Ageing Research Reviews 71 (2021) 101443

Contents lists available at ScienceDirect

Ageing Research Reviews

journal homepage: www.elsevier.com/locate/arr

Review

p21-Activated kinase 1 (PAK1) in aging and longevity: An overview

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ARTICLE INFO

Keywords:
p21-Activated kinases
RAC1/CDC42
PAK1
Caenorhabditis elegans
DAF-16/FOXO
Longevity

ABSTRACT

The p21-activated kinases (PAKs) belong to serine/threonine kinases family, regulated by ~21 kDa small signaling G proteins RAC1 and CDC42. The mammalian PAK family comprises six members (PAK1–6) that are classified into two groups (I and II) based on their domain architecture and regulatory mechanisms. PAKs are implicated in a wide range of cellular functions. PAK1 has recently attracted increasing attention owing to its involvement in oncogenesis, tumor progression, and metastasis as well as several life-limiting diseases and pathological conditions. In *Caenorhabditis elegans*, PAK1 functions limit the lifespan under basal conditions by inhibiting forkhead transcription factor DAF-16. Interestingly, PAK depletion extended longevity and attenuated the onset of age-related phenotypes in a premature-aging mouse model and delayed senescence in mammalian fibroblasts. These observations implicate PAKs as not only oncogenic but also aging kinases. Therefore, PAK-targeting genetic and/or pharmacological interventions, particularly PAK1-targeting, could be a viable strategy for developing cancer therapies with relatively no side effects and promoting healthy longevity. This review describes PAK family proteins, their biological functions, and their role in regulating aging and longevity using *C. elegans*. Moreover, we discuss the effect of small-molecule PAK1 inhibitors on the lifespan and healthspan of *C. elegans*.

1. Introduction

p21-activated kinases (PAKs) are members of a family of serine/threonine protein kinases that are central to cellular regulation and signal transduction (Hofmann, 2004; Kumar et al., 2006; Manser et al., 1994). PAKs regulate a wide range of cellular functions, including cytoskeletal actin assembly, neurite outgrowth, cell migration, apoptosis, cell cycle, survival, gene transcription, hormone signaling, and mitogen-activated protein kinase (MAPK) pathways (Bokoch, 2003; D rijard, 1995; Rudel, 1997; Sells et al., 1999; Zhang et al., 1995). In mammals, six PAK isoforms have been identified, and they are classified into two groups, namely group I and group II, based on their domain architecture and regulatory mechanisms. PAK1 (PAKα), PAK2 (PAKγ), and PAK3 (PAKβ) are group I PAKs, and PAK4, PAK5, and PAK6 are group II PAKs. Group I PAKs contain the CDC42/RAC interactive binding (CRIB) domain, whereas group II PAKs do not contain this domain (Bokoch, 2003; Hofmann, 2004). All six PAK isoforms contain a highly conserved C-terminal catalytic kinase domain and an N-terminal regulatory domain. The regulatory domain of group I PAKs contains an autoinhibitory switch domain (AID) in addition to the CRIB domain; however, group II PAKs do not contain a defined AID, specifying the absence of an identical regulatory mechanism. PAKs are activated by small GTPases known as Ras-related C3 botulinum toxin substrate 1 (RAC1) and cell division control protein 42 (CDC42) that are important mediators of Rho GTPase signaling; group I PAKs are activated by binding of RAC1 and CDC42, whereas group II PAKs are activated independent of Rho GTPases. Upon GTPase binding, PAK4 and PAK6 show no increase in kinase activity; however, whether the kinase activity of full-length PAK5 is regulated by active RAC1/CDC42 remains to be explored. Indeed, when compared to the full-length protein, the kinase domains of group II PAKs are reportedly more active in isolation. Taken together, these findings indicate that the kinase activity of group II PAKs is regulated through an intramolecular switch that is entirely different from the mode of activation of group I PAKs (Bokoch, 2003; Dummler et al., 2009; Hofmann, 2004; Kumar et al., 2006). Nevertheless, the complete regulatory mechanisms of group II PAKs remain to be fully understood.

The precise functions of group I and group II PAKs have been studied using loss-of-function and knockout mice models (Hofmann, 2004). The deletion of *pak2* or *pak4* results in early embryonic lethality, whereas

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https://doi.org/10.1016/j.arr.2021.101443
Received 5 April 2021; Received in revised form 26 July 2021; Accepted 10 August 2021
Available online 12 August 2021

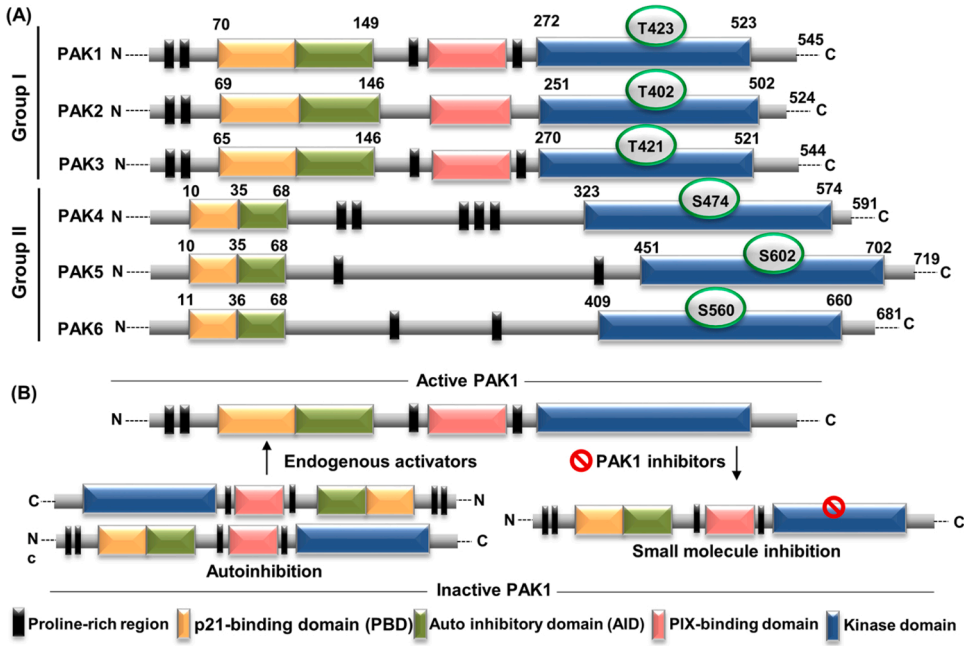

Fig. 1. Structural organization of group I and group II PAK domains.

pak1, *pak3*, and *pak5* null mutants are healthy, viable and fertile, indicating functional non-complementarity between group-I and -II PAKs. The embryonic lethality observed in the *pak2* and *pak4* null mutants is mainly due to fetal cardiac defects, abnormalities in axonal outgrowth, and abnormal neuronal development (Qu et al., 2003). Though *pak3* mutants are healthy, they exhibit learning and memory deficits (Hofmann, 2004). PAKs are expressed in several tissue types; PAK1, PAK3, and PAK5 are expressed in the brain, while PAK2 is expressed ubiquitously. High PAK4 and PAK6 expression has been observed in the testes, prostate, and large intestine (Hofmann, 2004). Apart from their indispensable role in vital cellular functions, PAKs are implicated in the progression of aging and age-related diseases. The dysregulated expression of PAKs is often associated with several types of cancer in humans. In particular, PAK1 and PAK4 genes are frequently upregulated in cancer, and their expression level is regulated by several intracellular signal transduction mechanisms (Kumar and Li, 2016; Radu et al., 2014; Rane and Minden, 2019). Among others, PAK1 has recently attracted increasing attention owing to its involvement in oncogenesis and the

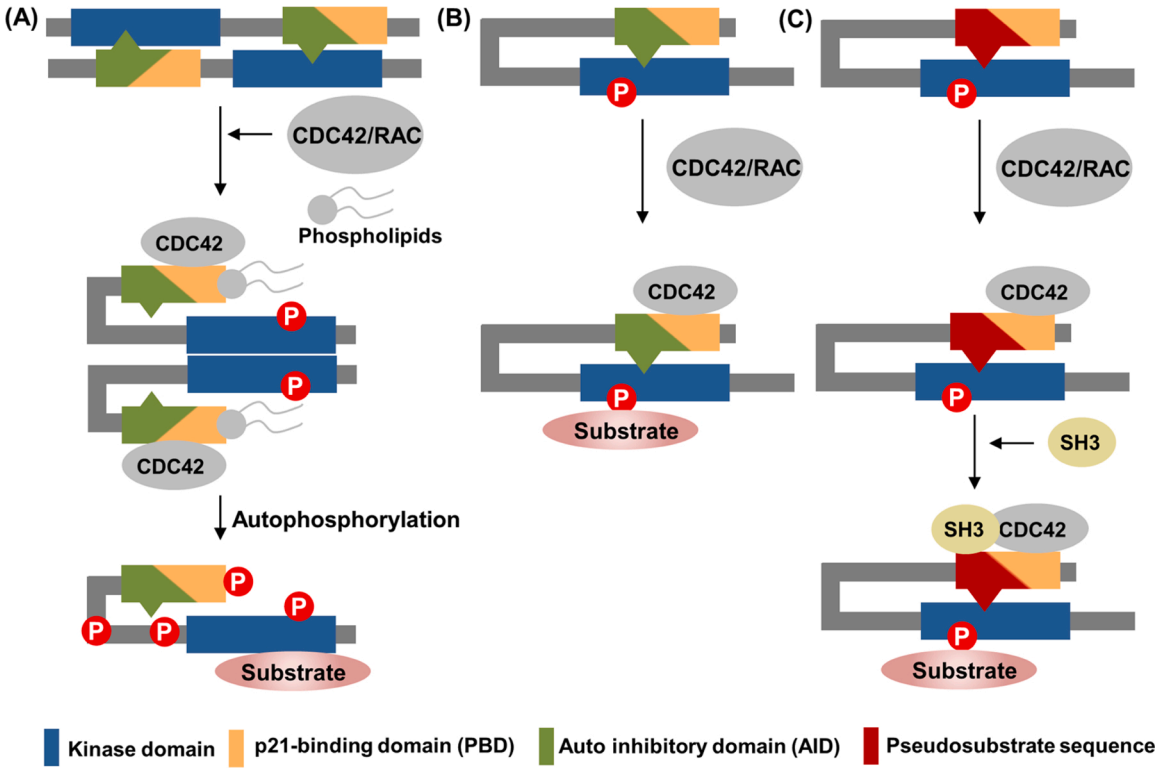

Fig. 2. Mechanisms of activation of (**A**) group I (*i.e.*, PAK1) and (**B, C**) group II (*i.e.*, PAK4) PAKs.

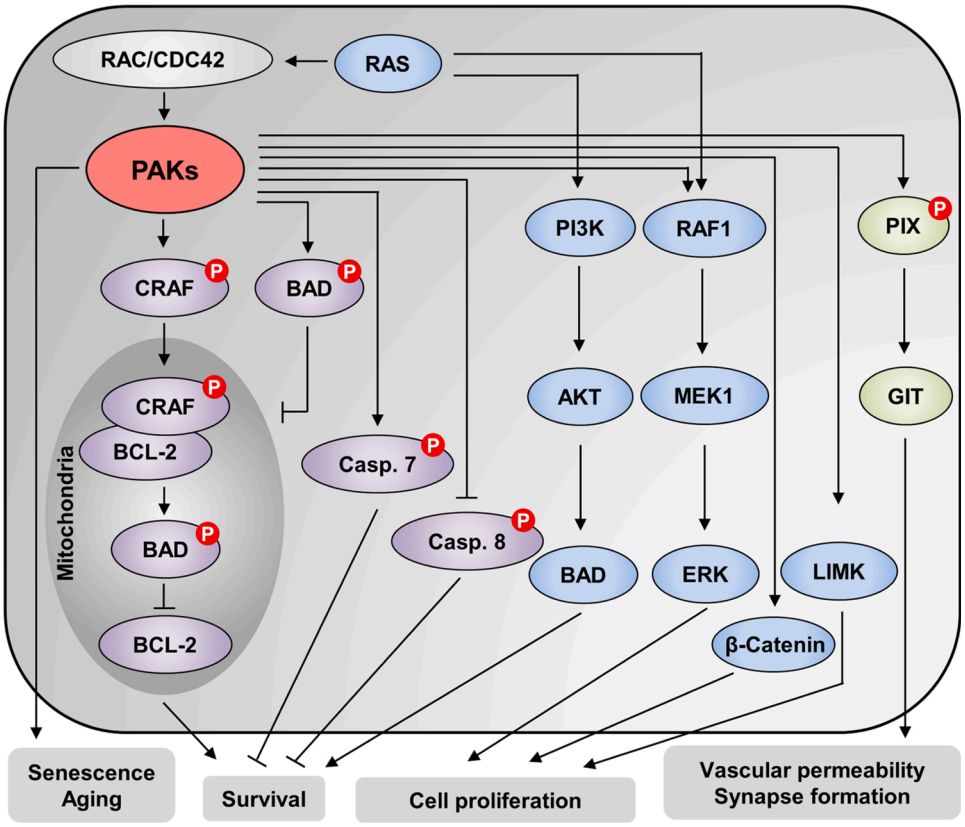

Fig. 3. The role of PAKs in regulating a wide range of cellular functions. Several components and overlapping are avoided for simplicity. P, phosphate; Casp. 7, caspase 7; Casp. 8, caspase 8.

aging process. In *C. elegans*, PAK1 functions limit the lifespan under basal conditions by inhibiting the expression of *daf-16* and its direct transcriptional readout *hsp-16.2*. The inhibition of *pak-1* extends the lifespan of *C. elegans* up to 60 % when compared to its wild-type counterparts (Yanase et al., 2013). In the mammalian model, deletion of *pak1* reduced the average tumor size, protected against the development of sensorineural hearing loss, and extended the lifespan of the genetically engineered mouse model of neurofibromatosis 2 (Hawley et al., 2021). Interestingly, it was found that PAK2 promotes cellular senescence and organismal aging (Lee et al., 2019b). *pak2* depletion delayed oxidative stress-induced senescence in mouse embryonic fibroblasts (IMR90) and oncogene-induced senescence in human fibroblasts, while overexpression of *pak2* accelerated the senescence phenotype in IMR90 cells. Moreover, *pak2* depletion prolongs the lifespan and delays the onset of age-associated phenotypes in the BubR1 hypomorphic (BubR1H/H) premature aging (progeria) mouse model. In addition, *pak2* deficiency increased the body fat mass and delayed cataract formation in progeroid mice (Lee et al., 2019b). In primary fibroblasts, activation of *pak4* promotes premature senescence, and it appears to be mediated by the ERK pathway (Cammarano et al., 2005). These observations suggest that PAK1 and PAK2 regulate the expression of several gerontogenes and thereby accelerate the aging process in *C. elegans* and mammals. The activity and expression rate of PAKs are also dysregulated in age-related neurodegenerative diseases (NDDs), including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). Both gain and loss of PAK activity have been detected in NDD mouse models and postmortem brains of patients with NDDs (Civiero et al., 2015; Civiero and Greggio, 2018; Kim et al., 2016b; Luo et al., 2008; Luo and Rubinsztein, 2009; Ma et al., 2013; Zhao et al., 2006), suggesting the involvement of PAKs in maintaining brain homeostasis and aging.

This review describes PAK family proteins, their biological functions, and their role in regulating aging and longevity. We have used the multifaceted *Caenorhabditis elegans*, a premier animal model in aging research, to provide an in-depth understanding of the roles of PAKs and suggestions for future research.

2. p21-Activated kinases (PAKs)

2.1. PAK's structural domains and activation mechanisms

From a structural point of view, all PAKs contain a p21-binding domain (PBD) at the amino-terminus for the GTPase association (also known as CRIB), an AID, and a kinase domain at the carboxyl-terminus (Fig. 1A). Group-I and -II PAKs have a highly conserved carboxyl-terminus serine/threonine kinase domain with a single phosphorylation site, and their regulatory regions (PBD and AID) are structurally distinct, specifying the absence of an identical regulatory mechanism. Among all PAKs, PAK1 is the most studied member, which comprises a total of 545 residues, including regulatory domain (PBD: residues 75–105; AID: residues 70–149) and kinase domain (residues 272–523) (Fig. 1A). Several endogenous activators and inhibitors (endogenous or small molecule) directly regulates the activity of PAKs (Fig. 1B) and are discussed in Section 2.4. The activation mechanism of PAK family members has been discussed extensively in several previous articles (Eswaran et al., 2008; Kumar et al., 2017; Radu et al., 2014; Rane and Minden, 2014; Yao et al., 2020); therefore, we have briefly summarized the mechanism of PAK activation here. Group I PAKs are regulated through a *trans* autoinhibition mechanism (Eswaran et al., 2008; Lei et al., 2000). The amino-terminus PBD overlaps with an AID, and the activity of group I PAKs (*i.e.*, PAK1; Fig. 2A) are modified through a reciprocal autoinhibitory mechanism; wherein two PAK molecules fold into an inactive homodimer. During folding, the PBD-AID complex binds to the kinase domain of another PAK monomer, occupies the cleft of the kinase domain, and stabilizes inactive catalytic sites. Upon binding of

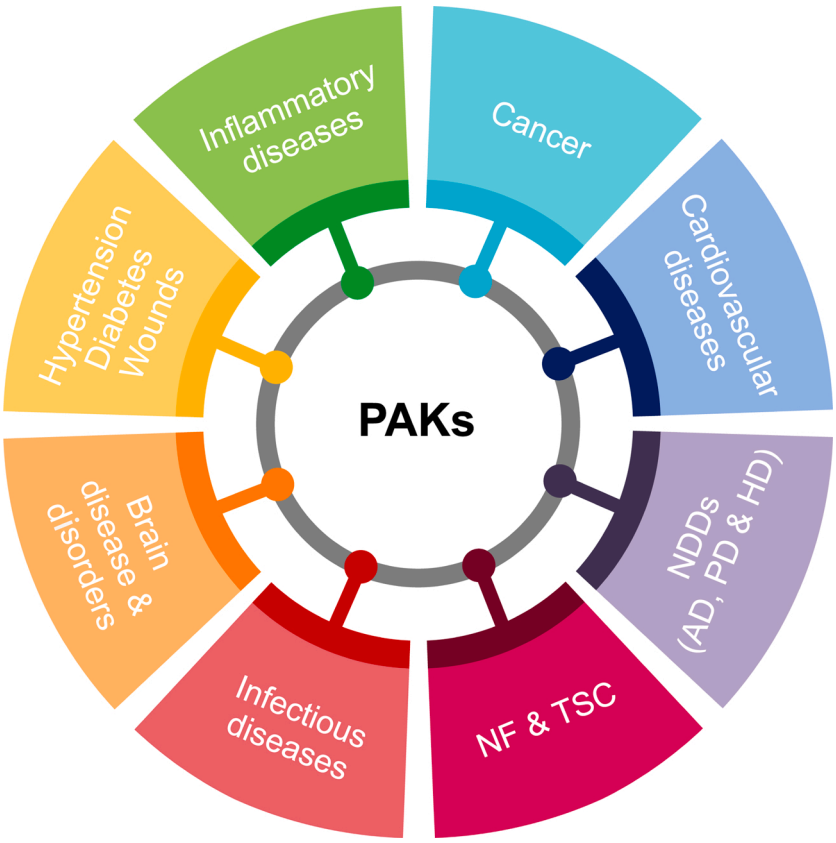

Fig. 4. PAKs in age-related diseases and disorders. AD, Alzheimer's disease; HD, Huntington's disease; NDD, neurodegenerative diseases; PD, Parkinson's disease; TSC, tuberous sclerosis complex.

the Rho GTPases, including CDC42 and Rac1, to the PBD and binding of phosphoinositide to an amino acid rich proximal segment disrupts the dimer that leads to dissociation of the AID from the kinase domain, induce conformational changes, and stimulates kinase activity (Lei et al., 2000). As a result, each PAK monomer undergoes autophosphorylation and becomes competent to phosphorylate their substrates. After binding to a substrate, PAKs can autophosphorylate at several sites, which could stabilize this catalytically active state and prevent the kinase from returning to an inactive conformation (Buchwald et al., 2001). The autophosphorylation residue of PAK1 (T423), PAK2 (T402), and PAK3 (T421) is found in the activation loop of the kinase domain and was known to act as a key determinant of PAK activation by RAC1/CDC42 GTPases or other GTPase-independent mechanisms (Zenke et al., 1999). The additional mechanisms reportedly regulate PAK activity are trans-phosphorylation by other kinases and the binding of SRC-homology 3 (SH3) domain-containing proteins, phospholipids, sphingosine, and β isoform of PAK-interactive exchange factor (β-PIX) (Bokoch et al., 1998; Buchwald et al., 2001; Lei et al., 2000).

By contrast, the activation mechanisms of group II PAKs are entirely different. At the N-terminus, group II PAKs contain the PBD followed by an AID-like pseudosubstrate sequence, in contrast to the group I, which inactivates the kinase domain in *cis* until CDC42-binds to PBD (Fig. 2B). As seen in Fig. 2C, an alternative model indicates that group II PAKs are inhibited by a pseudosubstrate domain located within the regulatory domain at the N-terminus. In this model, the binding of the SH3 motif-containing protein to the pseudosubstrate domain triggers the release of catalytic domain and stimulates the kinase activity of PAK (Ha et al., 2012). The autophosphorylation residue of PAK4 (S474), PAK5 (S602), and PAK6 (S560) is located within the activation loop of group II PAKs and play an essential role in regulating the functions and activity of these kinases (Fig. 1A). Similar to the model proposed for PAK4 activation, it is assumed that PAK5 and PAK6 follow the model; however, the exact mechanisms by which group II PAKs are activated rather unclear.

2.2. PAKs as multifunctional signal transducers

PAKs transmit signals from integrin cell-adhesion complexes, G-protein coupled receptors (GPCRs), bioactive lipids, and growth factor receptors to various signaling cascades that control dynamic physiological functions (Fig. 3). Among the PAK family members, PAK1 and PAK4 are key modulators of cytoskeletal reorganization through mutually operated overlapping signaling cascades. PAK1 regulates cytoskeletal reorganization through several reported substrates, including cofilin, filamin, and actin-related protein 2/3 complex41 kDa subunit (p41-ARC). Moreover, PAK1 induces membrane ruffle formation, filopodia, and lamellipodia through LIM domain kinase (LIMK) and mixed lineage kinase (MLK), thus directing cell motility during development (Arias-Romero and Chernoff, 2008; Bokoch, 2003). Additionally, PAK1 regulates, *via* phosphorylation, the chromatin interaction of integrin-linked kinase and its nucleo-cytoplasmic shuttling that is involved in cytoskeletal reorganization, adhesion-dependent signaling, cell motility, and tumor development (Bokoch, 2003; Lardennois et al., 2019). PAK4 regulates actin depolarization through cofilin family proteins, including LIMK, slingshot phosphatase, scaffolding 14-3-3 protein, and actin. In addition, PAK4-mediated phosphorylation of LIMK and slingshot phosphatase reciprocally controls the actin dynamics by inhibiting and stimulating their activity, respectively. PAK4 and PAK5 also control cytoskeletal alterations essential for promoting neurite outgrowth in the adrenergic cell line N1E-115 derived from mouse neuroblastoma, possibly by inhibiting the action of RAC1/CDC42 GTPases (Dan et al., 2002). Although RAC1 and CDC42 are known to be the most predominant upstream PAK regulators, accumulating evidence indicates the involvement of Rho guanine nucleotide exchange factors

(RhoGEFs) in PAK activation, at least in cytoskeletal remodeling. PAK1 phosphorylates neuroepithelial cell-transforming gene 1 protein and guanine nucleotide exchange factor H1 to suppress their activity; this subsequently downregulates stress fiber formation and RAC-, CDC42-, and Rho-mediated signaling, respectively (Bokoch, 2003; Hofmann, 2004). The α and β isoforms of PAK-interactive exchange factor (PIX) are well-established PAK1-interacting partners that regulate several cellular functions; in particular, α-PIX functions in association with PAK3 in synapse formation, dendritic spine morphogenesis and plasticity, memory, and epithelial wound healing (Zegers, 2003; Zhang, 2005).

In addition, most PAK family proteins regulate cell death, a dynamic process that suppresses tumor development through several mechanisms. Except PAK3 and PAK6, all PAKs promote the survival of cells through phosphorylation-induced inactivation of BCL2-associated agonist of cell death (BAD) (Dummler et al., 2009; Kumar et al., 2006; Radu et al., 2014). BAD is a pro-apoptotic member of the B-cell lymphoma 2 (Bcl-2) family and is directly involved in initiating apoptosis. PAK1 phosphorylates Raf-1 at Ser-338 and Ser-339, resulting in its translocation to the mitochondria where Raf-1 phosphorylates BAD at Ser-112. In general, Raf-1 prevents cell apoptosis by translocating to the mitochondria, where it binds to Bcl-2 and replaces BAD independent of ERK and MEK signaling. Upon phosphorylation by PAK1, the translocated Raf-1 initiates an interaction with BAD, disrupting the BAD–Bcl-xL and BAD–Bcl-2 interactions in the mitochondria (Jin et al., 2005). Among group II PAKs, PAK4 and PAK5 control apoptosis by phosphorylating BAD specifically at Ser-112, resulting in the decreased release of cytochrome c, a pivotal event in caspase-dependent apoptotic cell death. Furthermore, PAK4 promotes cell survival by antagonizing the activation of initiator caspase 8 in response to ultraviolet irradiation and tumor necrosis factor α (Gnesutta and Minden, 2003). In contrast to its anti-apoptotic role, PAK4 mediates premature senescence. PAK4 expression is upregulated by several stimuli that are known to promote cellular senescence, and loss of *pak4* leads to a decrease in senescence and an increase in cell growth. Premature senescence in response to PAK4 is mediated by ERK–MAPK and cell cycle regulatory proteins, including $p16^{INK4a}$, $p19^{ARF}$, p53, and $p21^{CIP}$ (Cammarano et al., 2005). PAK1 is the most studied PAK till date, and has recently attracted increasing attention, mainly because it is not only involved in oncogenesis, tumor progression, and metastasis, but also in several life-limiting diseases and pathological conditions.

2.3. PAKs in age-related diseases

Aging is an unavoidable natural phenomenon characterized by a gradual functional decline at the molecular to organismal levels. Several molecular and cellular declines, including cellular senescence, telomere erosion, genomic instability, epigenetic alterations, mitochondrial dysfunction, stem cell exhaustion, decreased intracellular communications, and loss of protein homeostasis, have been identified to cause aging. These deteriorations are the primary risk factors for several human ailments, including cancer, cardiovascular diseases, arthritis, diabetes, and NDDs (López-Otín et al., 2013). Accumulating evidence indicates the involvement of PAK family proteins in age-related diseases (Fig. 4). However, these kinases are most frequently associated with several cancer types. Although PAKs are not often mutated in cancers, their dysregulated expression, particularly overexpression, is associated with cancerous phenotypes in humans. *PAK1* and *PAK4* are frequently upregulated in cancer, and their expression levels are regulated by several signaling mechanisms. In cancer, PAK1 and PAK4 have been associated with uncontrolled cell proliferation, invasion, increased metastasis, immune regulation, altered intracellular signaling, and drug resistance. At present, PAK-related therapy seems to be a self-evident treatment option for cancer, as PAKs contribute to the onset, progression, and persistence of the disease. Notably, there are several published reports on PAKs and their role in cancer (Eswaran et al., 2008; He and Maruta, 2013; Kumar et al., 2017, 2006; Radu et al., 2014; Rane and Minden, 2019; Yao et al., 2020). In addition to its critical role in oncogenesis, PAK impairment has been implicated in cardiovascular diseases (Taglieri et al., 2014; Wang et al., 2018b), atherosclerosis (Singh et al., 2015), immune function and infectious diseases (John Von Freyend et al., 2017; Maruta, 2013; Pacheco and Chernoff, 2010), inflammatory diseases (Taglieri et al., 2014), neuronal physiology and brain function (Civiero and Greggio, 2018; Kreis and Barnier, 2009), wound healing (Zegers, 2008), and brain diseases and disorders, including mental retardation, autism, schizophrenia, depression, epilepsy, glioma, retinoblastoma, neurofibromatosis, and tuberous sclerosis (Maruta and Messerli, 2013). Recently, a new role of PAK1 in the maintenance of cardiac function and adipose tissue homeostasis in aged mice was reported (Batra et al., 2021). Wild-type female mice exhibited increased cardiac PAK1 expression, preserved systole and diastole parameters, an enlarged left atrium, and thickening of the posterior wall during the aging process compared to young females. In contrast, global deletion of PAK1 ($PAK1^{-/-}$) leads to atrial enlargement, cardiac hypertrophy, diastolic dysfunction, and increased adiposity as they grow to middle age. Another study demonstrated the novel role of PAK1 in counteracting stress-induced cardiac hypertrophy (Liu et al., 2011b). These results support the importance of PAK1 signaling in the regulation of cardiac function and adipose tissue homeostasis during aging.

Aging is the leading risk factor for the onset and development of most NDDs. Several genetic factors are involved in the regulation of aging and age-associated NDDs. Deregulated PAK activity has been observed in multiple brain disorders (Civiero and Greggio, 2018). Compelling evidence indicates the involvement of PAK family proteins in age-related NDDs, including AD (Krivinko et al., 2017; Ma et al., 2008; Zhao et al., 2006), PD (Civiero et al., 2015; Kim et al., 2016b; Won et al., 2016), and HD (Luo et al., 2008; Luo and Rubinsztein, 2009). Both gain and loss of PAK activity have been detected in NDD mouse models and postmortem brains of patients with NDDs, suggesting the involvement of PAKs in maintaining brain homeostasis (Civiero et al., 2015; Civiero and Greggio, 2018; Kim et al., 2016b; Luo et al., 2008; Luo and Rubinsztein, 2009; Ma et al., 2013; Zhao et al., 2006). This is not surprising considering the involvement of PAKs in several signal transduction pathways that regulate brain physiology and their high expression levels in the central nervous system (CNS) (Civiero and Greggio, 2018; Ma et al., 2013; Zhao et al., 2006). A missense mutation in PAK3 is linked to severe X-linked nonspecific mental retardation. The first evidence of altered PAK activity and their expression levels in NDDs was reported in a study on the postmortem hippocampus and temporal cortex tissues from the brains of patients with AD (Zhao et al., 2006). AD is an incurable and progressive neurodegenerative disorder characterized by the overproduction and deposition of extracellular plaques composed of amyloid-β peptides (Aβ) and insoluble aggregates of hyperphosphorylated microtubule-binding tau protein known as neurofibrillary tangles. AD is the leading cause of dementia in the elderly worldwide. In 2006, Zhao et al. reported the reduced activity of PAK1 and PAK3, redistributed and reduced phosphor-PAKs, and changes in cofilin and drebrin content in the hippocampus of patients with AD postmortem and higher Aβ-producing APPswe AD-mouse model. Moreover, pharmacological inhibition of PAK causes similar memory impairment, drebrin loss, and cofilin pathology in adult mice (Zhao et al., 2006). The same group demonstrated that PAK1 is aberrantly activated and translocated from the cytosol to the membrane in the brains of AD transgenic mice and patients with AD. In addition, $A\beta_{42}$ oligomer-treated cultured hippocampal neurons exhibited similar effects, accompanied by reduced dendrites that were protected by kinase-active PAK1. Notably, anti-Aβ antibody and curcumin ameliorate aberrant translocation of PAKs (PAK1−3) in $A\beta_{42}$ oligomer-treated cultured hippocampal neurons and aged transgenic mice (TgI2576) with AD pathology by targeting upstream factors, such as Src family Fyn kinase, phosphatidylinositol-3 kinase (PI3K), T-cell lymphoma invasion and metastasis 1 (Tiam1), calcium/calmodulin-dependent protein kinase II (CaMKII), and Rac (Ma et al., 2008). In addition, PAK

inactivation increased the phosphorylation ratio of tau protein, enhanced dendritic spine defects, impaired social behavior, and aggravated anxious behavior; however, it had no effect on $A\beta_{40}$ and $A\beta_{42}$ levels in a heterozygous triple-transgenic mouse model of AD (Bories et al., 2017). Furthermore, dominant-negative PAK mutations cause spinal defects and memory impairment in the AD mouse model. Several studies support the notion that dysfunction of PAK activity and its expression rate are involved in the pathological events of AD (Bories et al., 2017; Civiero and Greggio, 2018; Ma et al., 2013, 2008). In contrast, increased phosphorylation rate of PAKs, specifically group 1 PAKs, was observed in the brain tissue samples of APPswe/PSEN1dE9 transgenic mice, suggesting that pharmacological inhibition of group I PAKs is a possible therapeutic approach in AD (Krivinko et al., 2017).

The activity and expression rate of PAKs are also dysregulated in PD (MIM 168600), the second most common devastating neurological condition in the elderly after AD. Neuronal loss in the pars compacta of the substantia nigra and typical aggregation of intracytoplasmic inclusions (Lewy bodies), mainly comprising α-synuclein, in the brain tissues are the two pathological hallmarks of PD. The loss of dopaminergic (DAergic) neurotransmission induces severe motor dysfunction (postural instability, resting tremors, muscle rigidity, and bradykinesia) and behavioral and cognitive disorders, including anxiety, depression, dementia, and insomnia. Recently, Kim et al. discovered the role of PAK1 and the molecular mechanism of neuronal death involved in PD pathogenesis (Kim et al., 2016b). The authors proposed that the expression of a dominant-negative PAK1 mutation decreased the cell viability to 35 %, enhanced apoptotic cell death, and increased cell vulnerability to oxidative stressors, such as 6-hydroxydopamine (6-OHDA) and hydrogen peroxide (H_2O_2), thus confirming the role of PAK1 in apoptosis regulation. Inactivation of PAK1 reduced the Bcl-2 level, which is directly mediated by PAK1 and/or PAK1/ERK signaling. Conversely, the expression of the PAK1 active form (PAK1-CA, $PAK1^{T423E}$) recovered the 6-OHDA-induced behavioral deficits in hemiparkinsonian Sprague–Dawley rats as well as DAergic neurodegeneration in the substantia nigra (Kim et al., 2016b). The same group recently reported the key role of PAK4 in DAergic neuronal survival. The authors found, through immunohistochemical and western blot analyses, that PAK4 activity was markedly decreased in the brain of rodent models of PD as well as in patients with PD. They further demonstrated that the mutant form of constitutively active PAK4 ($PAK4^{S445N/S474E}$) significantly reduced motor dysfunction and protected DAergic neurons in both α-synuclein- and 6-OHDA-induced rat models of PD. This protective effect of the PAK4 active form was mediated by the phosphorylation of CREB (cyclic adenosine 3,5-monophosphate response element-binding protein)-regulated transcription coactivator 1 (CRCTC1) at SER215 (Won et al., 2016), suggesting a new avenue for developing novel therapeutic interventions for PD. Civiero et al. further reported that the interaction between PAK6 and leucine-rich repeat kinase 2 (LRRK2) controls neurite complexity in the mammalian brain; PAK6 positively regulates neurite outgrowth *via* LRRK2. In addition, PAK6 overexpression was observed in postmortem brain tissues from idiopathic and LRRK2 G2019S carriers, while LRRK2 mutation reduced PAK6 expression and phosphorylation of PAK6 substrates (Civiero et al., 2015).

