Genes & Diseases (2023) 10, 758–770

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.keaipublishing.com/en/journals/genes-diseases

REVIEW ARTICLE

New insights into the roles for DYRK family in mammalian development and congenital diseases

Saishu Yoshida, Kiyotsugu Yoshida*

Department of Biochemistry, The Jikei University School of Medicine, Minato-ku, Tokyo 105 8461, Japan

Received 26 July 2021; received in revised form 4 November 2021; accepted 6 December 2021
Available online 6 January 2022

KEYWORDS
Ciliopathies;
Development;
Down syndrome;
DYRKs;
Embryogenesis

Abstract The dual-specificity tyrosine-regulated kinase (DYRK) family is evolutionarily conserved from invertebrate to mammals. DYRKs regulate cell proliferation, apoptosis, survival, and differentiation by modifying the protein activation state, cellular localization, and turnover. In contrast to several studies in cellular models, the available evidence regarding the *in vivo* roles of DYRKs in mammalian development is limited. This review summarizes the *in vivo* studies on *Dyrks* which provide insight into their roles in mammalian tissue development and congenital diseases. *In vivo* evidence obtained using knockout and genetically modified animals helps to understand and develop novel clinical therapies and drug for human congenital diseases, such as Down syndrome and neuronal disorders (associated with DYRK1A) and skeletal ciliopathies (associated with DYRK2).

Introduction

Dual-specificity tyrosine-regulated kinase (DYRK) family belongs to the CMGC group which includes cyclin-dependent kinases (CDKs), mitogen-activated protein kinase (MAPK), CDK-like kinases (CLKs), and glycogen synthase kinase (GSK).[1] The five members of mammalian DYRKs (DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4) are phylogenetically categorized into two classes: class I (DYRK1A and DYRK1B) and class II (DYRK2, DYRK3, and DYRK4) based on sequence homology within the conserved kinase domain.[2,3]

Abbreviations: CDKs, cyclin-dependent kinases; CRMP4, collapsin response mediator protein 4; DS, Down syndrome; DSCR, Down syndrome-critical region; DYRK, dual-specificity tyrosine-regulated kinase; FOXO1, forkhead box O1; GSK, glycogen synthase kinase; Hh, Hedgehog; NFAT, nuclear factor.

* Corresponding author.
E-mail address: kyoshida@jikei.ac.jp (K. Yoshida).
Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2021.12.004

DYRKs exhibit the characteristic property of the DYRK family of having both tyrosine (Tyr) and serine/threonine (Ser/Thr) kinase activities. To attain full phosphorylation of the target substrates, DYRKs are activated by auto-phosphorylation of the second Tyr residue in a conserved Y-x(x)(x)-Y sequence within activation loops[2,4,5] (Fig. 1). Once Tyr residue is auto-phosphorylated, Tyr kinase activity is lost and DYRKs retain only Ser/Thr kinase activity.[6,7] Therefore, these kinases are termed dual-specificity tyrosine-regulated kinases. During phosphorylation of target proteins, DYRKs are known to phosphorylate Ser/Thr in the consensus sequence "Rx(x)[S/T]P" (Fig. 1).[5,8,9] In particular, proline (Pro) at the +1 position and arginine (Arg) at the −2 or −3 position relative to Ser/Thr seem to be preferred with a degree of divergence from this consensus.[5,8,9]

DYRKs are evolutionarily conserved and are known to regulate cell proliferation, apoptosis, survival, and differentiation. Accumulating studies in cellular models have revealed the molecular mechanisms of DYRKs, especially DYRK1A, DYRK1B, and DYRK2. In embryogenesis, DYRKs play important roles in development and cell differentiation in several species, especially flies (*Drosophila*),[10,11] and worms (*C. elegans*).[12–20] In contrast to invertebrate and cellular models, there are limited reports regarding the functions of DYRKs in mammalian development. This review summarizes the *in vivo* studies that show that DYRKs play a role in mammalian development. Moreover, using these studies, we present an overview promoting the understanding of its roles in mammalian tissue development and congenital diseases.

Representative molecular mechanisms of DYRKs

Numerous studies have demonstrated that phosphorylation by DYRKs regulates several biological processes by modifying protein activation, cellular localization, and turnover (stabilization and degradation) by the ubiquitin-proteasome system. In this section, we summarize the representative molecular mechanisms of DYRKs. For findings on DYRKs inhibitor and clinical implications, see the latest excellent reviews.[21–24]

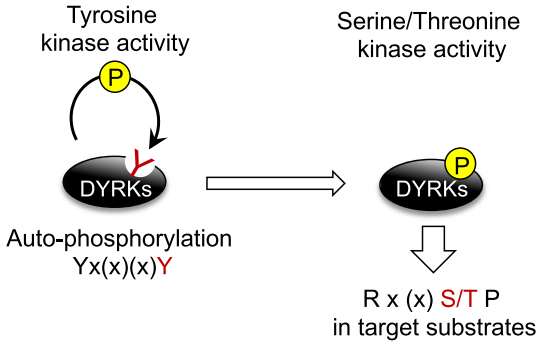

Figure 1 Conformational activation of DYRKs by auto-phosphorylation of the second Tyr residue in a conserved Y-x(x)(x)-Y sequence within the activation loop. After a conformational change, DYRKs retain only Ser/Thr kinase activity with the consensus sequence "Rx(x)[S/T]P".

DYRKs regulate protein turnover during the cell cycle

DYRKs phosphorylate several critical regulators of the cell cycle. The first evidence regarding the role of DYRKs in cell cycle control has been reported for DYRK1B via proteasome-dependent turnover.[25–28] Cyclin D1 (encoded by *CCND1*) is a key regulator of the G1/S-phase transition via interaction with CDK4 and CDK6, which phosphorylate Rb (retinoblastoma), resulting in de-repression of E2F-dependent transcription of S-phase genes.[29] DYRK1B directly phosphorylates cyclin D1 at Thr286 for proteasomal degradation (Fig. 2A).[25,28]

DYRK1B also phosphorylates another cell-cycle regulator, $p27^{Kip1}$ (encoded by the cyclin-dependent kinase inhibitor 1B; *CDKN1B*), which is a member of the Cip/Kip family of CKIs and maintains cells in a quiescent state.[30,31] DYRK1B co-localizes with nuclear $p27^{Kip1}$ and directly phosphorylates $p27^{Kip1}$ at Ser10 for stabilization (Fig. 2A).[26] Knockdown of *DYRK1B* by siRNA increased cell entry from G0 to G1 via a decrease in protein levels of $p27^{Kip1}$, but not mRNA levels, in C2C12 myoblasts.[26] Accordingly, DYRK1B is expressed in G0/G1 to maintain cell growth arrest. These reports show that DYRK1B maintains growth arrest and quiescence by depleting cyclin D1 and stabilizing $p27^{Kip1}$ in a kinase activity-dependent manner.

Similarly, cyclin D family members as well as $p27^{Kip1}$ are also phosphorylated by DYRK1A to regulate their protein stabilities,[32,33] thus affecting neurodevelopment, heart development, and lymphopoiesis,[34,35] as described below.

DYRK2 plays important roles in regulating the cell cycle by modifying protein turnover. DYRK2 phosphorylates and degrades CDC25A, which is essential for controlling the cell cycle as a mediator of checkpoint responses to DNA damage.[36] DYRK2 also phosphorylates the 19S subunit Rpt3 at Thr25 in a cell cycle-regulated manner, leading to the activation of proteasome activity and cell proliferation.[37] Another function of DYRK2 is to act as a scaffold protein for the EDD-DDB1-VprBP (EDVP) E3 ligase complex. In this complex, DYRK2 functions as an adaptor protein between EDD and DDB1 and directly phosphorylates Katanin p60 at Ser42, Ser109, and Thr133, resulting in ubiquitination and degradation of Katanin p60 by DYRK2-EDVP E3 ligase complex.[38] Furthermore, DYRK2 regulates the cell cycle as a priming kinase of c-Jun and c-Myc,[39] as described below.

