dFOXO phosphorylation. In the absence of phosphorylation, dFOXO remains in the nucleus, where it cooperatively binds with Aop to mediate the expression of dFOXO target genes. Aop, Anterior open; dFOXO, Forkhead box class O; ERK, extracellular signal-regulated kinase; ETS, E-twenty-six; GDP, guanosine diphosphate; GTP, guanosine triphosphate; MAPK, mitogen-activated protein kinase; P, phosphorylation; PI3K, phosphoinositide-3 kinase; Pnt, Pointed.
LIN-31 forms a complex with LIN-1, which inhibits vulval induction. However, LIN-31 is directly phosphorylated by MPK-1, the worm orthologue of ERK, which disrupts the LIN-1/LIN-31 complex and thus promotes vulval cell fates (Tan et al., 1998). Ubiquitous loss of LET-60 signalling in reduction-of-function mutants might therefore decrease worm lifespan through the loss of vulval integrity via disruptions to LIN-1/LIN-31 activity. Restricting the reduction of LET-60 signalling to specific tissues may therefore bypass the detrimental effects of LET-60 reduction of function in the vulva. Similar to observations in flies, the spatial activation of different transcriptional mediators of RAS/MAPK signalling could influence its capacity to extend lifespan (Dobson et al., 2019).

Mammals
A role for the inhibition of RAS signalling in mammalian ageing has yet to be determined. Mammals have four members of the RAS protein family, NRAS, HRAS, KRAS4A and KRAS4B, which are expressed from three genes. KRAS4A and KRAS4B are isoforms expressed from the same Kras gene (Fig. 3D). Knockout of the entire Kras locus in mice is embryonic lethal (Johnson et al., 1997). However, mouse knockouts for Nras, Hras and Kras4A, the latter of which still expresses a functional KRAS4B protein, are adult viable. In addition, lifespan experiments have only been reported for Kras4A deletion, which shows no obvious effects on survival (Umanoff et al., 1995; Ise et al., 2000; Plowman et al., 2003). Indirect inhibition of HRAS has been achieved by genetic knockout of Rasgrf1, which encodes the GEF RASGRF1 that stimulates the GDP to GTP exchange on and subsequent activation of HRAS, as well as members of the RAS-related protein (R-RAS) and RAC families of small GTPases (Fernández-Medarde and Santos, 2011). Rasgrf1 homozygous knockouts show ∼20% increases in both average and maximal lifespan compared to control animals (Borrás et al., 2011). Interestingly, lifespan extension was also observed in
tumour-free animals, suggesting that the increased survival of Rasgrp1 knockouts was not simply a consequence of reduced tumour incidence (Borràs et al., 2011).

In humans, mutations in HRAS are associated with Costello syndrome, a rare genetic condition characterised by short stature, developmental delay and distinctive facial features (Gripp et al., 2019). Costello syndrome patients also show multiple physiological disorders associated with premature ageing, including osteoporosis and osteopenia (Gripp et al., 2019). An allelic variant of HRAS1, consisting of a variable number of 28 bp tandem repeats downstream of the coding region, shows reduced frequency in centenarians compared to younger controls (Bonafe et al., 2002). Such alleles of HRAS1 are proposed to contain binding sites for NF-κB, a key stress-responsive transcription factor and inflammatory mediator (Bonafe et al., 2002). Analysis of copy-number variants associated with mortality at old age identified a deletion that encompasses HRAS1 that was consistently associated with higher mortality at older ages across three different populations (Kuningas et. al., 2011). However, this deletion is located in a gene-rich region, and other genes located here or in close proximity might also be relevant. In the search for longevity-determining genes in humans, genome-wide association studies have so far not found an association between allelic variants in RAS genes and longer lifespan. However, variants of HRAS1 are associated with exceptional longevity when they occur in combination with certain allelic variants of two other genes, APOE and LASS1 (also known as CERS1) (Jazwinski et al., 2010). Moreover, allelic combinations of the three genes were not only associated with longevity, but also with reduced incidences of self-reported functional deficits in study participants (Jazwinski et al., 2010).

Thus, genetic interactions between HRAS1 and other longevity-associated genes such as APOE may play an important role in the genetic effects on ageing in humans.