Dysregulated PAK activity has also been observed in HD, an autosomal dominant inherited NDD caused by abnormal expansion of CAG trinucleotide into exon 1 of the Huntington gene (*HTT*) that encodes a ubiquitously expressed protein whose functions are still a matter of debate. The mutant Huntington protein (mHTT) has an expanded polyglutamine tract (polyQ) at the N-terminus that causes protein aggregation and subsequent proteotoxicity, leading to progressive alterations in motor, psychiatric, and cognitive functions. However, the exact mechanism of cellular toxicity induced by polyQ repeats remains unclear. A team of investigators from the Cambridge Institute for Medical Research, UK, explored the interaction of wild-type HTT with PAK1 and PAK2 isoforms from a pathological and physiological perspective (Luo et al., 2008; Luo and Rubinsztein, 2009). PAK1 binds and colocalizes with mHTT aggregates in cultured striatal and cortical neurons, SK-N-SH neuroblastoma cells, and brain tissues from patients with HD. Moreover, PAK1 overexpression enhanced mHTT aggregation and HTT self-interaction. The authors showed that PAK1 overexpression further enhanced HTT toxicity in cell models and neurons, while its knockdown suppressed mHTT-induced cellular toxicity, aggregate formation, and cell death (Luo et al., 2008). Thus, pharmacological interventions that target PAK1 activation might suppress HTT aggregation and its associated pathologies, although in-depth investigations are essential to shed more light on the therapeutic potential and molecular mechanism of PAK1 in HD. Interestingly, wild-type HTT interacts with PAK2, and its overexpression inhibits caspase-3- and caspase-8-mediated cleavage of PAK2, thus promoting cell survival (Luo and Rubinsztein, 2009).

Collectively, PAK family proteins are highly expressed in the mammalian brain, indicating their importance in preserving CNS homeostasis. Biochemical, behavioral, and mechanistic evidence highlights the contribution of altered PAK activity and expression levels to the pathogenesis of age-related NDDs, indicating that these PAK proteins are targets for genetic and/or pharmacological interventions. Nevertheless, future studies are needed to investigate whether activation or inhibition of PAKs is a valuable solution to delay or halt the onset and progression of these NDDs.

2.4. Targeting PAKs—In search of a happy ending

The rationale for targeting specific PAK family proteins to treat diseases has been established considering their regulatory roles as molecular switches in multiple signaling pathways in health and disease. Therefore, the development of efficacious genetic and/or pharmacological inhibitors that suppress the aberrant activities of PAKs might be an effective strategy for treating PAK-associated pathological conditions. Protein kinases are attractive drug targets, and approximately 30 US FDA-approved small molecule kinase inhibitors are readily available in the market. Most PAK blockers are either ATP-competitive or allosteric inhibitors. ATP-competitive inhibitors bind to the active pocket of the kinase, whereas allosteric inhibitors bind to an allosteric pocket adjacent to the ATP-binding site (Wu et al., 2015). This section summarizes a few selective PAK blockers that have promising therapeutic applications against PAK-related diseases, including aging and age-associated diseases.

2.4.1. Group I PAK inhibitors

Among the group I PAK family members, PAK1 has emerged as a potential therapeutic target because of its involvement in many oncogenic signaling pathways and pathogenic conditions (Kumar et al., 2006; Radu et al., 2014). Considering the wide-spectrum biological role of PAK1, the last two decades have seen significant efforts by academic, industrial, and pharmaceutical research groups in the identification and development of natural/synthetic PAK1 blockers. The size and shape of the ATP-binding pocket in the kinase domain of PAK1 completely differ from those of other PAK proteins, thus making it a unique target for structure-based pharmacological inhibitors. Several approaches for designing PAK1 blockers have been demonstrated, and some fine-design ATP competitive blockers have been successfully identified. In 2008, Deacon et al. conducted high-throughput screening to identify allosteric (non-ATP-like, uncompetitive) PAK1 inhibitors, considering the conserved nature of the PAK1 catalytic pocket. They screened 33,000 structurally diverse small molecular weight compounds using full-length PAK1 protein activated *in vitro* with GTP-bound CDC42, and identified 2, 20-dihydroxy-1,10-dinaphthyldisulfide (IPA-3), an ATP non-competitive small-molecule inhibitor that selectively stabilizes the autoinhibitory conformation of PAK1 (Deacon et al., 2008). Despite being quite specific towards group I PAKs, IPA-3 is no longer useful as a therapeutic inhibitor owing to its unstable nature. Similarly, small-molecule pyridopyrimidinone compounds, FRAX486, FRAX597,

Table 1
Known endogenous inhibitors of group I and group II PAKs.

Endogenous inhibitor	PAK isoform inhibited	Mechanism	Reference
Cell division cycle 2 (CDC2)	PAK2	Phosphorylates PAK2 and inhibits its kinase activity	(Delsert et al., 2000)
C-terminal kinase domain of the PITSLRE proteins (p110C)	PAK1	p110C binds with regulatory and kinase domain in PAK1, thereby inhibiting its functions	(Chen et al., 2003)
Cysteine-rich inhibitor of Pak1 (CRIPak)	PAK1	CRIPak inhibits PAK1 by interacting with the N-terminal regulatory domain of PAK1. CRIPak inhibits PAK1-mediated LIM kinase activation and enhanced estrogen receptor transactivation	(Talukder et al., 2006)
Human PAK-interacting protein 1 (hPIP1)	PAK1	hPIP1 interaction abolishes the RAC1/CDC42-stimulated kinase activity through the N-terminal regulatory domains of PAK1	(Xia et al., 2001)
LKB1	PAK1	Inhibits PAK1 activation *via* Thr109 phosphorylation	(Deguchi et al., 2010)
Merlin/ neurofibromin 2 (NF2)	PAK1	Merlin binds to the PAK1 p21-binding domain (PBD) and inhibits its recruitment to focal adhesions	(Kissil et al., 2003)
Micro RNAs (miR-7, miR-34b, miR-96, miR-140-5p, miR-145, miR-485-5p, miR-494, miR-15b, miR29a-3p, miR142-3p, miR-142-3p, miR4715-5p, miR-130b, miR-146a, miR-194-3p, miR-302-367, and miR-331-3p)	PAK1	Inhibits PAK1 through translational regulation, RAC1/CDC42 suppression, and other mechanisms	(Yao et al., 2020)
Nischarin	PAK1, PAK4, and PAK5	The amino terminus of nischarin binds to the carboxy-terminal domain of PAKs and inhibits their activity	(Alahari et al., 2004)
Nudt21	PAK1	It inhibits PAK1 expression through its 3′-UTR alternative polyadenylation	(Chu et al., 2019)
p35/CDK5 (Cyclin-dependent kinase 5)	PAK1	Active p35/Cdk5 inhibits PAK1 activity by hyperphosphorylating PAK1 in an RAC-dependent manner	(Nikolic et al., 1998)
PAK18	PAK1	PAK18 blocks PIX–PAK1 interaction and RAS/RAC-induced membrane ruffling of fibroblasts	(Obermeier, 1998)
PP2C-like phosphatases, POPX1 and POPX1 (Partner of PIX1 and PIX2)	PAK1	POPX1 and POPX2 interact with PIX, dephosphorylate PAK, and downregulate the kinase	(Koh et al., 2002)
TAT-/WR-PAK18	PAK1	Blocks RAS/RAC-induced membrane ruffling and Ras-induced tumor development	(He et al., 2001)

and FRAX1036, were developed by Afraxis Inc., (La Jolla, CA) using high-throughput screening. FRAX597 strongly inhibits group I PAKs and was shown to have *in vitro* and *in vivo* anticancer activity in neurofibromatosis type 2 (NF2)-deficient cells, suggesting that inhibition of group I PAKs may be promising for neurofibromatosis treatment considering their indispensable roles in NF1 and NF2. FRAX486 and FRAX1036 showed strong anti-proliferative activity and inhibited the growth of peripheral never-sheath tumors and oncogenic RAC1 mutant melanoma cells. In addition, FRAX486 has had excellent pharmacokinetic properties as it crosses the blood–brain barrier, and single administration of FRAX486 (20 mg/kg) rescued the fragile X mental retardation 1 (*Fmr1*) gene knockdown-induced brain-related pathologies in adult mice (Dolan et al., 2013; Licciulli et al., 2013). Porchia et al. developed the PDK1 inhibitor OSU-03012, a derivative of the cyclooxygenase inhibitor celecoxib, that binds to the PAK1 ATP-binding site. Later, OSU-03012, at low concentrations, was shown to directly inhibit PAK1 phosphorylation in multiple cell lines (Porchia et al., 2007). Recently, OSU-03012 has been shown to inhibit the replication of SARS-CoV-2 by suppressing glucose regulating protein 78 (GRP78), an essential chaperone required for the attachment, entry, and life cycle of most mammalian viruses (Rayner et al., 2020). In 2008, Maksimoska et al. reported that a combination of structure-based design, small-molecule screening, and organoruthenium chemistry to develop specific and more potent PAK1 inhibitors led to the identification of a tetrahedral and octahedral ruthenium compounds DW12 and FL172, respectively. Both compounds showed significant PAK1 inhibition potency at nanomolar concentrations (Maksimoska et al., 2008).

Studies have revealed that certain endogenous proteins and microRNAs (miRNAs) can affect the activity of PAK1 (Kumar et al., 2006). In medical oncology, the regulation of target genes by miRNAs has become a research hotspot. Transcriptional regulation of PAK1 is largely controlled by miRNAs that directly or indirectly suppress PAK1 translation by recognizing PAK1 mRNA and guiding the RNA-induced silencing complex to degrade it. Studies have found that miR-7, miR-34b, miR-96, miR-140-5p, miR-145, miR-485-5p, and miR-494 can block PAK1 activity by directly degrading PAK1 mRNA (Yao et al., 2020). Several miRNAs targeting Rho GTPases RAC1 (miR-142-3p and miR4715-5p) and CDC42 (miR-15b, miR29a-3p, and miR142-3p) inhibit the activity of PAK1. Several other tumor-suppressing miRNAs, including miR-130b, miR-146a, miR-194-3p, miR-302-367, and miR-331-3p, have been shown to inhibit PAK1 through several mechanisms (Yao et al., 2020). Multiple endogenous proteins, other than RAC1 and CDC42, can also regulate PAK1 activity through phosphorylation; MLK 3 triggers the kinase activity of PAK1 *via* phosphorylation of Ser133 and Ser204, LKB1 directly suppresses PAK1 through phosphorylation of The109, and bFGF, CK2, cytoplasmic p27, HER2, Janus kinase 2 (JAK2), and PDK1 affect PAK1 activity *via* phosphorylation (Kumar et al., 2006). The protein–protein interaction network can also regulate PAK1 activity; in particular, CKIP-1, NCK1, PI3K, protein kinase (PKC) iota, P-REX1, and RIT1 activate PAK1 by regulating RAC1/CDC42. Additionally, a number of endogenous upstream regulators, such as BCAR3, BRAF, ELP3, ELP3, estrogen, Etk/Bmx, HLX, JMJD6, LINC00460, lncRNA MALAT1, lncRNA-H19, Net1, and STIL can trigger PAK1 activation. These proteins can regulate PAK1 mainly *via* acetylation, phosphorylation, protein–protein interactions, and transcriptional regulation (Yao et al., 2020). Table 1 summarizes the known endogenous inhibitors of group I and group II PAKs.

Natural products and bioactive phytomolecules are less toxic and possess better anticancer activity than conventional synthetic chemopreventive and chemotherapeutic agents. To reduce the toxic effects of synthetic drugs, scientists are looking for alternative and potentially useful natural products to treat PAK-related diseases. Frondoside A (a sulfated saponin extracted from the orange-footed sea cucumber *Cucumaria frondosa* Gunnerus), Okinawa propolis (the resinous substance from honeycombs found in Okinawa, Japan), and nymphaeols A and C (geranylated flavonoids found in Okinawa propolis) inhibited PAK1-dependent growth of A549 lung cancer cells with IC_{50} values of 2.5 μM, 10 μg/mL, 4 μM, and 7 μM, respectively, suggesting that these natural products could block PAK1 (Nguyen et al., 2017b; Taira et al., 2016). In addition, frondoside A directly inhibited PAK1, LIMK, and AKT *in vitro* at micromolar concentrations and blocked the growth of pancreatic cancer cells and A549 cells (Nguyen et al., 2017b). Moreover, other propolis ingredients, such as flavonoid apigenin, artepillin C,

caffeic acid, and caffeic acid phenethyl ester (CAPE; a natural ester of caffeic acid), blocked PAK1 *in vitro*; however, the biological mechanism underlying the PAK inhibitory potential of these phytochemicals has not yet been fully described. It is interesting to note that Takahashi et al. successfully developed highly cell-permeable 1,2,3-triazolyl esters of caffeic acid and artepillin C using "click chemistry" approach wherein a 1,2,3-triazole ring could be coupled to a COOH group in the presence of a copper catalyzer to form a water-soluble ester (Takahashi et al., 2017). Such 1,2,3-triazolyl esters of artepillin C (15A) and caffeic acid (15C) inhibited the growth of A549 cancer cells more potently (30-fold and 140-fold, respectively) than their parent compound, with IC_{50} values of approximately 250 nM and 225 nM, respectively. Moreover, this esterization process boosts the cell permeability of 15A and 15C up to 8-fold and 70-fold, respectively, compared to that of artepillin C and caffeic acid. A few other well-established anticancer molecules, such as astaxanthin, berberine, curcumin, cucurbitacins, emodin, capsaicin, honokiol, resveratrol, salidroside, and ursolic acid, inhibit the upstream regulators of PAK1 (Maruta, 2014; Maruta and Ahn, 2017); therefore, these phytomolecules may also inhibit PAK1 activity, and currently, we are testing these possibilities using a combination of *in silico*, *in vitro*, and *in vivo* methods. FK228 and CEP-1347 are natural derivatives that strongly block group I PAKs at micromolar concentrations. FK228 (also called romidepsin and marketed as Istodax) is a histone deacetylase inhibitor produced by the soil-dwelling bacterium *Chromobacterium violaceum* that inhibits the growth of RAS-transformed cells and blocks PAK1 activity in RAS cancer and breast cancer cells (Hirokawa et al., 2005). Among natural compounds, FK228 is the most potent PAK1 inhibitor identified to date and is approved by the FDA as a monotherapy for the treatment of cutaneous T-cell lymphoma, a rare malignant solid tumor in T lymphocytes. In an AD mouse model, administration of FK228 together with metformin synergistically reduced AD-associated pathologies and offers neuroprotective and memory-restoring effects (Zeng et al., 2021). CEP-1347, an indolocarbazole non-specific kinase inhibitor, is a synthetic derivative of the ATP antagonist K252a found in broths of *Narcodiopsis* bacterium. CEP-1347 was shown to block JNKs by inhibiting its upstream MLK family proteins in an ATP-competitive manner. *In vitro* conditions, CEP-1347 inhibited PAK1 and MLKs in RAS-transformed cells with an IC_{50} of approximately 1 μM and 20 nM, respectively (Nheu et al., 2002). However, although CEP-1347 displayed better PAK1 selectivity over a number of kinases, including PKA, PKC, PI3K, and myosin light-chain kinase, it was later found to show 100-fold higher selectivity towards MLK3 (Maroney et al., 2001). CEP-1347 suppressed the mHTT-mediated neurotoxicity in multiple model systems (cell line, *D. melanogaster*, and mouse). This compound exerts its beneficial effect by inhibiting pro-apoptotic JNK pathway and restoring the expression of brain-derived neurotrophic factor, a critical neurotrophic factor that is reduced in HD, in R6/2 mice (Apostol et al., 2008). In 2017, Nguyen et al. demonstrated the PAK1 blocking potential of ketorolac and ivermectin. Ketorolac is a non-steroidal anti-inflammatory drug used to relieve moderate-to-severe pain in adults. Ketorolac is a chiral molecule administered as a racemic mixture (50 % S-enantiomer and 50 % R-enantiomer), and blocks the oncogenic RAC1/PAK1/cyclooxygenase-2 activity (COX-2) signaling axis by directly inhibiting the COX enzyme (by S-ketorolac) and RAC1/CDC42 proteins (by R-ketorolac). However, ketorolac has a COOH moiety that hampers its cell permeability; therefore, the 1,2,3-triazolyl ester of ketorolac was synthesized using click chemistry to improve its water solubility. This 1,2,3-triazolyl ester of ketorolac (15 K) exhibited 5000 times more potent anticancer activity against PAK1-dependent growth of B16F10 melanoma cells and A549 cancer cells than ketorolac (Nguyen et al., 2017a). Additionally, the anthelmintic drug ivermectin inhibits PAK1 in human ovarian cancer cells and NF2-deficient Schwannoma cell line with an IC_{50} of approximately 5–20 μM (Hashimoto et al., 2009). Notably, Yoshikawa et al., confirmed that geranylgeranylacetone (GGA) inhibited PAK1-dependent growth of ovarian and colon cancer cells by inducing apoptosis and cell cycle arrest in

Table 2
Natural and synthetic PAK-inhibiting small-molecules.

Natural/synthetic inhibitor	PAK isoform inhibited	Mechanism	Reference
1,2,3-Triazolyl ester of artepillin C (15A) and caffeic acid (15C)	PAK1	Anticancer and anti-PAK1 activity	(Takahashi et al., 2017)
1,2,3-Triazolyl ester of ketorolac (15 K)	PAK1	RAC, COX-2, and PAK1 blocker. Exhibits strong anticancer activity	(Hennig et al., 2019; Nguyen et al., 2017a)
1A-116	PAK1	Inhibits RAC1	(Gonzalez et al., 2017)
2,20-Dihydroxy-1,1′-dinaphthyldisulfide (IPA-3)	Group I PAKs	IPA-3 inhibits group I PAKs (PAK1–3) when the enzyme activators sphingosine or CDC42-GTPγS are added. It does not act *via* cysteine residue nor competition with PAK ATP-binding; allosteric inhibitor	(Deacon et al., 2008)
2-arylamino-4-aryl-pyrimidines	PAK1	ATP competitive inhibitor	(Xu et al., 2013)
AG879	PAK1	Tyrosine kinase inhibitors specific for ErbB2 and FLK-1	(He et al., 2004)
AK963	PAK1	ATP competitive inhibitor	(Zhou et al., 2019)
AZA197	PAK1	CDC42 inhibitor	(Zins et al., 2013)
CEP-1347	Group I PAKs	ATP antagonist; non-selectively inhibits most kinases	(Nheu et al., 2002)
Compound 13	Group I PAKs	ATP competitive inhibitor	(Crawford et al., 2015)
Dibenzodiazepine derivative	PAK1	Allosteric kinase inhibitor	(Karpov et al., 2015)
DW12 and Λ-FL172	PAK1	ATP competitive inhibitor	(Maksimoska et al., 2008)
FK228	PAK1	Histone deacetylase and PAK1 inhibitor	(Hirokawa et al., 2005)
FRAX019, FRAX414, FRAX486, FRAX597, and FRAX1036	Group I PAKs and PAK4	Fragile X mental retardation protein (FMRP) and PAK antagonist; ATP competitive inhibitor	(Dolan et al., 2013; Han et al., 2020; Licciulli et al., 2013)
G-5555	PAK1	Selectively inhibits PAK1; ATP competitive inhibitor	(Ndubaku et al., 2015)
G-9791	Group I PAKs	ATP competitive inhibitor	(Rudolph et al., 2016)
Geranylgeranylacetone (GGA) or Selbex	PAK1	Attenuate Rho activation, and Ras-MAPK activation	(Yoshikawa et al., 2010)
GL-2003 (a water-soluble derivative of AG879)	PAK1	ATP antagonist; ERK inhibitor	(Hirokawa et al., 2007)
Ivermectin	PAK1	ATP competitive inhibitor	(Hashimoto et al., 2009)
K252a	PAK1	ATP competitive inhibitor	
MIA-602	PAK1	Growth hormone-releasing hormone (GHRH)	(Gan et al., 2016)

(*continued on next page*)

Table 2 (*continued*)

Natural/synthetic inhibitor	PAK isoform inhibited	Mechanism	Reference
		receptor antagonist	
Naphtho(hydro)quinone-based small molecules	PAK1 and PAK3	Allosterically inhibit PAK activity by interacting with p21-binding domains	(Kim et al., 2016a)
OSU-03012	PAK1	ATP competitive inhibitor	(Porchia et al., 2007)
PF3758309 (UnPAK309 or PanPAK309)	PAK1 and PAK4	ATP competitive inhibitor	(Murray et al., 2010)
PP1/PP2	PAK1	Inhibits Src family Tyr-kinases, in particular those specific for FYN and HCK	(Hanke et al., 1996; Ma et al., 2008)
Propolis and its active components, including apigenin, caffeic acid, and its ester, artepillin C, and nymphaeol A–C	PAK1	Directly inhibit PAK1 activation; ATP competitive inhibitors	(Messerli et al., 2009; Nguyen et al., 2017b; Taira et al., 2016; Way et al., 2004; Xu et al., 2005)
Ruthenium phthalimide complex	PAK1	ATP competitive inhibitor	(Blanck et al., 2012)
ST2001	PAK1	ATP antagonist	(Nheu et al., 2002)
Staurosporine	PAK1	ATP competitive inhibitor	(Karaman et al., 2008)
Triptolide and Minnelide (a water-soluble phosphoryl derivative of Triptolide)	PAK1	Inhibit RAC and JAK2	(Patil et al., 2015; Wang et al., 2009)
YM-155	PAK1	Suppress survivin	(Ahn et al., 2017)
ZMF-10	PAK1	ATP competitive inhibitor	(Zhang et al., 2020a)
CDK-1/2 inhibitor, Purvalanol A, K252, and SU11652	Group II PAKs	ATP mimetic	(Eswaran et al., 2007)
Compound 17	Group II PAKs	Binds to an open back pocket cavity of PAKs	(Staben et al., 2014)
GL-1196	PAK4	Inhibits PAK4-dependent signaling	(Zhang et al., 2016)
KPT-8752 and KPT-9274	PAK4	Allosteric kinase inhibitor	(Rane et al., 2017)
KY-04031	PAK4	ATP competitive inhibitor	(Ryu et al., 2014)
LCH-7749944	Group II PAKs	Blocks several PAK4-mediated signaling pathways	(Zhang et al., 2012)
PF-3758309	PAK4	ATP competitive inhibitor	(Murray et al., 2010)

2010. The induction of cell cycle arrest in the G1 phase is mediated by upregulation of p21 and p27, and apoptosis is induced by the activation of caspase-8 and caspase-9 signaling (Yoshikawa et al., 2010). GGA (6, 10,14,18-tetramethyl-5,9,13,17-nonadecatetraen-2-one), an oral anti-peptic ulcer drug developed by Eisai Inc. (Tokyo, Japan) and approved in Japan in 1984 (Murakami et al., 1981), inhibits *Helicobacter pylori* (a gram-negative bacterium colonizing the human stomach)-induced inflammatory reactions and tissue injury. In addition, GGA has reportedly activates several molecular chaperones (heat shock proteins [HSPs]) through heat shock factor 1 (HSF1), which offers conserved cytoprotection against a number of aging pathologies (Anckar and Sistonen, 2011; Katsuno et al., 2005).

Based on these observations, the considerable efforts devoted to the development of fine-design ATP-competitive/allosteric small-molecule inhibitors specific to PAK1 are evident, and some of these compounds have exhibited excellent activity. Although these small molecules have been found relatively safe and effective in mammalian cell lines and a few *in vivo* models, their behavior at the organismal level is still unclear. Importantly, these studies reported only a limited amount of experimental evidence, and future trials should be performed to test these molecules in appropriate mammalian models, the results of which will dictate whether such small molecules are amenable to further development. Table 2 summarizes the natural and synthetic compounds that block PAK1 activity directly or indirectly, and a few of these are commercially available.

2.4.2. Group II PAK inhibitors

Group II PAKs have fewer coding exons than group I PAKs, indicating potential structural and regulatory differences between the two groups. Group II PAKs structurally differ from group I PAKs in terms of their catalytic and regulatory domains, although they share some sequence homology with group I PAKs. Similar to PAK1, PAK4 is frequently linked to cancer and other age-related diseases (Bokoch, 2003). Interaction of RAC1/CDC42 GTPases with PAK4 directs its cellular localization, and it exhibits kinase-independent and kinase-dependent functions to promote cell growth. In addition, PAK4 has been shown to phosphorylate a larger set of substrates than PAK1, and several overlaps in substrate specificity have been reported (Hofmann, 2004; Kumar et al., 2017). PAK4 is mostly upregulated in human cancers, including basal-like breast cancer, endometrioid ovarian cancer, oral squamous cell carcinoma, and pancreatic cancer; its kinase activity supports various cancerous phenotypes (Bokoch, 2003; Kumar et al., 2017). Considering the differences in domain architecture and regulatory mechanisms between PAK1 and PAK4, molecules that inhibit PAK1 might not be as effective at targeting PAK4 activity. Therefore, the strategy for developing inhibitors that directly target PAK4 should be different from that used for classical PAK1 inhibitors. Glaucarubinone, a member of the quassinoid family isolated from the seeds of *Simarouba glauca* DC, is the first natural product that inhibits pancreatic and colon cancer cell growth by inhibiting PAK4 and PAK1 activity (Yeo et al., 2014). Glaucarubinone was originally developed as an antimalarial drug and its combination with the front-line chemotherapeutic agent gemcitabine synergistically reduced cancer cell growth by reducing the levels of active PAK4 and PAK1 protein levels and consequently inhibiting pancreatic cancer cell xenograft growth *in vivo*. Despite decreasing PAK1 activity, glaucarubinone did not markedly alter the phosphorylation levels of AKT or ERK that are suppressed by PAK1 inactivation in cancer cells (Yeo et al., 2014). These results indicate the complex nature of glaucarubinone, which is an undesirable characteristic for developing drug candidates. In *C. elegans*, glaucarubinone promotes mitochondrial metabolism, reduce fat deposition, and promotes healthy longevity at nanomolar concentration (Zarse et al., 2011). PF-3758309 is the first synthetic PAK4 inhibitor, developed by Pfizer (San Diego, CA) that binds to the PAK4 kinase domain in an ATP-competitive manner. PF-3758309 inhibited the phosphorylation of the PAK4 substrate GEF-H1 and anchorage-independent growth of HCT116 cells with an IC_{50} around 4.7 $\pm$ 3 nM. In tumor models (A549, colo205, HCT116, M24met, and MDA-MB-231), PF-3758309 inhibited cell proliferation, survival, and activation of PAK4-dependent pathways. Although it was rationally designed through a structure-based approach as a PAK4 inhibitor, PF-3758309 was found to actively target almost all PAK family members (PAK1, PAK4, PAK5, and PAK6) as well as other kinases, including ribosomal S6 kinase and AMP-activated kinase (Murray et al., 2010). Unfortunately, clinical trials of this drug have been withdrawn because of its adverse side effects, inadequate tumor response, and undesirable pharmacokinetic properties. In 2014, Genentech Inc. (San Francisco, CA) developed compound 17, another group II PAK-specific inhibitor that exhibits good solubility and passive permeability, through structure-based methods. Compound 17 was shown to be more potent and highly selective against PAK4 as well as other group II PAKs because of the presence of an accessible back pocket cavity. It enhanced

tamoxifen sensitivity in MCF-7 breast cancer cells and inhibited tumor cell migration and invasion in two triple-negative breast cancer cell lines, MDA-MB-436 and MCF10A PIK3CA (Staben et al., 2014). In 2012, Zhang et al. developed a novel and potent PAK4-specific inhibitor, LCH-7749944, that effectively suppressed PAK4-mediated behaviors, including proliferation, migration, and invasion of human gastric cancer cells (BGC823, MGC803, MKN-1, MKN-45, and SGC7901) *in vitro* by blocking PAK4/LIMK1/cofilin and PAK4/MEK-1/ERK1/2/MMP2 pathways. Although it was designed as PAK4-specific inhibitor, it displays inhibitory activity against other PAK family proteins, including PAK1, PAK5, and PAK6. More interestingly, LCH-7749944 inhibited cell elongation and filopodia formation in SGC7901 cells; however, it's *in vivo* properties have not yet been investigated (Zhang et al., 2012). A few other small-molecule inhibitors such as GL-1196 (Zhang et al., 2016), KY-04031 (Ryu et al., 2014), and a series of PAK4-inhibiting analogs (KPT-8752 and KPT-9274) (Rane et al., 2017) have also been identified. Among these, KPT-9274 has proven to be a promising clinical compound that reduces the steady-state level of cellular PAK4 and is found to block the activity of nicotinamide phosphoribosyltransferase, a key enzyme that initiates the nicotinamide adenine dinucleotide (NAD) biosynthetic pathway in mammals. Recent studies have shown that KPT-9274 can inhibit the growth of multiple cancer cells, including B cell acute lymphoblastic leukemia, triple-negative breast cancer, multiple myeloma, pancreatic ductal adenocarcinoma, and renal cell carcinoma. KPT-9274 and its closely related analog KPT-8752 are different from other PAK4 blockers because they reduce cellular PAK4 levels instead of inhibiting its catalytic activity. This orally bioavailable clinical drug candidate, combined with nivolumab, is currently in phase I clinical trials for patients with advanced solid malignancies and non-Hodgkin's lymphoma (ClinicalTrials.gov Identifier: NCT02702492). Another multi-center, open-label clinical study is currently investigating the safety, tolerability, and efficacy of KPT-9274, either alone or co-administrated with niacin ER, in patients with non-Hodgkin's lymphoma and advanced solid malignancies (ClinicalTrials.gov Identifier: NCT04281420). Furthermore, the use of miRNAs may be considered an alternative strategy for targeting PAK4 activity. Overexpression of miR-24 has been associated with the modulation of epithelial differentiation and actin adhesion dynamics in keratinocytes by suppressing PAK4 expression. Therefore, considering the pivotal role of PAK4 in epithelial morphogenesis and the hyperproliferative nature of skin cancers, it may represent a potential therapeutic target. Similarly, a few other miRNAs, including miR-9-5p, miR-145, miR-199a/b-3p, miR-224, miR-433, miR-485, and miR-663, suppress PAK4 expression and are reportedly downregulated in several cancer cells (Rupaimoole et al., 2016). Table 2 summarizes the natural and synthetic compounds that block the activity of group II PAKs.

3. *Caenorhabditis elegans*—a premier model in aging research

The ultimate goal of aging research is to increase disability-free, active, and healthy longevity. The lifespan can be extended by decreasing the rate of aging progression, delaying the onset of age-related diseases, and/or eliminating the root causes of mortality in the elderly population. Using complex multicellular experimental models (*i.e.*, mammals) for studying the aging process in detail is quite difficult, time-consuming, and involves ethical issues. Over the past few decades, *C. elegans*, a free-living non-pathogenic nematode, has helped expand our understanding of the molecular determinants of aging and is considered an excellent *in vivo* model system to investigate the process of aging (Kenyon, 2010). Since Sydney Brenner first introduced the *C. elegans* model in the 1960s, this nematode has been widely used in almost all areas of biological research. A new era in aging research began following the isolation of the first long-lived *C. elegans* strain by Klass at the University of Houston, USA in 1983 (Klass, 1983). *C. elegans* has several advantageous features in aging studies, and they have been extensively covered in several review articles (Mack et al., 2018; Murakami, 2007; Olsen et al., 2006; Zhang et al., 2020c). In addition, the availability of excellent online resources and databases on *C. elegans* anatomy, biology, and genetics, such as WormBase (wormbase.org) WormAtlas (wormatlas.org), WormBook (wormbook.org), SynergyAge (synergistic.aging-research.group), and GenAge (genomics.senescence.info/genes), makes it a premier model system for aging.

Table 3
Comparison between *C. elegans* and the human genomes.

	C. elegans	Human
Whole-genome		
Genome Size (bp)	100,286,401	3,257,319,537
Total number of genes	49,177	60,649
Protein coding genes	19,987	19,955
Non-coding RNA and pseudogenes	27,664	40,284[a]
Mitochondrial genome		
Genome size (bp)	13,794	16,569
Total number of genes	36	37
rRNA genes	02	02
tRNA genes	22	22
Respiratory chain genes	12	13

Comparison has been adapted from (Mack et al., 2018). Statistics are based on WormBase (WS281 https://wormbase.org/about/wormbase_release_WS281#0–10) and GENECODE (release version 38 https://www.gencodegenes.org/human/stats.html) as on July 23, 2021.

[a] Total non-coding RNA (long and small non-coding RNA) genes and pseudogenes (processed, unprocessed, unitary, polymorphic, and pseudogenes).