DYRKs induce apoptosis via p53 phosphorylation

Genotoxic stress induces the activation of p53 (encoded by *TRP53*), which is a transcription factor that acts as a master regulator of the cell cycle checkpoint system.[40,41] Phosphorylation of p53 at Ser46 is indispensable for apoptotic induction. Although DYRK2 is mostly cytosolic, upon genotoxic stress, phosphorylation of DYRK2 at Thr33 and Ser369 by ataxia telangiectasia-mutated (ATM) induces its activation and nuclear localization.[42] Notably, in response to genotoxic stress, DYRK2 induces apoptosis by phosphorylation of p53 at Ser46 in normal and tumor cells (Fig. 2B).[42]

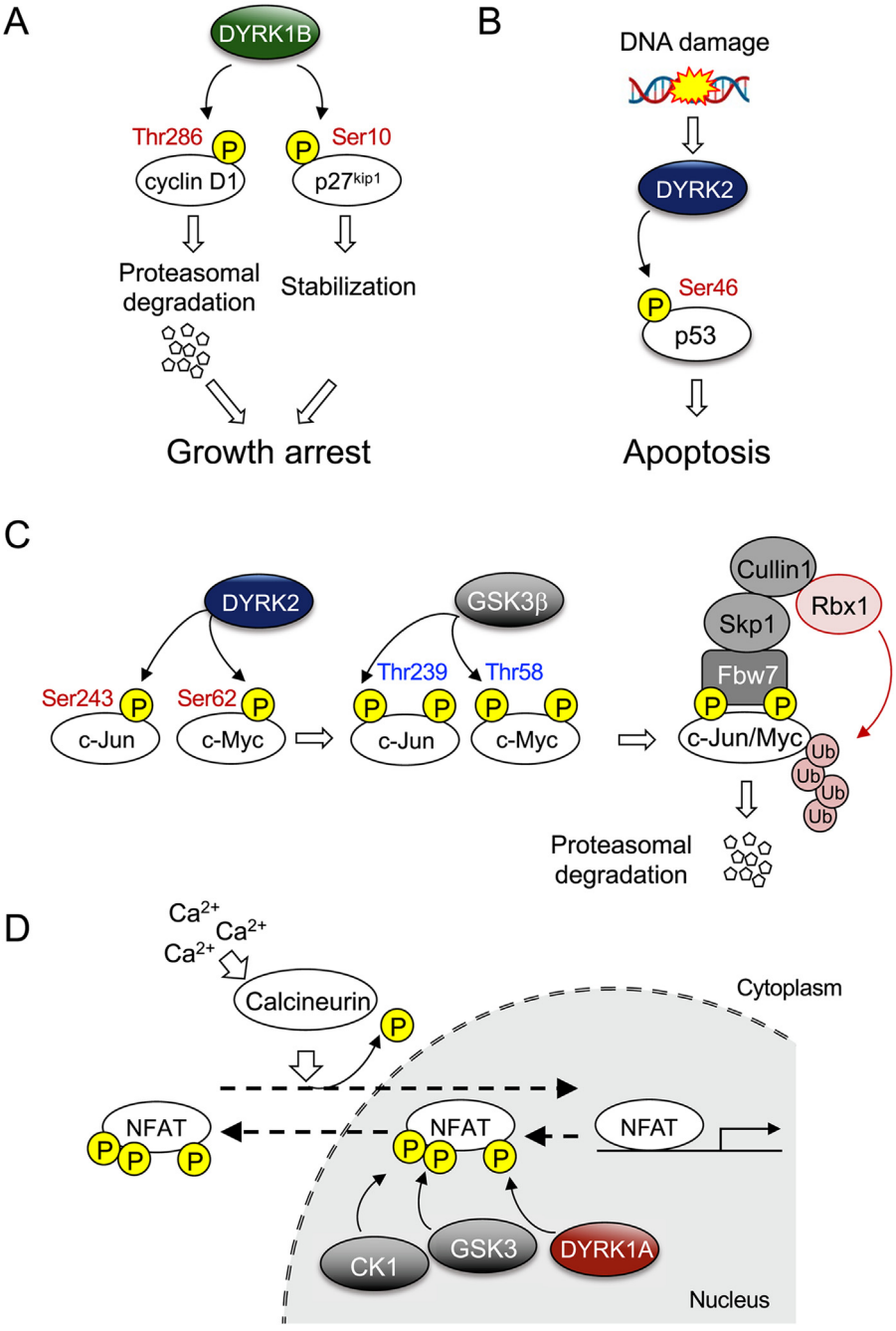

Figure 2 Molecular mechanisms of DYRKs. **(A)** DYRK1B-mediated growth arrest by protein turnover of cyclin D1 and $p27^{kip1}$. DYRK1B phosphorylates cyclin D1 at Thr286 for proteasomal degradation, and $p27^{kip1}$ at Ser10 for stabilization. **(B)** DYRK2-mediated apoptosis under DNA damage by p53-activation. Under genotoxic conditions, DYRK2 phosphorylates p53 at Ser46 and induces apoptosis. **(C)** DYRK2-mediated cell cycle regulation as a priming kinase for GSK3β. DYRK2-mediated phosphorylation of c-Jun at Ser243 and c-Myc at Ser62 induces sequential phosphorylation by GSK3β. Phosphorylated c-Jun and c-Myc are recognized by the Skp1-cullin-F-box-protein (SCF) complex and degraded by the ubiquitin-proteasome system. **(D)** DYRKs-mediated phosphorylation of NFAT induces sequential phosphorylation by GSK3β and CK1 (casein kinase 1). Phosphorylated NFAT is exported to the cytoplasm and its transcriptional activity is reduced.

Interestingly, DYRK1A and DYRK3 are also involved in p53-regulation in different manner. Sirtuin1, also known as NAD-dependent deacetylase sirtuin-1, deacetylates transcription factors that contribute to a variety of physiological processes including pro-survival activities.[43] DYRK1A and DYRK3 directly phosphorylate Sirtuin1 at Thr522 and induce its activation. Activated SIRT1 promotes deacetylation of p53, resulting in the promotion of cell survival upon genotoxic stress.[44]

Priming kinase activity of DYRKs induces protein degradation

DYRKs also function as priming kinases, meaning that the phosphorylation of a given residue by DYRKs is a prerequisite for the subsequent phosphorylation of a different residue by other protein kinases such as GSK3β, CK1 (casein kinase 1), or PLK-like kinase (PLK).[45]

DYRK2 acts as a priming kinase for GSK3β, which is a serine/threonine kinase.[39] DYRK2 directly phosphorylates oncogenic transcription factors c-Jun (at Ser243) and c-Myc (at Ser62), and regulates the G1/S transition and proliferation of tumor cells.[39] Phosphorylation at the +4 position by DYRK2 induced subsequent phosphorylation by GSK3β (Fig. 2C). Both priming kinase (DYRK2) and GSK3β-phosphorylated sites are essential for the recognition and ubiquitination of the Skp1-cullin-F-box-protein (SCF) complex, which is a ubiquitin ligase-mediated degradation system.[39]

DYRK2 also actively regulates epithelial-mesenchymal transition and metastasis via phosphorylation of the transcription factor SNAIL.[46] DYRK2 initially phosphorylates SNAIL at Ser104 as a GSK3β-priming kinase. Phosphorylated SNAIL is recognized and ubiquitinated by the SCF-TrCP complex and is degraded in breast cancer cells.[46] In neuronal cells, DYRK2 phosphorylates collapsin response mediator protein 4 (CRMP4) at Ser522 as a GSK3β−priming kinase, resulting in the regulation of axon elongation.[47]

DYRK1A and DYRK2 also function as priming kinases for GSK3β. Woods et al reported that phosphorylation of eukaryotic initiation factor 2B (eIF2B) at Ser539, by DYRK1A and DYRK2,[48] is required for phosphorylation of Ser535 by GSK3. More recently, the role of DYRK1A in controlling circadian rhythm via CRY2 was reported.[49] CRY2 plays a critical role as a potent inhibitor of E-box-dependent clock gene expression. CRY2 is rhythmically phosphorylated by DYRK1A (as a priming kinase) at Ser557 in the C-terminal tail for subsequent GSK3β mediated phosphorylation of CRY2 at Ser553, which leads to proteasomal degradation of CRY2.[49]