In summary, emerging evidence from genetic studies in both invertebrate and mammalian models of ageing and age-related disease, as well as genomic studies in human populations, have identified a role for RAS/MAPK signalling in ageing and age-related health. Further exploration of these functions of RAS/MAPK signalling may offer new therapeutic targets to improve healthy ageing. Furthermore, pharmacological inhibition of the pathway to improve healthy ageing will be critical for clinical translation.

From genetics to intervention
A better understanding of the genetic pathways that underlie ageing has helped to identify molecular targets for pharmacological interventions that might both extend lifespan and improve age-related health. Small molecule RAS pathway inhibitors have been developed as anti-cancer therapeutics to target hyperactivating mutations in key RAS/MAPK signalling components that drive unregulated cell proliferation, several of which are already in clinical use (Table 1). Here, we discuss the repurposing (Box 1) of these compounds, along with other molecular inhibitors of RAS/MAPK signalling, as geroprotectors. We also highlight the challenges and limitations that this repurposing raises. We have specifically chosen compounds that have demonstrated clear inhibition of RAS/MAPK signalling, either directly or indirectly, and that have also shown effects on ageing or age-related health in model systems.

Trametinib
Trametinib is a specific inhibitor of MEK and is currently used clinically to treat some forms of metastatic melanomas (Hoffner and Bench, 2018). Oral administration of trametinib to adult Drosophila is reported to extend their median lifespan by 8-12% when added directly to their food at concentrations of 1.56-15.6 μM (Slack et al., 2015). Moreover, feeding 15.6 μM of trametinib to flies in later life, from 30 days of age, was sufficient to extend adult flies’ lifespan, albeit to a smaller extent than continual exposure. Later-life onset of treatment increased median lifespan by ~4% (Slack et al., 2015). Trametinib is one of several clinical MEK inhibitors (Han et al., 2021), but it has yet to be determined whether its effects on Drosophila lifespan are drug specific. Recent structural and biochemical studies of trametinib have identified some important differences in the mechanism of action of trametinib compared to other allosteric inhibitors (Box 1) of MEK. For example, trametinib acts more potently on MEK when it is complexed with RAF rather than on free MEK (Gonzalez-Del Pino et al., 2021), whereas other MEK inhibitors induce the formation of these MEK/RAF complexes in KRAS-mutant lung cancer cell lines, which prevents durable inhibition of ERK (Lito et al., 2014). Crystal structures of trametinib bound to MEK-kinase suppressor of RAS (KSR) complexes have identified binding sites for trametinib not only in the typical allosteric binding pocket of MEK, but also where MEK interacts with KSR, an interaction that is not observed with other MEK inhibitors (Khan et al., 2020). It will therefore be interesting to determine whether the lifespan-extending effects of trametinib can be recapitulated by other MEK inhibitors.

The mechanisms by which trametinib extends lifespan in Drosophila are not fully understood, but recent work points to the maintenance of translational fidelity as one possibility. Loss of proteostasis is one of the widely accepted hallmarks of ageing and is affected by the accuracy of translation (López-Otín et al., 2013). In S. cerevisiae, treatment with paromomycin or nourseothricin, which both induce translational errors by increasing the frequencies of stop-codon read-through and amino acid misincorporation, also accelerates chronological ageing. Moreover, the same study showed that mutation of RPS2, encoding the 40S ribosomal protein S2, also showed increased translational errors and reduced lifespan (von der Haar et al., 2017). Mutation of the yeast MRPS12, which encodes the S12 orthologue of the mitoribosome, reduces the frequency of translational errors in both mitochondrial and cytosolic proteins and extends chronological lifespan (Suhm et al., 2018). Similarly, in worms and flies, mutation of orthologues encoding RPS23, a key protein in the ribosomal decoding centre, leads to more accurate translation and longer lifespan (Martinez-Miguel et al., 2021).