The *C. elegans* genome is just 1/30th the size of the human genome; however, approximately 80 % of *C. elegans* proteins have human orthologs (Lai, 2000). Interestingly, *C. elegans* was the first metazoan animal with a completely sequenced genome using shotgun Sanger sequencing in 1998, and the data has been refined continuously thereafter (The C. elegans Sequencing Consortium, 1998). A comparison of the genomes of *C. elegans* and human is presented in Table 3. Recently, Qin et al. identified 143 essential genes in *C. elegans* using combined genetic mapping, whole-genome sequencing, bioinformatics analysis, and experimental validation; of these, 108 were found to have putative human orthologs. Among the 108 genes, 90 % (n = 97) of genes were found to be associated with 1218 different human diseases (Qin et al., 2018). In addition, the genetic flexibility of *C. elegans* allows the transgenic expression of human genes associated with age-related NDDs, including α-synuclein, amyloid-β, polyglutamine, transthyretin, and TDP-43, fused with a green or yellow fluorescent protein (GFP or YFP) in body wall muscle cells offers a perfect platform for examining the quantitative aggregation of these toxic proteins with age (Alexander et al., 2014; Caldwell et al., 2020; Li and Le, 2013; Mohankumar et al., 2020; Van Pelt and Truttmann, 2020). It is important to highlight the discovery of a revolutionary and precise gene-editing technology, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR associated protein 9 (Cas9) or CRISPR/Cas9 (Jinek et al., 2012), that provides archaea and bacteria with adaptive immunity against invading plasmids and viruses through CRISPR RNAs (crRNAs) (Wiedenheft et al., 2012). In 2013, this precise genome-editing technology was first adapted for *C. elegans*. Since then, several different CRISPR approaches have been developed, especially for *C. elegans*, and researchers have produced increasingly sophisticated methods to tag, delete, and mutate genes using CRISPR/Cas9 technology (Dickinson et al., 2015, 2013; Friedland et al., 2013; Paix et al., 2016; Zhao et al., 2014). CRISPR/Cas9 genome engineering has greatly facilitated the fundamental goal of biological research, which is to identify and understand gene functions. For this game-changing gene-editing technology, Emmanuelle Charpentier (the Max Planck Unit for the Science of Pathogens, Berlin, Germany) and Jennifer A. Doudna (the University of California, Berkeley, USA) were awarded the Noble Prize in Chemistry in 2020 (nobelprize.org/prizes/chemistry/2020/). Collectively, the use of this advantageous *C. elegans* model has resulted in multiple breakthrough discoveries and innovations in aging research, and several

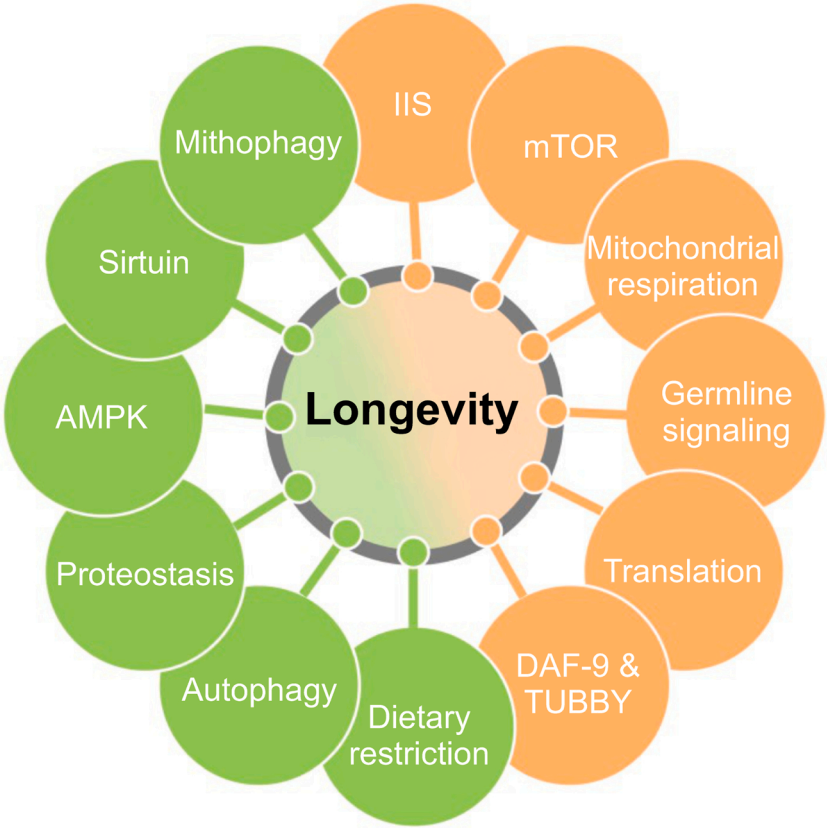

Fig. 5. Biological pathways that promote longevity in *C. elegans*. Green and orange colors indicate enhancement and reduction, respectively, in the signaling of these pathways to promote longevity. AMPK, AMP-activated protein kinase; IIS, insulin/insulin-like growth factor signaling; mTOR, mechanistic target of rapamycin.

laboratories across the globe still use this model to gain deeper insights into aging mechanisms, genes involved in aging processes, and identification of novel molecules with anti-aging properties to counteract aging and age-related ailments.

Using the *C. elegans* model, several genes and genetic networks that control lifespan have been identified. A new era in aging research has bloomed after the identification of the first long-lived *C. elegans* mutant by Klass (Klass, 1983) and another long-lived mutant, *age-1*, by Johnson and colleagues (Friedman and Johnson, 1988). Since then, several genetic determinants have been identified for controlling lifespan, and these determinants link environmental factors to the rate of aging. Subsequently, studies identified the insulin/insulin-like growth factor signaling (IIS) pathway, the first established pathway implicated in regulating the aging process in *C. elegans*, which constitutes DAF-2, AGE-1, and DAF-16 (Kenyon, 2010). A mutation in *daf*-2, the *C. elegans* homolog of insulin/insulin-like growth factor-1 receptor (IGF1R), approximately doubles the lifespan by affecting the activity of *age-1*, the *C. elegans* homolog of PI3K. The longevity induced by reduced IIS signaling is mediated by DAF-16, the *C. elegans* homolog of forkhead box O (FOXO) transcription factor (Kenyon et al., 1993). Reduced IIS signaling leads to dephosphorylation of DAF-16, which triggers its nuclear translocation where DAF-16 activates several genes involved in lifespan extension (Kenyon, 2010; Coleen T Murphy et al., 2003a). Similar to the IIS pathway, reduced mechanistic target of rapamycin (mTOR), mitochondrial respiration, DAF-9, TUBBY signaling, germline signaling, and mRNA translation were shown to extend the lifespan of *C. elegans*. In addition, enhanced activation of autophagy, AMP-activated protein kinase (AMPK), *sir-2.1* (ortholog of human sirtuin 1/SIRT1), mitophagy (a selective degradation of impaired mitochondria by autophagy), dietary restriction, proteostasis, and MAPKs, including p38, JNK, and ERK, promote longevity in *C. elegans* (Fig. 5). Uno and Nishida (Kyoto University, Japan) have comprehensively reviewed the lifespan-regulating genes and signaling pathways in *C. elegans* (Uno and Nishida, 2016). In conclusion, despite its limitations, *C. elegans* is a powerful model system to understand the genetic regulation of aging and a rapid, inexpensive, and high-throughput screening platform for anti-aging compounds.

4. PAK1 regulates longevity in *C. elegans*

4.1. PAKs in C. elegans and their functions

The *C. elegans* genome encodes three PAK family proteins, namely PAK-1, PAK-2, and MAX-2. The RAC and CDC42 GTPases, RAS superfamily GTPases of the RHO subtype, are upstream regulators of these PAKs and function as molecular switches to control cytoskeletal dynamics (Lardennois et al., 2019; Takai et al., 2001). Activated RAC/CDC42 GTPase binds to PAK-1 and stimulates PAK activity *via* the canonical GTPase/PAK pathway. PAK-1 shares all known sequence motifs with human group I PAKs, whereas PAK-2 is more similar to group II PAKs. Although MAX-2 closet in sequence identity to group I PAKs with respect to the p21-binding domain (PBD) and kinase regions, it does not share other N-terminal regulatory sequence motifs typical of group I PAKs (Chen et al., 1996; Hofmann, 2004). Loss of *max-2* causes moderate disruption of axonal pathfinding in *C. elegans* larval development, but *pak-1* loss-of-function significantly improves these defects. However, the loss of *pak-1* alone causes no apparent defects in pathfinding errors (Lucanic and Cheng, 2008). Surprisingly, worms carrying the *pak-1* deletion allele, *pak-1*(*ok448*), live significantly longer (approximately 60 %), and are healthier compared to the wild-type control groups. PAK-1 exhibits GTP binding and kinase activity in *C. elegans* and is involved in several biological functions, including motor neuron axon guidance, inductive cell migration, and hemidesmosome assembly. It localizes to several cellular components,

including hemidesmosomes, cytosol, and neuronal cell bodies, and colocalizes with intermediate filaments (Goh et al., 2012; He and Maruta, 2013; Iino and Yamamoto, 1998; Okada et al., 2013) (wormbase.org). The expression of *pak-2* is observed in the spermatheca, vulva, pharynx, and pharyngeal-intestinal valve. PAK-2 is predicated to enable ion binding and kinase activity, and is involved in larval development. *C. elegans* carrying *pak-2*(*ok332*) loss-of-function allele (a probable null allele) does not have an apparent mutant phenotype in isolation and does not affect the axonal guidance phenotype of *max-2*(*cy2*) worms. In contrast, the *pak-2* deletion shows some embryonic defects and L1 lethality as a double mutant with the the null *pak-1*(*ok448*) allele (Iino and Yamamoto, 1998; Lucanic et al., 2006; McKay et al., 2003) (wormbase.org). Peters et al. described the synergy between *pak-1* and *max-2* in distal tip cell (DTC) migration (Peters et al., 2013). They found that loss of *pak-1* and *max-2* (double mutation) results in pathfinding errors and incompletely extended gonad arms during early development in *C. elegans*, suggesting that these functionally redundant PAKs are critical for proper migration and appropriate direction of DTC migration. In addition, CED-10, an ortholog of human RAC1 and RAC2, is likely to function upstream of MAX-2. MAX-2 exhibits mitogen-activated protein kinase kinase (MEK) activity and RAC GTPase binding activity, and is involved in motor neuron guidance, JNK activation, and inductive cell migration. It is likely expressed ubiquitously in early embryos and then localized to the anterior embryo, where *max-2* is expressed in the ventral nerve cord, pharynx, and head neurons. Additionally, *max-2* seems to be expressed in body wall touch receptor neurons (mechanosensory neurons), including PLM, PVD, and ALM (Iino and Yamamoto, 1998; Locke et al., 2009; Lucanic and Cheng, 2008) (wormbase.org; wormatlas.org). Worms bearing a loss-of-function mutation in *max-2* exhibit a mild axonal guidance phenotype and with only subtle defects in movement. Further, *max-2* mutation suppresses *unc-5* overexpression and is dominantly enhanced by either *unc-5* or *unc-6* mutations, indicating that *max-2* functions downstream of *unc-5*, a gene that encodes a netrin receptor (Lucanic et al., 2006).

The CED-2/5/12 complex, constituent of the Dock/ELMO atypical Rac guanine nucleotide exchange factors, interacts with PAK-1 and MAX-2 similar to CED-10/RAC, suggesting that the Dock/ELMO complex controls DTC migration by promoting CED-10/RAC activity (Peters et al., 2013). Genetic analysis suggests a modest contribution of CED-10/RAC to PAK-1 branch activity, indicating partial RAC dependency of the GIT-1/PIX-1/PAK-1 complex (Peters et al., 2013). GIT-1 and PIX-1, orthologs of the human G-protein coupled receptor kinase interactor (OMIM: 608434) and PIX exchange factor (OMIM: 300267), respectively, are equally essential to the PAK-1 pathway and are required for PAK-1 activity. The GIT-1/PIX-1/PAK-1 axis is a highly conserved signaling module that controls cytoskeletal dynamics in all metazoans and regulates neuronal development and plasticity (Zhang, 2005). In *C. elegans*, the GTPase activator protein PIX-1 and the scaffolding protein GIT-1 form a highly conserved signaling complex with PAK-1 to control DTC migration and cell shape during morphogenesis of the gonad, independent of RAC/CDC42. This RAC/CDC42-independent PAK-1 pathway (GIT-1/PIX-1/PAK-1) functions in parallel to the RAC/CDC42–PAK1 pathway to regulate DTC migration and other cellular processes. However, PIX-1 and GIT-1 do not interact with the other PAK family members, namely PAK-1 and MAX-2 (Lucanic and Cheng, 2008; Martin et al., 2014; Okada et al., 2013; Peters et al., 2013). PIX and GIT have been shown to regulate multiple cellular processes *via* PAK activity regulation in diverse experimental models (Ku, 2001; Zhang, 2005; Zhao et al., 2000). As the GIT-1/PIX-1/PAK-1 pathway functions independently of classical RAC/CDC42, loss of *cdc-42* would exacerbate the pathologies resulting from a loss of the GIT-1/PIX-1/PAK-1 axis. This was supported by a study where double RNAi knockdown of *pix-1* and *cdc-42* caused severe defects in DTC migration compared to the RNAi knockdown of either *pix-1* or *cdc-42* alone. In contrast, *max-2* knockdown failed to enhance the defects caused by *cdc-42* RNAi, and *max-2*; *cdc-42* double RNAi did not resulted in enhancement of defects, suggesting that MAX-2 works with CDC-42 during gonad elongation (Lucanic and Cheng, 2008). RACs, GIT, PIX, and PAKs have been implicated in integrin-regulated processes in multiple experimental models (Webb et al., 2007; Zhao et al., 2000). The *C. elegans* genome contains two α subunits (*ina-1* and *pat*-2) and one β subunit (*pat-3*) integrins that function as heterodimers and are involved in controlling DTC migration (Baum and Garriga, 1997; Lee et al., 2001). Loss of function of either *ina-1*, *pat*-2, or *pat-3* displayed migration and guidance defects as identified in (*rac/max-2*); (*git-1/pix-1/pak-1*) double mutants and those with the bloated DTC morphology phenotype of the GIT-1/PIX-1/PAK-1 pathway, suggesting that the integrins may function with both RAC/PAK-1 and GIT-1/PIX-1/PAK-1 signaling pathways (Lucanic and Cheng, 2008). Notably, this GIT-1/PIX-1/PAK-1 pathway promotes the maturation of hemidesmosomes into junctions that can withstand mechanical stress and contribute to the coordination of epidermal and muscle tissue morphogenesis (Zhang et al., 2011). Nearly all metazoan PAKs contain a PIX-binding motif, and PIX-dependent activation of PAKs has evolved very early in these metazoans. PAK-1 null mutant worms reportedly produce lower progeny and live significantly longer than the wild-type (Yanase et al., 2013) similar to worms bearing longevity-promoting genetic mutation(s) (Uno and Nishida, 2016), suggesting that the GIT-1/PIX-1/PAK-1 signaling axis is not only essential for gonad morphogenesis in *C. elegans*, but also has a specific role in controlling longevity and reproductive functions.

The involvement of redundant PAKs (*max-2* and *pak-1*) in other biological functions was demonstrated by Peters et al. (Peters et al., 2013). *max-2* and *pak-1* were shown to have diverse roles in germline function and development; additionally, sperm development is clearly disrupted in the worms bearing *max-2*; *pak-1* double mutation, resulting in sterility. Surprisingly, tail development, one of the most complex morphogenetic events in *C. elegans*, was not disrupted by the loss of *pak-1* and *max-2*. In particular, *pak-1*(*tm403*); *max-2* and *pak-1*(*ok4448*); *max-2* males had male tail structures and wild-type morphology and spacing of sensory rays. However, *max-2*; *pak-1* double mutants exhibited moderate defects in vulval morphogenesis (Peters et al., 2013). In 2010, Fujiki et al. demonstrated the novel role of *max-2* kinase in regulating stress response in *C. elegans* (Fujiki et al., 2010). They found that when the worms carrying *pak-1*(*ok448*) and *pak-2*(*ok332*) deletion mutations were exposed to copper ions (Cu^{2+}) they did not exhibit sensitivity to heavy metals, as seen in their wild-type counterparts; however, *max-2*(*nv162*) mutant worms displayed a partial heavy metal-sensitive phenotype. In addition, the growth rate of *max-2* mutants was poor in the presence of Cu^{2+}, whereas *pak-1* mutants, *pak-2* mutants, and wild-type worms grew normally and became adults on day 4. Interestingly, *max-2*(*nv162*); *pak-2*(*ok332*) double mutation as well as RNAi knockdown of *pak-1* in *max-2*(*nv162*) mutant and *max-2*(*nv162*); *pak-2*(*ok332*) double mutant worms failed to enhance their sensitivity to heavy metals. Moreover, RNAi-mediated inhibition of *vhp-1* markedly suppressed the stress sensitivity observed in *max-2*-deficient worms. *vhp-1* encodes a MAPK phosphatase that negatively regulates the KGB-1/JNK-like MAPK-mediated stress response pathway (Mizuno et al., 2004). Notably, phosphorylated levels of KGB-1 declined moderately in *max-2*(*nv162*) mutants under Cu^{2+} treatment, indicating that KGB-1 requires functional MAX-2 for full activation. Genetic and mechanistic studies have shown that MAX-2 interacts with and phosphorylates MLK-1 MAPK kinase kinase at the Ser-355 residue, functioning upstream in the KGB-1 pathway, similar to MEK-1. Fujiki et al. investigated the involvement of *mig-2* of the KGB-1 pathway in mediating sensitivity towards heavy metals (Fujiki et al., 2010). *mig-2* encodes a member of the RhoG family of GTP-binding proteins, functioning in parallel to the two PAK (*max-2* and *pak-1*) pathways regulating DTC migration (Peters et al., 2013). Studies found that the Cu^{2+}-sensitive phenotype observed in *max-2*(*nv162*) worms was not altered by *mig-2*(*mu28*) mutation, and overexpression of *max-2* in *mig-2* (*mu28*) worms partially suppressed the heavy-metal sensitivity in a MAX-2-dependent manner, indicating that MIG-2 and MAX-1 appear to

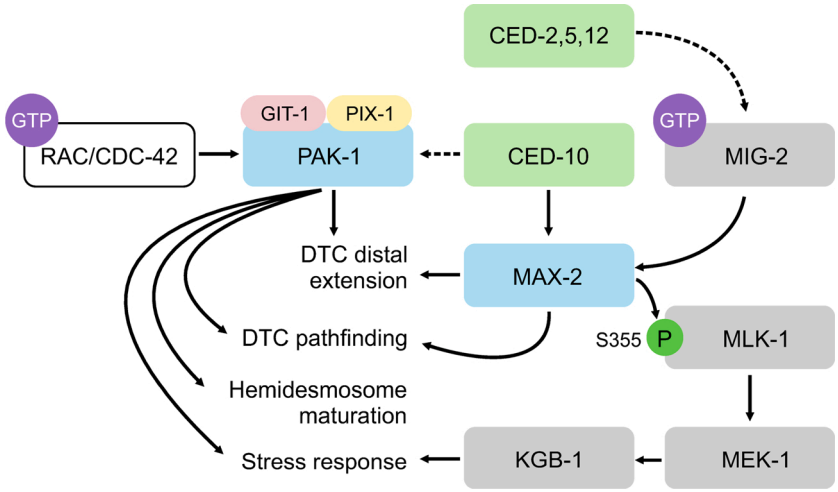

Fig. 6. Overview and biological functions of PAKs in *C. elegans* [adapted from (Fujiki et al., 2010; Lucanic and Cheng, 2008; Mizuno et al., 2004; Peters et al., 2013; Zhang et al., 2011)]. Dotted line indicates a modest effect. DTC, distal tip cell; P, phosphate.

function in the same pathway, with MIG-2 functioning upstream of MAX-2 to activate the KGB-1 pathway (Fig. 6) (Fujiki et al., 2010).

4.2. Role of PAK-1 in regulating longevity

Yanase et al. first identified the relationship between PAK-1 and longevity in the non-vertebrate model *C. elegans* using genetic manipulation (Yanase et al., 2013). They compared the lifespan of worms carrying the *pak-1*(*ok448*) loss-of-function allele (RB689) with wild-type worms (Bristol-N2) grown under standard laboratory conditions and found that *pak-1*(*ok448*) worms live significantly longer than wild-type worms. The N2 worms lived an average of 17 days, whereas PAK-1-deficient worms survived for 27 days, approximately 60 % longer. These findings clearly indicate that PAK-1 functions limit the lifespan of *C. elegans* under basal conditions similar to its upstream regulators DAF-2/IGFR1 (Dorman et al., 1995), AGE-1/PI3K (Friedman and Johnson, 1988), and mTOR (Jia et al., 2004). The hyper-function theory of aging proposes that over-activation of biological processes, such as reproduction and development, leads to aging as a result of hypertrophy-associated pathologies (Gems and de la Guardia, 2012). The relationship between longevity and developmental rates is intriguing and has been studied using experimental models. Genetic studies using multiple model organisms have shown that long-lived mutants exhibit delayed development and reduced fecundity rates (Mukhopadhyay and Tissenbaum, 2007). This indicates that longevity is negatively correlated with reproduction and development. Consistent with these findings, the brood size was smaller in *pak-1*(*ok448*) worms than in wild-type worms, and the *pak-1* mutant progeny showed no noticeable defects. These results strongly suggest that the reduction in brood size is associated with reduced PAK-1 activity in *C. elegans*. The long-lived worms deficient in components of the IIS and TOR signaling pathways also displayed a significantly reduced brood size (Dorman et al., 1995; Friedman and Johnson, 1988; Jia et al., 2004). In other words, PAK-1 typically promotes reproduction in *C. elegans*, thereby accelerating the aging process, at least partially.

In human cells, PAK1 directly interacts with the forkhead transcription factor FKHR and inhibits its function *via* phosphorylation (Mazumdar and Kumar, 2003). In *C. elegans*, many transcription factors and classical cellular signaling pathways that modulate the aging process have been identified (Uno and Nishida, 2016). Among these, the transcription factors SKN-1, HSF-1, and DAF-16 have been extensively studied and are suggested to control multiple genes involved in species longevity and stress resistance. The activity of SKN-1, an Nrf2-like transcription factor, and HSF-1 up- or down-regulates several target genes involved in a wide range of biological functions, including homeostatic functions, oxidative/xenobiotic defense, and longevity (Brunquell et al., 2016; Tullet et al., 2008). Additionally, enhanced activity of DAF-16, a *C. elegans* homolog of a forkhead transcription factor, significantly extended lifespan by regulating a wide range of geroprotective genes (Kenyon, 2010; Murphy et al., 2003a). Furthermore, the activity of these transcription factors is entirely controlled by the IIS pathway; activated IIS inhibits the functions of SKN-1, HSF-1, and DAF-16 *via* phosphorylation-dependent cytoplasmic sequestration. Genetic analysis identified DAF-16 as the major transcriptional output of IIS, and translocation of DAF-16 from the cytoplasm to the nucleus is necessary for its transcriptional activity (Kenyon and Murphy, 2006; Murphy et al., 2003a, 2003b). However, whether PAK-1 inactivates DAF-16 in *C. elegans*, as in mammals, is unclear. To clarify this

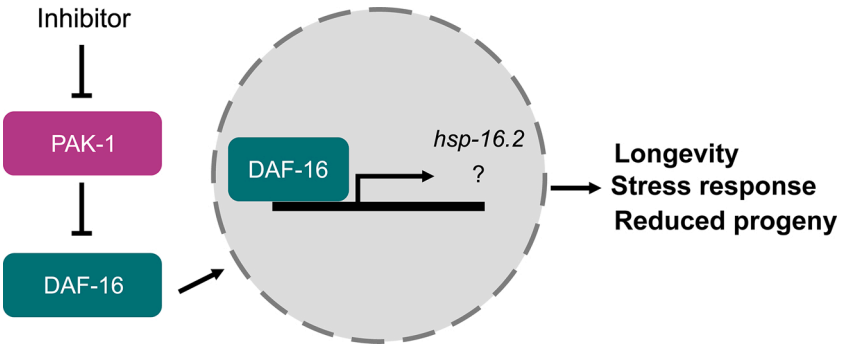

Fig. 7. The role of PAK-1 in regulating lifespan and healthspan of *C. elegans*.

possibility, Yanase et al. examined the nuclear localization pattern of DAF-16 using microinjection of pGP30 vector carrying *daf-16* transcript a2 (*da-16*a2) fused with GFP cDNA into *pak-1*(*ok448*) worms. They found significantly increased nuclear accumulation of DAF-16 in *pak-1*-deficient *C. elegans* strain when compared to that in wild-type worms (Yanase et al., 2013). Once activated, DAF-16 translocates to the cell nucleus and triggers specific DAF-16-depended gene expression required for lifespan extension (Lee et al., 2003). In particular, *hsp-16.2*, an important transcriptional readout of DAF-16, which is categorized as a small HSP, is only expressed in organisms facing pernicious environmental conditions, such as protein damage-causing oxidative and thermal stresses (Burnaevskiy et al., 2019; Lithgow et al., 1994; Walker and Lithgow, 2003). Interestingly, worms bearing a loss-of-function mutation in *pak-1* displayed enhanced survival under thermal stress conditions compared to wild-type worms exposed to 35 °C for 4 h. In addition, worms bearing *pak-1* mutation showed increased expression of *hsp-16.2::GFP* after a short heat shock. These results confirm that the aging kinase PAK-1 normally functions to inactivate DAF-16, thereby hindering the expression of stress-protective HSP-16.2 and thus reducing longevity in *C. elegans* (Fig. 7). Therefore, interventions may be developed to target this oncogenic/aging kinase to attenuate aging, cancer, and age-related diseases. Nevertheless, a clear biological mechanism underlying PAKs in regulating the lifespan of C. *elegans* remains largely unclear and is an important issue that needs to be validated in the future.

4.3. Does dietary restriction regulate longevity through PAK-1?

Dietary restriction (DR) is the most robust environmental manipulation known to significantly increase healthy longevity in *C. elegans*, as well as in diverse species across taxa. Mounting pre-clinical and clinical evidence indicates the significance of nutrient-sensing pathways, including IIS, sirtuins, and mTOR, in mediating the effects of DR (Kapahi et al., 2017). Multiple global transcriptional regulators, including DAF-16/FOXO (Greer et al., 2007), SKN-1/Nrf2 (Bishop and Guarente, 2007), HSF-1/HSF1 (Steinkraus et al., 2008), HIF-1/HIF1 (Chen et al., 2009), and PHA-1/FOXA (Panowski et al., 2007), are required to ensure the healthy longevity of diet-restricted *C. elegans*. Several types of diluted foods are used to subject worms to DR; these include dilution of bacteria in a liquid known as IDR or bDR, seeding diluted bacteria onto agar media referred to as sDR and msDR (seeding diluted bacteria onto agar media with reduced peptone concentration), complete dietary deprivation (DD), and intermittent fasting (IF) (Bishop and Guarente, 2007; Chen et al., 2009; Greer et al., 2007; Honjoh et al., 2009; Kaeberlein et al., 2006; Panowski et al., 2007). Multiple genetic factors and conserved cellular signaling pathways reportedly mediate the longevity-promoting effects of DR. Genes associated with the IIS pathway were the first genes to be directly linked to aging; a few components of this pathway, including the Nrf2 transcription factor SKN-1 and FOXO transcription factor DAF-16, have also been associated with DR (Kenyon, 2010; Pang et al., 2014). bDR fails to extend the lifespan of *skn-1* mutants under normal culture conditions showing that SKN-1/Nrf2 is indispensable for DR-mediated longevity (Pang et al., 2014). The DR regimens IF and sDR require the transcriptional activity of DAF-16 to extend the lifespan. During sDR, the nutrient sensor *aak-2*, an AMPK, activates *daf-16* to extend the lifespan and healthspan of the organism (Greer et al., 2009). *aak-2* is, in turn, directly regulated by an upstream kinase *par-4*, a *C. elegans* ortholog of mammalian LKB1 (Lee et al., 2008; Narbonne and Roy, 2009). In *C. elegans*, six *par* genes (*par-1* to *par-6*) have been identified, and mutation in any of these genes results in impaired anterior-posterior asymmetries during embryonic cell division, ultimately leading to embryonic lethality (Kemphues et al., 1988). This suggests that the asymmetric cell division in the *C. elegans* embryo is controlled by *par* genes. *par-1* and *par-4* encode putative serine/threonine kinases, *par-2* encodes a C3HC4-type ring-finger protein, *par-3* and *par-6* encode a PDZ domain-containing protein, and *par-5* encodes a 14-3-36 protein (Gomes and Bowerman, 2002). Notably, *par-4*, a LKB1 or STK11 (serine/threonine kinase 11) ortholog, exhibits protein serine/threonine kinase and calmodulin-binding activities and is involved in multiple processes, including cell fate determination, asymmetric protein localization, positive regulation of dauer larval development, and adult lifespan determination. Studies on *C. elegans par* gene have improved our understanding of how this kinase coordinates cell division, cell polarity, cell growth, survival, and lifespan of mammalian cells. Additionally, the human ortholog of this gene, LKB1, has been implicated in pancreatic carcinoma, testicular germ cell tumor, and Peutz–Jeghers syndrome (Alessi et al., 2006). The serine/threonine kinase LKB1 regulates several cellular processes, including energy homeostasis, cell division and proliferation, and cell polarity, and is often downregulated in various cancers. Interestingly, the tumor-suppressing LKB1 inactivated the oncogenic kinase PAK1 in mammalian cells. In metazoans, LKB1 activates FOXO transcription factors by either inhibiting PAK1 or activating AMPK. LKB1 directly phosphorylates PAK1 at Thr109 in the p21-binding domain to inhibit its activation (Deguchi et al., 2010). This clearly indicates that PAK1 is a direct downstream target of LKB1 and plays a pivotal role in LKB1-mediated suppression of cancer cell migration. Thus, DR regimens IF and sDR promotes longevity in *C. elegans* by activating the DAF-16 transcription factor through two distinct mechanisms involving the activation of *aak-2* and suppression of *pak-1*, both of which are common targets of the serine/threonine-protein kinase PAR-4/LKB1. Nevertheless, whether DR inactivates PAK-1 in *C. elegans* to extend organismal lifespan remains unclear and needs to be validated in the near future.

4.4. Role of mTOR–S6K–PAK-1 in regulating longevity

The DR-mediated LKB1-AMPK pathway has been shown to inhibit mTOR in fruit flies, *C. elegans*, and mammals (Burkewitz et al., 2014; Kapahi et al., 2017). mTOR, another nutrient-sensing protein kinase, mediates the metabolic response to DR, and inhibition of mTOR activity extends organismal lifespan in several experimental models (Kapahi et al., 2010). Downregulation of mTOR expression in adult *C. elegans* results in lifespan extension, and this effect is independent of the forkhead transcription factor DAF-16 (Vellai et al., 2003). It forms two complexes, namely, mTOR complex (mTORC)1 (containing LET-363/mTOR and DAF-15/Raptor) and mTORC2 (containing LET-363/mTOR and RICT-1/Rictor) (Blackwell et al., 2019). mTORC1 integrates mitogen and nutrient signals to control cell size and proliferation, while mTORC2 regulates cell shape in mammalian cells (Blackwell et al., 2019; Laplante and Sabatini, 2012). The activity of mTORC1 is regulated by diverse extracellular stimuli, and the tuberous sclerosis complex (TSC), comprising TSC1 (hamartin) and TSC2 (tuberin) heterodimer, is a major upstream regulator. The IF-mediated inhibition of RHEB-1, an upstream regulator of mTOR, suppresses *ins-7* that activates *daf-16* to promote longevity, whereas sDR triggers the nutrient sensor *aak-2*, which again activates *daf-16,* thereby extending the lifespan *C. elegans* (Honjoh et al., 2009). Strong inhibition of mTORC1 in the early stage of development drastically slows or even halts the development of *C. elegans*. In contrast, inhibition of mTOR during late stage (adulthood) extends the lifespan. These results support the antagonistic pleiotropy theory, in which the genes important for fitness early in life limit survival later in life (Williams, 1957). mTORC2 plays a negligible role in modulating the longevity of *C. elegans*. Notably, mTOR-deficient *C. elegans* exhibited a marked reduction in progeny production and increased resistance to thermal stress, as observed in worms with PAK-1 mutation (Hansen et al., 2007). Activation of ribosomal protein p70 S6 kinase (S6K) through direct phosphorylation by mTORC1 is one of the key downstream events in the mTOR pathway. A deletion mutation in *rsks-1*/S6K results in lifespan extension and reduced body size and growth in *C. elegans*, similar to mTORC1; however, *rsks-1* is not required for development, unlike mTORC1 (Hansen et al., 2007). Recently, S6K was found to stimulate the rapid activation

of RAC1/CDC42 and their downstream effector PAK1. Thus, inhibition of the mTORC1–S6K pathway through genetic, dietetic, or pharmacological interventions might inactivate PAK-1 activity and thus promote healthy longevity.

In addition, AMPK acts downstream of S6K and mediates the S6K-dependent effects on lifespan and body size, and the DAF-16 transcription factor is required for AMPK-mediated lifespan extension, which is downregulated by PAK-1 (Kapahi et al., 2010). A genetic study identified that inhibition of either mTORC1 or mTORC2 increased the lifespan in an SKN-1-dependent manner. However, genetic inhibition of mTORC1 requires DAF-16 for lifespan extension, whereas mTORC2 does not (Robida-Stubbs et al., 2012). Based on this, we speculate that PAK-1 hyperactivates mTOR and reduce lifespan by inactivating both DAF-16 and SKN-1 transcription factors. To study the interaction between the mTOR pathway and insulin-like signaling, Chen et al. (2013) constructed a *daf-2 rsks-1* double mutant and examined their lifespan. They found that mutations in both *daf-2* and *rsks-1* synergistically extended the lifespan by 5-fold through positive feedback regulation of DAF-16 transcription factor by AMPK (Chen et al., 2013). In 2019, Lan et al. investigated the significant longevity-promoting phenotype caused by *daf-2 rsks-1* mutation in more detail. Functional studies revealed that *daf-2* and *rsks-1* double mutations cause translational repression of cytochrome c gene *cyc-2.1* by RNA binding protein GLD-1 in the germline; this non-autonomously activates mitochondrial unfolded protein response and AMPK in the intestine *via* germline-produced mitokine, leading to significant lifespan extension (Lan et al., 2019). Together, these findings highlight the importance of interactions between the IIS and mTOR, the two major conserved longevity-promoting mechanisms, in more complex organisms. A better understanding of these interactions will pave the way for the development of much-needed therapeutics (PAK-1 inhibitors) to extend healthy longevity. Moreover, PAK1 appears to control the interaction of raptor (regulatory associated protein of mTOR) with the mTORC1 complex. Raptor positively regulates mTORC1 functions, acts as a scaffold, and is essential for the full activation of mTORC1. *daf-15* encodes the *C. elegans* ortholog of raptor. A heterozygous *daf-15* mutation extends both the mean and maximum lifespan of *C. elegans,* similar to the results achieved by *let-363*/mTOR inhibition. Interestingly, DAF-16 negatively regulates *daf-15* transcription by directly binding PEPCK-like insulin response sequences (IRS) in the regulatory region of *daf-15* (Jia et al., 2004). These findings demonstrate that activation of PAK1 encourages tumor cell growth and metastasis and may also reduce longevity by promoting the activation of the mTOR pathway. Thus, tumor growth and aging progression depend not only on mTOR, but also on PAK1.