DYRKs regulate cellular localization of the transcription factor NFAT

The priming kinase activity of DYRKs also regulates cellular localization and the activation of nuclear factor of activated T cells (NFAT), a transcription factor essential for vertebrate development.[50] The inactive phosphorylated NFAT proteins reside in the cytoplasm. Increased intercellular Ca^{2+} levels induce de-phosphorylation of NFATs by the calmodulin-dependent phosphatase calcineurin, resulting in NFATs translocating to the nucleus and acting as a transcription factor (Fig. 2D). DYRK1A and DYRK2 have been identified as regulators of this NFAT process in vertebrate neurons[51] and Drosophila,[52] respectively. DYRK2 directly phosphorylates NFAT1 as a GSK3β- and CK1-priming kinase.[52] DYRK1A also functions as a GSK3β-priming kinase for NFATc4.[51] GSK3β phosphorylates NFATc4 at a site different from that of the c-Myc/c-Jun priming at +4 position (as described above). Phosphorylation at both the priming and GSK3β sites acts as export signals and drive translocation to the cytoplasm, resulting in inactivation of NFAT transcriptional activity (Fig. 2D).[53–55] Alternatively, phosphorylation of NFAT by DYRKs has also been reported to affect the stability of NFATs, that is, DYRK1A contributes to stabilization and destabilization of NFATc1 and NFATc2, respectively.[56,57]

Functions of DYRKs in mammalian development, revealed by *in vivo* approaches

Accumulating evidence in cellular models demonstrates that DYRKs regulate protein turnover, activation, and cellular localization. DYRKs play indispensable roles in cell differentiation and patterning during tissue development. Although knowledge on DYRKs expression profile in various developmental tissue is limited, data from RNA-seq analysis in adult human tissues are available from public databases. Expression profiles in adult human tissues showed some differences among DYRKs, that is, *DYRK1A* and *DYRK2* tend to be ubiquitously expressed, but expression of *DYRK1B* in the testis and muscle, and *DYRK3* and *DYRK4* in the testis were higher than those in other tissues (Fig. S1).

In comparison to cellular model-based analysis on DYRKs, studies on their roles in mammalian development using *in vivo* models are limited. In this section, we summarize the available literature on DYRKs in vertebrate development, obtained using *in vivo* approaches, with a view to reconfirm their role in mammalian tissue development. We also describe the knockout studies involving the various mice *Dyrk* types, including the latest findings on the *Dyrk2* depletion.

In vivo developmental roles of DYRK1A

DYRK1A is an orthologous gene of *minibrain* (*mnb*) in Drosophila.[58] *Mnb* mutant ($Mnb^{-/-}$ flies) shows a smaller brain due to abnormal arrangement of neuroblasts in the outer proliferation layers of the larval brain and causes specific behavioral abnormalities in learning, memory, visual, and olfactory abilities.[11,58] In mammals, DYRK1A has been most extensively studied *in vivo* among DYRKs, because DYRK1A is located in the human Down syndrome (DS)-critical region (DSCR; chromosome 21q22.2), and its overexpression in trisomy 21 is known to contribute to the neuropathological traits of DS.[21] In addition, a human *DYRK1A*-haploinsufficiency syndrome (*DYRK1A*-Related Intellectual Disability Syndrome), which is characterized by intellectual disability, including impaired speech development, autism spectrum disorder including anxiety and/or stereotypic behavior problems, and microcephaly, has been reported.[59–61] This rare neurodevelopmental disorder is phenocopied by the heterozygous *Dyrk1A* ($Dyrk1A^{+/-}$) mice models.[62,63]

In mice, *Dyrk1A* is highly expressed in the neural tube in E9.5 embryos[64] and transiently expressed in cycling neuronal progenitor cells of the ventricular (VZ) and subventricular zone (SVZ) at E14.5.[65] DYRK1A protein is also expressed in several early postnatal tissues, such as the brain, heart, lung, liver, spleen, and skeletal muscle.[66] A number of studies using genetically modified mice have demonstrated that DYRK1A dosage is a key factor for neurogenesis and neuronal maturation.[21]

Dyrk1A null (*Dyrk1A*$^{-/-}$) mutants are lethal *in utero* at the embryonic stage between E10.5 and E13.5, with only 20% surviving at E13.5 (Table 1). *Dyrk1A*$^{-/-}$ embryos show significant growth delay (with 1/3 to 1/2 size reduction compared to wild type) with hypoplasia of several primitive tissues such as the heart, liver, branchial arches, and brain vesicles.[64] In contrast, *dyrk1a*-deficient (*dyrk1a*$^{-/-}$) zebrafish shows hydrocephalus with small brain size caused by increased apoptosis and impaired social behavior, but develops into adulthood,[67] and kinase-dead mutations exhibit cerebral hemorrhage and defects in angiogenesis of central arteries in the developing hindbrain (Table 1).[68]

Notably, *Dyrk1A*$^{+/-}$ mice, which are a model of *DYRK1A*-Related Intellectual Disability Syndrome, survived but showed decreased neonatal viability (approximately 30% of *Dyrk1A*$^{+/-}$ mice survive), lower body weight (approximately 30% lower), and a reduced number of neurons in some brain regions such as the superior colliculus.[64] *Dyrk1A*$^{+/-}$ mice show behavioral abnormalities such as impairment in spatial learning, delay in the appearance of the righting reflex, Preyer's reflex, eye lid opening, and autistic traits.[62,64] Conversely, transgenic mice overexpressing *Dyrk1A* (*Dyrk1A*-Tg) mice showed normal reproductive performance and litter viability. *Dyrk1A*-Tg mice, however, exhibit retardation in neurodevelopmental delay, and motor and cognitive deficits.[19,69,70] These phenotypes of *Dyrk1A*-Tg mice are similar to those of DS people.[19]

In addition to neurodevelopment, several studies have shown that DYRK1A regulates tissue development (described in the next sections).

In vivo molecular mechanisms: DYRK1A acts as a dosage-sensitive kinase in developmental pathways

Molecular mechanism of DYRK1A's role in neurodevelopment

The function of DYRK1A in neuronal development has been studied *in vivo*, since *DYRK1A* is located in the human DS-critical region. Genetically modified mice studies have revealed that DYRK1A dosage is indispensable for neurogenesis and neuronal maturation *in vivo* (Fig. 3A). DYRK1A regulates neuronal development via dynamic spatiotemporal expression patterns. *Dyrk1A*-expression is transiently upregulated before the transition from proliferating to neurogenic divisions and is maintained in neural progenitor cells at a low level.[71]

As a representative mechanism, DYRK1A regulates proliferation and differentiation via phosphorylation of cyclin D1 and p27^{Kip}, which are the main regulators of the cell cycle in neurogenesis and neuronal maturation (Fig. 3A). DYRK1A directly phosphorylates cyclin D1 at Thr286 for proteasomal degradation in mice and humans.[32] *In utero* electroporation of *Dyrk1A* in the embryonic mouse neocortex inhibits cell proliferation by inducing nuclear export and degradation of cyclin D1. *Dyrk1A*-Tg mouse embryos show an increase in the G1-phase in stem/progenitor cells due to cyclin D1 phosphorylation and degradation to produce a deficit in cortical projection neurons.[34] Supporting these data, DYRK1A regulates the G1-phase of trisomic fibroblast cells through the direct Thr286 phosphorylation of cyclin D1 and induces its nuclear export and degradation.[33] *Dyrk1A*-overexpression induces cell cycle arrest with an increased number of cells expressing p27^{Kip1} via phosphorylation at Ser10, resulting in protein stabilization. Conversely, inhibition of *Dyrk1A* induces proliferation through a decrease in p27^{Kip1}.[32,71]

Other DYRK1A downstream molecules that contribute to neurogenesis include NOTCH, REST1, STAT, and WNT. DYRK1A directly phosphorylates and suppresses NOTCH1 in a kinase activity dependent manner.[72] NOTCH is a single-pass transmembrane receptor activated by Delta and Serrate ligand families. This activation leads to the proteolytic cleavage and release of the NOTCH intracellular domain (NICD),[73,74] which in turn acts as a transcriptional regulator in the nucleus. NOTCH signaling is essential for maintaining the proliferation of neural progenitor cells, and tightly regulates the decision of neural progenitors to stop or commit to differentiation.[75] Fernandez-Martinez et al have shown that *Dyrk1A* is co-expressed with components of the NOTCH signaling pathway (*Notch1*, *Dll1*, and a target gene, *Hes1*) in the ventricular zone of the developing cortex.[72] Moreover, DYRK1A physically interacts with the NICD, inducing the ankyrin domain, phosphorylating multiple sites in the NICD, and reducing its transcriptional activity in cultured cells and *in vivo*.

Dosage imbalance in DYRK1A contributes to the reduction in RE1 silencing transcription factor (REST), which is a neuron-restrictive silencer factor that suppresses the transcription of its target genes.[76] Although the phosphorylation sites have not yet been identified, DYRK1A may phosphorylate the C terminus of REST and induce degradation, resulting in reduced REST expression.[77] Kurabayashi et al have shown that overexpression of DYRK1A promotes the activity of the astrogliogenic transcription factor STAT in neuronal progenitors, resulting in aberrant astrogliogenesis in DS.[78] Overexpression of DYRK1A in DS negatively regulates hippocampal WNT/β-catenin signaling.[79] These insights into the molecular mechanisms of DYRK1A in neurogenesis lead to an understanding of the pathogenesis of DS and Alzheimer's disease.