Martinez-Miguel and colleagues analysed stop codon read-through and amino acid misincorporations in Drosophila S2R+ cells to show that 5 nM and 10 nM trametinib can reduce translational errors in vitro (Martinez-Miguel et al., 2021). However, the authors did not test whether these same doses of trametinib had any effect on lifespan, or examine translational error rates in flies treated with trametinib at doses previously determined to be conducive to lifespan extension. Moreover, the direct consequences of fewer translational errors during ageing are not well understood, and the effects of trametinib on translational error have yet to be determined in mammals.

Pharmacological inhibition of MEK using trametinib has been shown to reduce insulin resistance in obese but genetically wild-type mice and in genetically obese (ob/ob) mice, which suggests that RAS inhibition might affect metabolic regulation during ageing (Banks et al., 2015). Trametinib treatment is also associated with reduced cellular senescence in vitro, another key hallmark of ageing (López-Otín et al., 2013). Laterre and colleagues have shown that the...
treatment of senescent normal human dermal fibroblasts (NHDF) cells with either 1 µM or 10 µM trametinib resulted in a robust, dose-dependent decrease in the proportion of senescent cells in culture. Furthermore, treatment with trametinib was able to negate the prosenescent effects of the ERK agonist ceramide in these cells (Latorre et al., 2017). The expression of mRNA splicing factors shows age-dependent changes in human peripheral blood leukocytes (Harries et al., 2011), and similar gene expression changes were observed by Latorre and colleagues in senescent NHDF cells (Latorre et al., 2017). Interestingly, alongside its effects on cellular senescence, trametinib treatment in NHDF cells also restored the expression of multiple splicing factors to levels seen in early-passage cultures (Latorre et al., 2017). Investigations into the effects of trametinib on cellular senescence and translational fidelity during ageing in vivo would therefore be interesting to explore further.

**Dihydromyricetin**
The flavonoid dihydromyricetin (DHM), extracted from the *Ampelopsis* plant, has a range of antioxidant, anti-inflammatory and anti-carcinogenic effects, and has been used in traditional Chinese medicine to treat pyretic fever, cough, pain and jaundice (Li et al., 2017). Its administration in rodent models of age-related neurodegeneration improved multiple phenotypes associated with disease. For example, in both rat and mouse models of Alzheimer’s disease, DHM improved cognitive function and reduced neuronal cell loss (Liang et al., 2014; Sun et al., 2019). Similarly, in mouse models of Parkinson’s disease, treatment with DHM protected against loss of dopaminergic neurons and associated behavioural deficits (Ren et al., 2016; Wu et al., 2019).

In *Drosophila*, feeding adult flies with 40 µM DHM can extend median lifespan by ~16% and maximum lifespan by ~6%. These effects were associated with decreased levels of phosphorylated ERK and AKT, and activation of both Aop and dFOXO (Fan et al., 2021). Furthermore, similarly to trametinib, late-life exposure to 40 µM DHM from 30 days of age was also found to increase fly lifespan, although to a lesser extent than chronic exposure to the same dose throughout adulthood (Fan et al., 2021).

Autophagy is an evolutionarily conserved cellular recycling mechanism that maintains protein and organelle quality by targeting cellular components for lysosomal degradation. In worms and flies, upregulation of autophagy by overexpression of key regulatory factors increases lifespan (Barbosa et al., 2019). Pharmacological inhibition of RAS/MAPK signalling using the covalent KRASG12C inhibitor ARS-853, the MEK inhibitors trametinib or cobimetinib,