4.5. Does PAK-1 regulate MAPKs to shorten lifespan?

PAK1 is hyperactivated and upregulated in various cancer tissues to promote cancer cell migration and proliferation (Dummler et al., 2009; Eswaran et al., 2012). PAK-1 activates the ERK–MAPK pathway *via* MEK in a kinase-independent manner, most likely by facilitating MEK recruitment to the cell membrane. The ERK–MAPK pathway, an evolutionarily conserved signaling module in eukaryotes, transduces signals from the cell surface to the nucleus, and controls diverse cellular processes, including cell differentiation and proliferation (Nishida and Gotoh, 1993). Approximately 30 % of human cancers, including breast cancer, colorectal cancer, and neurofibromatosis, show hyperactivation of the MAPK pathway, represented by the high phosphorylation rate of ERK1/2 (Dumesic et al., 2009). Accumulating evidence indicates that PAK1 regulates both MEK1 and C-RAF by directly phosphorylating these proteins at Ser-298 and Ser-338, respectively (Higuchi et al., 2008). *In vitro* and *in vivo* studies have shown that RAC1 promotes ERK-dependent hyperproliferation of keratinocytes *via* PAK1-mediated MEK activation, independent of MEK1 phosphorylation at Ser-298 (Wang et al., 2010). However, depletion of PAK1 expression markedly reduced AKT and ERK activities, thereby inhibiting cell proliferation, invasion, and survival; this suggests the key regulatory role of PAK1 in transmitting signals from PI3K and RAS by activating downstream AKT and MEK pathways (Beeser et al., 2005). Importantly, it has been established that relocalization of active ERK, an impaired event in senescent cells, results from the activation of the ERK signaling pathway and can increase the replicative lifespan of diploid human cells by 61 % (Tresini et al., 2006). In fruit flies, direct reduction of ERK activity through genetic intervention and administration of the highly specific ERK inhibitor trametinib in adults extends their lifespan (Slack et al., 2015). Moreover, increased levels of phosphorylated ERK were found in Snell dwarf mice (Madsen et al., 2004) (mice carrying a *pit-1* mutation that results in a long-living phenotype), type 5 adenylyl cyclase knockout mice (Yan et al., 2007), and long-lived calorie-restricted mice (Ikeyama et al., 2002). In 2010, Okuyama et al. identified a novel role of ERK–MAPK signaling in longevity promotion in *C. elegans* (Okuyama et al., 2010). In this model worm, the ERK–MAPK cascade transduces signals through three main components, LIN-45/RAF1, MEK-2/MEK1/2, and MPK-1/ERK1/2, that are essential for several developmental events. MPK-1 directly phosphorylates SKN-1 at the key sites (Ser-74 and Ser-340) required for translocation of SKN-1 into the nuclei of intestinal cells and its expression in ASI neurons. The activation of SKN-1 by MPK-1, in turn, represses the expression of insulin-like peptides *ins-39* and *daf-28*, which act as DAF-2 agonists, through a positive feedback mechanism. Thus, MPK-1/ERK may function to extend the lifespan of *C. elegans* by phosphorylating SKN-1. In addition, ERK phosphorylates FOXO1 transcription factor at various sites (Asada et al., 2007; Klotz et al., 2015) and promotes longevity in *C. elegans via* activation of DAF-16/FOXO (Okuyama et al., 2010), suggesting that the ERK–MAPK signaling pathway may play a pivotal role in FOXO regulation. Based on these observations, it is apparent that PAK1 mediates hyperactivation of the ERK–MAPK cascade, promotes cancer cell proliferation and tumorigenesis, and directly or indirectly regulates ERK to shorten the lifespan of *C. elegans,* possibly by blocking DAF-16 and SKN-1 transcription factors.

Two other well-characterized subfamilies of MAPK, representatives of all eukaryotes, are p38 and JNK MAPK. Similar to ERK–MAPK, the p38 MAPK cascade confers resistance against pathogenic infection, neuronal development, and longevity (Troemel et al., 2006), while the JNK MAPK cascade plays an important role in the regulation of lifespan and stress resistance in *C. elegans* through direct interaction with and phosphorylation of DAF-16 (Oh et al., 2005a). Thus, evolutionarily conserved MAPK cascades might play a distinct and overlapping role in regulating organismal lifespan. All three subtypes of MAPKs (ERK, p38, and JNK) are targets of PAK1. In particular, constitutively activated PAK1 leads to the selective activation of the p8 MAPK pathway in response to various environmental stimuli (D rijard, 1995). The p38 pathway has also been reported to be aberrantly activated by aging in various tissues and organs, including the brain, skin, and liver, suggesting a strong link between aging and p38 signaling. In addition, p38 MAPK was abnormally activated in fibroblasts derived from patients with premature aging syndrome, and inhibition of p38 using SB203580, a selective inhibitor of p38, reversed the features of accelerated replicative decline and helped cells retain their normal lifespan (DAVIS et al., 2007). Moreover, p38 MAPK activity was markedly lower in the liver tissue of a long-lived Snell dwarf mouse, indicating decreased developmental rates and lower oxidative stress levels (Hsieh and Papaconstantinou, 2004).

Progression of aging resulted in impaired p38 MAPK signaling pathway in the brains of 24-month-old Fischer 344 rats when compared to that in young adults. This suggests that age-induced impairments in MAPK signaling pathways may be a result of attenuated upstream regulators that are responsible for activation of the MAPKs. Furthermore, calorie restriction (CR) without malnutrition inhibits the age-related decline in basal activity of p38 MAPK in the brain (Zhen et al., 1999). CR also markedly suppresses the age-related increase in MAPK activity, triggered by increased reactive oxygen species (ROS) production

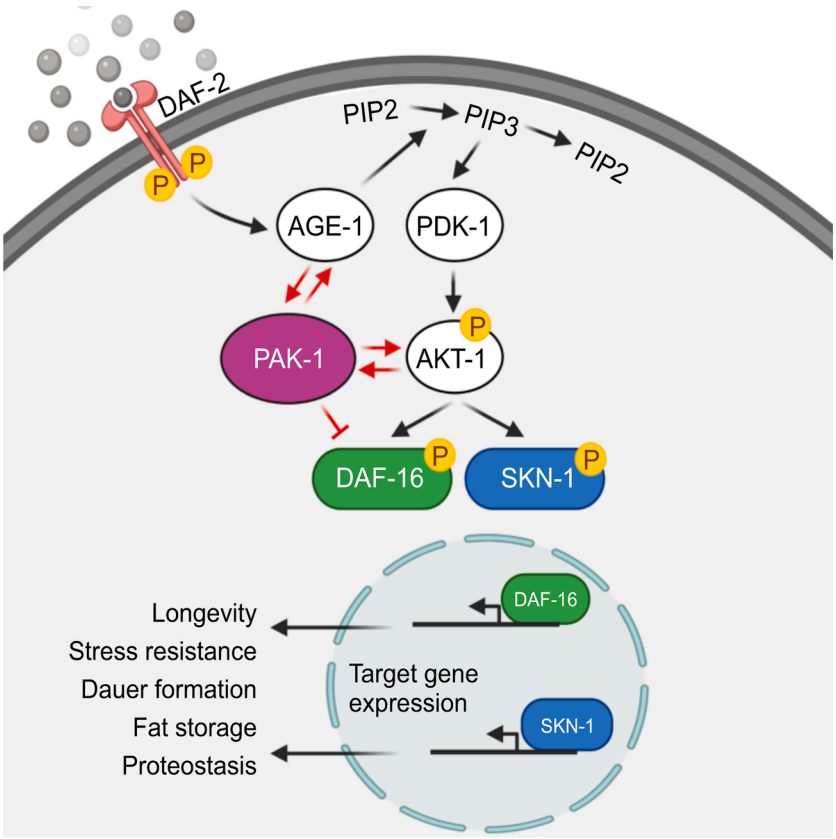

Fig. 8. The role of PAK-1 in PI3K-AKT pathway regulation. Red arrowheads indicate the interaction between AGE-1/PI3K or AKT-1/AKT and PAK-1/PAK1, as identified in higher models; however, their role in aging and longevity remains unidentified in *C. elegans*. P, phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate.

resulting from failed antioxidant defense system (Kim et al., 2002). In this context, it should be noted that CR suppressed an age-related increase in MAPK activity associated with increased ROS production through antioxidative action and by blocking PAK1 functions. PMK-1, a *C. elegans* homolog of p38 MAPK, directly phosphorylates SKN-1 at Ser-74 and Ser-340, leading to activation and nuclear translocation of SKN-1 in response to oxidative stress (Inoue, 2005), thus contributing to the enhanced lifespan of long-lived *daf-2* mutants (Troemel et al., 2006). A recent study uncovered the novel role of p38 MAPK in ensuring DR-mediated longevity *via* regulation of cytoprotective gene expression in *C. elegans* (Chamoli et al., 2020). Another study investigated the interrelationship between nutrient uptake, innate immunity, and longevity in *C. elegans* and found that the evolutionarily conserved p38 kinase plays a crucial role in regulating the lifespan of worms based on food intake. Additionally, DR was found to extend lifespan by modulating the innate immune pathway that is regulated by intact p38 MAPK signaling and ATF-7 transcription factor, which functions downstream of p38 MAPK (Wu et al., 2019). However, the precise role of PAK-1 in regulating p38 MAPK in *C. elegans* and other metazoans to influence longevity is still unexplored and is an important area for future study.

The regulatory role of PAK1 is not limited to p38 MAPK. PAK1 and its upstream regulators, RAC and CDC42, directly stimulate JNK activity (Minden et al., 1995). Activation of RAC and/or CDC42 by various environmental stimuli leads to an increase in the activity of PAKs. Constitutively activated PAK1 stimulates the JNK MAPK signaling pathway in a Ras-dependent manner, leading to transcriptional control (D rijard, 1995). However, PAK does not directly phosphorylate JNK and p38 MAPKs because these require phosphorylation of Tyr and Thr residues, which is mediated by upstream MAP kinase kinase (MKK4, also designated as SEK1/JNKK), which is in turn controlled by MAP kinase kinase kinase (MEKK1) in a typical MAPK regulatory network (Minden et al., 1994). In *C. elegans*, several orthologs of JNK MAPK pathway components have been characterized, including *unc-16* (JIP3 scaffold protein ortholog), *jkk-1* and *mek-1* (MKK4/7 orthologs), and *jnk-1* (mammalian JNK ortholog). The *C. elegans* JNK MAPK pathway comprises two types: JNK-like MAPKs (KGB-1 and KGB-2) and JNK (JNK-1) pathways (Gerke et al., 2014). Previously, *mig-2*-mediated *max-2* activation was shown to trigger the KGB-1 pathway, which offers conserved protection against heavy metals in *C. elegans* (Fujiki et al., 2010). JKK-1 is an upstream component that transmits the signal to JNK-1 in response to several stimuli, resulting in phosphorylation of the forkhead transcription factor DAF-16, thus extending longevity (Oh et al., 2005b); this suggests a possible crosstalk between JNK MAPK and insulin/IGF-1 signaling. Likewise, MEK-1 also activates JNK-1, triggering the nuclear translocation of DAF-16 and contributing to lifespan extension (Neumann-Haefelin et al., 2008). In contrast, the IIS pathway-mediated phosphorylation of DAF-16/FOXO passively retained DAF-16 in the cytoplasm of cells. Therefore, PAK-1 may also interact with JNK MAPK, similar to that in mammals. However, whether PAK-1 controls lifespan by regulating the JNK-1 MAPK cascade in *C. elegans* remains to be determined. We speculated that functional interaction between PAK-1 and MAPKs, such as MPK-1/ERK1, PMK-1/p38, and JNK-1/JNK, might alter the transcriptional activity of SKN-1 and DAF-16 in *C. elegans* to modulate aging and longevity.

4.6. Role of PAK1 in PI3K–PDK1–AKT signaling axis

Aberrant activation and overexpression of PAKs have been identified in various solid cancers, including prostate, colon, breast, and pancreatic ductal adenocarcinoma. Emerging scientific evidence highlights a link between the PI3K pathway and PAK activity in these cancers. Phosphorylation of PAK family members, in particular PAK1, by PI3K, PDK1,

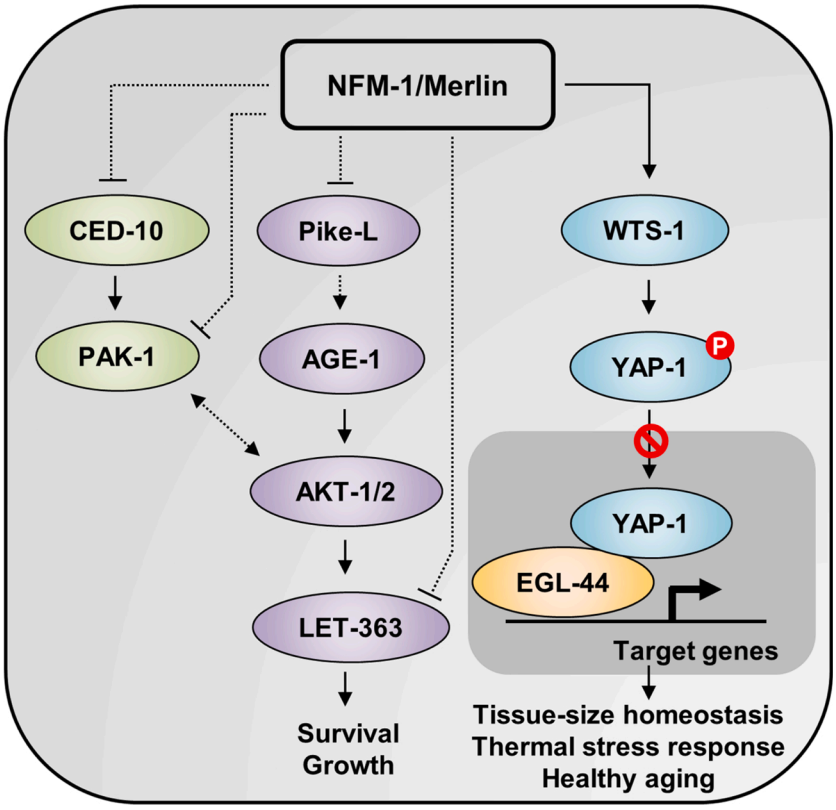

Fig. 9. Merlin signaling in aging and longevity. Black arrows indicate stimulatory interaction, while T-bars indicate repressive interaction. Dashed lines indicate that the mechanism is uncertain in *C. elegans.*

and AKT can trigger various downstream signaling events, including a number of cellular functions that, when dysregulated, contribute to the hallmarks of aging and cancer (Kaletsky and Murphy, 2010; Tsakiridis et al., 1996). The aberrantly activated PI3K–PDK1–AKT axis, an intricate signaling pathway, promotes oncogenesis by regulating growth, migration, metabolism, survival, and angiogenesis. Additionally, the kinase domain of PAK1 serves as a scaffold, allowing AKT activation by PDK1, and aids the recruitment of AKT to the cell membrane (Thillai et al., 2017). In *C. elegans*, the IIS pathway contains four major components, namely, DAF-2, AGE-1, PDK-1, and AKT-1/2 (Murphy, 2013). Inhibition of the *C. elegans* IGF1R ortholog, DAF-2, approximately doubles the lifespan by activating the FOXO transcription factor DAF-16 (Kenyon et al., 1993). Previous studies have shown that worms carrying the *age-1* loss-of-function allele, a gene encoding a catalytic subunit of PI3K, exhibit lifespan extension in a DAF-16-dependent manner as seen in *daf-2* mutants (Ayyadevara et al., 2008). Moreover, an *akt-1* deficiency caused robust lifespan extension, and the worms carrying mutations in *akt-1* lived approximately 50 % longer than the wild-type worms, similar to *age-1* mutants (Hamilton, 2005). AGE-1 and AKT-1 are well-established downstream targets of DAF-2 in the IIS pathway. Since PI3K was shown to activate both AKT and PAK1, the lifespan discrepancies observed in *age-1* and *akt-1* deficiencies may be attributed to PAK-1 deficiency in *C. elegans*. In contrast, PAK1 was also shown to activate AKT, a crucial factor for cell growth, migration, metabolism, survival, and tumorigenesis, in a kinase-independent manner by aiding the recruitment of AKT to the membrane (Higuchi et al., 2008). In colorectal cancer cells, decreased PAK1 expression inhibited the phosphorylation of AKT, which correlated with a subsequent reduction in cell survival, proliferation, and invasion (Huynh et al., 2010). A link between PAK1 and AKT has also been demonstrated in fibroblast-like cells of monkeys, where AKT phosphorylation was inhibited by the expression of the dominant-negative PAK1 mutant (Higuchi et al., 2008). Indeed, silencing PAK1 or PAK2 decreased the activation of endogenous AKT, indicating that PAK1 and PAK2 are required for AKT phosphorylation. Based on these observations, it is apparent that the association between PAK-1 and AGE-1/PI3K and/or AKT-1/AKT shortens the lifespan of *C. elegans*. One plausible explanation is that either AGE-1 or AKT-1 directly interacts with PAK-1, shortening the lifespan, at least in part, by inhibiting DAF-16 activity. Another possibility is that PAK-1 may trigger AGE-1 and AKT-1 activity, resulting in phosphorylation of DAF-16 and SKN-1, which controls the subcellular and nuclear localization of these transcription factors (Fig. 8). However, the precise relationship between these two theories has not been explored in *C. elegans* and should be investigated in future studies.

4.7. *PAK1 regulation by merlin and its impact on longevity*

Merlin or moesin-ezrin-radixin-like protein (OMIM: 607379) is an *NF2* gene product that acts as a tumor suppressor in humans whose dysfunction causes type 2 neurofibromatosis (NF2; OMIM: 101000). Merlin is a direct inhibitor of PAK1 and this inhibitory function is mediated by its binding to the PBD and by inhibiting PAK1 recruitment to focal adhesions (Kissil et al., 2003). Merlin is a cytoskeletal linker protein that limits tumor growth and progression through increased apoptosis, decreased proliferation, and contact-dependent growth inhibition (Morrow and Shevde, 2012). Loss of merlin expression leads to abnormal activation of *PAK1* and a group of mitogenic signaling cascades that mediate cell survival, adhesion, motility, size, morphology, and proliferation. Several key signaling pathways such as mTORC1/2 (James et al., 2012, 2009), PI3K/Akt (Rong et al., 2004), receptor tyrosine kinase (RTK) (McClatchey and Fehon, 2009), Ras/Rac (Morrison et al., 2007), epidermal growth factor receptor (EGFR) (Curto et al., 2007), Hippo (Zhao et al., 2018a), CD44 (Morrison, 2001), and cMET (Zhao et al., 2018b) are deregulated following the loss of merlin. Among others, mTOR, PI3K/Akt, RTK, and EGFR signaling are directly implicated in aging and pathogenesis of many age-related diseases. Though

merlin regulates these aging signaling, the involvement of merlin in extending healthy life expectancy remains unknown. In *C. elegans*, merlin is encoded by the *nfm-1* gene and is predicted to enable actin-binding activity. *nfm-1* expressed in intestinal cells, pharyngeal muscles, gonadal sheath, and excretory and rectal gland cells (wormbase.org). NFM-1/Merlin is the most well-identified upstream regulator of the Hippo signaling pathway in *C. elegans* and is conserved across species. The Hippo pathway in *C. elegans* consists of NFM-1–WTS-1–YAP-1–EGFL-44 that regulates tissue-size homeostasis and maintains apicobasal polarity in the growing intestine (Lee et al., 2019a). NFM-1 has been shown to inhibit the activity and nuclear accumulation of the Yes-associated protein homolog YAP-1 by phosphorylation *via* the activation of WTS-1 in the Hippo pathway (Lee et al., 2019a). *C. elegans* strain carrying loss-of-function *yap-1*(*tm1416*) allele exhibited extended lifespan and healthspan in the middle age of life (Iwasa et al., 2013), which provides indirect evidence for the involvement of *nfm-1* in aging (Fig. 9). Future research is therefore needed to delineate the role of *nfm-1*/merlin in aging and longevity. Based on these observations, we believe that *nfm-1*/merlin may downregulate the activity of *pak-1*, *yap-1*, and other gerontogenes (*i.e.*, *age-1*/PI3K, *akt-1*/AKT, *let-363*/mTOR) in *C. elegans* to extend healthy longevity.

4.8. Advanced glycation end products (AGEs) and PAK1

Advanced glycation end products (AGEs) are a complex, potentially harmful, and heterogeneous group of irreversible compounds that are mainly formed in the classical Maillard reaction. The Maillard reaction (or glycation) occurs when the reactive carbonyl group of a reducing sugar reacts in a non-enzymatic way with amino groups of proteins, lipids, and/or nucleic acids (Singh et al., 2001). AGEs can be produced from exogenous or endogenous sources and their accumulation in various tissues has been evidenced in several species during the normal aging process (Chaudhuri et al., 2018). Various forms of AGEs are the potential drivers of aging and are implicated in the development and progression of chronic age-related diseases (Chaudhuri et al., 2018; Luevano-Contreras and Chapman-Novakofski, 2010; Monnier and Taniguchi, 2016; Rungratanawanich et al., 2021). In *C. elegans*, the accumulation of AGEs was found to be decreasing the lifespan and healthspan (Morcos et al., 2008). The formation of intracellular AGEs in *C. elegans* is controlled by *glod-4* gene, which encodes glyoxalase, and its overexpression prolongs lifespan and decreases the glycolysis-derived dicarbonyl metabolite methylglyoxal (MG; a precursor of AGEs)-induced modification of mitochondrial proteins and ROS production. On the other hand, *glod-4* mutation decreases lifespan (Morcos et al., 2008). Moreover, high dietary AGEs suppress the expression of *glod-4* and *gst-4*; they function to prevent the formation of more AGEs (Kingsley et al., 2021). A recent study showed that a low dose of exogenous MG or MG produced during inactivation of *gcat*, a gene that encodes glycine-C-acetyltransferase involved in threonine metabolism, increases *C. elegans* healthspan through proteohormesis, which is regulated by HSF-1 and SKN-1 transcription factors (Ravichandran et al., 2018). Pharmacological interventions such as FDA-approved drug rifampicin and ferulsinaic acid have been shown to reduce the AGE-mediated glycation and extend the lifespan in *C. elegans* (Golegaonkar et al., 2015; Sayed, 2011). This evidence suggests that genetic and pharmacological therapeutics that lowers AGE accumulation could be an effective strategy to retard aging and age-related metabolic and degenerative diseases. Similar to MG, reactive α-dicarbonyls (α-DCs) accumulate with age, and their elevated levels showed age-related disease pathologies in *C. elegans*. The conserved TRPA-1 sense α-DCs and detoxify them by activating SKN-1/Nrf2 *via* CaMKII. In addition, α-lipoic acid and podocarpic acid were found to activates TRPA1-Nrf2 pathway that ameliorates the deleterious consequences of α-DCs and MG deposition in *C. elegans* and mammalian cells (Chaudhuri et al., 2016). These findings imply that the genetic and/or pharmacological inhibitions of AGE accumulation can delay or even halt the onset and progression of aging and age-related complications. Although the PAK1 mutation retard aging in *C. elegans* and mice, the direct involvement of PAK1 and PAK1 blockers on AGE accumulation has remained unknown. We believe that the formation and accumulation of AGEs in long-lived *pak-1* mutants can be altered by established or novel detoxification mechanisms. Moreover, potent PAK1 blockers (natural or synthetic) may exert dual effects that extend organismal lifespan and reduce glycation *in vivo*. Future interventional studies on *C. elegans* and higher models are therefore required to address this hypothesis experimentally.

4.9. Epidermal growth factor (EGF) signaling and PAK1 in healthy longevity

Epidermal growth factor (EGF) is known for its role in regulating cell division and differentiation. Activation of the EGF signaling pathway has been shown to promote both the health and longevity of *C. elegans* independently of IIS pathway (Iwasa et al., 2010). In *C. elegans*, *let-23* encodes the only member of the EGF receptor (EGFR/ERBB4) family transmembrane tyrosine kinase that regulates male spicule formation, larval viability, the inductive signal during vulva development, ovulation, posterior development of the epidermis, and stress-induced sleep (wormbase.org). EGFR/*let-23* gain-of-function mutants survived more robustly in the middle adulthood stage, retained swimming vigor and muscle integrity, and exhibited low age pigment (lipofuscin) levels later in life, revealing a role for *let-23* in promoting healthy aging. Conversely, the worms carrying a reduction-of-function mutation in *let-23* exhibit shortened lifespan and reduced swimming vigor in mid-adulthood; as does mutation of *lin-3*/EGF (Iwasa et al., 2010). Activation of EGF signaling not only regulates the lifespan and healthspan in *C. elegans* but is also directly linked to the maintenance of organismal-wide protein homeostasis (proteostasis). The EGF signaling triggers the RAS–RAF–MAPK pathway to regulate the expression of Skp1-like protein SKR-5 and HSP16-type chaperones through the PLZF transcription factors EOR-1 and EOR-2, which determine the time of activation of the ubiquitin-proteosome system (UPS) in adulthood worms (Liu et al., 2011a). Interestingly, *skr-5* is also a transcriptional readout of DAF-16, signifying that it might be a common link between the EGF and IIS signaling pathways (Murphy et al., 2003a; Safra et al., 2014). LET-23–mediated Ras–ERK signaling cascade phosphorylates SKN-1/Nrf2 transcription factor and it triggers the expression of several antioxidant and detoxification genes, which help to minimizes protein oxidation and aberrant aggregation of insoluble proteins (Rongo, 2011). Recently, it was shown that augmented EGF signaling enhanced the expression of *gst-4* independently of the transcriptional activity of SKN-1 (Detienne et al., 2016), indicating that *gst-4* is not under exclusive transcriptional readout of the SKN-1. The binding of *lin-3*/EGF to its receptor (*let-23*/EGFR) can be directly regulated by putative EGF-binding proteins HPA-1 and HPA-2. HPA-1/2 normally functions as a negative regulator of EGF signaling pathway and acted largely through the EGFR/IP3R axis to confer healthy aging (Iwasa et al., 2010). However, some details of this mechanism remain to be explored, including the questions of which isoform of LIN-3 (isoform a-h) modulates lifespan and whether HAP-1 or HPA-2 binds to LIN-3. Finally, upon activation, EGFR can promote PI3K activity directly or indirectly with p85 interaction, and activated Ras can also trigger the PI3K. It is unknown whether EGF signaling in *C. elegans* activates PI3K or Akt. Some phytomolecules, including royalactin (Detienne et al., 2014) and santalol isomers (Mohankumar et al., 2020), have been shown to extend the healthy life expectancy of *C. elegans* by modulating EGF signaling, at least in part.

Apart from the activation of highly conserved RAS–RAF–MAPK pathway, *let-23*/EGFR activation triggers phospholipase Cγ (PLC-γ encoded by *plc-3*) and IP3-inositol (1,4,5) triphosphate receptor (IP3R, encoded by *itr-1*) pathway to affect defecation cycles and ovulation (Clandinin et al., 1998). It was also shown that EGF signaling act through PLC-γ–IP3R axis to extend *C. elegans* healthspan and lifespan

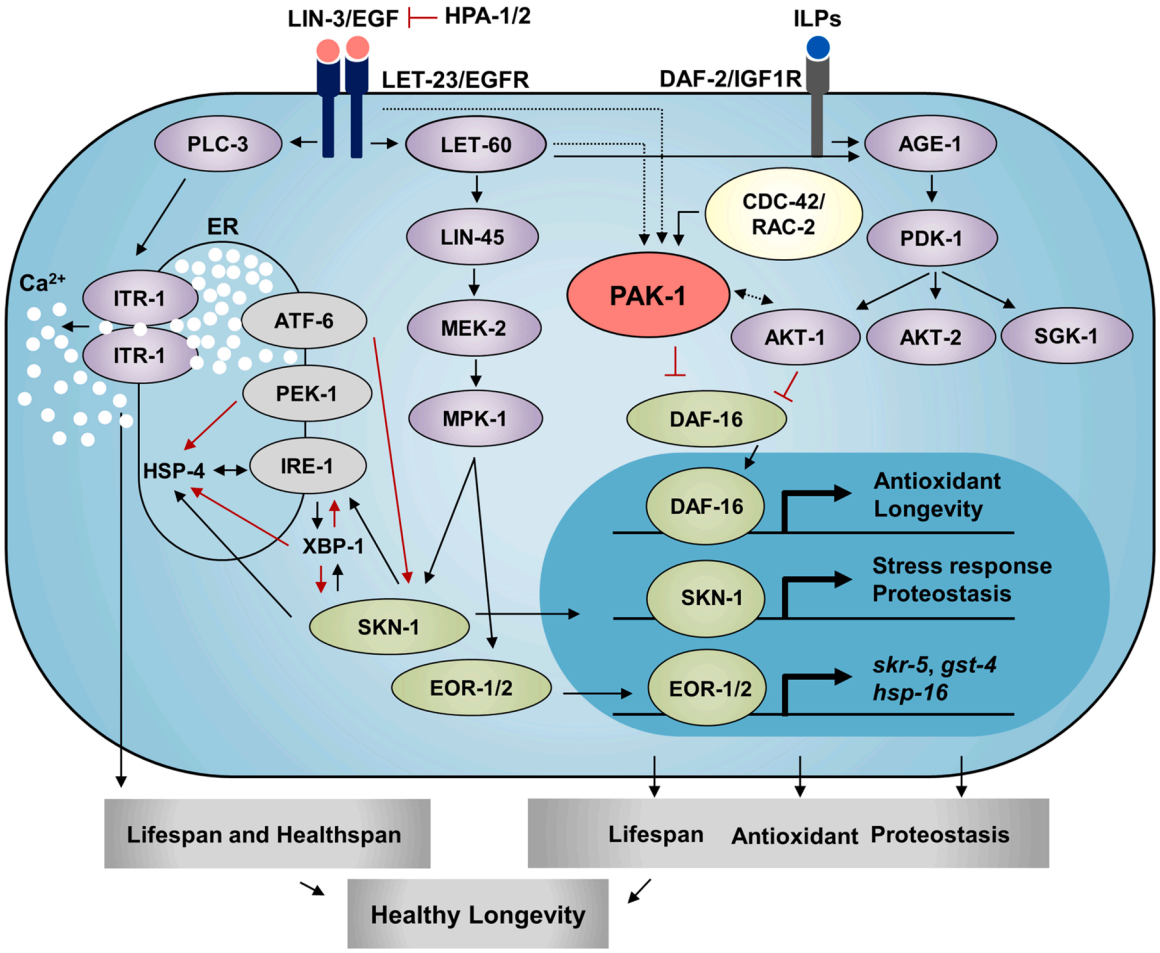

Fig. 10. Several signaling modules interact with EGF pathway in regulating health and longevity. Black arrows indicate stimulatory interaction, while T-bars indicate repressive interaction. Dashed lines indicate that the mechanism is uncertain in *C. elegans*, and red arrows indicate the direct transcriptional effect of the canonical UPR. Some additional reviews describe this pathway and its overlap in more detail (Blackwell et al., 2015; Rajalingam and Dikic, 2011; Rongo, 2011; Yu and Driscoll, 2011).

(Iwasa et al., 2010). Iwasa et al. found that knockdown of either *plc-3* or *itr-1* suppressed the youthful swimming phenotype seen with the *let-23* gain-of-function mutation. Reduction-of-function mutation in *itr-1* accelerates swimming decline and shortens median and maximum lifespan by 11 % and 31 %, respectively. However, worms carrying a gain-of-function mutation in *itr-1* exhibit reduced lipofuscin accumulation, increased lifespan and relatively youthful swimming vigor. The activity of IP3R was found to be downstream of (or in parallel with) *lin-3*/EGF, since *itr-1*(*gf*);*lin-3*(*rf*) double mutants displayed healthspan extension. After *let-23*/EGFR activation, the downstream signaling through PLC-γ–IP3R promotes healthy aging by enhancing the release of intracellular calcium from the endoplasmic reticulum (ER) and influencing cellular calcium homeostasis (Iwasa et al., 2010); nevertheless, how calcium release promotes healthspan and longevity is not known. Undoubtedly, calcium homeostasis has been suggested to be modulated in the long-lived Klotho mouse and plays an essential role in adult maintenance, particularly in non-dividing tissues (Imura et al., 2007). This evidence indicated that the components of the RTK–PLC-γ–IP3R signaling axis as a plausible therapeutic target for lifespan and healthspan extension. To date, there is no experimental evidence directly addressed the involvement of EGF signaling in the control of PAK-1 activation in *C. elegans*. However, we speculated that EGFR-mediated Ras activation might regulate the PAK-1 in *C. elegans*. Future efforts to understand how the EGF signaling pathway affects PAK-1 will have important implications not only on the aging process itself but also on cancer, neurodegeneration, and other age-related diseases.

A team of researchers from the Sanford Burnham Prebys Medical Discovery Institute, USA, identified that nutrient depletion could trigger the activation of PAKs through EGFR signaling in pancreatic ductal adenocarcinoma (PDAC) tumor cells. They found that glutamine starvation and EGF stimulation can increase the phosphorylation of PAK1/2 and that the inhibition of Rac1 partially attenuates PAK activation in PDAC cells upon glutamine depletion. Metabolic activation of PAK is efficiently suppressed by inhibiting EGFR and Rac1 (Lee and Commisso, 2020). The dominant-negative form of Rac1 abolished the metabolic activation of PAK1/2; however, nutrient depletion increased PAK phosphorylation in the presence of constitutively active Rac1. This indicates that EGFR could directly contribute to the activation of PAK1/2 (Bokoch, 2003). In addition, EGF signaling through PAK controls the extent of macropinocytosis in these cells (Lee et al., 2019c) and stimulates Rac1, PAK, and JNK in vascular smooth muscle cells (Beier et al., 2008). These *in vitro* and *in vivo* evidence suggests that EGF signaling-induced PAK activation is required for the survival of proliferating or cancer cells under normal and nutrient stress conditions, while activation of EGF signaling in *C. elegans*, particularly in non-dividing tissues, extends healthspan (Fig. 10). Based on these observations, it is evident that preciously modulated EGF signaling branches somehow interact with PAK1 to promote healthy longevity and effective repair processes; caution should be exercised, however, as activated EGF signaling has been implicated in epithelial and other cancers. To date, the role of EGF signaling in promoting cellular longevity (especially in non-dividing tissues or postmitotic cells) has not

been addressed in other experimental models. Given the conserved nature of EGF signaling, we believe that it should have a similar impact on healthy aging in higher models.