Molecular mechanism of DYRK1A's role in pancreatic β cell development

Rachdi et al have reported that DYRK1A regulates pancreatic β-cell development (Fig. 3B).[80] In the pancreas, *Dyrk1A* is highly expressed during development and is enriched in adult islets.[81] *Dyrk1A*$^{+/-}$ mice show reduced β-cell mass by reduction of β-cell proliferation, resulting in defective insulin secretion and impaired glucose tolerance.[81] Conversely, transgenic mice overexpressing *Dyrk1A* (*Dyrk1A*-Tg mice) show expansion of β-cell mass through increased proliferation and cell size and improved glucose tolerance.[80] Mechanistically, accumulation of p27^{Kip1} is observed in the pancreas of *Dyrk1A*$^{+/-}$ mice. Since the expression of p27^{Kip1} in β-cells is regulated by the transcription factor forkhead box O1 (FOXO1), which is a substrate for DYRK1A, β-cell mass and proliferation may be controlled via a DYRK1A-FOXO1-p27 pathway (Fig. 3B). However, an inhibitor of DYRK1A, harmine, induces β-cell proliferation, increases islet mass, and improves glycemic control in human pancreatic β-cells.[82] Further analysis is required to reach a conclusion on the function of DYRK1A in the pancreas.

Table 1 Phenotypes and associated diseases of *Dyrks* knockout animals.

Knockout animal	Defects in knockout animal	Associated diseases	Ref.
DYRK1A	Lethal at E10.5 to 13.5		19,59–61,64,65,69,70
 mouse	Smaller body and hypoplasia of several primitive tissues such as heart, liver, branchial arches, and brain vesicles		
	Dosage-dependent	Down syndrome	
 zebrafish	Cerebral hemorrhage and defects in angiogenesis of central arteries in the developing hindbrain	DYRK1A-Related Intellectual Disability Syndrome	67,68
	Hydrocephalus with small brain size and impaired social behavior		
DYRK1B  mouse	No obvious developmental defects (Detailed phenotype is not available)		89,90,92
	Lethal before 72 hpf	Abdominal obesity-metabolic syndrome 3	98
 zebrafish	Shortened and curved body axis, visible loss of somites, and cyclopic eyes		
	Loss of paraxial *myoD*-expression		
DYRK2  mouse	Lethal at or close to birth	Skeletal ciliopathies	109,110
	Smaller body and hypoplasia of multi organs with especially skeletal abnormality		
	Suppression of Hedgehog signaling via abnormal ciliogenesis		
	Dosage-independent		
DYRK3  mouse	No obvious developmental defects		124,126,128
	Increase numbers of red blood cells under conditions of anemia (postnatal)		
DYRK4  mouse	No obvious developmental defects		125

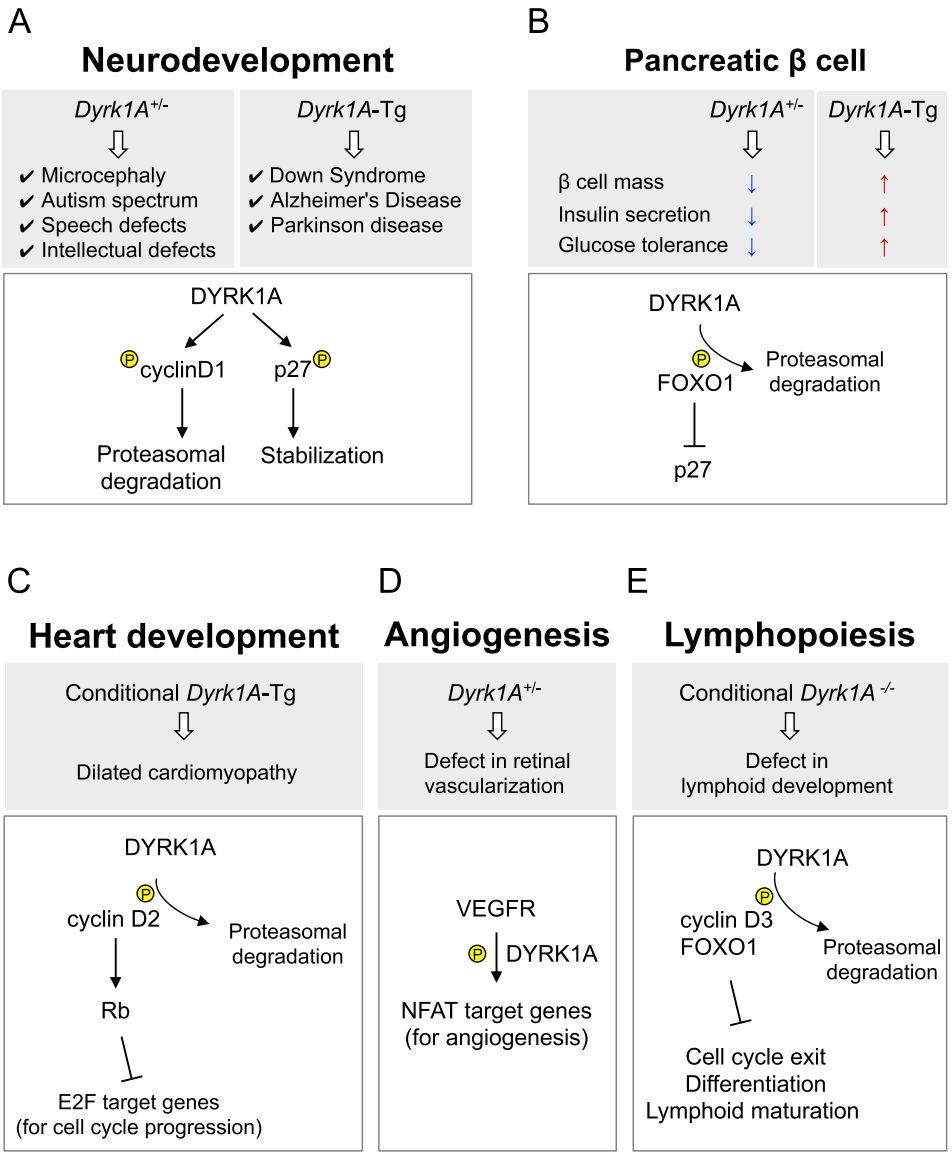

Figure 3 *In vivo* phenotypes and representative molecular mechanism of DYRK1A during development by genetically modified mice. The phenotypes of haploinsufficiency (*Dyrk1A*$^{+/-}$ mice), conditional knockout mice (*Dyrk1A*$^{-/-}$), and/or transgenic mice overexpressing *Dyrk1A* (*Dyrk1A*-Tg) are shown in each upper panel with its representative molecular mechanism in the lower panel; **(A)** In neurodevelopment, DYRK1A phosphorylates cyclin D1 and p27^{Kip1} for proteasomal degradation and stabilization, respectively; **(B)** In pancreatic β-cells, DYRK1A phosphorylates and degrades FOXO1, which is a negative regulator of a cyclin-dependent kinase inhibitor, p27^{Kip1}; **(C)** In heart development, DYRK1A phosphorylates and degrades cyclin D2, which is a positive regulator of Rb (retinoblastoma); **(D)** In angiogenesis, DYRK1A positively regulates VEGF-induced NFAT target genes in a kinase activity-dependent manner; **(E)** In lymphopoiesis, DYRK1A phosphorylates and degrades cyclin D3 and FOXO1, which are negative regulators of B cell development.