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**Table 1. Small molecule inhibition of RAS/MAPK signalling and effects on lifespan and age-related conditions**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>RAS pathway effect</th>
<th>Effect in model organism</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Trametinib</td>
<td>MEK ↓</td>
<td>↑ Lifespan</td>
<td>Slack et al., 2015</td>
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<td></td>
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<td>↓ Translational error</td>
<td>Matrinez-Miguel et al., 2021</td>
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<td>↓ Senescence</td>
<td>Latorre et al., 2017</td>
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<td>Acarbose</td>
<td>RAS ↓</td>
<td>↑ Lifespan</td>
<td>Harrison et al., 2019</td>
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<td>↓ Inflammation, atherosclerosis</td>
<td>Chan et al., 2016b</td>
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<td>↓ Myocardial infarction</td>
<td>Zhang et al., 2021</td>
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<td>↓ All-cause death in type II diabetics</td>
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<tr>
<td>Dihydromyricetin</td>
<td>ERK ↓</td>
<td>↑ Lifespan, autophagy</td>
<td>Fan et al., 2021</td>
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<tr>
<td>Metformin</td>
<td>?</td>
<td>↑ Lifespan</td>
<td>Martin-Montalvo et al., 2013</td>
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<tr>
<td>ERK ↓</td>
<td></td>
<td>↓ Huntington’s disease phenotype</td>
<td>Sanchis et al., 2019</td>
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<tr>
<td>RAS ↓</td>
<td></td>
<td>↓ Cancer growth</td>
<td>Iglesias et al., 2013</td>
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<td>↑ Lifespan</td>
<td>Cabreiro et al., 2013</td>
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<tr>
<td>Statins</td>
<td>RAS ↓</td>
<td>↑ Lifespan</td>
<td>Liao and Laufs, 2005; Spindler et al., 2012</td>
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<td>↓ Arhythmias</td>
<td>Spindler et al., 2012</td>
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Small molecule inhibitors that directly or indirectly inhibit RAS/MAPK signalling, as discussed in this Review, have been shown to extend lifespan or alleviate age-associated pathological conditions in different model organisms and, to a limited extent, patients.
or the ERK1/2 (also known as MAPK3/1) inhibitor SCH772984 increases autophagic flux (Bryant et al., 2019; Kinsey et al., 2019). These long-lived DHM-treated flies also showed increased resistance to starvation, a reduction in age-related intestinal dysfunction, and increased expression of the autophagy-related genes Atg1, Atg5 and Atg8a, indicating that DHM might promote longevity and improve healthspan in part by upregulating autophagy (Fan et al., 2021).

**Statins**

Statins lower circulating levels of low-density lipoprotein and cholesterol and prevent dyslipidaemia (Liao and Laufs, 2005), and so are often used clinically to prevent cardiovascular disease. Statins are highly specific inhibitors of HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Such isoprenoids are required for the post-translational farnesylation and geranylgeranylation (Box 1) of proteins, and especially of small signalling GTPases, including RAS itself (Bonetti, 2003). These post-translational modifications are crucial for RAS activation and membrane anchoring via prenylation (Box 1). Statins reduce the prenylation of both RAS and Rho in endothelial cell culture, leading to the accumulation of inactive forms of both proteins in the cytoplasm (Liao and Laufs, 2005).

Oral administration of 240 µM simvastatin to adult male flies increased their mean lifespan by ~25%, an effect that was associated with decreased levels of active farnesylated and geranylgeranylated forms of both RAS and Rab4 (Spindler et al., 2012). Furthermore, the direct inhibition of protein prenylation by L744832, a specific farnesyl transferase inhibitor, and by GGTI-298, a specific geranylgeranyl transferase inhibitor, also increased fly lifespan to a similar extent (Spindler et al., 2012). Simvastatin not only increased lifespan but also improved healthspan in *Drosophila* by decreasing heart arrhythmias in old male flies. Thus, RAS signalling inhibition by simvastatin may increase longevity by improving heart health (Spindler et al., 2012).

Simvastatin and a closely related analogue, lovastatin, also extend the lifespan of *C. elegans* at relatively low doses of 25 µM, 50 µM and 100 µM (Jahn et al., 2020). Ageing in worms is associated with the accumulation of fluorescent pigments such as lipofuscin, formed from oxidised and cross-linked proteins, lipids and carbohydrates, and advanced glycation end-products formed from the non-enzymatic addition of sugar to free amino groups of proteins (Son et al., 2019). Treatment with lovastatin significantly reduced the accumulation of these fluorescent pigments during ageing, demonstrating that statins act on biomarkers of ageing in worms, not just on lifespan (Jahn et al., 2020). In this study, the reduction of ageing pigment accumulation and lifespan extension induced by statins depended on DAF-16 activity (Jahn et al., 2020). However, direct effects on RAS prenylation in response to these treatments have yet to be established in worms.