5. Bioactive PAK1 blockers in *C. elegans*

5.1. Propolis

Propolis is a mixture of resinous substances collected from plants and processed by bees to defend the colony from pathogenic invaders and maintain the hive. The name "propolis" is derived from Greek terms "pro" and "polis" meaning "protection" and "city (comb)," respectively. Propolis has been used for over 5000 years, and ancient Egyptians used it for embalming, religious rituals, and medicinal purposes. Propolis is classified into seven groups based on the collection area and vegetation source: brich, Clusia, green, Mediterranean, Pacific, poplar, and red. Although all propolis groups appear to share similar pharmacological properties, biotic and abiotic factors can directly influence their phytochemical composition and bioactivity. For example, Brazilian green propolis contains artepillin C, New Zealand propolis (Bio30) derived from young buds of willow and poplar trees contains CAPE, and propolis from subtropical areas of the Pacific, such as Hawaii, Okinawa, and Taiwan, are rich in prenylflavonoids, including nymphaeols, derived from *Macaranga tanarius* L. (Euphorbiaceae) trees (Sforcin, 2016; Shahinozzaman et al., 2021). Nevertheless, all propolis products block PAK1 activity and suppress tumor growth. In particular, CAPE-based propolis (Bio30), Brazilian green propolis, and Okinawa propolis completely suppressed the growth of human neurofibromatosis tumors and lung cancer cells (A549 cell line) *via* PAK1 inactivation (Demestre et al., 2009; Messerli et al., 2009; Taira et al., 2016). In 2016, we demonstrated the longevity-promoting ability of Okinawa propolis using the multifaceted *in vivo C. elegans* model for the first time (Taira et al., 2016). We found that, among the tested pharmacological doses (0.5, 1, and 5 μg/mL), 1 μg/mL Okinawa propolis enhanced the mean lifespan of wild-type worms by 33 % (24 ± 0.02 days) compared with that of untreated controls (18 ± 1.1 days) in a PAK1-dependent manner. Importantly, even the lowest concentration of Okinawa propolis extended the lifespan significantly higher than the positive control resveratrol (10 μg/mL). In addition, Okinawa propolis administration upregulated the expression of *hsp-16.2* by more than 30 % in the transcriptional reporter strain CL2070 carrying the *hsp-16.2::GFP* transgene. *hsp-16.2* offers conserved protection against stress and is a key regulator of lifespan in *C. elegans* that is inactivated by PAK-1 to an extent. Moreover, exposure to the study doses did not adversely affect the survival rate of *C. elegans,* but reduced the brood size as seen in worms carrying the *pak-1(ok448)* loss-of-function allele. Currently, there are no reports on the longevity-promoting effects of other propolis groups; however, Hawaiian and Taiwanese propolis could be explored as safe anti-aging therapeutics because they are highly similar to Okinawa propolis in terms of phytochemistry (Shahinozzaman et al., 2021). Moreover, the major active prenylated flavonoids in Okinawa and Taiwanese propolis, including nymphaeol-A, nymphaeol-B, nymphaeol-C, 3′-geranyl-naringenin, and isonymphaeol-B, were found to be nontoxic and displayed several pharmacological properties (Shahinozzaman et al., 2018, 2021). Notably, nymphaeols A–C from Okinawa propolis suppress the growth of A459 lung cancer cells and directly inhibit the oncogenic kinase PAK1 *in vitro* (Nguyen et al., 2017b); however, their anti-aging effects remain to be clarified.

5.2. Caffeic acid and its natural ester (CAPE)

Caffeic acid and its ester (CAPE) have also been shown to increase stress resistance and enhance the lifespan of *C. elegans*. Caffeic acid directly reduces RAC1 GTPase protein and activity levels, followed by the downregulation of NADPH oxidase activity, leading to inactivation of PAK1 (Xu et al., 2005). Caffeic acid inhibited the growth of A549 lung cancer cells at 100 μM concentration. Exposure to caffeic acid enhances lifespan and stress tolerance and reduces intestinal fat deposition and lipofuscin (age pigment) accumulation in *C. elegans*. The lifespan and stress tolerance enhancing properties of caffeic acid were found to rely on the genes *osr-1*, *unc-43*, *sek-1*, *sir-2.1*, and *daf-16*. Caffeic acid also modestly affects pharyngeal pumping rates, reproduction, and locomotion (Pietsch et al., 2011). Recently, caffeic acid was reported to extend the lifespan of worms expressing $A\beta_{1-42}$ (CL4176) and PolyQ40 (AM141) in body wall muscle cells by activating DAF-16 and HSF-1 transcription factors and their direct transcriptional readouts *sod-3*, *gst-4*, and *hsp-16.2*. In addition, caffeic acid enhances the expression of *lgg-1*, a gene that positively regulates autophagy, and alters the metabolism-related pathways in the *C. elegans* AD model (Li et al., 2021). Furthermore, caffeic acid prevented the reduction of survival in wild-type *C. elegans* and protected them against motor alterations induced by the dopaminergic toxin 6-OHDA, the pro-oxidant ferrous sulfate, and the excitotoxin quinolinic acid through SKN-1/Nrf2 activation (Colonnello et al., 2020). However, the presence of the COOH moiety in caffeic acid limits its permeability through the negatively charged plasma membrane, and this might be one of the reasons for its relatively higher IC_{50} values. To increase the cell permeability of caffeic acid, Hiroshi Maruta's group in 2017 esterized caffeic acid with water-soluble 1,2,3-triazolyl alcohol through click chemistry. The esterization was found to boost the anticancer activity of caffeic acid (15C), by over 400-fold, against the PAK1-dependent growth of A549 cells, while increasing its cell permeability by more than 70-fold (Takahashi et al., 2017).

Similar to caffeic acid, CAPE downregulates the PAK1 effector RAC1 GTPase; however, its anticancer activity is approximately 10 times higher than that of caffeic acid. CAPE is a major active constituent of propolis and a natural ester of caffeic acid that is widely used in traditional medicine. Interestingly, 100 μM CAPE increased the median and maximum lifespan of *C. elegans* by 9 % and 13 %, respectively, compared with that of the untreated control group. CAPE increased the resistance to thermal stress and diminished the thermal stress-induced intracellular ROS production by approximately 50 %. This increase in lifespan and stress resistance was mediated by DAF-16, but not SKN-1, transcription factor in *C. elegans* (Havermann et al., 2014). In 2013, Hiroshi Maruta's group demonstrated that CAPE reduced brood size and upregulated the expression of *hsp-16.2* through DAF-16/FOXO (Yanase et al., 2013). Eleftherios Mylonakis and colleagues found *pak-1* mutant worms to be highly susceptible to infection by the pathogenic fungus *Candida albicans*. However, CAPE supplementation increased the survival of wild-type *C. elegans* challenged with *C. albicans*. CAPE requires the CED-10/PAK-1 pathway to perform this function, indicating the need for PAK-1 activity to elicit the immune response against *C. albicans* infection and induce the immunomodulatory activity of CAPE (Coleman et al., 2016). However, PAK-1 activity reduces the longevity of *C. elegans* under basal conditions (Yanase et al., 2013). Therefore, identifying the precise relationship between these seemingly conflicting observations is essential for future research.

5.3. Apigenin and artepillin C

Apigenin (5,7,4′-trihydroxyflavone) is a common flavonoid found in a wide range of plants, including chamomile flowers (*Matricaria recutita* L.) and CAPE-based propolis. Apigenin directly inhibits PAK1 activity by downregulating PI3K/Akt. In *C. elegans*, apigenin extends the lifespan in a DAF-16/FOXO-dependent manner and enhances the expression of its effector *sod-3*. It also inhibits the expression of *daf-2*, an essential gene in the IIS pathways that directly controls DAF-16 activity in *C. elegans* (Kawasaki et al., 2010). Interestingly, apigenin is structurally related to the well-established PAK1 inhibitors called nymphaeols; therefore, it is quite possible that other flavonoids, including quercetin, kaempferol, myricetin, taxifolin, naringenin, fisetin, nobiletin, isorhamnetin, liquiritigenin, isoliquiritigenin, and sakuranetin, may also inhibit PAK-1

activity in *C. elegans*. Moreover, quercetin, kaempferol, naringenin, nobiletin, and fisetin supplementation have been shown to extend the lifespan and healthspan of *C. elegans* by promoting DAF-16 activity (Pallauf et al., 2017). These results indicate an interaction between these flavonoids and PAK-1 to extend lifespan, at least in part.

Artepillin C, a constituent of the Brazilian green propolis, is a major anticancer agent and has been shown to inhibit PAK1 activity with an IC_{50} of 25 μM. In *C. elegans*, artepillin C reduces the brood size, protects against heat-induced paralysis phenotype, and activates *hsp-16.2* in a DAF-16-dependent manner (Yanase et al., 2013). However, the precise biological mechanism underlying its life-promoting ability under basal and stress conditions remains unclear. Similar to caffeic acid, artepillin C possesses a COOH moiety that limits its cell permeability. Interestingly, triazolyl esterification of artepillin C was found to enhance its cell permeability eight-fold, leading to a 100-fold increase in its anticancer activity (Takahashi et al., 2017).

5.4. Curcumin

Curcumin (diferuloylmethane) is a hydrophobic polyphenol constituent of the dietary spice turmeric (*Curcuma longa* L.), and has a broad spectrum of biological activities. Curcumin directly inhibited PAK1 activity under *in vitro* conditions, with an IC_{50} of approximately 16 μM (Nguyen et al., 2017b). Curcumin retarded aging and age-related declines (*i.e.*, body size and pharyngeal pumping rate) and increased the survival rate during juglone-induced oxidative stress conditions in *C. elegans*. The lifespan extension induced by curcumin was found to rely on gerontogenes *age-1*, *sir-2.1*, *osr-1*, *sek-1*, *mek-1*, *skn-1*, and *unc-43*, but not on *daf-16* and *mev-1* (Liao et al., 2011). In addition, curcumin required *age-1*, *akt-1*, *pdk-1*, *osr-1*, *unc-43*, *sek-1*, *mev-1*, *sir-2.1*, and *skn-1* to protect the worms from oxidative stress-induced reduction in survival (Yu et al., 2014). Notably, the effect of curcumin on lifespan and stress tolerance depended on both *age-1* (PI3K) and *akt-1* (AKT1) in addition to other gerontogenes. The involvement of PI3K and AKT1 in PAK1 inhibition has been established (Higuchi et al., 2008; Huynh et al., 2010); therefore, curcumin may extend lifespan by blocking PAK-1 activity in *C. elegans*. However, its poor aqueous solubility, lower pharmacokinetic profile, and chemical instability limit its use as an effective therapeutic agent and compromise its clinical application. Liposome-mediated delivery of curcumin is considered a viable strategy to overcome these limitations, and was shown to remarkably improve its solubility and enhance its systemic bioavailability. In 2014, a group led by Michael Wolzt at the Medical University of Vienna conducted a completely randomized phase I clinical trial to investigate the safety, tolerability, and pharmacokinetics of intravenously administered liposomal curcumin (10–400 mg/m^2) in healthy individuals (18–45 years). They observed that a single dose of liposomal curcumin appeared to be safe up to 120 mg/m^2. Increasing concentrations were found to be toxic, as represented by the altered morphology of red blood cells (ClinicalTrials.gov Identifier: NCT01403545).

5.5. Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) is a naturally occurring polyphenol that was first isolated by Takaoka from *Veratrum grandiflorum* Loes. fil. (white hellebore) (Takaoka, 1939). Subsequently, resveratrol was detected in grapevines (*Vitis vinifera* L.), and a relatively higher abundance of resveratrol was observed in red wine (5 mg/L on average). In 2014, we confirmed that resveratrol directly inhibits PAK1 activity with an IC_{50} of approximately 15 μM under *in vitro* conditions (Taira et al., 2016), and it was promoting longevity in several model organisms. In addition to extending the lifespan of *Saccharomyces cerevisiae* up to 70 % (Howitz et al., 2003), early adulthood supplementation with resveratrol extended the mean lifespan of *Drosophila melanogaster* and *C. elegans* without affecting the fecundity rate (Wood et al., 2004). In all cases, the life-promoting ability of resveratrol was originally shown to be sirtuin-dependent. In contrast, a study from a group of investigators at University College London, UK, claimed that resveratrol supplementation only marginally increases the lifespan of *D. melanogaster* and *C. elegans*, and that this minimal effect is regulated by the sirtuin gene (Bass et al., 2007). Recently Yoon et al. demonstrated that the ERK homolog MPK-1 contributes to resveratrol-mediated longevity extension by inducing nuclear translocation of the SKN-1/Nrf2 transcription factor independent of the SIR-2.1/DAF-16 pathway. In addition, resveratrol-induced life prolongation was entirely abolished by *mpk-1* RNAi in *daf-16* and *sir-2.1* mutants, strongly indicating the involvement of MPK-1 in resveratrol-mediated longevity in *C. elegans* (Yoon et al., 2019). Moreover, resveratrol directly inhibits COX-2 that requires PAK1 for its activation. Therefore, given the established role of PAK1 in regulating ERK and COX-2, we believe that resveratrol may extend the lifespan of *C. elegans* by blocking PAK-1 activity, thereby leading to MPK-1 activation and COX-2 inhibition. However, the mechanism underlying the direct action of resveratrol on PAK-1 activity in *C. elegans* remains to be elucidated and detailed mechanistic studies are currently underway in our laboratory.

5.6. Ursolic acid

Ursolic acid (3β-hydroxy-12-urs-2-en-28-oic acid) is a pentacyclic triterpene acid that belongs to the cyclosqualenenoid family and is found in several medicinal plants, fruits, and spices, including apple, cranberry, bilberry, prune, blueberry, thyme, lavender, oregano, basil, rosemary, and olive. Ursolic acid inhibits the PAK1-dependent growth of A549 lung cancer cells with an IC_{50} of approximately 33 ± 0.03 μM (Rashid et al., 2013) as well as suppresses the activation of JAK2 (Shanmugam et al., 2011), an important tyrosine kinase that phosphorylates PAK1 (Rider et al., 2007). Although ursolic acid has many pharmacological effects, its poor bioavailability (due to the presence of a COOH moiety) under *in vivo* conditions hampers its clinical application. In 2013, a highly cell-permeable ester of ursolic acid was synthesized, through a click chemistry-based approach, by coupling a 1,2,3-triazolyl ring to the COOH moiety at C28. The esters of ursolic acid, namely 7 g, 7p, and 7 r, were found to be most effective in their study with IC_{50} values less than 0.1 μM against certain cancer cell lines (Rashid et al., 2013). Interestingly, an Indian group led by Rakesh Pandey found that ursolic acid at 25 μM promotes longevity, stress resistance, and proteostasis in *C. elegans*; they further identified the precise mechanism underlying its anti-aging effects (Negi et al., 2017, 2016). Mechanistic studies have shown that ursolic acid-mediated longevity depends on JNK-1 and mimics DR by enhancing SKN-1/Nrf2 nuclear accumulation. The longevity mechanism is independent of PHA-4/FOXA and IIS signaling and partially depends on *sir-2.1*. Furthermore, ursolic acid reduces the aggregation of polyQ and preserves impaired proteostasis with age *via* JNK-1 activation. Ursolic acid treatment also reduced the brood size and prolonged the reproduction span as in *pak-1* deficient worms. Considering the role of PAK1 in regulating DR and JNK1 activity, ursolic acid possibly blocks PAK-1 to enhance the lifespan and stress tolerance in *C. elegans*. More recently, Naß and Efferth from Johannes Gutenberg University, Germany, identified that ursolic acid ameliorates stress-induced ROS generation, improves lifespan, and upregulates the expression of *dop-1* and *dop-3* genes exhibiting dopamine neurotransmitter receptor activity in *C. elegans* (Naß and Efferth, 2021).

5.7. Caffeine

Caffeine (1,3,7-trimethylxanthine) is a highly consumed psychoactive alkaloid in the xanthine family and is a widely studied phytomolecule owing to its pronounced biological properties. It is present in many stimulating beverages, such as coffee, tea, and soft drinks, and chocolates. Gabrielli's group at the University of Queensland in Australia found that caffeine triggers apoptosis in mitotic checkpoint-

arrested cells by directly inhibiting PAK1 activity. It also promoted a quick apoptotic response, in which >40 % of cells underwent apoptosis within 5 h of caffeine treatment (Gabrielli et al., 2007). In the *C. elegans* model, chronic exposure to caffeine (5 mM) during adulthood extends the healthspan and lifespan by activating the FOXO transcription factor DAF-16 in a manner similar to that in the reduced IIS pathway. Caffeine appears to act independently of sirtuin ortholog *sir-2.1*, p53 ortholog *cep-1*, and hypoxia-inducible factor ortholog *hif-1*. In addition, caffeine exposure delayed the polyQ-induced paralysis phenotype in the *C. elegans* model of HD, independent of DAF-16 (Sutphin et al., 2012) and protected against DAergic neurodegeneration (Manalo and Medina, 2018). In contrast, Bridi et al. found that the caffeine-induced lifespan extension was partially dependent on DAF-2 but not on DAF-16. Moreover, they demonstrated the role of adenosine signaling pathway in caffeine-induced lifespan extension (Bridi et al., 2015), as it is a known inhibitor of cAMP phosphodiesterase and adenosine receptor antagonist. This inconsistency in results might be related to the differences in the methodology and laboratory environment. Notably, caffeine-treated worms exhibited *pak-1* deficiency-associated phenotypes, such as reduced reproduction, body length, and delayed larval development (Bridi et al., 2015).

Coffee and its active ingredients have similar effects as that of caffeine on *C. elegans*. Thus, coffee and its ingredients may block PAK-1 activity, leading to lifespan extension. Indeed, green coffee bean extract was shown to increase the mean lifespan of *C. elegans* by 46.1 %, and the extract-treated worms were more resistant to oxidative stress. The major activity demonstrated by green coffee extract was mediated by 5-O-caffeoylquinic acid, an isomer of chlorogenic acid. Green coffee beans consist of 50 % chlorogenic acids, of which 5-O-caffeoylquinic acid is the most studied chlorogenic acid ester (Amigoni et al., 2017). Chlorogenic acid extends healthy longevity in *C. elegans* mainly by activating the FOXO transcription factor in the IIS pathway (Zheng et al., 2016). In 2018, Farias-Pereira et al. investigated the effect of green coffee bean extract and 5-O-caffeoylquinic acid on the lipid profile of *C. elegans* and found that both treatments reduced the triglyceride content by 29 % and 23 %, respectively. This lipid-lowering effect was mediated by *sbp-1* (sterol regulatory element-binding protein), *far-6* (fatty acid/retinol-binding protein), and *daf-16* (Farias-Pereira et al., 2018).

5.8. Epigallocatechin gallate (EGCG)

EGCG (epigallocatechin-3-gallate) is the most abundant and potent flavonoid in green tea. EGCG reportedly has numerous health-promoting and therapeutic effects against a broad range of diseases, including cancer, HIV, neurodegeneration, cardiovascular diseases, chronic fatigue syndrome, and cerebrovascular insult. Importantly, EGCG passes through the blood–brain barrier and, therefore, can be used to treat brain tumors, NDDs, and other CNS disorders and diseases. It was shown to inhibit PAK1 directly (Tepedelen et al., 2017) as well as through the activation of the tumor suppressor protein Merlin (Tsukamoto et al., 2014). Merlin, encoded by the NF2 gene, directly blocks PAK1 activity and RAS transformation. This green tea flavonoid has been demonstrated to improve longevity under oxidative and thermal stress conditions; however, it failed to extend the lifespan of *C. elegans* under basal conditions. The longevity-promoting effect of EGCG may be attributed to its antioxidant activity and its upregulation of gerontogenes, including *daf-16*, *skn-1*, *sod-3*, and *hsp-16.2* (Zhang et al., 2009). Subsequently, EGCG was found to promote healthy lifespan *in C. elegans,* mainly during early to mid-adulthood. EGCG administration triggered AAK-2 activation by transiently increasing ROS production. AAK-2 alters NAD^+ metabolism, activating SIR-2.1 that in turn activates the FOXO transcription factor, which upregulates various stress-protective and geroprotective genes. Together, these results suggest that EGCG extends the lifespan of *C. elegans via* mitohormesis and activation of the AAK-2/SIR-2.1/DAF-16 signaling axis (Xiong et al., 2018). In addition, EGCG was reported to activate the LKB1/AMPK pathway in mammalian cell lines and in mice. Interestingly, LKB1 inactivates the oncogenic/aging kinase PAK1 and activates AMPK simultaneously. Therefore, EGCG may trigger the activation of *par-4*, a *C. elegans* ortholog of mammalian LKB1, which in turn upregulates *aak-2* and inhibits *pak-1* to exert anti-aging effects.

5.9. Urolithin-A

Urolithin-A (3,8-dihydroxy-6H-benzo[c]chromen-6-one) is produced by the colonic microbiota from ellagic acid and ellagitannins found in pomegranate, blackberry, raspberry, strawberry, Indian gooseberry, and nuts. Among the four identified urolithins (urolithin A–D), urolithin-A is one of the most predominant isoform found in urine and blood plasma samples of the species investigated to date, including humans. Thus, urolithin-A is generally regarded as the bioactive molecule underlying the health-promoting properties of pomegranate and other food products rich in ellagic acid and ellagitannins. More importantly, urolithin-A can cross the blood–brain barrier (Gasperotti et al., 2015; Hearne et al., 2020; Kujawska et al., 2019; Yuan et al., 2016) and has been reported to be a potent neuroprotectant *in vivo* against brain aging (Chen et al., 2019; Fang et al., 2019b; Gong et al., 2019), NDDs and its associated pathologies (Fang et al., 2019b; Kujawska et al., 2019; Shen et al., 2021; Yuan et al., 2016), ischemic neuronal injury (Ahsan et al., 2019), and other CNS disorders (D'Amico et al., 2021; Shen et al., 2021). Urolithin-A treatment directly inhibited the transcript levels and activities of RAC1 and PAK1, with subsequent decreases in migration and actin polymerization in cancer cells. Additionally, it blocked the activity of epidermal growth factor, a well-known activator of RAC1/-PAK1 signaling (Alauddin et al., 2020). In *C. elegans*, urolithin-A extends lifespan and improves fitness by inducing mitophagy, the selective removal of damaged and superfluous mitochondria by the autophagic machinery. Lifespan extension mediated by urolithin-A was partially dependent on *aak-2* (AMPK homolog), and it improved the age-related decline of muscle functions in mouse models (Ryu et al., 2016). In addition, it extends the lifespan and improves the pharyngeal pumping rate in the Werner syndrome *C. elegans* model (*wrn-1*[*gk99*]) by inducing mitophagy (Fang et al., 2019a). Werner syndrome is an autosomal recessive premature aging disorder (progeria) caused by mutations in the gene encoding the Werner DNA helicase. Administration of urolithin-A increased the survival, skeletal muscle respiration, and muscle function in Duchenne muscular dystrophy (DMD) mouse models and rescued impaired mitophagy in mice and *C. elegans* models of DMD, as well as in primary myoblasts from individuals with DMD (Luan et al., 2021). Interestingly, urolithin-A appears to activate AMPK by either directly binding to a subunit of the enzyme complex or by activating the upstream protein kinase LKB1 (Kim et al., 2016c). Thus, there is a possibility that urolithin-A may block the activity of PAK-1 through the LKB1/AMPK pathway, leading to lifespan and healthspan extension.

5.10. Marine compounds

Here, we summarize the PAK1 blocking potential and life-promoting ability of three marine compounds: astaxanthin, fucoidan, and frondoside A. Astaxanthin (3,3-dihydroxy-β,β-carotene-4,4′-dione) is a xanthophyll carotenoid that is naturally and widely distributed in certain marine animals (salmon, trout, crab, shrimp, lobster, and others), edible red or brown algae, bacteria, and yeast. It was found to block the PAK1-dependent growth of A549 lung cancer cells (IC_{50} = 40 μM) (Maruta and Ahn, 2017). Astaxanthin and its three geometrical isomers (*all-trans*, *13-cis*, and *9-cis*) extend the mean lifespan of *C. elegans* in a DAF-16/FOXO-dependent manner (Liu et al., 2018). Fucoidan is a complex sulfated polysaccharide (containing a substantial percentage of L-fucose and sulfate ester groups) mainly found in brown seaweed and certain marine invertebrates, such as sea cucumbers and sea urchins. Fucoidan extracted from orange-footed sea cucumber *C. frondosa* exhibited anti-metastatic potential by inhibiting the activation of

RAC1/PAK1 mediated LIMK1/cofilin signaling axis in U2OS osteosarcoma cells (Zhang et al., 2020b). It also extended the lifespan of wild-type worms challenged with *H. pylori* (Palacios-Gorba et al., 2020) and inhibited Aβ-induced toxicity by reducing Aβ deposits and ROS production in AD model of *C. elegans* (Wang et al., 2018a). Frondoside A, an anti-cancer sulfated saponin derived from *C. frondosa*, inhibited the PAK1-dependent growth of A549 cancer cells (1.5 μM) (Dyshlovoy et al., 2016). We recently found that frondoside A directly inhibits PAK1 activity under *in vitro* conditions, with an IC_{50} of approximately 1 μM (Nguyen et al., 2017b). Moreover, a low concentration of frondoside A (1 μM) strongly protected the worms from Aβ-induced toxicity and all other pathological phenotypes observed in AD models (Tangrodchanapong et al., 2020). The molecular mechanism by which fucoidan and frondoside A exert their anti-AD activity remains unexplored, but we believe that these biomolecules interact with PAK-1 to do so. These marine compounds that block PAK1 *in vitro*, may exhibit similar effects on *C. elegans*, which is an important issue to be validated in the near future.

5.11. Ketorolac

Ketorolac is a non-narcotic and non-steroidal anti-inflammatory drug that belongs to a family of carboxylic acid derivatives. It has been administered through oral and parenteral routes to control mild-to-moderate pain, cancer pain, and postoperative pain. Ketorolac is a racemic mixture of the S-form and R-form that directly inhibit RAC and COX-2, respectively. In 2017, we synthesized a 1,2,3-triazolyl ester of ketorolac (15 K) through click chemistry to enhance its cell permeability and water solubility. The resultant "azo" derivative was found to inactivate PAK1 *in vitro* (IC_{50} = 65 nM), block the PAK1-dependent growth of A549 cells (IC_{50} = 24 nM), and inhibit COX-2 (IC_{50} = 6 nM) (Nguyen et al., 2017a). In addition, the 1,2,3-triazolyl ester of ketorolac at 50 nM extended the lifespan of *C. elegans* by inhibiting *pak-1* and upregulating *hsp-16.2*. Similar to the phenotype observed in worms bearing *pak-1* loss-of-function mutation, 15 K treatment decreased the brood size in wild-type worms. Detailed mechanistic studies on mammalian models are currently underway in Maruta's laboratory (Nguyen et al., 2018).

6. Concluding remarks and future perspectives

In recent years, significant efforts have been made to understand the essential cellular functions of PAK1 under normal and diseased conditions. As an oncogenic kinase, PAK1 is considered a cancer hallmark that regulates cytoskeletal remodeling, growth, proliferation, invasion, metastasis, cancer cell immune evasion, metabolism reprogramming, and cancer drug resistance; as an aging kinase, it shortens organismal lifespan and healthspan. Given that PAK1 is a well-characterized culprit in cancer promotions and aging progression, it exerts distinct as well as overlapping functions in these processes. The functional interaction between PAK1 and DR, MAPKs, PI3K/Akt, mTOR, and AMPK has been described in several cancers; however, its role in regulating aging and longevity is still unclear. Although PAK1 has been extensively studied in cancer biology, the molecular mechanisms of *C. elegans* PAKs (PAK-1, PAK-2, and MAX-2) remain to be elucidated, and the involvement of PAK-2 and MAX-2 in lifespan regulation has not yet been described. Moreover, the mechanism by which PAK-1 extends longevity in *C. elegans* remains elusive; therefore, an in-depth scientific investigation is needed to fully understand its mechanism of action, which will pave the way for the development of more potent and target-specific pharmacological drug candidates. Currently, we are investigating such possibilities using combined *in vitro*, *in silico*, and *in vivo* techniques.

Aging, cancer, and age-related diseases are multifactorial in origin; therefore, polypharmacological approaches are needed to inhibit their onset and progression, instead of the one drug–one target–one disease approach. Targeting the different nodes of evolutionarily conserved gene regulatory networks that inhibit the activity of oncogenic/aging kinase PAK1 using genetic synergism and/or a combination of established longevity-promoting drugs might be an effective way to prolong healthy longevity. Moreover, using *C. elegans* as a versatile animal model to identify potentially useful genetic and pharmacological interventions that target PAK1 to extend a healthy lifespan would be a viable strategy. We further suggest that future investigations should be directed toward understanding the long-term benefits of identified PAK1 blocking candidate drug(s) in a broader range of experimental models and evaluate the potential translation of a combination of these interventions to the clinic. More importantly, repurposing the US FDA-approved drugs for targeting PAK1 will significantly reduce the development time and cost and advance the application of new therapeutics to reset the aging clock. Therefore, it is not surprising that the identification and development of safe, more potent, and clinically useful PAK1 blockers that could potentially help extend healthy longevity and inhibit cancer cell proliferation will hold a significant place in the pharmaceutical armory. Table 4 lists the future directions and open questions that are relevant in this area and should be addressed in the future. Detailed mechanistic studies on PAKs with respect to their regulation of aging and longevity are currently underway in our laboratory.

Table 4
Future directions and open questions for the investigation of PAKs in regulating aging and longevity.

Future directions
PAK-specific novel inhibitors should be developed to target PAK1/2 to achieve healthy longevity and potentially useful cancer therapy.
Previously established and new PAK-blocking interventions should be tested using the genetic tractability of the *C. elegans* model in the context of aging.
The uncertainty regarding the roles of PAK-1, PAK-2, and MAX-2 in several aspects of aging and longevity need to be addressed.
Other mechanisms that regulate PAK-1 activity in *C. elegans* should be investigated.
Open Questions
How does PAK1 inhibition extend lifespan?
Does PAK-1 regulate transcription factors other than DAF-16 in *C. elegans*?
Are there any other genetic factors that mediate longevity in worms bearing *pak-1* loss-of-function allele [*pak-1*(*ok448*)]?
Does *pak1* mutation-induced lifespan extension transmit to the next generation?
Do DR, mTOR, MAPKs, and PI3K/AKT regulate lifespan through PAK1?
Do EGFR, AGEs, and merlin modulates aging through PAK1?
How does PAK1 (or PAKs) modulate NDDs and associated pathologies?

Acknowledgments

We thank Paul Davis (European Molecular Biology Laboratory, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SD, UK) for his advice and technical expertise. We apologize to all researchers whose work could not be discussed in this review due to space limitations.

References

Ahn, M.-R., Bae, J.-Y., Jeong, D.-H., Takahashi, H., Uto, Y., Maruta, H., 2017. Both triazolyl ester of ketorolac (15K) and YM155 inhibit the embryonic angiogenesis in ovo (fertilized eggs) via their common PAK1-survivin/VEGF signaling pathway. Drug Discov. Ther. 11, 300–306. https://doi.org/10.5582/ddt.2017.01058.

Ahsan, A., Zheng, Y., Wu, X., Tang, W., Liu, M., Ma, S., Jiang, L., Hu, W., Zhang, X., Chen, Z., 2019. Urolithin A-activated autophagy but not mitophagy protects against ischemic neuronal injury by inhibiting ER stress in vitro and in vivo. CNS Neurosci. Ther. 25, 976–986. https://doi.org/10.1111/cns.13136.

Alahari, S.K., Reddig, P.J., Juliano, R.L., 2004. The integrin-binding protein Nischarin regulates cell migration by inhibiting PAK. EMBO J. 23, 2777–2788. https://doi.org/10.1038/sj.emboj.7600291.

Alauddin, M., Okumura, T., Rajaxavier, J., Khozooei, S., Pöschel, S., Takeda, S., Singh, Y., Brucker, S.Y., Wallwiener, D., Koch, A., Salker, M.S., 2020. Gut bacterial metabolite urolithin a decreases actin polymerization and migration in cancer cells. Mol. Nutr. Food Res. 64, 1900390 https://doi.org/10.1002/mnfr.201900390.

Alessi, D.R., Sakamoto, K., Bayascas, J.R., 2006. LKB1-dependent signaling pathways. Annu. Rev. Biochem. 75, 137–163. https://doi.org/10.1146/annurev.biochem.75.103004.142702.

Alexander, A.G., Marfil, V., Li, C., 2014. Use of *Caenorhabditis elegans* as a model to study Alzheimerâ€TMs disease and other neurodegenerative diseases. Front. Genet. 5 https://doi.org/10.3389/fgene.2014.00279.

Amigoni, L., Stuknytė, M., Ciaramelli, C., Magoni, C., Bruni, I., De Noni, I., Airoldi, C., Regonesi, M.E., Palmioli, A., 2017. Green coffee extract enhances oxidative stress resistance and delays aging in *Caenorhabditis elegans*. J. Funct. Foods 33, 297–306. https://doi.org/10.1016/j.jff.2017.03.056.

Anckar, J., Sistonen, L., 2011. Regulation of HSF1 function in the heat stress response: implications in aging and disease. Annu. Rev. Biochem. 80, 1089–1115. https://doi.org/10.1146/annurev-biochem-060809-095203.

Apostol, B.L., Simmons, D.A., Zuccato, C., Illes, K., Pallos, J., Casale, M., Conforti, P., Ramos, C., Roarke, M., Kathuria, S., Cattaneo, E., Marsh, J.L., Thompson, L.M., 2008. CEP-1347 reduces mutant huntingtin-associated neurotoxicity and restores BDNF levels in R6/2 mice. Mol. Cell. Neurosci. 39, 8–20. https://doi.org/10.1016/j.mcn.2008.04.007.

Arias-Romero, L.E., Chernoff, J., 2008. A tale of two Paks. Biol. Cell 100, 97–108. https://doi.org/10.1042/BC20070109.

Asada, S., Daitoku, H., Matsuzaki, H., Saito, T., Sudo, T., Mukai, H., Iwashita, S., Kako, K., Kishi, T., Kasuya, Y., Fukamizu, A., 2007. Mitogen-activated protein kinases, Erk and p38, phosphorylate and regulate Foxo1. Cell. Signal. 19, 519–527. https://doi.org/10.1016/j.cellsig.2006.08.015.

Ayyadevara, S., Alla, R., Thaden, J.J., Shmookler Reis, R.J., 2008. Remarkable longevity and stress resistance of nematode PI3K-null mutants. Aging Cell 7, 13–22. https://doi.org/10.1111/j.1474-9726.2007.00348.x.

Bass, T.M., Weinkove, D., Houthoofd, K., Gems, D., Partridge, L., 2007. Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. Mech. Ageing Dev. 128, 546–552. https://doi.org/10.1016/j.mad.2007.07.007.