Molecular mechanism of DYRK1A's role in heart development

Dyrk1A, along with dysregulation of NFAT signaling, is involved in heart development (Fig. 3C). Knockdown of *Dyrk1A* by siRNA sensitized agonist-stimulated cardiomyocyte hypertrophy.[83] *Dyrk1A*$^{+/-}$ mice with calcineurin overexpression show an exaggerated pathological cardiac growth response.[84] Moreover, in this process, *Dyrk1A* was downregulated by miR-199b.[84] Later, Hille et al reported a study using transgenic mice with cardiac-specific overexpression of *DYRK1A* under the control of the cardiac-specific αMHC promoter.[85] Although newborn *Dyrk1A*-Tg mice do not show obvious phenotypes, *Dyrk1A*-Tg mice are generally smaller than their wild-type littermates until they invariably die at a young age with marked dilation of the atria and ventricles. DYRK1A interacts with all members of the cyclin D family and represses their protein levels in cellular models and *in vivo* (Fig. 3C). In particular, DYRK1A phosphorylates cyclin D2 at Thr280 and promotes its proteasomal degradation, and consequently

impairs the formation of the phospho-active cyclin D-Cdk4/6 complex. This phospho-active cyclin D-Cdk4/6 complex induces hypo-phosphorylated Rb1, resulting in suppression of Rb/E2f-signaling and expression of E2f-target genes for cell cycle progression (Fig. 3C).[85] This negative regulation of cyclin D-mediated Rb/E2f-signalling via DYRK1A-overexpression impairs cardiomyocyte proliferation, leading to cardiomyopathy.

Molecular mechanism of DYRK1A's role in angiogenesis

Recently, the importance of DYRK1A in angiogenesis has been revealed.[86] Vascular endothelial growth factor (VEGF) signaling in endothelial cells is an important regulator of angiogenesis. In VEGF signaling, NFATs are key downstream effectors. Rozen et al showed that DYRK1A positively regulates VEGF-induced NFAT target genes in primary endothelial cells in a kinase activity-dependent manner (Fig. 3D).[86] In fact, the loss of DYRK1A impairs VEGF-stimulated Ca^{2+} mobilization in human umbilical vein endothelial cells. In this process, DYRK1A induces VEGFR2 accumulation and stabilization in response to VEGF. Moreover, in postnatal retinal development, *Dyrk1A*$^{+/-}$ mice show defects in retinal vascularization via fine-tuning of endothelial cell proliferation and angiogenesis (Fig. 3D).[86] Although the possibility of a combination of endothelial and non-endothelial effects cannot be ruled out, these data support a role for DYRK1A in physiological angiogenesis.

Molecular mechanism of DYRK1A's role in lymphopoiesis

DYRK1A is required for normal lymphopoiesis. *Dyrk1A* conditional knockout in hematopoietic cells of mice showed that B and T cell precursors failed to enter a quiescent state and a cell-autonomous defect emerged in lymphoid development (Fig. 3E).[35] During lymphopoiesis, DYRK1A phosphorylates cyclin D3 (at Thr283) for its degradation, thereby promoting cell cycle exit and subsequent lymphoid maturation (Fig. 3E). More recently, the same group showed that DYRK1A is required for the growth of B cell acute lymphoblastic leukemia cells in a kinase-dependent manner using *Dyrk1A* conditional knockout mice and a small-molecule inhibitor, EHT 1610.[87] Mechanistically, DYRK1A phosphorylates FOXO1 and STAT3, which are indispensable transcription factors for B cell development in B lymphopoiesis via the regulation of DNA damage and ROS, respectively (Fig. 3E).[87] These data suggest that DYRK1A is a candidate therapeutic target for B cell acute lymphoblastic leukemia. Other groups showed that DYRK1A regulates the balance between Th17 and T regulatory (Treg) cells, which critically modulates immune homeostasis.[88] Inhibition of DYRK1A by harmine induces differentiation into Tregs and suppresses differentiation into Th17 without affecting the pathways of Treg/Th17 differentiation.[88] The molecular mechanisms by which DYRK1A regulates Th differentiation remain to be elucidated.

In vivo developmental roles of DYRK1B

In humans, *DYRK1B* mutations are related to abdominal obesity and metabolic syndrome 3 (AOMS3), which is characterized by abdominal obesity, type 2 diabetes, hypertension, and early onset coronary artery disease.[89,90] Mutations H90P and R102C affect the structural element DYRK homology box and interfere with the maturation of DYRK1B by tyrosine auto-phosphorylation, thereby compromising the conformational stability of the catalytic domain and resulting in loss-of-function.

In normal human tissues, *DYRK1B* mRNA is highly expressed in the skeletal muscle and testes (Fig. S1).[91] Since higher expression of DYRK1B is observed in the muscle cells and testes during normal development,[92] DYRK1B is assumed to play key roles in growth arrest in a quiescent state, differentiation, and survival during myogenesis[93–95] and spermatogenesis.[96]

In myogenesis, DYRK1B is strongly expressed in differentiated skeletal muscle but does not dividing myoblasts, indicating a physiological role in muscle development and function. In a study using cellular models, expression of DYRK1B was highly increased in non-dividing cells such as serum-starved fibroblasts and myoblasts that exit the cell cycle to undergo terminal differentiation. Moreover, DYRK1B overexpression promotes differentiation of myoblasts into skeletal muscle cells (terminal differentiation), whereas knockdown of *Dyrk1B* by siRNA suppresses differentiation.[94] Notably, DYRK1B is also involved in skeletal muscle regeneration after injury in adult tissue.[94] DYRK1B is expressed at low levels in satellite cells (stem cells); however, it is upregulated when satellite cells are activated and re-enter the cell cycle.[94]

In spermatogenesis, DYRK1B regulates proliferation and differentiation by phosphorylating $p27^{Kip1}$ for stabilization and cyclin D1 for degradation, and its kinase activity is negatively regulated by interaction with cold-inducible RNA-binding protein.[96]

Unexpectedly, *Dyrk1B* knockout (*Dyrk1b*$^{-/-}$) mice show no obvious developmental defects and survive several weeks after birth (Table 1).[97] Detailed phenotypes of *Dyrk1b*$^{-/-}$ mice are not available. The International Mouse Phenotyping Consortium (https://www.mousephenotype.org/) provides information that *Dyrk1B*$^{-/-}$ mice show preweaning lethality, and *Dyrk1B*$^{+/-}$ adult mice show decreased body weight, retinal thickness, bone mineral density, lymphocyte cell number, and abnormal auditory brainstem response. To reach a conclusion on the function of DYRK1B in mammalian development, further analysis is needed.

More recently, a study of CRISPR/Cas9-mediated *dyrk1b* knockout zebrafish using two different guide RNAs was conducted (Table 1).[98] Homozygous knockout (*dyrk1b*$^{-/-}$) zebrafish exhibit embryonic lethality and do not survive beyond 72 hpf (hours post fertilization) with shortened and curved body axis, visible loss of somites, and cyclopic eye. *Dyrk1b* is expressed in the somitic mesoderm, which is the precursor of skeletal muscles cells, and *dyrk1b*$^{-/-}$ zebrafish showed loss of paraxial *myoD*-expression, which is a key basic helix-loop-helix transcription factor for myogenic differentiation. In addition, knockdown analysis shows that *dyrk1b* may be involved in endoderm formation and craniofacial patterning via the induction of endodermal markers *cas* and *sox17* expression in zebrafish.[99] These data suggest that DYRK1B is indispensable for survival and embryogenesis, at least in zebrafish.

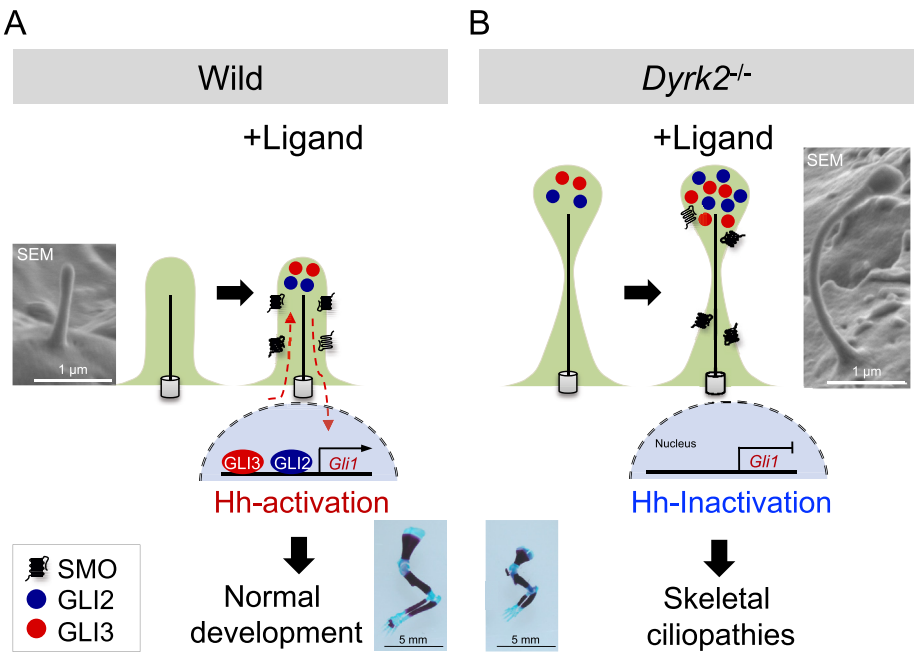

Figure 4 DYRK2 in ciliogenesis and Hedgehog (Hh) signaling. A schematic model of ciliogenesis and response to stimulation with Hh ligand in wild-type **(A)**; and *Dyrk2*$^{-/-}$ mice **(B)**; *Dyrk2*$^{-/-}$ embryo shows Hh-inactivation and skeletal abnormalities via abnormal ciliary morphology (showing by scanning electron microscopy; SEM) and abnormal ciliary trafficking of Hh pathway components (GLI2 and GLI3).