Statins may also improve healthspan by preventing cancer. Statins activate endoplasmic reticulum stress via inhibition of RAS prenylation, leading to enhanced immune responses against KRAS-mutant cancer cell lines and against KRAS-mutant tumours in the lung and liver of genetically engineered mouse models (Nam et al., 2021). The exposure of blood cancer cells to simvastatin has been shown to decrease phosphorylated ERK levels and to suppress RAS prenylation in human acute promyelocytic leukaemia (HL-60) cells and human histiocytic lymphoma (U937) cells in culture, subsequently inducing cell death (Fujiwara et al., 2017). Furthermore, a recent epidemiological study found an association between statin use and reduced risk of cancer incidences and cancer-related morbidities in patients with heart failure (Ren et al., 2021).

**Acarbose**

Acarbose is a complex oligosaccharide that acts as an inhibitor of alpha-glucosidase and alpha-amyrase, digestive enzymes that are responsible for the breakdown of complex carbohydrates in the intestine. By inhibiting these enzymes, acarbose limits the absorption of dietary carbohydrates, thereby limiting associated increases in blood glucose levels and subsequent insulin release. Owing to these properties, acarbose is routinely used in conjunction with metformin, exercise and diet to treat type 2 diabetes (Martin and Montgomery, 1996; Hsu et al., 2018).

Activated RAS supports the proliferation and migration of vascular smooth muscle cells, thereby propagating atherosclerosis (Yu et al., 2018). Treating vascular smooth muscle cells (A7r5) in culture with increasing doses of acarbose for 24 h and 48 h resulted in both dose- and time-dependent inhibition of their proliferation (Yu et al., 2018). These effects on cell proliferation were associated with decreased levels of RAS protein, as well as with a reduction in phosphorylated ERK levels, indicating that acarbose decreases atherosclerosis by targeting RAS signalling (Yu et al., 2018). Acarbose is thought to regulate RAS protein expression through microRNA (miRNA)-dependent regulation of gene expression. Acarbose upregulates the expression of miR-143, a specific miRNA that targets transcripts directly to regulate expression of RAS proteins (Wang et al., 2014; Akao et al., 2018).

Confirming *in vitro* findings (Yu et al., 2018), acarbose also inhibits RAS and the development of atherosclerosis in rabbits fed a high-cholesterol diet. Oral administration of acarbose to these rabbits at 2.5 mg/kg and 5 mg/kg resulted in a significant decrease in the levels of the inflammatory marker TNF-α, as well as a dose-dependent decrease in RAS protein levels (Chen et al., 2016b). Acarbose also increases the median lifespan of male mice by 16-17% and that of female mice by 4-5% (Harrison et al., 2019). In humans, acarbose therapy significantly reduced the incidence of myocardial infarctions and all-cause deaths in patients with type 2 diabetes, suggesting that acarbose can improve health and thus potentially extend lifespan (Zhang et al., 2021). Currently, acarbose is in Phase 2 clinical trials to study its effects during ageing in individuals aged 60-100 years (Garay, 2021).

**Metformin**

Metformin is a first-line medication for the treatment of type 2 diabetes, particularly in overweight individuals, and is also frequently used to treat polycystic ovary syndrome and to prevent nephrotoxicity ( Nasri and Rafieian-Kopaei, 2014). Metformin functions as an anti-hyperglycaemic drug by lowering blood glucose concentration without causing hypoglycaemia. Metformin also has anti-tumour properties in RAS-dependent cancers. Exposure of human Hec1A endometrial carcinoma cells or Panc1 pancreatic cancer cells, which both carry RAS-hyperactivating mutations, to metformin *in vitro* results in the removal of the constitutively active RAS from the plasma membrane and translocation into the cytoplasm, thereby inhibiting RAS signalling and cell proliferation (Iglesias et al., 2013; Nair et al., 2014). Metformin also downregulates specificity protein (Sp) transcription factors in Panc1 cells *in vitro* to inhibit the EGF-dependent activation of RAS (Nair et al., 2014).
Recently, metformin treatment was shown to extend lifespan in several model systems. In C. elegans, exposure to metformin at concentrations of 50 mM and 100 mM resulted in a 40% longer median lifespan but no increase in maximum lifespan (Onken and Driscoll, 2010; Cabreiro et al., 2013). Similarly, exposure to phenformin, a closely related but more potent bioguanide, also extended lifespan in worms when used at doses of 3 mM and 4.5 mM (Cabreiro et al., 2013). The effects of metformin on worm lifespan were dependent on AMP-activated protein kinase (AMPK) activation. AMPK is a major regulator of energy metabolism and is activated in response to changes in the cellular ATP:AMP ratio (Long, 2006). Activated ERK can directly phosphorylate AMPK on Ser485 to inhibit its activity (López-Cotarelo et al., 2015; Owens et al., 2021), while dephosphorylation of AMPK at Thr172, an activation site, is associated with stress-induced ERK activation (Kodíha et al., 2007). Thus, the effects of metformin on lifespan in C. elegans may result from relieving ERK-dependent AMPK inhibition.