Batra, A., Warren, C.M., Ke, Y., McCann, M., Halas, M., Capote, A.E., Liew, C.W., Solaro, R.J., Rosas, P.C., 2021. Deletion of P21-activated kinase-1 induces age-dependent increased visceral adiposity and cardiac dysfunction in female mice. Mol. Cell. Biochem. 476, 1337–1349. https://doi.org/10.1007/s11010-020-03993-3.

Baum, P.D., Garriga, G., 1997. Neuronal migrations and axon fasciculation are disrupted in ina-1 integrin mutants. Neuron 19, 51–62. https://doi.org/10.1016/S0896-6273(00)80347-5.

Beeser, A., Jaffer, Z.M., Hofmann, C., Chernoff, J., 2005. Role of group a p21-activated kinases in activation of extracellular-regulated kinase by growth factors. J. Biol. Chem. 280, 36609–36615. https://doi.org/10.1074/jbc.M502306200.

Beier, I., Düsing, R., Vetter, H., Schmitz, U., 2008. Epidermal growth factor stimulates Rac1 and p21-activated kinase in vascular smooth muscle cells. Atherosclerosis 196, 92–97. https://doi.org/10.1016/j.atherosclerosis.2007.02.003.

Bishop, N.A., Guarente, L., 2007. Two neurons mediate diet-restriction-induced longevity in C. elegans. Nature 447, 545–549. https://doi.org/10.1038/nature05904.

Blackwell, T.K., Steinbaugh, M.J., Hourihan, J.M., Ewald, C.Y., Isik, M., 2015. SKN-1/Nrf, stress responses, and aging in Caenorhabditis elegans. Free Radic. Biol. Med. 88, 290–301. https://doi.org/10.1016/j.freeradbiomed.2015.06.008.

Blackwell, T.K., Sewell, A.K., Wu, Z., Han, M., 2019. TOR signaling in Caenorhabditis elegans development, metabolism, and aging. Genetics 213, 329–360. https://doi.org/10.1534/genetics.119.302504.

Blanck, S., Maksimoska, J., Baumeister, J., Harms, K., Marmorstein, R., Meggers, E., 2012. The art of filling protein pockets efficiently with octahedral metal complexes. Angew. Chem. Int. Ed. 51, 5244–5246. https://doi.org/10.1002/anie.201108865.

Bokoch, G.M., 2003. Biology of the p21-activated kinases. Annu. Rev. Biochem. 72, 743–781. https://doi.org/10.1146/annurev.biochem.72.121801.161742.

Bokoch, G.M., Reilly, A.M., Daniels, R.H., King, C.C., Olivera, A., Spiegel, S., Knaus, U.G., 1998. A GTPase-independent mechanism of p21-activated kinase activation. J. Biol. Chem. 273, 8137–8144. https://doi.org/10.1074/jbc.273.14.8137.

Bories, C., Arsenault, D., Lemire, M., Tremblay, C., De Koninck, Y., Calon, F., 2017. Transgenic autoinhibition of p21-activated kinase exacerbates synaptic impairments and fronto-dependent behavioral deficits in an animal model of Alzheimer's disease. Aging (Albany. NY) 9, 1386–1403. https://doi.org/10.18632/aging.101239.

Bridi, J.C., Barros, A.Gde A., Sampaio, L.R., Ferreira, J.C.D., Antunes Soares, F.A., Romano-Silva, M.A., 2015. Lifespan extension induced by caffeine in Caenorhabditis elegans is partially dependent on adenosine signaling. Front. Aging Neurosci. 7 https://doi.org/10.3389/fnagi.2015.00220.

Brunquell, J., Morris, S., Lu, Y., Cheng, F., Westerheide, S.D., 2016. The genome-wide role of HSF-1 in the regulation of gene expression in Caenorhabditis elegans. BMC Genomics 17, 559. https://doi.org/10.1186/s12864-016-2837-5.

Buchwald, G., Hostinova, E., Rudolph, M.G., Kraemer, A., Sickmann, A., Meyer, H.E., Scheffzek, K., Wittinghofer, A., 2001. Conformational switch and role of phosphorylation in PAK activation. Mol. Cell. Biol. 21, 5179–5189. https://doi.org/10.1128/MCB.21.15.5179-5189.2001.

Burkewitz, K., Zhang, Y., Mair, W.B., 2014. AMPK at the Nexus of energetics and aging. Cell Metab. 20, 10–25. https://doi.org/10.1016/j.cmet.2014.03.002.

Burnaevskiy, N., Sands, B., Yun, S., Tedesco, P.M., Johnson, T.E., Kaeberlein, M., Brent, R., Mendenhall, A., 2019. Chaperone biomarkers of lifespan and penetrance track the dosages of many other proteins. Nat. Commun. 10, 5725. https://doi.org/10.1038/s41467-019-13664-7.

Caldwell, K.A., Willicott, C.W., Caldwell, G.A., 2020. Modeling neurodegeneration in Caenorhabditis elegans. Dis. Model. Mech. 13 https://doi.org/10.1242/dmm.046110.

Cammarano, M.S., Nekrasova, T., Noel, B., Minden, A., 2005. Pak4 induces premature senescence via a pathway requiring p16INK4/p19ARF and mitogen-activated protein kinase signaling. Mol. Cell. Biol. 25, 9532–9542. https://doi.org/10.1128/MCB.25.21.9532-9542.2005.

Chamoli, M., Goyala, A., Tabrez, S.S., Siddiqui, A.A., Singh, A., Antebi, A., Lithgow, G.J., Watts, J.L., Mukhopadhyay, A., 2020. Polyunsaturated fatty acids and p38-MAPK link metabolic reprogramming to cytoprotective gene expression during dietary restriction. Nat. Commun. 11, 4865. https://doi.org/10.1038/s41467-020-18690-4.

Chaudhuri, J., Bose, N., Gong, J., Hall, D., Rifkind, A., Bhaumik, D., Peiris, T.H., Chamoli, M., Le, C.H., Liu, J., Lithgow, G.J., Ramanathan, A., Xu, X.Z.S., Kapahi, P., 2016. A Caenorhabditis elegans model elucidates a conserved role for TRPA1-Nrf signaling in reactive α-dicarbonyl detoxification. Curr. Biol. 26, 3014–3025. https://doi.org/10.1016/j.cub.2016.09.024.

Chaudhuri, J., Bains, Y., Guha, S., Kahn, A., Hall, D., Bose, N., Gugliucci, A., Kapahi, P., 2018. The role of advanced glycation end products in aging and metabolic diseases: bridging association and causality. Cell Metab. 28, 337–352. https://doi.org/10.1016/j.cmet.2018.08.014.

Chen, W., Chen, S., Yap, S.F., Lim, L., 1996. The Caenorhabditis elegans p21-activated kinase (CePAK) colocalizes with CeRac1 and CDC42Ce at hypodermal cell boundaries during embryo elongation. J. Biol. Chem. 271, 26362–26368. https://doi.org/10.1074/jbc.271.42.26362.

Chen, S., Yin, X., Zhu, X., Yan, J., Ji, S., Chen, C., Cai, M., Zhang, S., Zong, H., Hu, Y., Yuan, Z., Shen, Z., Gu, J., 2003. The C-terminal kinase domain of the p34cdc2-related PITSLRE protein kinase (p110C) associates with p21-activated kinase 1 and inhibits its activity during anoikis. J. Biol. Chem. 278, 20029–20036. https://doi.org/10.1074/jbc.M300818200.

Chen, D., Thomas, E.L., Kapahi, P., 2009. HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in Caenorhabditis elegans. PLoS Genet. 5, e1000486 https://doi.org/10.1371/journal.pgen.1000486.

Chen, D., Li, P.W.-L., Goldstein, B.A., Cai, W., Thomas, E.L., Chen, F., Hubbard, A.E., Melov, S., Kapahi, P., 2013. Germline signaling mediates the synergistically prolonged longevity produced by double mutations in daf-2 and rsks-1 in C. elegans. Cell Rep. 5, 1600–1610. https://doi.org/10.1016/j.celrep.2013.11.018.

Chen, P., Chen, F., Lei, J., Li, Q., Zhou, B., 2019. Activation of the miR-34a-mediated SIRT1/mTOR signaling pathway by urolithin a attenuates d-galactose-induced brain aging in mice. Neurotherapeutics 16, 1269–1282. https://doi.org/10.1007/s13311-019-00753-0.

Chu, Y., Elrod, N., Wang, C., Li, L., Chen, T., Routh, A., Xia, Z., Li, W., Wagner, E.J., Ji, P., 2019. Nudt21 regulates the alternative polyadenylation of Pak1 and is predictive in the prognosis of glioblastoma patients. Oncogene 38, 4154–4168. https://doi.org/10.1038/s41388-019-0714-9.

Civiero, L., Greggio, E., 2018. PAKs in the brain: function and dysfunction. Biochim. Biophys. Acta - Mol. Basis Dis. 1864, 444–453. https://doi.org/10.1016/j.bbadis.2017.11.005.

Civiero, L., Cirnaru, M.D., Beilina, A., Rodella, U., Russo, I., Belluzzi, E., Lobbestael, E., Reyniers, L., Hondhamuni, G., Lewis, P.A., Van den Haute, C., Baekelandt, V., Bandopadhyay, R., Bubacco, L., Piccoli, G., Cookson, M.R., Taymans, J.-M., Greggio, E., 2015. Leucine-rich repeat kinase 2 interacts with p21-activated kinase 6 to control neurite complexity in mammalian brain. J. Neurochem. 135, 1242–1256. https://doi.org/10.1111/jnc.13369.

Clandinin, T.R., DeModena, J.A., Sternberg, P.W., 1998. Inositol trisphosphate mediates a RAS-Independent response to LET-23 receptor tyrosine kinase activation in C. elegans. Cell 92, 523–533. https://doi.org/10.1016/S0092-8674(00)80945-9.

Coleman, J.J., Komura, T., Munro, J., Wu, M.P., Busanelli, R.R., Koehler, A.N., Thomas, M., Wagner, F.F., Holson, E.B., Mylonakis, E., 2016. Activity of caffeic acid phenethyl ester in Caenorhabditis elegans. Future Med. Chem. 8, 2033–2046. https://doi.org/10.4155/fmc-2016-0085.

Colonnello, A., Aguilera-Portillo, G., Rubio-López, L.C., Robles-Bañuelos, B., Rangel-López, E., Cortez-Núñez, S., Evaristo-Priego, Y., Silva-Palacios, A., Galván-Arzate, S., García-Contreras, R., Túnez, I., Chen, P., Aschner, M., Santamaría, A., 2020. Comparing the neuroprotective effects of caffeic acid in rat cortical slices and Caenorhabditis elegans: involvement of Nrf2 and SKN-1 signaling pathways. Neurotox. Res. 37, 326–337. https://doi.org/10.1007/s12640-019-00133-8.

Crawford, J.J., Lee, W., Aliagas, I., Mathieu, S., Hoeflich, K.P., Zhou, W., Wang, W., Rouge, L., Murray, L., La, H., Liu, N., Fan, P.W., Cheong, J., Heise, C.E., Ramaswamy, S., Mintzer, R., Liu, Y., Chao, Q., Rudolph, J., 2015. Structure-guided design of group I selective p21-activated kinase inhibitors. J. Med. Chem. 58 (12), 5121–5136. https://doi.org/10.1021/acs.jmedchem.5b00572.

Curto, M., Cole, B.K., Lallemand, D., Liu, C.-H., McClatchey, A.I., 2007. Contact-dependent inhibition of EGFR signaling by Nf2/Merlin. J. Cell Biol. 177, 893–903. https://doi.org/10.1083/jcb.200703010.

D rijard, B., 1995. Cdc42 and PAK-mediated signaling leads to Jun Kinase and p38 mitogen-activated protein kinase activation. J. Biol. Chem. 270, 27995–27998. https://doi.org/10.1074/jbc.270.47.27995.

D'Amico, D., Andreux, P.A., Valdés, P., Singh, A., Rinsch, C., Auwerx, J., 2021. Impact of the natural compound urolithin a on health, disease, and aging. Trends Mol. Med. 27, 687–699. https://doi.org/10.1016/j.molmed.2021.04.009.

Dan, C., Nath, N., Liberto, M., Minden, A., 2002. PAK5, a new brain-specific kinase, promotes neurite outgrowth in N1E-115 cells. Mol. Cell. Biol. 22, 567–577. https://doi.org/10.1128/MCB.22.2.567-577.2002.

Davis, T., Wyllie, F.S., Rokicki, M.J., Bagley, M.C., Kipling, D., 2007. The role of cellular senescence in werner syndrome: toward therapeutic intervention in human premature aging. Ann. N. Y. Acad. Sci. 1100, 455–469. https://doi.org/10.1196/annals.1395.051.

Deacon, S.W., Beeser, A., Fukui, J.A., Rennefahrt, U.E.E., Myers, C., Chernoff, J., Peterson, J.R., 2008. An isoform-selective, small-molecule inhibitor targets the autoregulatory mechanism of p21-activated kinase. Chem. Biol. 15, 322–331. https://doi.org/10.1016/j.chembiol.2008.03.005.

Deguchi, A., Miyoshi, H., Kojima, Y., Okawa, K., Aoki, M., Taketo, M.M., 2010. LKB1 suppresses p21-activated kinase-1 (PAK1) by phosphorylation of thr 109 in the p21-binding domain. J. Biol. Chem. 285, 18283–18290. https://doi.org/10.1074/jbc.M109.079137.

Delsert, C., Cau, J., Faure, S., Vigneron, S., Labbé, J.C., Morin, N., 2000. Regulation of Xenopus p21-activated kinase (X-PAK2) by Cdc42 and maturation-promoting factor

controls Xenopus oocyte maturation. J. Biol. Chem. 275, 2367–2375. https://doi.org/10.1074/jbc.275.4.2367.
Demestre, M., Messerli, S.M., Celli, N., Shahhossini, M., Kluwe, L., Mautner, V., Maruta, H., 2009. CAPE (caffeic acid phenethyl ester)-based propolis extract (Bio 30) suppresses the growth of human neurofibromatosis (NF) tumor xenografts in mice. Phyther. Res. 23, 226–230. https://doi.org/10.1002/ptr.2594.
Detienne, G., De Haes, W., Ernst, U.R., Schoofs, L., Temmerman, L., 2014. Royalactin extends lifespan of Caenorhabditis elegans through epidermal growth factor signaling. Exp. Gerontol. 60, 129–135. https://doi.org/10.1016/j.exger.2014.09.021.
Detienne, G., Van de Walle, P., De Haes, W., Schoofs, L., Temmerman, L., 2016. SKN-1-independent transcriptional activation of glutathione S-transferase 4 (GST-4) by EGF signaling. Worm. https://doi.org/10.1080/21624054.2016.1230585, 00–00.
Dickinson, D.J., Ward, J.D., Reiner, D.J., Goldstein, B., 2013. Engineering the Caenorhabditis elegans genome using Cas9-triggered homologous recombination. Nat. Methods 10, 1028–1034. https://doi.org/10.1038/nmeth.2641.
Dickinson, D.J., Pani, A.M., Heppert, J.K., Higgins, C.D., Goldstein, B., 2015. Streamlined genome engineering with a self-excising drug selection cassette. Genetics 200, 1035–1049. https://doi.org/10.1534/genetics.115.178335.
Dolan, B.M., Duron, S.G., Campbell, D.A., Vollrath, B., Rao, B.S.S., Ko, H.-Y., Lin, G.G., Govindarajan, A., Choi, S.-Y., Tonegawa, S., 2013. Rescue of fragile X syndrome phenotypes in Fmr1 KO mice by the small-molecule PAK inhibitor FRAX486. Proc. Natl. Acad. Sci. 110, 5671–5676. https://doi.org/10.1073/pnas.1219383110.
Dorman, J.B., Albinder, B., Shroyer, T., Kenyon, C., 1995. The age-1 and daf-2 genes function in a common pathway to control the lifespan of Caenorhabditis elegans. Genetics 141, 1399–1406.
Dumesic, P.A., Scholl, F.A., Barragan, D.I., Khavari, P.A., 2009. Erk1/2 MAP kinases are required for epidermal G2/M progression. J. Cell Biol. 185, 409–422. https://doi.org/10.1083/jcb.200804038.
Dummler, B., Ohshiro, K., Kumar, R., Field, J., 2009. Pak protein kinases and their role in cancer. Cancer Metastasis Rev. 28, 51–63. https://doi.org/10.1007/s10555-008-9168-1.
Dyshlovoy, S.A., Menchinskaya, E.S., Venz, S., Rast, S., Amann, K., Hauschild, J., Otte, K., Kalinin, V.I., Silchenko, A.S., Avilov, S.A., Alsdorf, W., Madanchi, R., Bokemeyer, C., Schumacher, U., Walther, R., Aminin, D.L., Fedorov, S.N., Shubina, L.K., Stonik, V.A., Balabanov, S., Honecker, F., von Amsberg, G., 2016. The marine triterpene glycoside frondoside A exhibits activity in vitro and in vivo in prostate cancer. Int. J. Cancer 138, 2450–2465. https://doi.org/10.1002/ijc.29977.
Eswaran, J., Lee, W.H., Debreczeni, J.É., Filippakopoulos, P., Turnbull, A., Fedorov, O., Deacon, S.W., Peterson, J.R., Knapp, S., 2007. Crystal structures of the p21-activated kinases PAK4, PAK5, and PAK6 reveal catalytic domain plasticity of active group II PAKs. Structure 15, 201–213. https://doi.org/10.1016/j.str.2007.01.001.
Eswaran, J., Soundararajan, M., Kumar, R., Knapp, S., 2008. UnPAKing the class differences among p21-activated kinases. Trends Biochem. Sci. 33, 394–403. https://doi.org/10.1016/j.tibs.2008.06.002.
Eswaran, J., Li, D.-Q., Shah, A., Kumar, R., 2012. Molecular pathways: targeting P21-activated kinase 1 signaling in cancer–opportunities, challenges, and limitations. Clin. Cancer Res. 18, 3743–3749. https://doi.org/10.1158/1078-0432.CCR-11-1952.
Fang, E.F., Hou, Y., Lautrup, S., Jensen, M.B., Yang, B., SenGupta, T., Caponio, D., Khezri, R., Demarest, T.G., Aman, Y., Figueroa, D., Morevati, M., Lee, H.-J., Kato, H., Kassahun, H., Lee, J.-H., Filippelli, D., Okur, M.N., Mangerich, A., Croteau, D.L., Maezawa, Y., Lyssiotis, C.A., Tao, J., Yokote, K., Rusten, T.E., Mattson, M.P., Jasper, H., Nilsen, H., Bohr, V.A., 2019a. NAD+ augmentation restores mitophagy and limits accelerated aging in Werner syndrome. Nat. Commun. 10, 5284. https://doi.org/10.1038/s41467-019-13172-8.
Fang, E.F., Hou, Y., Palikaras, K., Adriaanse, B.A., Kerr, J.S., Yang, B., Lautrup, S., Hasan-Olive, M.M., Caponio, D., Dan, X., Rocktäschel, P., Croteau, D.L., Akbari, M., Greig, N.H., Fladby, T., Nilsen, H., Cader, M.Z., Mattson, M.P., Tavernarakis, N., Bohr, V.A., 2019b. Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. Nat. Neurosci. 22, 401–412. https://doi.org/10.1038/s41593-018-0332-9.
Farias-Pereira, R., Oshiro, J., Kim, K.-H., Park, Y., 2018. Green coffee bean extract and 5-O-caffeoylquinic acid regulate fat metabolism in Caenorhabditis elegans. J. Funct. Foods 48, 586–593. https://doi.org/10.1016/j.jff.2018.07.049.
Friedland, A.E., Tzur, Y.B., Esvelt, K.M., Colaiácovo, M.P., Church, G.M., Calarco, J.A., 2013. Heritable genome editing in C. elegans via a CRISPR-Cas9 system. Nat. Methods 10, 741–743. https://doi.org/10.1038/nmeth.2532.
Friedman, D.B., Johnson, T.E., 1988. A mutation in the age-1 gene in Caenorhabditis elegans lengthens life and reduces hermaphrodite fertility. Genetics 118, 75–86.
Fujiki, K., Mizuno, T., Hisamoto, N., Matsumoto, K., 2010. The Caenorhabditis elegans Ste20-related kinase and rac-type small GTPase regulate the c-Jun N-terminal kinase signaling pathway mediating the stress response. Mol. Cell. Biol. 30, 995–1003. https://doi.org/10.1128/MCB.01131-09.
Gabrielli, B., Chau, Y.Q., Giles, N., Harding, A., Stevens, F., Beamish, H., 2007. Caffeine promotes apoptosis in mitotic spindle checkpoint-arrested cells*. J. Biol. Chem. 282, 6954–6964. https://doi.org/10.1074/jbc.M610104200.
Gan, J., Ke, X., Jiang, J., Dong, H., Yao, Z., Lin, Y., Lin, W., Wu, X., Yan, S., Zhuang, Y., Chu, W.K., Cai, R., Zhang, X., Cheung, H.S., Block, N.L., Pang, C.P., Schally, A.V., Zhang, H., 2016. Growth hormone-releasing hormone receptor antagonists inhibit human gastric cancer through downregulation of PAK1–STAT3/NF-κB signaling. Proc. Natl. Acad. Sci. 113, 14745–14750. https://doi.org/10.1073/pnas.1618582114.
Gasperotti, M., Passamonti, S., Tramer, F., Masuero, D., Guella, G., Mattivi, F., Vrhovsek, U., 2015. Fate of microbial metabolites of dietary polyphenols in rats: is the brain their target destination? ACS Chem. Neurosci. 6, 1341–1352. https://doi.org/10.1021/acschemneuro.5b00051.
Gems, D., de la Guardia, Y., 2012. Alternative perspectives on ageing in the nematode Caenorhabditis elegans: reactive oxygen species or hyperfunction? Free Radic. Biol. Med. 53, S14–S15. https://doi.org/10.1016/j.freeradbiomed.2012.08.100.
Gerke, P., Keshet, A., Mertenskötter, A., Paul, R.J., 2014. The JNK-Like MAPK KGB-1 of Caenorhabditis elegans promotes reproduction, lifespan, and gene expressions for protein biosynthesis and germline homeostasis but interferes with hyperosmotic stress tolerance. Cell. Physiol. Biochem. 34, 1951–1973. https://doi.org/10.1159/000366392.
Gnesutta, N., Minden, A., 2003. Death receptor-induced activation of initiator caspase 8 is antagonized by serine/threonine kinase PAK4. Mol. Cell. Biol. 23, 7838–7848. https://doi.org/10.1128/MCB.23.21.7838-7848.2003.
Goh, K.Y., Ng, N.W., Hagen, T., Inoue, T., 2012. p21-Activated kinase interacts with Wnt signaling to regulate tissue polarity and gene expression. Proc. Natl. Acad. Sci. 109, 15853–15858. https://doi.org/10.1073/pnas.1120795109.
Golegaonkar, S., Tabrez, S.S., Pandit, A., Sethurathinam, S., Jagadeeshaprasad, M.G., Bansode, S., Sampathkumar, S.-G., Kulkarni, M.J., Mukhopadhyay, A., 2015. Rifampicin reduces advanced glycation end products and activates DAF-16 to increase lifespan in Caenorhabditis elegans. Aging Cell 14, 463–473. https://doi.org/10.1111/acel.12327.
Gomes, J.-E., Bowerman, B., 2002. Caenorhabditis elegans par genes. Curr. Biol. 12, R444. https://doi.org/10.1016/S0960-9822(02)00939-9.
Gong, Z., Huang, J., Xu, B., Ou, Z., Zhang, L., Lin, X., Ye, X., Kong, X., Long, D., Sun, X., He, X., Xu, L., Li, Q., Xuan, A., 2019. Urolithin A attenuates memory impairment and neuroinflammation in APP/PS1 mice. J. Neuroinflammation 16, 62. https://doi.org/10.1186/s12974-019-1450-3.
Gonzalez, N., Cardama, G.A., Comin, M.J., Segatori, V.I., Pifano, M., Alonso, D.F., Gomez, D.E., Menna, P., 2017. Pharmacological inhibition of Rac1-PAK1 axis restores tamoxifen sensitivity in human resistant breast cancer cells. Cell. Signal. 30, 154–161. https://doi.org/10.1016/j.cellsig.2016.12.002.
Greer, E.L., Dowlatshahi, D., Banko, M.R., Villen, J., Hoang, K., Blanchard, D., Gygi, S.P., Brunet, A., 2007. An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr. Biol. 17, 1646–1656. https://doi.org/10.1016/j.cub.2007.08.047.
Greer, E.L., Banko, M.R., Brunet, A., 2009. AMP-activated protein kinase and FoxO transcription factors in dietary restriction-induced longevity. Ann. N. Y. Acad. Sci. 1170, 688–692. https://doi.org/10.1111/j.1749-6632.2009.04019.x.
Ha, B.H., Davis, M.J., Chen, C., Lou, H.J., Gao, J., Zhang, R., Krauthammer, M., Halaban, R., Schlessinger, J., Turk, B.E., Boggon, T.J., 2012. Type II p21-activated kinases (PAKs) are regulated by an autoinhibitory pseudosubstrate. Proc. Natl. Acad. Sci. 109, 16107–16112. https://doi.org/10.1073/pnas.1214447109.
Hamilton, B., 2005. A systematic RNAi screen for longevity genes in C. elegans. Genes Dev. 19, 1544–1555. https://doi.org/10.1101/gad.1308205.
Han, D., Wang, H., Cui, W., Zhang, B., Chen, B.-Z., 2020. Computational insight into the mechanisms of action and selectivity of Afraxis PAK inhibitors. Future Med. Chem. 12, 367–385. https://doi.org/10.4155/fmc-2019-0273.
Hanke, J.H., Gardner, J.P., Dow, R.L., Changelian, P.S., Brissette, W.H., Weringer, E.J., Pollok, B.A., Connelly, P.A., 1996. Discovery of a novel, potent, and src family-selective tyrosine kinase inhibitor. J. Biol. Chem. 271, 695–701. https://doi.org/10.1074/jbc.271.2.695.
Hansen, M., Taubert, S., Crawford, D., Libina, N., Lee, S.-J., Kenyon, C., 2007. Lifespan extension by conditions that inhibit translation in Caenorhabditis elegans. Aging Cell 6, 95–110. https://doi.org/10.1111/j.1474-9726.2006.00267.x.
Hashimoto, H., Messerli, S.M., Sudo, T., Maruta, H., 2009. Ivermectin inactivates the kinase PAK1 and blocks the PAK1-dependent growth of human ovarian cancer and NF2 tumor cell lines. Drug Discov. Ther. 3, 243–246.
Havermann, S., Chovolou, Y., Humpf, H.-U., Wätjen, W., 2014. Caffeic acid phenethylester increases stress resistance and enhances lifespan in Caenorhabditis elegans by modulation of the insulin-like DAF-16 signalling pathway. PLoS One 9, e100256. https://doi.org/10.1371/journal.pone.0100256.
Hawley, E., Gehlhausen, J., Karchugina, S., Chow, H.-Y., Araiza-Olivera, D., Radu, M., Smith, A., Burks, C., Jiang, L., Li, X., Bessler, W., Masters, A., Edwards, D., Burgin, C., Jones, D., Yates, C., Clapp, D.W., Chernoff, J., Park, S.-J., 2021. PAK1 inhibition reduces tumor size and extends the lifespan of mice in a genetically engineered mouse model of Neurofibromatosis Type 2 (NF2). Hum. Mol. Genet. https://doi.org/10.1093/hmg/ddab106 ddab106. Online ahead of print.
He, H., Maruta, H., 2013. Oncogenicity of PAKs and their substrates. Paks, Rac/Cdc42 (P21)-Activated Kinases. Elsevier, pp. 23–51. https://doi.org/10.1016/B978-0-12-407198-8.00002-3.
He, H., Hirokawa, Y., Manser, E., Lim, L., Levitzki, A., Maruta, H., 2001. Signal therapy for RAS-induced cancers in combination of AG 879 and PP1, specific inhibitors for ErbB2 and Src family kinases, that block PAK activation. Cancer J. 7, 191–202.
He, H., Hirokawa, Y., Gazit, A., Yamashita, Y., Mano, H., Kawakami, Y., Kawakami, T., Hsieh, C.-Y., Kung, H.-J., Lessene, G., Baell, J., Levitzki, A., Maruta, H., 2004. The tyr-kinase inhibitor AG879, that blocks the ETK-PAK1 interaction, suppresses the RAS-induced PAK1 activation and malignant transformation. Cancer Biol. Ther. 3, 96–101. https://doi.org/10.4161/cbt.3.1.643.
Hearne, A.L., Brockway, D., Wu, S., Li, Y., 2020. Abstract 2972: Urolithin A decreases cell proliferation of three genetically diverse glioblastoma multiforme cell lines. Experimental and Molecular Therapeutics. American Association for Cancer Research, p. 2972. https://doi.org/10.1158/1538-7445.AM2020-2972.
Hennig, R., Albawardi, A., Almarzooqi, S., Haneefa, S., Imbaraj, E., Zaaba, N.E., Nemmar, A., Subramanya, S., Maruta, H., Adrian, T.E., 2019. 1,2,3-Triazolyl ester of ketorolac (15K), a potent PAK1 blocker, inhibits both growth and metastasis of