In vivo developmental roles of DYRK2

In *C. elegans*, *Mbk2* has been identified as an ortholog gene of DYRK2. Null *Mbk2* mutants in *C. elegans* are lethal due to various defects in cytokinesis during early embryonic development.[100] Phosphorylation by MBK2 initiates the degradation of MEI1 via a ubiquitin-dependent mechanism involving the CUL3/MEL26-E3 ligase ubiquitin complex.[12–14] *In vivo* studies clearly show that MBK2 plays a critical role in the regulation of maternal protein degradation during the oocyte-to-embryo transition.[101] In zebrafish, morpholino-mediated knockdown of *dyrk2* reduces the expression levels of *myoD* in muscle progenitor cells.[102] Double knockdown of *dyrk2* with *cdk5* causes abnormal positioning of Rohon-Beard neurons[103] and caudal primary neurons[104] in the dorsal part of the spinal cord. In mammals, DYRK2 functions as a tumor suppressor in breast,[39,105] liver,[106] and colon[107,108] by regulating cell survival, proliferation, and epithelial-mesenchymal transition/migration via p53 (Fig. 2B),[42] c-Jun/c-Myc (Fig. 2C),[39] and SNAIL[46] as described above. Although evidence indicates that DYRK2 plays important roles in the development of invertebrates and tumor suppression, little is known about the functions of DYRK2 in mammalian development because no study with *Dyrk2* knockout mice has been carried out.

Recently, our group revealed for the first time the function of DYRK2 in mammalian development by analyzing knockout mice (Fig. 4 and Table 1).[109,110] Although heterozygous *Dyrk2* knockout showed no obvious defects, homozygous *Dyrk2* knockout (*Dyrk2*$^{-/-}$) mice died at or close to birth due to respiratory disorders[110] with a smaller body and hypoplasia of multiple organs. In particular, skeletal abnormalities were remarkable, including the limbs, sternebrae, vertebra, cleft palate, tracheal cartilage, and cranial base.[109] These phenotype defects exhibited by *Dyrk2*$^{-/-}$ mice are similar to those seen in Hedgehog (Hh) signaling.[111] Indeed, *Dyrk2* deficiency causes suppression of *Gli1*-expression, which is a transcription factor and indicator of Hh signal activation,[112] in the embryo tissues. Supporting this *in vivo* evidence, primary mouse embryonic fibroblasts derived from *Dyrk2*$^{-/-}$ mice or transient knockdown of *Dyrk2* by siRNA showed that DYRK2 is essential for responding to the Hh ligand in a kinase activity-dependent manner.[109] Primary cilia are essential organelles required for signal transduction in vertebrate Hh signaling.[113] Notably, deletion of *Dyrk2* induced abnormal ciliary morphology, that is, cilia were significantly elongated, bulged at the tips, and twisted (Fig. 4).[109] During Hh activation, key regulators of Hh signaling (such as seven-spanner Smoothened (SMO), GLI2, and GLI3) are recruited and activated at the cilia via intraflagellar transport.[114–117] Disorders in the ciliary trafficking of Hh components cause Hh signaling dysfunction.[118] Deletion of *Dyrk2* induces abnormal ciliary trafficking of Hh pathway components, GLI2 and GLI3, but not SMO (Fig. 4).[109] While this study did not include any evidence to identify a potential substrate for phosphorylation of DYRK2, the *in vivo* evidence from *Dyrk2*$^{-/-}$ mice shows that DYRK2 is a novel regulator of ciliogenesis and is required for normal embryogenesis via activation of Hh signaling during development. Cilia are known to be indispensable for tissue development, and their defects cause diseases termed "ciliopathies" in humans and mice.[119] Although regulators of ciliogenesis have been identified using genetic screening in

Chlamydomonas (a model organism for ciliogenesis),[120] this study shows for the first time that *DYRK2* is a ciliogenesis-related gene. Additionally, DYRK2 is a ciliary protein that is primarily localized at the basal body and the transition zone,[109] which contains a growing number of ciliopathy proteins.[121] These findings using *Dyrk2*$^{-/-}$ mice suggest the possibility that *DYRK2* is involved in human skeletal ciliopathies, such as short-rib thoracic dysplasia, short-rib polydactyly syndrome, and Jeune asphyxiating thoracic dystrophy (Table 1).[122,123]

In vivo developmental roles of DYRK3 and DYRK4

As described above, *DYRK3* and *DYRK4* in human and mice are expressed at higher levels in the testes than in other tissues (Fig. S1).[124,125] Among the *DYRK* family genes, however, literature on *DYRK3* and *DYRK4* involvement in mammalian development is limited.

Dyrk3 is strongly expressed in hematopoietic cells, especially erythroid progenitor cells and the testis,[126–128] and is assumed to be involved in erythropoiesis and spermatogenesis. Surprisingly, *Dyrk3*$^{-/-}$ mice survived and showed no obvious developmental defects and essentially had normal hematological phenotype (Table 1).[124] An increase in the number of red blood cells under conditions of anemia in postnatal *Dyrk3*$^{-/-}$ mice indicated that DYRK3 functions as a negative regulator of erythropoiesis.[124]

Dyrk4 mRNA is also expressed at a higher level in the testis.[125] Notably, DYRK4 expression is restricted to stage VIII post-meiotic spermatids during spermatogenesis. *Dyrk4* knockout mice show, however, no aberrant sperm phenotype or defects in male fertility (Table 1).[125] Using *in situ* hybridization, Sacher et al showed that *Dyrk1B*, *Dyrk2*, and *Dyrk4* are expressed in stage VIII post-meiotic spermatids.[125] One explanation might be that other molecules such as *Dyrk1B* and *Dyrk2* functionally compensate for the lack of *Dyrk4* during spermatogenesis. This possibility remains to be analyzed further.

Conclusion

Identification of spatio-temporal proteins expression patterns with evidence from knockout animals are important in understanding tissue development and congenital diseases. This review highlights the fact that among the DYRKs, DYRK1A is the best characterized and has been identified to have important roles in DS and *DYRK1A*-haploinsufficiency syndrome. *Dyrk2* knockout mice provide novel insights into the dosage-independent relationship between ciliogenesis and the congenital disease called ciliopathies. These *in vivo* studies help to understand and develop novel clinical therapies and drugs for human congenital diseases such as DS (*DYRK1A*-associated) and skeletal ciliopathies (*DYRK2*-associated). Absence of developmental defects in some knockout mice (*Dyrk1B*, *Dyrk3*, and *Dyrk4*), might suggest a functional redundancy among DYRKs. Further *in vivo* studies and tissue-specific depletion via a Cre-loxP recombination system will provide novel insights into the functions of DYRKs in several diseases, including cancer progression, as well as in maintaining post-natal tissue homeostasis.

Author contributions

Saishu Yoshida: Conceptualization, Funding acquisition, Writing-original draft, Writing-review and editing.
Kiyotsugu Yoshida: Conceptualization, Funding acquisition, Writing-review and editing.

Conflict of interests

Authors declare no conflict of interests.

Funding

This work was partially supported by JSPS KAKENHI (No. 21K06192 to S.Y. and 17H03584, 18K19484, and 20H03519 to K.Y.), the Jikei University Research Fund (to K.Y.), the Takeda Science Foundation (to S.Y.), and the Uehara Memorial Foundation (to S.Y. and K. Y.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2021.12.004.