However, these metformin effects on lifespan appear not to be conserved in Drosophila. Even though metformin activates AMPK, and the genetic activation of AMPK increases lifespan in Drosophila, metformin-treated flies did not show extended lifespan at any dose, and exhibited perturbations in intestinal homeostasis and toxicity at high doses (Stensen et al., 2013; Slack et al., 2012). In rodent models, metformin has been shown to extend lifespan in both male and female mice, and the effects were generally greater in female mice (Anisimov et al., 2008). Similarly, chronic treatment of female C3H/Sn mice with phenformin extended both mean and maximum lifespan by ~20% and reduced the incidence of spontaneous mammary tumours in these animals (Anisimov et al., 2003).

However, the mouse strains used in these studies were relatively short-lived and prone to developing cancer. More recent studies using longer-lived and genetically outbred strains have provided less conclusive results, suggesting that metformin’s effects on lifespan might be context-dependent, with more dramatic effects observed under conditions that shortened lifespan (Martin-Montalvo et al., 2013; Strong et al., 2016). Metformin might also protect against age-related disease in animal models. In a mouse model of Huntington’s disease, 20 mg/kg oral metformin per day prevented the upregulation of phosphorylated ERK in the cortex (Sanchis et al., 2019). Thus, metformin might alleviate Huntington’s disease phenotypes in mice, at least in part by preventing RAS/MAPK signal induction (Sanchis et al., 2019).

Conclusions and future perspectives
The significant increase in life expectancy within the past century poses important socioeconomic concerns, requiring novel strategies to maintain health and well-being amongst an increasingly older global population. Ageing research in model organisms has led to the rapid identification and genetic dissection of key ageing pathways, including the RAS/MAPK signalling pathway. Genetic interventions that modulate signalling through RAS proteins and their downstream effectors have been shown to increase lifespan in these model systems and to improve multiple parameters of health, both during normal ageing and in animal models of age-related disease.

RAS/MAPK signalling therefore adds to an emerging theme that manipulating cancer-promoting pathways, either by inhibiting the function of oncogenes or by increasing the activity of tumour suppressors, can affect healthy ageing. Examples from rodent models that extend lifespan include Myc haploinsufficiency (Hofmann et al., 2015), extra genomic copies of the Ink4/Arf locus, that elevate expression of its encoded tumour suppressor proteins p16 and p14 (Matheu et al., 2009), and increased gene dosage of the tumour suppressor Pten (Ortega-Molina et al., 2012), or mimicking its activation through genetic inhibition of its direct target, PI3K (Foukas et al., 2013). Interestingly, although some these models had reduced cancer incidence, researchers also observed lifespan extension in cancer-free animals, suggesting that delayed ageing may not simply be a consequence of treatment against cancer.

Nevertheless, the prominent role of RAS/MAPK signalling in cancer has led to the isolation of several small molecule inhibitors of the pathway, and some are already in clinical use. As discussed in this Review, recent work with model organisms suggests that these same compounds may provide beneficial effects on age-related health. Thus, repurposing these anti-cancer treatments could provide a useful strategy to develop novel interventions to promote healthy ageing. Moreover, other pro-longevity pharmacological interventions may elicit their effects on ageing and age-related health, at least in part through perturbations in RAS/MAPK signal transduction. It should be noted, though, that compounds like metformin, acarbose, DHM and statins have much broader effects, and, although they have an impact on RAS/MAPK outputs, they do not exclusively target this pathway.