orthotopic human pancreatic cancer xenografts in mice. Drug Discov. Ther. 13, 248–255. https://doi.org/10.5582/ddt.2019.01068.
Higuchi, M., Onishi, K., Kikuchi, C., Gotoh, Y., 2008. Scaffolding function of PAK in the PDK1–Akt pathway. Nat. Cell Biol. 10, 1356–1364. https://doi.org/10.1038/ncb1795.
Hirokawa, Y., Arnold, M., Nakajima, H., Zalcberg, J., Maruta, H., 2005. Signal therapy of breast cancers by the HDAC inhibitor FK228 that blocks the activation of PAK1 and abrogates the tamoxifen-resistance. Cancer Biol. Ther. 4, 956–960. https://doi.org/10.4161/cbt.4.9.1911.
Hirokawa, Y., Levitzki, A., Lessene, G., Baell, J., Xiao, Y., Zhu, H., Maruta, H., 2007. Signal therapy of human pancreatic cancer and NF1-deficient breast cancer xenograft in mice by a combination of PP1 and GL-2003, anti-PAK1 drugs (Tyr-kinase inhibitors). Cancer Lett. 245, 242–251. https://doi.org/10.1016/j.canlet.2006.01.018.
Hofmann, C., 2004. The genetics of Pak. J. Cell. Sci. 117, 4343–4354. https://doi.org/10.1242/jcs.01392.
Honjoh, S., Yamamoto, T., Uno, M., Nishida, E., 2009. Signalling through RHEB-1 mediates intermittent fasting-induced longevity in C. elegans. Nature 457, 726–730. https://doi.org/10.1038/nature07583.
Howitz, K.T., Bitterman, K.J., Cohen, H.Y., Lamming, D.W., Lavu, S., Wood, J.G., Zipkin, R.E., Chung, P., Kisielewski, A., Zhang, L.-L., Scherer, B., Sinclair, D.A., 2003. Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan. Nature 425, 191–196. https://doi.org/10.1038/nature01960.
Hsieh, C.-C., Papaconstantinou, J., 2004. Akt/PKB and p38 MAPK signaling, translational initiation and longevity in Snell dwarf mouse livers. Mech. Ageing Dev. 125, 785–798. https://doi.org/10.1016/j.mad.2004.07.008.
Huynh, N., Liu, K.H., Baldwin, G.S., He, H., 2010. P21-activated kinase 1 stimulates colon cancer cell growth and migration/invasion via ERK- and AKT-dependent pathways. Biochim. Biophys. Acta - Mol. Cell Res. 1803, 1106–1113. https://doi.org/10.1016/j.bbamcr.2010.05.007.
Iino, Y., Yamamoto, M., 1998. Expression pattern of theC. elegansp21-activated protein kinase. CePAK. Biochem. Biophys. Res. Commun. 245, 177–184. https://doi.org/10.1006/bbrc.1998.8380.
Ikeyama, S., Kokkonen, G., Shack, S., Wang, X.-T., Holbrook, N.J., 2002. Loss in oxidative stress tolerance with aging linked to reduced extracellular signal-regulated kinase and Akt kinase activities. FASEB J. 16, 1–16. https://doi.org/10.1096/fj.01-0409fje.
Imura, A., Tsuji, Y., Murata, M., Maeda, R., Kubota, K., Iwano, A., Obuse, C., Togashi, K., Tominaga, M., Kita, N., Tomiyama, K., Iijima, J., Nabeshima, Yoko, Fujioka, M., Asato, R., Tanaka, S., Kojima, K., Ito, J., Nozaki, K., Hashimoto, N., Ito, T., Nishio, T., Uchiyama, T., Fujimori, T., Nabeshima, Yo-ichi, 2007. Alpha-Klotho as a regulator of calcium homeostasis. Science 316, 1615–1618. https://doi.org/10.1126/science.1135901.
Inoue, H., 2005. The C. elegans p38 MAPK pathway regulates nuclear localization of the transcription factor SKN-1 in oxidative stress response. Genes Dev. 19, 2278–2283. https://doi.org/10.1101/gad.1324805.
Iwasa, H., Yu, S., Xue, J., Driscoll, M., 2010. Novel EGF pathway regulators modulate C. elegans healthspan and lifespan via EGF receptor, PLC-γ, and IP3R activation. Aging Cell 9, 490–505. https://doi.org/10.1111/j.1474-9726.2010.00575.x.
Iwasa, H., Maimaiti, S., Kuroyanagi, H., Kawano, S., Inami, K., Timalsina, S., Ikeda, M., Nakagawa, K., Hata, Y., 2013. Yes-associated protein homolog, YAP-1, is involved in the thermotolerance and aging in the nematode Caenorhabditis elegans. Exp. Cell Res. 319, 931–945. https://doi.org/10.1016/j.yexcr.2013.01.020.
James, M.F., Han, S., Polizzano, C., Plotkin, S.R., Manning, B.D., Stemmer-Rachamimov, A.O., Gusella, J.F., Ramesh, V., 2009. NF2/Merlin is a novel negative regulator of mTOR complex 1, and activation of mTORC1 is associated with meningioma and schwannoma growth. Mol. Cell. Biol. 29, 4250–4261. https://doi.org/10.1128/MCB.01581-08.
James, M.F., Stivison, E., Beauchamp, R., Han, S., Li, H., Wallace, M.R., Gusella, J.F., Stemmer-Rachamimov, A.O., Ramesh, V., 2012. Regulation of mTOR complex 2 signaling in neurofibromatosis 2–deficient target cell types. Mol. Cancer Res. 10, 649–659. https://doi.org/10.1158/1541-7786.MCR-11-0425-T.
Jia, K., Chen, D., Riddle, D.L., 2004. The TOR pathway interacts with the insulin signaling pathway to regulate C. elegans larval development, metabolism and life span. Development 131, 3897–3906. https://doi.org/10.1242/dev.01255.
Jin, S., Zhuo, Y., Guo, W., Field, J., 2005. p21-activated kinase 1 (Pak1)-dependent phosphorylation of Raf-1 regulates its mitochondrial localization, phosphorylation of BAD, and Bcl-2 association. J. Biol. Chem. 280, 24698–24705. https://doi.org/10.1074/jbc.M413374200.
Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E., 2012. A programmable Dual-RNA-Guided DNA endonuclease in adaptive bacterial immunity. Science (80-.) 337, 816–821. https://doi.org/10.1126/science.1225829.
John Von Freyend, S., Kwok-Schuelein, T., Netter, H., Haqshenas, G., Semblat, J.-P., Doerig, C., 2017. Subverting host cell P21-activated kinase: a case of convergent evolution across pathogens. Pathogens 6, 17. https://doi.org/10.3390/pathogens6020017.
Kaeberlein, T.L., Smith, E.D., Tsuchiya, M., Welton, K.L., Thomas, J.H., Fields, S., Kennedy, B.K., Kaeberlein, M., 2006. Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell 5, 487–494. https://doi.org/10.1111/j.1474-9726.2006.00238.x.
Kaletsky, R., Murphy, C.T., 2010. The role of insulin/IGF-like signaling in C. elegans longevity and aging. Dis. Model. Mech. 3, 415–419. https://doi.org/10.1242/dmm.001040.
Kapahi, P., Chen, D., Rogers, A.N., Katewa, S.D., Li, P.W.-L., Thomas, E.L., Kockel, L., 2010. With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging. Cell Metab. 11, 453–465. https://doi.org/10.1016/j.cmet.2010.05.001.
Kapahi, P., Kaeberlein, M., Hansen, M., 2017. Dietary restriction and lifespan: lessons from invertebrate models. Ageing Res. Rev. 39, 3–14. https://doi.org/10.1016/j.arr.2016.12.005.
Karaman, M.W., Herrgard, S., Treiber, D.K., Gallant, P., Atteridge, C.E., Campbell, B.T., Chan, K.W., Ciceri, P., Davis, M.I., Edeen, P.T., Faraoni, R., Floyd, M., Hunt, J.P., Lockhart, D.J., Milanov, Z.V., Morrison, M.J., Pallares, G., Patel, H.K., Pritchard, S., Wodicka, L.M., Zarrinkar, P.P., 2008. A quantitative analysis of kinase inhibitor selectivity. Nat. Biotechnol. 26, 127–132. https://doi.org/10.1038/nbt1358.
Karpov, A.S., Amiri, P., Bellamacina, C., Bellance, M.-H., Breitenstein, W., Daniel, D., Denay, R., Fabbro, D., Fernandez, C., Galuba, I., Guerro-Lagasse, S., Gutmann, S., Hinh, L., Jahnke, W., Klopp, J., Lai, A., Lindvall, M.K., Ma, S., Möbitz, H., Pecchi, S., Rummel, G., Shoemaker, K., Trappe, J., Voliva, C., Cowan-Jacob, S.W., Marzinzik, A. L., 2015. Optimization of a dibenzodiazepine hit to a potent and selective allosteric PAK1 inhibitor. ACS Med. Chem. Lett. 6, 776–781. https://doi.org/10.1021/acsmedchemlett.5b00102.
Katsuno, M., Sang, C., Adachi, H., Minamiyama, M., Waza, M., Tanaka, F., Doyu, M., Sobue, G., 2005. Pharmacological induction of heat-shock proteins alleviates polyglutamine-mediated motor neuron disease. Proc. Natl. Acad. Sci. 102, 16801–16806. https://doi.org/10.1073/pnas.0506249102.
Kawasaki, I., Jeong, M.-H., Oh, B.-K., Shim, Y.-H., 2010. Apigenin inhibits larval growth of Caenorhabditis elegans through DAF-16 activation. FEBS Lett. 584, 3587–3591. https://doi.org/10.1016/j.febslet.2010.07.026.
Kemphues, K.J., Priess, J.R., Morton, D.G., Cheng, N., 1988. Identification of genes required for cytoplasmic localization in early C. elegans embryos. Cell 52, 311–320. https://doi.org/10.1016/S0092-8674(88)80024-2.
Kenyon, C.J., 2010. The genetics of ageing. Nature 464, 504–512. https://doi.org/10.1038/nature09047.
Kenyon, C., Murphy, C.T., 2006. Enrichment of regulatory motifs upstream of predicted DAF-16 targets. Nat. Genet. 38, 397–398. https://doi.org/10.1038/ng0406-397.
Kenyon, C., Chang, J., Gensch, E., Rudner, A., Tabtiang, R., 1993. A C. elegans mutant that lives twice as long as wild type. Nature 366, 461–464. https://doi.org/10.1038/366461a0.
Kim, H.J., Jung, K.J., Yu, B.P., Cho, C.G., Chung, H.Y., 2002. Influence of aging and calorie restriction on MAPKs activity in rat kidney. Exp. Gerontol. 37, 1041–1053. https://doi.org/10.1016/S0531-5565(02)00082-7.
Kim, D.-J., Choi, C.-K., Lee, C.-S., Park, M.-H., Tian, X., Kim, N.D., Lee, K.-I., Choi, J.-K., Ahn, J.H., Shin, E.-Y., Shin, I., Kim, E.-G., 2016a. Small molecules that allosterically inhibit p21-activated kinase activity by binding to the regulatory p21-binding domain. Exp. Mol. Med. 48, e229. https://doi.org/10.1038/emm.2016.13.
Kim, H., Oh, J.-Y., Choi, S.-L., Nam, Y.-J., Jo, A., Kwon, A., Shin, E.-Y., Kim, E.-G., Kim, H.K., 2016b. Down-regulation of p21-activated serine/threonine kinase 1 is involved in loss of mesencephalic dopamine neurons. Mol. Brain 9, 45. https://doi.org/10.1186/s13041-016-0230-6.
Kim, J., Yang, G., Kim, Y., Kim, Jin, Ha, J., 2016c. AMPK activators: mechanisms of action and physiological activities. Exp. Mol. Med. 48, e224. https://doi.org/10.1038/emm.2016.16.
Kingsley, S.F., Seo, Y., Allen, C., Ghanta, K.S., Finkel, S., Tissenbaum, H.A., 2021. Bacterial processing of glucose modulates C. elegans lifespan and healthspan. Sci. Rep. 11, 5931. https://doi.org/10.1038/s41598-021-85046-3.
Kissil, J.L., Wilker, E.W., Johnson, K.C., Eckman, M.S., Yaffe, M.B., Jacks, T., 2003. Merlin, the product of the Nf2 tumor suppressor gene, is an inhibitor of the p21-activated kinase, Pak1. Mol. Cell 12, 841–849. https://doi.org/10.1016/S1097-2765(03)00382-4.
Klass, M.R., 1983. A method for the isolation of longevity mutants in the nematode Caenorhabditis elegans and initial results. Mech. Ageing Dev. 22, 279–286. https://doi.org/10.1016/0047-6374(83)90082-9.
Klotz, L.-O., Sánchez-Ramos, C., Prieto-Arroyo, I., Urbánek, P., Steinbrenner, H., Monsalve, M., 2015. Redox regulation of FoxO transcription factors. Redox Biol. 6, 51–72. https://doi.org/10.1016/j.redox.2015.06.019.
Koh, C.-G., Tan, E.-J., Manser, E., Lim, L., 2002. The p21-Activated kinase PAK is negatively regulated by POPX1 and POPX2, a pair of serine/threonine phosphatases of the PP2C family. Curr. Biol. 12, 317–321. https://doi.org/10.1016/S0960-9822(02)00652-8.
Kreis, P., Barnier, J.-V., 2009. PAK signalling in neuronal physiology. Cell. Signal. 21, 384–393. https://doi.org/10.1016/j.cellsig.2008.11.001.
Krivinko, J.M., Erickson, S.L., Abrahamson, E.E., Wills, Z.P., Ikonomovic, M.D., Penzes, P., Sweet, R.A., 2017. Kalirin reduction rescues psychosis-associated behavioral deficits in APPswe/PSEN1dE9 transgenic mice. Neurobiol. Aging 54, 59–70. https://doi.org/10.1016/j.neurobiolaging.2017.02.006.
Ku, G.M., 2001. A PAK1-PIX-PKL complex is activated by the T-cell receptor independent of Nck, Slp-76 and LAT. EMBO J. 20, 457–465. https://doi.org/10.1093/emboj/20.3.457.
Kujawska, M., Jourdes, M., Kurpik, M., Szulc, M., Szaefer, H., Chmielarz, P., Kreiner, G., Krajka-Kuźniak, V., Mikołajczak, P.Ł., Teissedre, P.-L., Jodynis-Liebert, J., 2019. Neuroprotective effects of pomegranate juice against Parkinson's disease and presence of ellagitannins-derived metabolite—urolithin A—in the brain. Int. J. Mol. Sci. 21, 202. https://doi.org/10.3390/ijms21010202.
Kumar, R., Li, D.-Q., 2016. PAKs in human cancer progression: from inception to cancer therapeutic to future oncobiology. Adv. Cancer Res. 130, 137–209. https://doi.org/10.1016/bs.acr.2016.01.002.
Kumar, R., Gururaj, A.E., Barnes, C.J., 2006. p21-activated kinases in cancer. Nat. Rev. Cancer 6, 459–471. https://doi.org/10.1038/nrc1892.
Kumar, R., Sanawar, R., Li, X., Li, F., 2017. Structure, biochemistry, and biology of PAK kinases. Gene 605, 20–31. https://doi.org/10.1016/j.gene.2016.12.014.

Lai, C.-H., 2000. Identification of novel human genes evolutionarily conserved in Caenorhabditis elegans by comparative proteomics. Genome Res. 10, 703–713. https://doi.org/10.1101/gr.10.5.703.

Lan, J., Rollins, J.A., Zang, X., Wu, D., Zou, L., Wang, Z., Ye, C., Wu, Z., Kapahi, P., Rogers, A.N., Chen, D., 2019. Translational regulation of non-autonomous mitochondrial stress response promotes longevity. Cell Rep. 28, 1050–1062. https://doi.org/10.1016/j.celrep.2019.06.078 e6.

Laplante, M., Sabatini, D.M., 2012. mTOR signaling in growth control and disease. Cell 149, 274–293. https://doi.org/10.1016/j.cell.2012.03.017.

Lardennois, A., Pásti, G., Ferraro, T., Llense, F., Mahou, P., Pontabry, J., Rodriguez, D., Kim, S., Ono, S., Beaurepaire, E., Gally, C., Labouesse, M., 2019. An actin-based viscoplastic lock ensures progressive body-axis elongation. Nature 573, 266–270. https://doi.org/10.1038/s41586-019-1509-4.

Lee, S.-W., Commisso, C., 2020. Rac1 and EGFR cooperate to activate Pak in response to nutrient stress. Biochem. Biophys. Res. Commun. 533, 437–441. https://doi.org/10.1016/j.bbrc.2020.09.043.

Lee, M., Cram, E.J., Shen, B., Schwarzbauer, J.E., 2001. Roles for βpat-3 integrins in development and function of Caenorhabditis elegans muscles and gonads. J. Biol. Chem. 276, 36404–36410. https://doi.org/10.1074/jbc.M105795200.

Lee, S.S., Kennedy, S., Tolonen, A.C., Ruvkun, G., 2003. DAF-16 target genes that control C. elegans life-span and metabolism. Science 300, 644–647. https://doi.org/10.1126/science.1083614.

Lee, H., Cho, J.S., Lambacher, N., Lee, J., Lee, S.-J., Lee, T.H., Gartner, A., Koo, H.-S., 2008. The Caenorhabditis elegans AMP-activated protein kinase AAK-2 is phosphorylated by LKB1 and is required for resistance to oxidative stress and for normal motility and foraging behavior. J. Biol. Chem. 283, 14988–14993. https://doi.org/10.1074/jbc.M709115200.

Lee, H., Kang, J., Ahn, S., Lee, J., 2019a. The hippo pathway is essential for maintenance of apicobasal polarity in the growing intestine of Caenorhabditis elegans. Genetics 213, 501–515. https://doi.org/10.1534/genetics.119.302477.

Lee, J.-S., Mo, Y., Gan, H., Burgess, R.J., Baker, D.J., van Deursen, J.M., Zhang, Z., 2019b. Pak2 kinase promotes cellular senescence and organismal aging. Proc. Natl. Acad. Sci. 116, 13311–13319. https://doi.org/10.1073/pnas.1903847116.

Lee, S.-W., Zhang, Y., Jung, M., Cruz, N., Alas, B., Commisso, C., 2019c. EGFR-pak signaling selectively regulates glutamine deprivation-induced macropinocytosis. Dev. Cell 50, 381–392. https://doi.org/10.1016/j.devcel.2019.05.043 e5.

Lei, M., Lu, W., Meng, W., Parrini, M.-C., Eck, M.J., Mayer, B.J., Harrison, S.C., 2000. Structure of PAK1 in an autoinhibited conformation reveals a multistage activation switch. Cell 102, 387–397. https://doi.org/10.1016/S0092-8674(00)00043-X.

Li, J., Le, W., 2013. Modeling neurodegenerative diseases in Caenorhabditis elegans. Exp. Neurol. 250, 94–103. https://doi.org/10.1016/j.expneurol.2013.09.024.

Li, H., Yu, X., Li, C., Ma, L., Zhao, Z., Guan, S., Wang, L., 2021. Caffeic acid protects against Aβ toxicity and prolongs lifespan in Caenorhabditis elegans models. Food Funct. 12, 1219–1231. https://doi.org/10.1039/D0FO02784G.

Liao, V.H.-C., Yu, C.-W., Chu, Y.-J., Li, W.-H., Hsieh, Y.-C., Wang, T.-T., 2011. Curcumin-mediated lifespan extension in Caenorhabditis elegans. Mech. Ageing Dev. 132, 480–487. https://doi.org/10.1016/j.mad.2011.07.008.

Licciulli, S., Maksimoska, J., Zhou, C., Troutman, S., Kota, S., Liu, Q., Duron, S., Campbell, D., Chernoff, J., Field, J., Marmorstein, R., Kissil, J.L., 2013. FRAX597, a small molecule inhibitor of the p21-activated kinases, inhibits tumorigenesis of neurofibromatosis type 2 (NF2)-associated schwannomas. J. Biol. Chem. 288, 29105–29114. https://doi.org/10.1074/jbc.M113.510933.

Lithgow, G.J., White, T.M., Hinerfeld, D.A., Johnson, T.E., 1994. Thermotolerance of a long-lived mutant of Caenorhabditis elegans. J. Gerontol. 49, B270–B276. https://doi.org/10.1093/geronj/49.6.B270.

Liu, G., Rogers, J., Murphy, C.T., Rongo, C., 2011a. EGF signalling activates the ubiquitin proteasome system to modulate C. elegans lifespan. EMBO J. 30, 2990–3003. https://doi.org/10.1038/emboj.2011.195.

Liu, W., Zi, M., Naumann, R., Ulm, S., Jin, J., Taglieri, D.M., Prehar, S., Gui, J., Tsui, H., Xiao, R.-P., Neyses, L., Solaro, R.J., Ke, Y., Cartwright, E.J., Lei, M., Wang, X., 2011b. Pak1 as a novel therapeutic target for antihypertrophic treatment in the heart. Circulation 124, 2702–2715. https://doi.org/10.1161/CIRCULATIONAHA.111.048785.

Liu, X., Chen, X., Liu, H., Cao, Y., 2018. Antioxidation and anti-aging activities of astaxanthin geometrical isomers and molecular mechanism involved in Caenorhabditis elegans. J. Funct. Foods 44, 127–136. https://doi.org/10.1016/j.jff.2018.03.004.

Locke, C.J., Kautu, B.B., Berry, K.P., Lee, S.K., Caldwell, K.A., Caldwell, G.A., 2009. Pharmacogenetic analysis reveals a post-developmental role for rac GTPases in Caenorhabditis elegans GABAergic neurotransmission. Genetics 183, 1357–1372. https://doi.org/10.1534/genetics.109.106880.

López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039.

Luan, P., D'Amico, D., Andreux, P.A., Laurila, P.-P., Wohlwend, M., Li, H., Imamura de Lima, T., Place, N., Rinsch, C., Zanou, N., Auwerx, J., 2021. Urolithin A improves muscle function by inducing mitophagy in muscular dystrophy. Sci. Transl. Med. 13, eabb0319. https://doi.org/10.1126/scitranslmed.abb0319.

Lucanic, M., Cheng, H.-J., 2008. A RAC/CDC-42–Independent GIT/PIX/PAK signaling pathway mediates cell migration in C. elegans. PLoS Genet. 4, e1000269 https://doi.org/10.1371/journal.pgen.1000269.

Lucanic, M., Kiley, M., Ashcroft, N., L'Etoile, N., Cheng, H.-J., 2006. The Caenorhabditis elegans P21-activated kinases are differentially required for UNC-6/netrin-mediated commissural motor axon guidance. Development 133, 4549–4559. https://doi.org/10.1242/dev.02648.

Luevano-Contreras, C., Chapman-Novakofski, K., 2010. Dietary advanced glycation end products and aging. Nutrients 2, 1247–1265. https://doi.org/10.3390/nu2121247.

Luo, S., Rubinsztein, D.C., 2009. Huntingtin promotes cell survival by preventing Pak2 cleavage. J. Cell. Sci. 122, 875–885. https://doi.org/10.1242/jcs.050013.

Luo, S., Mizuta, H., Rubinsztein, D.C., 2008. p21-activated kinase 1 promotes soluble mutant huntingtin self-interaction and enhances toxicity. Hum. Mol. Genet. 17, 895–905. https://doi.org/10.1093/hmg/ddm362.

Ma, Q.-L., Yang, F., Calon, F., Ubeda, O.J., Hansen, J.E., Weisbart, R.H., Beech, W., Frautschy, S.A., Cole, G.M., 2008. p21-activated kinase-aberrant activation and translocation in Alzheimer disease pathogenesis. J. Biol. Chem. 283, 14132–14143. https://doi.org/10.1074/jbc.M708034200.

Ma, Q.-L., Yang, F., Frautschy, S.A., Cole, G.M., 2013. PAK1 in Alzheimer's and Huntington's diseases. Paks, Rac/Cdc42 (P21)-Activated Kinases. Elsevier, pp. 107–124. https://doi.org/10.1016/B978-0-12-407198-8.00006-0.

Mack, H.I.D., Heimbucher, T., Murphy, C.T., 2018. The nematode Caenorhabditis elegans as a model for aging research. Drug Discov. Today Dis. Model. 27, 3–13. https://doi.org/10.1016/j.ddmod.2018.11.001.

Madsen, M.A., Hsieh, C.-C., Boylston, W.H., Flurkey, K., Harrison, D., Papaconstantinou, J., 2004. Altered oxidative stress response of the long-lived Snell dwarf mouse. Biochem. Biophys. Res. Commun. 318, 998–1005. https://doi.org/10.1016/j.bbrc.2004.04.126.

Maksimoska, J., Feng, L., Harms, K., Yi, C., Kissil, J., Marmorstein, R., Meggers, E., 2008. Targeting large kinase active site with rigid, bulky octahedral ruthenium complexes. J. Am. Chem. Soc. 130, 15764–15765. https://doi.org/10.1021/ja805555a.

Manalo, R.V.M., Medina, P.M.B., 2018. Caffeine protects dopaminergic neurons from dopamine-induced neurodegeneration via synergistic adenosine-dopamine D2-Like receptor interactions in transgenic Caenorhabditis elegans. Front. Neurosci. 12 https://doi.org/10.3389/fnins.2018.00137.

Manser, E., Leung, T., Salihuddin, H., Zhao, Z., Lim, L., 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. Nature 367, 40–46. https://doi.org/10.1038/367040a0.

Maroney, A.C., Finn, J.P., Connors, T.J., Durkin, J.T., Angeles, T., Gessner, G., Xu, Z., Meyer, S.L., Savage, M.J., Greene, L.A., Scott, R.W., Vaught, J.L., 2001. CEP-1347 (KT7515), a semisynthetic inhibitor of the mixed lineage kinase family. J. Biol. Chem. 276, 25302–25308. https://doi.org/10.1074/jbc.M011601200.

Martin, E., Harel, S., Nkengfac, B., Hamiche, K., Neault, M., Jenna, S., 2014. pix-1 controls early elongation in parallel with mel-11 and let-502 in Caenorhabditis elegans. PLoS One 9, e94684. https://doi.org/10.1371/journal.pone.0094684.

Maruta, H., 2013. PAK1–3 in infectious diseases. Paks, Rac/Cdc42 (P21)-Activated Kinases. Elsevier, pp. 77–86. https://doi.org/10.1016/B978-0-12-407198-8.00004-7.

Maruta, H., 2014. Herbal therapeutics that block the oncogenic kinase PAK1: a practical approach towards PAK1-dependent diseases and longevity. Phyther. Res. 28, 656–672. https://doi.org/10.1002/ptr.5054.

Maruta, H., Ahn, M.-R., 2017. From bench (laboratory) to bed (hospital/home): how to explore effective natural and synthetic PAK1-blockers/longevity-promoters for cancer therapy. Eur. J. Med. Chem. 142, 229–243. https://doi.org/10.1016/j.ejmech.2017.07.043.

Maruta, H., Messerli, S.M., 2013. PAK1 in brain diseases or disorders. Paks, Rac/Cdc42 (P21)-Activated Kinases. Elsevier, pp. 87–106. https://doi.org/10.1016/B978-0-12-407198-8.00005-9.

Mazumdar, A., Kumar, R., 2003. Estrogen regulation of Pak1 and FKHR pathways in breast cancer cells. FEBS Lett. 535, 6–10. https://doi.org/10.1016/S0014-5793(02)03846-2.

McClatchey, A.I., Fehon, R.G., 2009. Merlin and the ERM proteins – regulators of receptor distribution and signaling at the cell cortex. Trends Cell Biol. 19, 198–206. https://doi.org/10.1016/j.tcb.2009.02.006.

Mckay, S.J., Johnsen, R., Khattra, J., Asano, J., Baillie, D.L., Chan, S., Dube, N., Fang, L., Goszczynski, B., Ha, E., Halfnight, E., Hollebakken, R., Huang, P., Hung, K., Jensen, V., Jones, S.J.M., Kai, H., Li, D., Mah, A., Marra, M., Mcghee, J., Newbury, R., Pouzyrev, A., Riddle, D.L., Sonnhammer, E., Tian, H., Tu, D., Tyson, J. R., Vatcher, G., Warner, A., Wong, K., Zhao, Z., Moerman, D.G., 2003. Gene expression profiling of cells, tissues, and developmental stages of the nematode C. elegans. Cold Spring Harb. Symp. Quant. Biol. 68, 159–170. https://doi.org/10.1101/sqb.2003.68.159.

Messerli, S.M., Ahn, M.-R., Kunimasa, K., Yanagihara, M., Tatefuji, T., Hashimoto, K., Mautner, V., Uto, Y., Hori, H., Kumazawa, S., Kaji, K., Ohta, T., Maruta, H., 2009. Artepillin C (ARC) in Brazilian green propolis selectively blocks oncogenic PAK1 signaling and suppresses the growth of NF tumors in mice. Phyther. Res. 23, 423–427. https://doi.org/10.1002/ptr.2658.

Minden, A., Lin, A., McMahon, M., Lange-Carter, C., Derijard, B., Davis, R., Johnson, G., Karin, M., 1994. Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. Science (80-.) 266, 1719–1723. https://doi.org/10.1126/science.7992057.

Minden, A., Lin, A., Claret, F.-X., Abo, A., Karin, M., 1995. Selective activation of the JNK signaling cascadeand c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. Cell 81, 1147–1157. https://doi.org/10.1016/S0092-8674(05)80019-4.

Mizuno, T., Hisamoto, N., Terada, T., Kondo, T., Adachi, M., Nishida, E., Kim, D.H., Ausubel, F.M., Matsumoto, K., 2004. The Caenorhabditis elegans MAPK phosphatase VHP-1 mediates a novel JNK-like signaling pathway in stress response. EMBO J. 23, 2226–2234. https://doi.org/10.1038/sj.emboj.7600226.

Mohankumar, A., Kalaiselvi, D., Thiruppathi, G., Muthusaravanan, S., Nivitha, S., Levenson, C., Tawata, S., Sundararaj, P., 2020. A- and β-Santalols delay aging in Caenorhabditis elegans via preventing oxidative stress and protein aggregation. ACS Omega 5, 32641–32654. https://doi.org/10.1021/acsomega.0c05006.

Monnier, V.M., Taniguchi, N., 2016. Advanced glycation in diabetes, aging and age-related diseases: editorial and dedication. Glycoconj. J. 33, 483–486. https://doi.org/10.1007/s10719-016-9704-0.

Morcos, M., Du, X., Pfisterer, F., Hutter, H., Sayed, A.A.R., Thornalley, P., Ahmed, N., Baynes, J., Thorpe, S., Kukudov, G., Schlotterer, A., Bozorgmehr, F., El Baki, R.A., Stern, D., Moehrlen, F., Ibrahim, Y., Oikonomou, D., Hamann, A., Becker, C., Zeier, M., Schwenger, V., Miftari, N., Humpert, P., Hammes, H.-P., Buechler, M., Bierhaus, A., Brownlee, M., Nawroth, P.P., 2008. Glyoxalase-1 prevents mitochondrial protein modification and enhances lifespan in Caenorhabditis elegans. Aging Cell 7, 260–269. https://doi.org/10.1111/j.1474-9726.2008.00371.x.

Morrison, H., 2001. The NF2 tumor suppressor gene product, merlin, mediates contact inhibition of growth through interactions with CD44. Genes Dev. 15, 968–980. https://doi.org/10.1101/gad.189601.

Morrison, H., Sperka, T., Manent, J., Giovannini, M., Ponta, H., Herrlich, P., 2007. Merlin/neurofibromatosis type 2 suppresses growth by inhibiting the activation of Ras and rac. Cancer Res. 67, 520–527. https://doi.org/10.1158/0008-5472.CAN-06-1608.

Morrow, K.A., Shevde, L.A., 2012. Merlin: the wizard requires protein stability to function as a tumor suppressor. Biochim. Biophys. Acta - Rev. Cancer 1826, 400–406. https://doi.org/10.1016/j.bbcan.2012.06.005.

Mukhopadhyay, A., Tissenbaum, H.A., 2007. Reproduction and longevity: secrets revealed by C. elegans. Trends Cell Biol. 17, 65–71. https://doi.org/10.1016/j.tcb.2006.12.004.

Murakami, S., 2007. Caenorhabditis elegans as a model system to study aging of learning and memory. Mol. Neurobiol. 35, 85–94. https://doi.org/10.1007/BF02700625.

Murakami, M., Oketani, K., Fujisaki, H., Wakabayashi, T., Ohgo, T., 1981. Antiulcer effect of geranylgeranylacetone, a new acyclic polyisoprenoid on experimentally induced gastric and duodenal ulcers in rats. Arzneimittelforschung. 31, 799–804.

Murphy, C.T., 2013. Insulin/insulin-like growth factor signaling in C. elegans. WormBook, pp. 1–43. https://doi.org/10.1895/wormbook.1.164.1.

Murphy, Coleen T., McCarroll, Sa, Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., Kenyon, C., 2003a. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424, 277–283. https://doi.org/10.1038/nature01789.

Murphy, Coleen T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., Kenyon, C., 2003b. Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424, 277–284. https://doi.org/10.1038/nature01789.

Murray, B.W., Guo, C., Piraino, J., Westwick, J.K., Zhang, C., Lamerdin, J., Dagostino, E., Knighton, D., Loi, C.-M., Zager, M., Kraynov, E., Popoff, I., Christensen, J.G., Martinez, R., Kephart, S.E., Marakovits, J., Karlicek, S., Bergqvist, S., Smeal, T., 2010. Small-molecule p21-activated kinase inhibitor PF-3758309 is a potent inhibitor of oncogenic signaling and tumor growth. Proc. Natl. Acad. Sci. 107, 9446–9451. https://doi.org/10.1073/pnas.0911863107.

Narbonne, P., Roy, R., 2009. Caenorhabditis elegans dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. Nature 457, 210–214. https://doi.org/10.1038/nature07536.

Naß, J., Efferth, T., 2021. Ursolic acid ameliorates stress and reactive oxygen species in C. elegans knockout mutants by the dopamine Dop1 and Dop3 receptors. Phytomedicine 81, 153439. https://doi.org/10.1016/j.phymed.2020.153439.

Ndubaku, C.O., Crawford, J.J., Drobnick, J., Aliagas, I., Campbell, D., Dong, P., Dornan, L.M., Duron, S., Epler, J., Gazzard, L., Heise, C.E., Hoeflich, K.P., Jakubiak, D., La, H., Lee, W., Lin, B., Lyssikatos, J.P., Maksimoska, J., Marmorstein, R., Murray, L.J., O'Brien, T., Oh, A., Ramaswamy, S., Wang, W., Zhao, X., Zhong, Y., Blackwood, E., Rudolph, J., 2015. Design of selective PAK1 inhibitor G-5555: improving properties by employing an unorthodox low-p K a polar moiety. ACS Med. Chem. Lett. 6, 1241–1246. https://doi.org/10.1021/acsmedchemlett.5b00398.

Negi, H., Shukla, A., Khan, F., Pandey, R., 2016. 3β-Hydroxy-urs-12-en-28-oic acid prolongs lifespan in C. elegans by modulating JNK-1. Biochem. Biophys. Res. Commun. 480, 539–543. https://doi.org/10.1016/j.bbrc.2016.10.073.

Negi, H., Saikia, S.K., Pandey, R., 2017. 3β-hydroxy-urs-12-en-28-oic acid modulates dietary restriction mediated longevity and ameliorates toxic protein aggregation in C. elegans. Journals Gerontol. Ser. A 72, 1614–1619. https://doi.org/10.1093/gerona/glx118.

Neumann-Haefelin, E., Qi, W., Finkbeiner, E., Walz, G., Baumeister, R., Hertweck, M., 2008. SHC-1/p52Shc targets the insulin/IGF-1 and JNK signaling pathways to modulate life span and stress response in C. elegans. Genes Dev. 22, 2721–2735. https://doi.org/10.1101/gad.478408.

Nguyen, B.C.Q., Takahashi, H., Uto, Y., Shahinozzaman, Md., Tawata, S., Maruta, H., 2017a. 1,2,3-Triazolyl ester of Ketorolac: a "Click Chemistry"-based highly potent PAK1-blocking cancer-killer. Eur. J. Med. Chem. 126, 270–276. https://doi.org/10.1016/j.ejmech.2016.11.038.

Nguyen, B.C.Q., Yoshimura, K., Kumazawa, S., Tawata, S., Maruta, H., 2017b. Frondoside A from sea cucumber and nymphaeols from Okinawa propolis: natural anti-cancer agents that selectively inhibit PAK1 in vitro. Drug Discov. Ther. 11, 110–114. https://doi.org/10.5582/ddt.2017.01011.

Nguyen, B.C., Kim, S.-A., Won, S.-M., Park, S.-K., Uto, Y., Maruta, H., 2018. 1,2,3-Triazolyl ester of ketorolac (15K): boosting both heat-endurance and lifespan of C. elegans by down-regulating PAK1 at nM levels. Drug Discov. Ther. 12, 92–96. https://doi.org/10.5582/ddt.2018.01018.

Nheu, T.V., He, H., Hirokawa, Y., Tamaki, K., Florin, L., Schmitz, M.L., Suzuki-Takahashi, I., Jorissen, R.N., Burgess, A.W., Nishimura, S., Wood, J., Maruta, H., 2002. The K252a derivatives, inhibitors for the PAK/MLK kinase family, selectively block the growth of HAS transformants. Cancer J. 8, 328–336. https://doi.org/10.1097/00130404-200207000-00009.

Nikolic, M., Chou, M.M., Lu, W., Mayer, B.J., Tsai, L.-H., 1998. The p35/Cdk5 kinase is a neuron-specific Rac effector that inhibits Pak1 activity. Nature 395, 194–198. https://doi.org/10.1038/26034.

Nishida, E., Gotoh, Y., 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. Trends Biochem. Sci. 18, 128–131. https://doi.org/10.1016/0968-0004(93)90019-J.

Obermeier, A., 1998. PAK promotes morphological changes by acting upstream of Rac. EMBO J. 17, 4328–4339. https://doi.org/10.1093/emboj/17.15.4328.

Oh, Seung Wook, Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., Tissenbaum, H.A., 2005a. JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. Proc. Natl. Acad. Sci. 102, 4494–4499. https://doi.org/10.1073/pnas.0500749102.

Oh, S.W., Mukhopadhyay, A., Svrzikapa, N., Jiang, F., Davis, R.J., Tissenbaum, H.A., 2005b. JNK regulates lifespan in Caenorhabditis elegans by modulating nuclear translocation of forkhead transcription factor/DAF-16. Proc. Natl. Acad. Sci. 102, 4494–4499. https://doi.org/10.1073/pnas.0500749102.

Okada, M., Cote, G., Jha, R.K., Maruta, H., 2013. Functional maturation of PAKs from unicellular to multicellular organisms. Paks, Rac/Cdc42 (P21)-Activated Kinases. Elsevier, pp. 1–21. https://doi.org/10.1016/B978-0-12-407198-8.00001-1.

Okuyama, T., Inoue, H., Ookuma, S., Satoh, T., Kano, K., Honjoh, S., Hisamoto, N., Matsumoto, K., Nishida, E., 2010. The ERK-MAPK pathway regulates longevity through SKN-1 and insulin-like signaling in Caenorhabditis elegans. J. Biol. Chem. 285, 30274–30281. https://doi.org/10.1074/jbc.M110.146274.

Olsen, A., Vantipalli, M.C., Lithgow, G.J., 2006. Using Caenorhabditis elegans as a model for aging and age-related diseases. Ann. N. Y. Acad. Sci. 120–128. https://doi.org/10.1196/annals.1354.015.

Pacheco, A., Chernoff, J., 2010. Group I p21-activated kinases: emerging roles in immune function and viral pathogenesis. Int. J. Biochem. Cell Biol. 42, 13–16. https://doi.org/10.1016/j.biocel.2009.09.006.