References

1. Manning G, Whyte DB, Martinez R, et al. The protein kinase complement of the human genome. *Science*. 2002;298(5600):1912–1934.
2. Aranda S, Laguna A, de la Luna S. DYRK family of protein kinases: evolutionary relationships, biochemical properties, and functional roles. *Faseb J*. 2011;25(2):449–462.
3. Becker W, Weber Y, Wetzel K, Eirmbter K, et al. Sequence characteristics, subcellular localization, and substrate specificity of DYRK-related kinases, a novel family of dual specificity protein kinases. *J Biol Chem*. 1998;273(40):25893–25902.
4. Soppa U, Becker W. DYRK protein kinases. *Curr Biol*. 2015;25(12):R488–R489.
5. Soundararajan M, Roos AK, Savitsky P, et al. Structures of Down syndrome kinases, DYRKs, reveal mechanisms of kinase activation and substrate recognition. *Structure*. 2013;21(6):986–996.
6. Lochhead PA, Sibbet G, Morrice N, et al. Activation-loop autophosphorylation is mediated by a novel transitional intermediate form of DYRKs. *Cell*. 2005;121(6):925–936.
7. Alvarez M, Altafaj X, Aranda S, et al. DYRK1A autophosphorylation on serine residue 520 modulates its kinase activity via 14-3-3 binding. *Mol Biol Cell*. 2007;18(4):1167–1178.
8. Campbell LE, Proud CG. Differing substrate specificities of members of the DYRK family of arginine-directed protein kinases. *FEBS Lett*. 2002;510(1–2):31–36.
9. Himpel S, Tegge W, Frank R, et al. Specificity determinants of substrate recognition by the protein kinase DYRK1A. *J Biol Chem*. 2000;275(4):2431–2438.
10. Luebbering N, Charlton-Perkins M, Kumar JP, et al. Drosophila Dyrk2 plays a role in the development of the visual system. *PLoS One*. 2013;8(10):e76775.
11. Tejedor F, Zhu XR, Kaltenbach E, et al. minibrain: a new protein kinase family involved in postembryonic neurogenesis in Drosophila. *Neuron*. 1995;14(2):287–301.
12. Lu C, Mains PE. The C. elegans anaphase promoting complex and MBK-2/DYRK kinase act redundantly with CUL-3/MEL-26

ubiquitin ligase to degrade MEI-1 microtubule-severing activity after meiosis. *Dev Biol*. 2007;302(2):438—447.
13. Pang KM, Ishidate T, Nakamura K, et al. The minibrain kinase homolog, mbk-2, is required for spindle positioning and asymmetric cell division in early C. elegans embryos. *Dev Biol*. 2004;265(1):127—139.
14. Pellettieri J, Reinke V, Kim SK, et al. Coordinate activation of maternal protein degradation during the egg-to-embryo transition in C. elegans. *Dev Cell*. 2003;5(3):451—462.
15. Shirayama M, Soto MC, Ishidate T, et al. The conserved kinases CDK-1, GSK-3, KIN-19, and MBK-2 promote OMA-1 destruction to regulate the oocyte-to-embryo transition in C. elegans. *Curr Biol*. 2006;16(1):47—55.
16. Nishi Y, Lin R. DYRK2 and GSK-3 phosphorylate and promote the timely degradation of OMA-1, a key regulator of the oocyte-to-embryo transition in C. elegans. *Dev Biol*. 2005;288(1):139—149.
17. Stitzel ML, Pellettieri J, Seydoux G. The C. elegans DYRK kinase MBK-2 marks oocyte proteins for degradation in response to meiotic maturation. *Curr Biol*. 2006;16(1):56—62.
18. Nishi Y, Rogers E, Robertson SM, et al. Polo kinases regulate C. elegans embryonic polarity via binding to DYRK2-primed MEX-5 and MEX-6. *Development*. 2008;135(4):687—697.
19. Ahn KJ, Jeong HK, Choi HS, et al. DYRK1A BAC transgenic mice show altered synaptic plasticity with learning and memory defects. *Neurobiol Dis*. 2006;22(3):463—472.
20. Mack HID, Zhang P, Fonslow BR, et al. The protein kinase MBK-1 contributes to lifespan extension in daf-2 mutant and germline-deficient Caenorhabditis elegans. *Aging*. 2017;9(5):1414—1432.
21. Arbones ML, Thomazeau A, Nakano-Kobayashi A, et al. DYRK1A and cognition: a lifelong relationship. *Pharmacol Ther*. 2019;194:199—221.
22. Boni J, Rubio-Perez C, López-Bigas N, et al. The DYRK family of kinases in cancer: molecular functions and therapeutic opportunities. *Cancers*. 2020;12(8):2106.
23. Correa-Sáez A, Jiménez-Izquierdo R, Garrido-Rodríguez M, Calzado MA, et al. Updating dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2): molecular basis, functions and role in diseases. *Cell Mol Life Sci*. 2020;77(23):4747—4763.
24. Laham AJ, Saber-Ayad M, El-Awady R. DYRK1A: a down syndrome-related dual protein kinase with a versatile role in tumorigenesis. *Cell Mol Life Sci*. 2021;78(2):603—619.
25. Zou Y, Ewton DZ, Deng X, et al. Mirk/dyrk1B kinase destabilizes cyclin D1 by phosphorylation at threonine 288. *J Biol Chem*. 2004;279(26):27790—27798.
26. Deng X, Mercer SE, Shah S, et al. The cyclin-dependent kinase inhibitor p27Kip1 is stabilized in G(0) by Mirk/dyrk1B kinase. *J Biol Chem*. 2004;279(21):22498—22504.
27. Takahashi-Yanaga F, Mori J, Matsuzaki E, et al. Involvement of GSK-3beta and DYRK1B in differentiation-inducing factor-3-induced phosphorylation of cyclin D1 in HeLa cells. *J Biol Chem*. 2006;281(50):38489—38497.
28. Ashford AL, Oxley D, Kettle J, et al. A novel DYRK1B inhibitor AZ191 demonstrates that DYRK1B acts independently of GSK3β to phosphorylate cyclin D1 at Thr(286), not Thr(288). *Biochem J*. 2014;457(1):43—56.
29. Goel S, DeCristo MJ, McAllister SS, et al. CDK4/6 inhibition in cancer: beyond cell cycle arrest. *Trends Cell Biol*. 2018;28(11):911—925.
30. Polyak K, Lee MH, Erdjument-Bromage H, et al. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell*. 1994;78(1):59—66.
31. Abbastabar M, Kheyrollah M, Azizian K, et al. Multiple functions of p27 in cell cycle, apoptosis, epigenetic modification and transcriptional regulation for the control of cell growth: a double-edged sword protein. *DNA Repair*. 2018;69:63—72.
32. Soppa U, Schumacher J, Florencio Ortiz V, et al. The Down syndrome-related protein kinase DYRK1A phosphorylates p27(Kip1) and Cyclin D1 and induces cell cycle exit and neuronal differentiation. *Cell Cycle*. 2014;13(13):2084—2100.
33. Chen JY, Lin JR, Tsai FC, et al. Dosage of Dyrk1a shifts cells within a p21-cyclin D1 signaling map to control the decision to enter the cell cycle. *Mol Cell*. 2013;52(1):87—100.
34. Najas S, Arranz J, Lochhead PA, et al. DYRK1A-mediated cyclin D1 degradation in neural stem cells contributes to the neurogenic cortical defects in Down syndrome. *EBioMedicine*. 2015;2(2):120—134.
35. Thompson BJ, Bhansali R, Diebold L, et al. DYRK1A controls the transition from proliferation to quiescence during lymphoid development by destabilizing Cyclin D3. *J Exp Med*. 2015;212(6):953—970.
36. Lara-Chica M, Correa-Sáez A, Jiménez-Izquierdo R, et al. A novel CDC25A/DYRK2 regulatory switch modulates cell cycle and survival. *Cell Death Differ*. 2022;29(1):105—117.
37. Guo X, Wang X, Wang Z, et al. Site-specific proteasome phosphorylation controls cell proliferation and tumorigenesis. *Nat Cell Biol*. 2016;18(2):202—212.
38. Maddika S, Chen J. Protein kinase DYRK2 is a scaffold that facilitates assembly of an E3 ligase. *Nat Cell Biol*. 2009;11(4):409—419.
39. Taira N, Mimoto R, Kurata M, et al. DYRK2 priming phosphorylation of c-Jun and c-Myc modulates cell cycle progression in human cancer cells. *J Clin Invest*. 2012;122(3):859—872.
40. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell*. 1997;88(3):323—331.
41. Taira N, Yoshida K. Post-translational modifications of p53 tumor suppressor: determinants of its functional targets. *Histol Histopathol*. 2012;27(4):437—443.
42. Taira N, Nihira K, Yamaguchi T, et al. DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage. *Mol Cell*. 2007;25(5):725—738.
43. Bishop NA, Guarente L. Genetic links between diet and lifespan: shared mechanisms from yeast to humans. *Nat Rev Genet*. 2007;8(11):835—844.
44. Guo X, Williams JG, Schug TT, et al. DYRK1A and DYRK3 promote cell survival through phosphorylation and activation of SIRT1. *J Biol Chem*. 2010;285(17):13223—13232.
45. Becker W. Emerging role of DYRK family protein kinases as regulators of protein stability in cell cycle control. *Cell Cycle*. 2012;11(18):3389—3394.
46. Mimoto R, Taira N, Takahashi H, et al. DYRK2 controls the epithelial-mesenchymal transition in breast cancer by degrading Snail. *Cancer Lett*. 2013;339(2):214—225.
47. Cole AR, Knebel A, Morrice NA, et al. GSK-3 phosphorylation of the Alzheimer epitope within collapsin response mediator proteins regulates axon elongation in primary neurons. *J Biol Chem*. 2004;279(48):50176—50180.
48. Woods YL, Cohen P, Becker W, et al. The kinase DYRK phosphorylates protein-synthesis initiation factor eIF2Bepsilon at Ser539 and the microtubule-associated protein tau at Thr212: potential role for DYRK as a glycogen synthase kinase 3-priming kinase. *Biochem J*. 2001;355(Pt 3):609—615.
49. Kurabayashi N, Hirota T, Sakai M, et al. DYRK1A and glycogen synthase kinase 3beta, a dual-kinase mechanism directing proteasomal degradation of CRY2 for circadian timekeeping. *Mol Cell Biol*. 2010;30(7):1757—1768.
50. Crabtree GR, Olson EN. NFAT signaling: choreographing the social lives of cells. *Cell*. 2002;109(Suppl):S67—S79.
51. Arron JR, Winslow MM, Polleri A, et al. NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. *Nature*. 2006;441(7093):595—600.