However, cancer therapeutics, including small molecule inhibitors of RAS/MAPK signalling, are notoriously toxic and can elicit severe side effects when administered for cancer treatment, particularly in older patients. Older cancer patients do not tolerate anti-cancer treatments well and experience increased occurrences of adverse events and side effects, like poor appetite and anaemia (de Glas, 2020). Furthermore, accelerated ageing phenotypes, including secondary cancers, frailty, cognitive impairment and premature mortality, have been reported in cancer survivors across all age groups after treatment (Wang et al., 2021). In addition, the prolonged use of MEK inhibitors, in particular during cancer treatment, can lead to adaptive drug resistance (Kun et al., 2021), with studies reporting that chronic exposure to MEK inhibitors leads to drug resistance in tumour cells associated with upregulation of oncogenic gene expression (Vergani et al., 2020).

Effective strategies to limit these adverse effects will therefore be essential for clinical implementation. Determining the critical time periods during the life course when RAS/MAPK inhibition modulates ageing, and assessing the effect of intermittent dosing, could be one such approach to minimise side effects. Careful titration will also help to provide the geroprotective effects of these drugs without side effects or drug resistance. For example, the effective dose of trametinib that produces geroprotective effects in Drosophila is estimated to be lower than those used clinically for cancer treatment (Slack et al., 2015). Additionally, trametinib administration in mice can promote healthy metabolic effects at much lower doses than those used in tumour models (Banks et al., 2015). The effective doses of these compounds as geroprotectors may therefore be much lower than those for cancer treatment. Calibration of therapeutic drug dosage for ageing may not, however, be straightforward. The degree of RAS/MAPK inhibition needed to affect ageing and healthspan is currently unclear. In cancer models, loss of ERK phosphorylation post-treatment is the primary readout for drug potency, and a high degree of ERK inhibition correlates well with treatment efficacy. The same correlation between degree of ERK inhibition and geroprotection has yet to be established in pre-clinical models of ageing.
Identifying the tissues for which RAS/MAPK signalling is limiting for lifespan may also allow for more targeted treatments to reduce side effects. Similarly, further characterisation of the downstream effector targets that mediate the geroprotective response of RAS/MAPK inhibition will provide important evidence of the extent to which inhibiting this pathway could improve health during ageing. Recently, the co-administration of three longevity-promoting compounds, lithium, ramipril and trametinib, at doses that maximised lifespan extension, extended lifespan in an additive fashion in Drosophila. This might be because they produce stronger inhibition of the same targets or because they function at least in part through non-overlapping mechanisms (Castillo-Quan et al., 2019). It would also be interesting to investigate the effect of modulating the function of GAPS to inactivate RAS during ageing, because currently RAS GAPS that promote the RAS-GTP to RAS-GDP exchange have primarily been studied in cancer (Prieto-Dominguez et al., 2019; Gray et al., 2020).

Intriguingly, the pro-longevity effects observed upon genetic inhibition of other cancer promoting pathways suggest that repurposing existing cancer therapeutics as geroprotective agents could extend beyond RAS/MAPK inhibitors. Indeed, recent studies have shown that pharmacological inhibition of AKT using oridonin, a diterpenoid isolated from Rabdosia rubescens, prolongs yeast lifespan, extends lifespan and improves multiple parameters of healthspan in mice, including grip strength, cardiac health and kidney function, and inhibits cellular senescence in cultured human fibroblasts (An et al., 2022). A small molecule inhibitor of AKT, GSK690693, also extends Drosophila lifespan (Cheng et al., 2022).

Pre-clinical investigations using models of ageing could therefore lead to the development of new drugs or to the repurposing of existing drugs as novel geroprotec tors. That either direct or indirect pharmacological inhibition of RAS/MAPK signalling has already been shown to improve healthspan suggests that this approach offers promising new clinical strategies to improve late-life health in humans. Addressing the challenges of clinical translation of these findings, however, is imperative to ensure their safety and success.

Competing interests
The authors declare no competing or financial interests.

References
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