Paix, A., Schmidt, H., Seydoux, G., 2016. Cas9-assisted recombineering in C. elegans: genome editing using in vivo assembly of linear DNAs. Nucleic Acids Res. gkw502. https://doi.org/10.1093/nar/gkw502.

Palacios-Gorba, C., Pina, R., Tortajada-Girbés, M., Jiménez-Belenguer, A., Siguemoto, É., Ferrús, M.A., Rodrigo, D., Pina-Pérez, M.C., 2020. Caenorhabditis elegans as an in vivo model to assess fucoidan bioactivity preventing Helicobacter pylori infection. Food Funct. 11, 4525–4534. https://doi.org/10.1039/D0FO00768D.

Pallauf, K., Duckstein, N., Rimbach, G., 2017. A literature review of flavonoids and lifespan in model organisms. Proc. Nutr. Soc. 76, 145–162. https://doi.org/10.1017/S0029665116000720.

Pang, S., Lynn, D.A., Lo, J.Y., Paek, J., Curran, S.P., 2014. SKN-1 and Nrf2 couples proline catabolism with lipid metabolism during nutrient deprivation. Nat. Commun. 5, 5048. https://doi.org/10.1038/ncomms6048.

Panowski, S.H., Wolff, S., Aguilaniu, H., Durieux, J., Dillin, A., 2007. PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature 447, 550–555. https://doi.org/10.1038/nature05837.

Patil, S., Lis, L.G., Schumacher, R.J., Norris, B.J., Morgan, M.L., Cuellar, R.A.D., Blazar, B.R., Suryanarayanan, R., Gurvich, V.J., Georg, G.I., 2015. Phosphonooxymethyl prodrug of triptolide: synthesis, physicochemical characterization, and efficacy in human colon adenocarcinoma and ovarian cancer xenografts. J. Med. Chem. 58, 9334–9344. https://doi.org/10.1021/acs.jmedchem.5b01329.

Peters, E.C., Gossett, A.J., Goldstein, B., Der, C.J., Reiner, D.J., 2013. Redundant canonical and noncanonical Caenorhabditis elegans p21-activated kinase signaling governs distal tip cell migrations. G3 3, 181–195. https://doi.org/10.1534/g3.112.004416.

Pietsch, K., Saul, N., Chakrabarti, S., Stürzenbaum, S.R., Menzel, R., Steinberg, C.E.W., 2011. Hormetins, antioxidants and prooxidants: defining quercetin-, caffeic acid- and rosmarinic acid-mediated life extension in C. elegans. Biogerontology 12, 329–347. https://doi.org/10.1007/s10522-011-9334-7.

Porchia, L.M., Guerra, M., Wang, Y.-C., Zhang, Y., Espinosa, A.V., Shinohara, M., Kulp, S. K., Kirschner, L.S., Saji, M., Chen, C.-S., Ringel, M.D., 2007. 2-Amino- N -{4-[5-(2-phenanthrenyl)-3-(trifluoromethyl)-1 H -pyrazol-1-yl]-phenyl} acetamide (OSU-03012), a celecoxib derivative, directly targets p21-activated kinase. Mol. Pharmacol. 72, 1124–1131. https://doi.org/10.1124/mol.107.037556.

Qin, Z., Johnsen, R., Yu, S., Chu, J.S.-C., Baillie, D.L., Chen, N., 2018. Genomic identification and functional characterization of essential genes in Caenorhabditis elegans. G3 8, 981–997. https://doi.org/10.1534/g3.117.300338.

Qu, J., Li, X., Novitch, B.G., Zheng, Y., Kohn, M., Xie, J.-M., Kozinn, S., Bronson, R., Beg, A.A., Minden, A., 2003. PAK4 kinase is essential for embryonic viability and for proper neuronal development. Mol. Cell. Biol. 23, 7122–7133. https://doi.org/10.1128/MCB.23.20.7122-7133.2003.

Radu, M., Semenova, G., Kosoff, R., Chernoff, J., 2014. PAK signalling during the development and progression of cancer. Nat. Rev. Cancer 14, 13–25. https://doi.org/10.1038/nrc3645.

Rajalingam, K., Dikic, I., 2011. Healthy ageing through regulated proteostasis. EMBO J. 30, 2983–2985. https://doi.org/10.1038/emboj.2011.237.

Rane, C.K., Minden, A., 2014. P21 activated kinases. Small GTPases 5, e28003. https://doi.org/10.4161/sgtp.28003.

Rane, C.K., Minden, A., 2019. P21 activated kinase signaling in cancer. Semin. Cancer Biol. 54, 40–49. https://doi.org/10.1016/j.semcancer.2018.01.006.

Rane, C., Senapedis, W., Baloglu, E., Landesman, Y., Crochiere, M., Das-Gupta, S., Minden, A., 2017. A novel orally bioavailable compound KPT-9274 inhibits PAK4, and blocks triple negative breast cancer tumor growth. Sci. Rep. 7, 42555. https://doi.org/10.1038/srep42555.

Rashid, S., Dar, B.A., Majeed, R., Hamid, A., Bhat, B.A., 2013. Synthesis and biological evaluation of ursolic acid-triazolyl derivatives as potential anti-cancer agents. Eur. J. Med. Chem. 66, 238–245. https://doi.org/10.1016/j.ejmech.2013.05.029.

Ravichandran, M., Priebe, S., Grigolon, G., Rozanov, L., Groth, M., Laube, B., Guthke, R., Platzer, M., Zarse, K., Ristow, M., 2018. Impairing L-threonine catabolism promotes healthspan through methylglyoxal-mediated proteohormesis. Cell Metab. 27, 914–925. https://doi.org/10.1016/j.cmet.2018.02.004 e5.

Rayner, J.O., Roberts, R.A., Kim, J., Poklepovic, A., Roberts, J.L., Booth, L., Dent, P., 2020. AR12 (OSU-03012) suppresses GRP78 expression and inhibits SARS-CoV-2 replication. Biochem. Pharmacol. 182, 114227 https://doi.org/10.1016/j.bcp.2020.114227.

Rider, L., Shatrova, A., Feener, E.P., Webb, L., Diakonova, M., 2007. JAK2 tyrosine kinase phosphorylates PAK1 and regulates PAK1 activity and functions. J. Biol. Chem. 282, 30985–30996. https://doi.org/10.1074/jbc.M701794200.

Robida-Stubbs, S., Glover-Cutter, K., Lamming, D.W., Mizunuma, M., Narasimhan, S.D., Neumann-Haefelin, E., Sabatini, D.M., Blackwell, T.K., 2012. TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab. 15, 713–724. https://doi.org/10.1016/j.cmet.2012.04.007.

Rong, R., Tang, X., Gutmann, D.H., Ye, K., 2004. Neurofibromatosis 2 (NF2) tumor suppressor merlin inhibits phosphatidylinositol 3-kinase through binding to PIKE-L. Proc. Natl. Acad. Sci. 101, 18200–18205. https://doi.org/10.1073/pnas.0405971102.

Rongo, C., 2011. Epidermal growth factor and aging: a signaling molecule reveals a new eye opening function. Aging (Albany. NY) 3, 896–905. https://doi.org/10.18632/aging.100384.

Rudel, T., 1997. Membrane and morphological changes in apoptotic cells regulated by caspase-mediated activation of PAK2. Science (80-.) 276, 1571–1574. https://doi.org/10.1126/science.276.5318.1571.

Rudolph, J., Murray, L.J., Ndubaku, C.O., O'Brien, T., Blackwood, E., Wang, W., Aliagas, I., Gazzard, L., Crawford, J.J., Drobnick, J., Lee, W., Zhao, X., Hoeflich, K.P., Favor, D.A., Dong, P., Zhang, H., Heise, C.E., Oh, A., Ong, C.C., La, H., Chakravarty, P., Chan, C., Jakubiak, D., Epler, J., Ramaswamy, S., Vega, R., Cain, G., Diaz, D., Zhong, Y., 2016. Chemically diverse group I p21-activated kinase (PAK) inhibitors impart acute cardiovascular toxicity with a narrow therapeutic window. J. Med. Chem. 59, 5520–5541. https://doi.org/10.1021/acs.jmedchem.6b00638.

Rungratanawanich, W., Qu, Y., Wang, X., Essa, M.M., Song, B.-J., 2021. Advanced glycation end products (AGEs) and other adducts in aging-related diseases and alcohol-mediated tissue injury. Exp. Mol. Med. 53, 168–188. https://doi.org/10.1038/s12276-021-00561-7.

Rupaimoole, R., Calin, G.A., Lopez-Berestein, G., Sood, A.K., 2016. miRNA deregulation in cancer cells and the tumor microenvironment. Cancer Discov. 6, 235–246. https://doi.org/10.1158/2159-8290.CD-15-0893.

Ryu, B.J., Kim, S., Min, B., Kim, K.Y., Lee, J.S., Park, W.J., Lee, H., Kim, S.H., Park, S., 2014. Discovery and the structural basis of a novel p21-activated kinase 4 inhibitor. Cancer Lett. 349, 45–50. https://doi.org/10.1016/j.canlet.2014.03.024.

Ryu, D., Mouchiroud, L., Andreux, P.A., Katsyuba, E., Moullan, N., Nicolet-dit-Félix, A.A., Williams, E.G., Jha, P., Lo Sasso, G., Huzard, D., Aebischer, P., Sandi, C., Rinsch, C., Auwerx, J., 2016. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nat. Med. 22, 879–888. https://doi.org/10.1038/nm.4132.

Safra, M., Fickentscher, R., Levi-Ferber, M., Danino, Y.M., Haviv-Chesner, A., Hansen, M., Juven-Gershon, T., Weiss, M., Henis-Korenblit, S., 2014. The FOXO transcription factor DAF-16 bypasses ire-1 requirement to promote endoplasmic reticulum homeostasis. Cell Metab. 20, 870–881. https://doi.org/10.1016/j.cmet.2014.09.006.

Sayed, A.A.R., 2011. Ferulsinaic acid attenuation of advanced glycation end products extends the lifespan of Caenorhabditis elegans. J. Pharm. Pharmacol. 63, 423–428. https://doi.org/10.1111/j.2042-7158.2010.01222.x.

Sells, M.A., Boyd, J.T., Chernoff, J., 1999. p21-activated kinase 1 (Pak1) regulates cell motility in mammalian fibroblasts. J. Cell Biol. 145, 837–849. https://doi.org/10.1083/jcb.145.4.837.

Sforcin, J.M., 2016. Biological properties and therapeutic applications of Propolis. Phyther. Res. 30, 894–905. https://doi.org/10.1002/ptr.5605.

Shahinozzaman, M., Taira, N., Ishii, T., Halim, M., Hossain, M., Tawata, S., 2018. Anti-inflammatory, anti-diabetic, and anti-alzheimer's effects of prenylated flavonoids from Okinawa Propolis: an investigation by experimental and computational studies. Molecules 23, 2479. https://doi.org/10.3390/molecules23102479.

Shahinozzaman, M., Obanda, D.N., Tawata, S., 2021. Chemical composition and pharmacological properties of Macaranga -type Pacific propolis: a review. Phyther. Res. 35, 207–222. https://doi.org/10.1002/ptr.6819.

Shanmugam, M.K., Rajendran, P., Li, F., Nema, T., Vali, S., Abbasi, T., Kapoor, S., Sharma, A., Kumar, A.P., Ho, P.C., Hui, K.M., Sethi, G., 2011. Ursolic acid inhibits multiple cell survival pathways leading to suppression of growth of prostate cancer xenograft in nude mice. J. Mol. Med. 89, 713–727. https://doi.org/10.1007/s00109-011-0746-2.

Shen, P.-X., Li, X., Deng, S.-Y., Zhao, L., Zhang, Y.-Y., Deng, X., Han, B., Yu, J., Li, Y., Wang, Z.-Z., Zhang, Y., 2021. Urolithin A ameliorates experimental autoimmune encephalomyelitis by targeting aryl hydrocarbon receptor. EBioMedicine 64, 103227. https://doi.org/10.1016/j.ebiom.2021.103227.

Singh, R., Barden, A., Mori, T., Beilin, L., 2001. Advanced glycation end-products: a review. Diabetologia 44, 129–146. https://doi.org/10.1007/s001250051591.

Singh, N.K., Kotla, S., Dyukova, E., Traylor, J.G., Orr, A.W., Chernoff, J., Marion, T.N., Rao, G.N., 2015. Disruption of p21-activated kinase 1 gene diminishes atherosclerosis in apolipoprotein E-deficient mice. Nat. Commun. 6, 7450. https://doi.org/10.1038/ncomms8450.

Slack, C., Alic, N., Foley, A., Cabecinha, M., Hoddinott, M.P., Partridge, L., 2015. The Ras-Erk-ETS-signaling pathway is a drug target for longevity. Cell 162, 72–83. https://doi.org/10.1016/j.cell.2015.06.023.

Staben, S.T., Feng, J.A., Lyle, K., Belvin, M., Boggs, J., Burch, J.D., Chua, C., Cui, H., DiPasquale, A.G., Friedman, L.S., Heise, C., Koeppen, H., Kotey, A., Mintzer, R., Oh, A., Roberts, D.A., Rouge, L., Rudolph, J., Tam, C., Wang, W., Xiao, Y., Young, A., Zhang, Y., Hoeflich, K.P., 2014. Back pocket flexibility provides group II p21-activated kinase (PAK) selectivity for type I 1/2 kinase inhibitors. J. Med. Chem. 57, 1033–1045. https://doi.org/10.1021/jm401768t.

Steinkraus, K.A., Smith, E.D., Davis, C., Carr, D., Pendergrass, W.R., Sutphin, G.L., Kennedy, B.K., Kaeberlein, M., 2008. Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in Caenorhabditis elegans. Aging Cell 7, 394–404. https://doi.org/10.1111/j.1474-9726.2008.00385.x.

Sutphin, G.L., Bishop, E., Yanos, M.E., Moller, R.M., Kaeberlein, M., 2012. Caffeine extends life span, improves healthspan, and delays age-associated pathology in Caenorhabditis elegans. Longev. Heal. 1, 9. https://doi.org/10.1186/2046-2395-1-9.

Taglieri, D.M., Ushio-Fukai, M., Monasky, M.M., 2014. P21-activated kinase in inflammatory and cardiovascular disease. Cell. Signal. 26, 2060–2069. https://doi.org/10.1016/j.cellsig.2014.04.020.

Taira, N., Nguyen, B.C.Q., Be Tu, P.T., Tawata, S., 2016. Effect of Okinawa Propolis on PAK1 activity, Caenorhabditis elegans longevity, melanogenesis, and growth of cancer cells. J. Agric. Food Chem. 64, 5484–5489. https://doi.org/10.1021/acs.jafc.6b01785.

Takahashi, H., Nguyen, B.C.Q., Uto, Y., Shahinozzaman, M., Tawata, S., Maruta, H., 2017. 1,2,3-Triazolyl esterization of PAK1-blocking propolis ingredients, artepillin C (ARC) and caffeic acid (CA), for boosting their anti-cancer/anti-PAK1 activities along with cell-permeability. Drug Discov. Ther. 11, 104–109. https://doi.org/10.5582/ddt.2017.01009.

Takai, Y., Sasaki, T., Matozaki, T., 2001. Small GTP-binding proteins. Physiol. Rev. 81, 153–208. https://doi.org/10.1152/physrev.2001.81.1.153.

Takaoka, M., 1939. Resveratrol, a new phenolic compound, from Veratrum grandiflorum. J. Chem. Soc. Japan 60, 1090–1100.

Talukder, A.H., Meng, Q., Kumar, R., 2006. CRIPak, a novel endogenous Pak1 inhibitor. Oncogene 25, 1311–1319. https://doi.org/10.1038/sj.onc.1209172.

Tangrodchanapong, T., Sobhon, P., Meemon, K., 2020. Frondoside A attenuates amyloid-β proteotoxicity in transgenic Caenorhabditis elegans by suppressing its formation. Front. Pharmacol. 11 https://doi.org/10.3389/fphar.2020.553579.

Tepedelen, B.E., Soya, E., Korkmaz, M., 2017. Epigallocatechin-3-gallate reduces the proliferation of benign prostatic hyperplasia cells via regulation of focal adhesions. Life Sci. 191, 74–81. https://doi.org/10.1016/j.lfs.2017.10.016.

The C. elegans Sequencing Consortium, 1998. Genome sequence of the nematode C. elegans: a platform for investigating biology. Science (80-.) 282, 2012–2018. https://doi.org/10.1126/science.282.5396.2012.

Thillai, K., Lam, H., Sarker, D., Wells, C.M., 2017. Deciphering the link between PI3K and PAK: an opportunity to target key pathways in pancreatic cancer? Oncotarget 8, 14173–14191. https://doi.org/10.18632/oncotarget.13309.

Tresini, M., Lorenzini, A., Torres, C., Cristofalo, V.J., 2006. Modulation of replicative senescence of diploid human cells by nuclear ERK signaling. J. Biol. Chem. 282, 4136–4151. https://doi.org/10.1074/jbc.M604955200.

Troemel, E.R., Chu, S.W., Reinke, V., Lee, S.S., Ausubel, F.M., Kim, D.H., 2006. p38 MAPK regulates expression of immune response genes and contributes to longevity in C. elegans. PLoS Genet. 2, e183. https://doi.org/10.1371/journal.pgen.0020183.

Tsakiridis, T., Taha, C., Grinstein, S., Klip, A., 1996. Insulin activates a p21-activated kinase in muscle cells via phosphatidylinositol 3-kinase. J. Biol. Chem. 271, 19664–19667. https://doi.org/10.1074/jbc.271.33.19664.

Tsukamoto, S., Huang, Y., Umeda, D., Yamada, S., Yamashita, S., Kumazoe, M., Kim, Y., Murata, M., Yamada, K., Tachibana, H., 2014. 67-kDa laminin receptor-dependent protein phosphatase 2A (PP2A) activation elicits melanoma-specific antitumor activity overcoming drug resistance. J. Biol. Chem. 289, 32671–32681. https://doi.org/10.1074/jbc.M114.604983.

Tullet, J.M.A., Hertweck, M., An, J.H., Baker, J., Hwang, J.Y., Liu, S., Oliveira, R.P., Baumeister, R., Blackwell, T.K., 2008. Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell 132, 1025–1038. https://doi.org/10.1016/j.cell.2008.01.030.

Uno, M., Nishida, E., 2016. Lifespan-regulating genes in C. elegans. NPJ Aging Mech. Dis. 2, 16010. https://doi.org/10.1038/npjamd.2016.10.

Van Pelt, K.M., Truttmann, M.C., 2020. Caenorhabditis elegans as a model system for studying aging-associated neurodegenerative diseases. Transl. Med. Aging 4, 60–72. https://doi.org/10.1016/j.tma.2020.05.001.

Vellai, T., Takacs-Vellai, K., Zhang, Y., Kovacs, A.L., Orosz, L., Müller, F., 2003. Influence of TOR kinase on lifespan in C. elegans. Nature 426, 620. https://doi.org/10.1038/426620a.

Walker, G.A., Lithgow, G.J., 2003. Lifespan extension in C. elegans by a molecular chaperone dependent upon insulin-like signals. Aging Cell 2, 131–139. https://doi.org/10.1046/j.1474-9728.2003.00045.x.

Wang, Z., Jin, H., Xu, R., Mei, Q., Fan, D., 2009. Triptolide downregulates Rac1 and the JAK/STAT3 pathway and inhibits colitis-related colon cancer progression. Exp. Mol. Med. 41, 717. https://doi.org/10.3858/emm.2009.41.10.078.

Wang, Z., Pedersen, E., Basse, A., Lefever, T., Peyrollier, K., Kapoor, S., Mei, Q., Karlsson, R., Chrostek-Grashoff, A., Brakebusch, C., 2010. Rac1 is crucial for Ras-dependent skin tumor formation by controlling Pak1-Mek-Erk hyperactivation and hyperproliferation in vivo. Oncogene 29, 3362–3373. https://doi.org/10.1038/onc.2010.95.

Wang, X., Yi, K., Zhao, Y., 2018a. Fucoidan inhibits amyloid-β-induced toxicity in transgenic Caenorhabditis elegans by reducing the accumulation of amyloid-β and decreasing the production of reactive oxygen species. Food Funct. 9, 552–560. https://doi.org/10.1039/C7FO00662D.

Wang, Y., Wang, S., Lei, M., Boyett, M., Tsui, H., Liu, W., Wang, X., 2018b. The p21-activated kinase 1 (Pak1) signalling pathway in cardiac disease: from mechanistic study to therapeutic exploration. Br. J. Pharmacol. 175, 1362–1374. https://doi.org/10.1111/bph.13872.

Way, T.-D., Kao, M.-C., Lin, J.-K., 2004. Apigenin induces apoptosis through proteasomal degradation of HER2/neu in HER2/neu-overexpressing breast cancer cells via the phosphatidylinositol 3-kinase/Akt-dependent pathway. J. Biol. Chem. 279, 4479–4489. https://doi.org/10.1074/jbc.M305529200.

Webb, D.J., Zhang, H., Majumdar, D., Horwitz, A.F., 2007. α5 integrin signaling regulates the formation of spines and synapses in hippocampal neurons. J. Biol. Chem. 282, 6929–6935. https://doi.org/10.1074/jbc.M610981200.

Wiedenheft, B., Sternberg, S.H., Doudna, J.A., 2012. RNA-guided genetic silencing systems in bacteria and archaea. Nature 482, 331–338. https://doi.org/10.1038/nature10886.

Williams, G.C., 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution (N. Y). 11, 398. https://doi.org/10.2307/2406060.

Won, S.-Y., Park, M.-H., You, S.-T., Choi, S.-W., Kim, H.-K., McLean, C., Bae, S.-C., Kim, S. R., Jin, B.K., Lee, K.H., Shin, E.-Y., Kim, E.-G., 2016. Nigral dopaminergic PAK4 prevents neurodegeneration in rat models of Parkinsons disease. Sci. Transl. Med. 8, 367ra170. https://doi.org/10.1126/scitranslmed.aaf1629.

Wood, J.G., Rogina, B., Lavu, S., Howitz, K., Helfand, S.L., Tatar, M., Sinclair, D., 2004. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430, 686–689. https://doi.org/10.1038/nature02789.

Wu, P., Nielsen, T.E., Clausen, M.H., 2015. FDA-approved small-molecule kinase inhibitors. Trends Pharmacol. Sci. 36, 422–439. https://doi.org/10.1016/j.tips.2015.04.005.

Wu, Z., Isik, M., Moroz, N., Steinbaugh, M.J., Zhang, P., Blackwell, T.K., 2019. Dietary restriction extends lifespan through metabolic regulation of innate immunity. Cell Metab. 29, 1192–1205. https://doi.org/10.1016/j.cmet.2019.02.013 e8.

Xia, C., Ma, W., Stafford, L.J., Marcus, S., Xiong, W.-C., Liu, M., 2001. Regulation of the p21-activated kinase (PAK) by a human G -like WD-repeat protein, hPIP1. Proc. Natl. Acad. Sci. 98, 6174–6179. https://doi.org/10.1073/pnas.101137298.

Xiong, L.-G., Chen, Y.-J., Tong, J.-W., Gong, Y.-S., Huang, J.-A., Liu, Z.-H., 2018. Epigallocatechin-3-gallate promotes healthy lifespan through mitohormesis during early-to-mid adulthood in Caenorhabditis elegans. Redox Biol. 14, 305–315. https://doi.org/10.1016/j.redox.2017.09.019.

Xu, J.-W., Ikeda, K., Kobayakawa, A., Ikami, T., Kayano, Y., Mitani, T., Yamori, Y., 2005. Downregulation of Rac1 activation by caffeic acid in aortic smooth muscle cells. Life Sci. 76, 2861–2872. https://doi.org/10.1016/j.lfs.2004.11.015.

Xu, Y., Foulks, J.M., Clifford, A., Brenning, B., Lai, S., Luo, B., Parnell, K.M., Merx, S., McCullar, M.V., Kanner, S.B., Ho, K.-K., 2013. Synthesis and structure–activity relationship of 2-arylamino-4-aryl-pyrimidines as potent PAK1 inhibitors. Bioorg. Med. Chem. Lett. 23, 4072–4075. https://doi.org/10.1016/j.bmcl.2013.05.059.

Yan, L., Vatner, D.E., O'Connor, J.P., Ivessa, A., Ge, H., Chen, W., Hirotani, S., Ishikawa, Y., Sadoshima, J., Vatner, S.F., 2007. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. Cell 130, 247–258. https://doi.org/10.1016/j.cell.2007.05.038.

Yanase, S., Luo, Y., Maruta, H., 2013. PAK1-deficiency/down-regulation reduces brood size, activates HSP16.2 gene and extends lifespan in Caenorhabditis elegans. Drug Discov. Ther. 7, 29–35. https://doi.org/10.5582/ddt.2013.v7.1.29.

Yao, D., Li, C., Rajoka, M.S.R., He, Z., Huang, J., Wang, J., Zhang, J., 2020. P21-Activated Kinase 1: emerging biological functions and potential therapeutic targets in cancer. Theranostics 10, 9741–9766. https://doi.org/10.7150/thno.46913.

Yeo, D., Huynh, N., Beutler, J.A., Christophi, C., Shulkes, A., Baldwin, G.S., Nikfarjam, M., He, H., 2014. Glaucarubinone and gemcitabine synergistically reduce pancreatic cancer growth via down-regulation of P21-activated kinases. Cancer Lett. 346, 264–272. https://doi.org/10.1016/j.canlet.2014.01.001.

Yoon, D.S., Cha, D.S., Choi, Y., Lee, J.W., Lee, M.-H., 2019. MPK-1/ERK is required for the full activity of resveratrol in extended lifespan and reproduction. Aging Cell 18, e12867. https://doi.org/10.1111/acel.12867.

Yoshikawa, N., Tsuno, N.H., Okaji, Y., Kawai, K., Shuno, Y., Nagawa, H., Oshima, N., Takahashi, K., 2010. Isoprenoid geranylgeranylacetone inhibits human colon cancer cells through induction of apoptosis and cell cycle arrest. Anticancer Drugs 21, 850–860. https://doi.org/10.1097/CAD.0b013e32833e53cf.

Yu, S., Driscoll, M., 2011. EGF signaling comes of age: promotion of healthy aging in C. elegans. Exp. Gerontol. 46, 129–134. https://doi.org/10.1016/j.exger.2010.10.010.

Yu, C.-W., Wei, C.-C., Liao, V.H.C., 2014. Curcumin-mediated oxidative stress resistance in Caenorhabditis elegans is modulated by age-1, akt-1, pdk-1, osr-1, unc-43, sek-1, skn-1, sir-2.1, and mev-1. Free Radic. Res. 48, 371–379. https://doi.org/10.3109/10715762.2013.872779.

Yuan, T., Ma, H., Liu, W., Niesen, D.B., Shah, N., Crews, R., Rose, K.N., Vattem, D.A., Seeram, N.P., 2016. Pomegranate's neuroprotective effects against Alzheimer's disease are mediated by urolithins, its ellagitannin-gut microbial derived metabolites. ACS Chem. Neurosci. 7, 26–33. https://doi.org/10.1021/acschemneuro.5b00260.

Zarse, K., Bossecker, A., Müller-Kuhrt, L., Siems, K., Hernandez, M.A., Berendsohn, W.G., Birringer, M., Ristow, M., 2011. The phytochemical glaucarubinone promotes mitochondrial metabolism, reduces body fat, and extends lifespan of Caenorhabditis elegans. Horm. Metab. Res. 43, 241–243. https://doi.org/10.1055/s-0030-1270524.

Zegers, M.M.P., 2003. Pak1 and PIX regulate contact inhibition during epithelial wound healing. EMBO J. 22, 4155–4165. https://doi.org/10.1093/emboj/cdg398.

Zegers, M., 2008. Roles of P21-activated kinases and associated proteins in epithelial wound healing. Int. Rev. Cell Mol. Biol. 253–298. https://doi.org/10.1016/S1937-6448(08)00606-0.

Zeng, H., Xu, L., Zou, Y., Wang, S., 2021. Romidepsin and metformin nanomaterials delivery on streptozocin for the treatment of Alzheimer's disease in animal model. Biomed. Pharmacother. 141, 111864 https://doi.org/10.1016/j.biopha.2021.111864.

Zenke, F.T., King, C.C., Bohl, B.P., Bokoch, G.M., 1999. Identification of a central phosphorylation site in p21-activated kinase regulating autoinhibition and kinase activity. J. Biol. Chem. 274, 32565–32573. https://doi.org/10.1074/jbc.274.46.32565.

Zhang, H., 2005. A GIT1/PIX/Rac/PAK signaling module regulates spine morphogenesis and synapse formation through MLC. J. Neurosci. 25, 3379–3388. https://doi.org/10.1523/JNEUROSCI.3553-04.2005.

Zhang, S., Han, J., Sells, M.A., Chernoff, J., Knaus, U.G., Ulevitch, R.J., Bokoch, G.M., 1995. Rho family GTPases regulate p38 mitogen-activated protein kinase through the downstream mediator Pak1. J. Biol. Chem. 270, 23934–23936. https://doi.org/10.1074/jbc.270.41.23934.

Zhang, L., Jie, G., Zhang, J., Zhao, B., 2009. Significant longevity-extending effects of EGCG on Caenorhabditis elegans under stress. Free Radic. Biol. Med. 46, 414–421. https://doi.org/10.1016/j.freeradbiomed.2008.10.041.

Zhang, H., Landmann, F., Zahreddine, H., Rodriguez, D., Koch, M., Labouesse, M., 2011. A tension-induced mechanotransduction pathway promotes epithelial morphogenesis. Nature 471, 99–103. https://doi.org/10.1038/nature09765.

Zhang, J., Wang, J., Guo, Q., Wang, Y., Zhou, Y., Peng, H., Cheng, M., Zhao, D., Li, F., 2012. LCH-7749944, a novel and potent p21-activated kinase 4 inhibitor, suppresses proliferation and invasion in human gastric cancer cells. Cancer Lett. 317, 24–32. https://doi.org/10.1016/j.canlet.2011.11.007.

Zhang, J., Zhang, H.-Y., Wang, J., You, L.-H., Zhou, R.-Z., Zhao, D.-M., Cheng, M.-S., Li, F., 2016. GL-1196 suppresses the proliferation and invasion of gastric cancer cells via targeting PAK4 and inhibiting PAK4-Mediated signaling pathways. Int. J. Mol. Sci. 17, 470. https://doi.org/10.3390/ijms17040470.

Zhang, J., Zou, L., Tang, P., Pan, D., He, Z., Yao, D., 2020a. Design, synthesis and biological evaluation of 1H-pyrazolo [3,4-d]pyrimidine derivatives as PAK1 inhibitors that trigger apoptosis, ER stress and anti-migration effect in MDA-MB-231 cells. Eur. J. Med. Chem. 194, 112220 https://doi.org/10.1016/j.ejmech.2020.112220.

Zhang, M., Chen, L., Liu, Y., Chen, M., Zhang, S., Kong, D., 2020b. Sea cucumber Cucumaria frondosa fucoidan inhibits osteosarcoma adhesion and migration by regulating cytoskeleton remodeling. Oncol. Rep. 44, 469–476. https://doi.org/10.3892/or.2020.7614.

Zhang, S., Li, F., Zhou, T., Wang, G., Li, Z., 2020c. Caenorhabditis elegans as a useful model for studying aging mutations. Front. Endocrinol. (Lausanne) 11. https://doi.org/10.3389/fendo.2020.554994.

Zhao, Z., Manser, E., Loo, T.-H., Lim, L., 2000. Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. Mol. Cell. Biol. 20, 6354–6363. https://doi.org/10.1128/MCB.20.17.6354-6363.2000.

Zhao, L., Ma, Q.-L., Calon, F., Harris-White, M.E., Yang, F., Lim, G.P., Morihara, T., Ubeda, O.J., Ambegaokar, S., Hansen, J.E., Weisbart, R.H., Teter, B., Frautschy, S.A., Cole, G.M., 2006. Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. Nat. Neurosci. 9, 234–242. https://doi.org/10.1038/nn1630.

Zhao, P., Zhang, Z., Ke, H., Yue, Y., Xue, D., 2014. Oligonucleotide-based targeted gene editing in C. elegans via the CRISPR/Cas9 system. Cell Res. 24, 247–250. https://doi.org/10.1038/cr.2014.9.

Zhao, F., Yang, Z., Chen, Y., Zhou, Q., Zhang, J., Liu, J., Wang, B., He, Q., Zhang, L., Yu, Y., Liu, P., 2018a. Deregulation of the hippo pathway promotes tumor cell proliferation through YAP activity in human sporadic vestibular schwannoma. World Neurosurg. 117, e269–e279. https://doi.org/10.1016/j.wneu.2018.06.010.

Zhao, Y., Liu, P., Zhang, N., Chen, J., Landegger, L.D., Wu, L., Zhao, F., Zhao, Yanxia, Zhang, Y., Zhang, J., Fujita, T., Stemmer-Rachamimov, A., Ferraro, G.B., Liu, H., Muzikansky, A., Plotkin, S.R., Stankovic, K.M., Jain, R.K., Xu, L., 2018b. Targeting the cMET pathway augments radiation response without adverse effect on hearing in NF2 schwannoma models. Proc. Natl. Acad. Sci. 115, E2077–E2084. https://doi.org/10.1073/pnas.1719966115.

Zhen, X., Uryu, K., Cai, G., Johnson, G.P., Friedman, E., 1999. Age-associated impairment in brain MAPK signal pathways and the effect of caloric restriction in fischer 344 rats. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 54, B539–B548. https://doi.org/10.1093/gerona/54.12.B539.

Zheng, S.-Q., Huang, X.-B., Xing, T.-K., Ding, A.-J., Wu, G.-S., Luo, H.-R., 2016. Chlorogenic acid extends the lifespan of Caenorhabditis elegans via Insulin/IGF-1 signaling pathway. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 72, 464–472. https://doi.org/10.1093/gerona/glw105.

Zhou, Y., Zhang, J., Wang, J., Cheng, M., Zhao, D., Li, F., 2019. Targeting PAK1 with the small molecule drug AK963/40708899 suppresses gastric cancer cell proliferation and invasion by downregulation of PAK1 activity and PAK1-Related signaling pathways. Anat. Rec. 302, 1571–1579. https://doi.org/10.1002/ar.24095.

Zins, K., Gunawardhana, S., Lucas, T., Abraham, D., Aharinejad, S., 2013. Targeting Cdc42 with the small molecule drug AZA197 suppresses primary colon cancer growth and prolongs survival in a preclinical mouse xenograft model by downregulation of PAK1 activity. J. Transl. Med. 11, 295. https://doi.org/10.1186/1479-5876-11-295.