52. Gwack Y, Sharma S, Nardone J, et al. A genome-wide Drosophila RNAi screen identifies DYRK-family kinases as regulators of NFAT. *Nature*. 2006;441(7093):646—650.
53. Beals CR, Sheridan CM, Turck CW, et al. Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. *Science*. 1997; 275(5308):1930—1934.
54. Zhu J, Shibasaki F, Price R, et al. Intramolecular masking of nuclear import signal on NF-AT4 by casein kinase I and MEKK1. *Cell*. 1998;93(5):851—861.
55. Okamura H, Garcia-Rodriguez C, Martinson H, et al. A conserved docking motif for CK1 binding controls the nuclear localization of NFAT1. *Mol Cell Biol*. 2004;24(10): 4184—4195.
56. Liu H, Wang K, Chen S, et al. NFATc1 phosphorylation by DYRK1A increases its protein stability. *PLoS One*. 2017;12(2): e0172985.
57. Liu H, Sun Q, Chen S, et al. DYRK1A activates NFATC1 to increase glioblastoma migration. *Cancer Med*. 2021;10(18): 6416—6427.
58. Guimerá J, Casas C, Pucharcòs C, et al. A human homologue of Drosophila minibrain (MNB) is expressed in the neuronal regions affected in Down syndrome and maps to the critical region. *Hum Mol Genet*. 1996;5(9):1305—1310.
59. van Bon BW, Coe BP, Bernier R, et al. Disruptive de novo mutations of DYRK1A lead to a syndromic form of autism and ID. *Mol Psychiatr*. 2016;21(1):126—132.
60. Earl RK, Turner TN, Mefford HC, et al. Clinical phenotype of ASD-associated DYRK1A haploinsufficiency. *Mol Autism*. 2017; 8:54.
61. Courraud J, Chater-Diehl E, Durand B, et al. Integrative approach to interpret DYRK1A variants, leading to a frequent neurodevelopmental disorder. *Genet Med*. 2021;23(11): 2150—2159.
62. Arranz J, Balducci E, Arató K, et al. Impaired development of neocortical circuits contributes to the neurological alterations in DYRK1A haploinsufficiency syndrome. *Neurobiol Dis*. 2019;127:210—222.
63. Dang T, Duan WY, Yu B, et al. Autism-associated Dyrk1a truncation mutants impair neuronal dendritic and spine growth and interfere with postnatal cortical development. *Mol Psychiatr*. 2018;23(3):747—758.
64. Fotaki V, Dierssen M, Alcántara S, et al. Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice. *Mol Cell Biol*. 2002;22(18):6636—6647.
65. Hämmerle B, Elizalde C, Tejedor FJ. The spatio-temporal and subcellular expression of the candidate Down syndrome gene Mnb/Dyrk1A in the developing mouse brain suggests distinct sequential roles in neuronal development. *Eur J Neurosci*. 2008;27(5):1061—1074.
66. Okui M, Ide T, Morita K, et al. High-level expression of the Mnb/Dyrk1A gene in brain and heart during rat early development. *Genomics*. 1999;62(2):165—171.
67. Kim OH, Cho HJ, Han E, et al. Zebrafish knockout of Down syndrome gene, DYRK1A, shows social impairments relevant to autism. *Mol Autism*. 2017;8:50.
68. Cho HJ, Lee JG, Kim JH, et al. Vascular defects of DYRK1A knockouts are ameliorated by modulating calcium signaling in zebrafish. *Dis Model Mech*. 2019;12(5):dmm037044.
69. Smith DJ, Stevens ME, Sudanagunta SP, et al. Functional screening of 2 Mb of human chromosome 21q22.2 in transgenic mice implicates minibrain in learning defects associated with Down syndrome. *Nat Genet*. 1997;16(1):28—36.
70. Altafaj X, Dierssen M, Baamonde C, et al. Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down's syndrome. *Hum Mol Genet*. 2001; 10(18):1915—1923.
71. Hämmerle B, Vera-Samper E, Speicher S, et al. Mnb/Dyrk1A is transiently expressed and asymmetrically segregated in neural progenitor cells at the transition to neurogenic divisions. *Dev Biol*. 2002;246(2):259—273.
72. Fernandez-Martinez J, Vela EM, Tora-Ponsioen M, et al. Attenuation of notch signalling by the down-syndrome-associated kinase DYRK1A. *J Cell Sci*. 2009;122(Pt 10): 1574—1583.
73. Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature*. 1998;393(6683):382—386.
74. Struhl G, Adachi A. Nuclear access and action of notch in vivo. *Cell*. 1998;93(4):649—660.
75. Moore R, Alexandre P. Delta-Notch signaling: the long and the short of a neuron's influence on progenitor fates. *J Dev Biol*. 2020;8(2):8.
76. Canzonetta C, Mulligan C, Deutsch S, et al. DYRK1A-dosage imbalance perturbs NRSF/REST levels, deregulating pluripotency and embryonic stem cell fate in Down syndrome. *Am J Hum Genet*. 2008;83(3):388—400.
77. Lu M, Zheng L, Han B, et al. REST regulates DYRK1A transcription in a negative feedback loop. *J Biol Chem*. 2011; 286(12):10755—10763.
78. Kurabayashi N, Nguyen MD, Sanada K. DYRK1A overexpression enhances STAT activity and astrogliogenesis in a Down syndrome mouse model. *EMBO Rep*. 2015;16(11):1548—1562.
79. Granno S, Nixon-Abell J, Berwick DC, et al. Downregulated Wnt/β-catenin signalling in the down syndrome hippocampus. *Sci Rep*. 2019;9(1):7322.
80. Rachdi L, Kariyawasam D, Aïello V, et al. Dyrk1A induces pancreatic β cell mass expansion and improves glucose tolerance. *Cell Cycle*. 2014;13(14):2221—2229.
81. Rachdi L, Kariyawasam D, Guez F, et al. Dyrk1a haploinsufficiency induces diabetes in mice through decreased pancreatic beta cell mass. *Diabetologia*. 2014;57(5):960—969.
82. Wang P, Alvarez-Perez JC, Felsenfeld DP, et al. A high-throughput chemical screen reveals that harmine-mediated inhibition of DYRK1A increases human pancreatic beta cell replication. *Nat Med*. 2015;21(4):383—388.
83. Kuhn C, Frank D, Will R, et al. DYRK1A is a novel negative regulator of cardiomyocyte hypertrophy. *J Biol Chem*. 2009; 284(25):17320—17327.
84. da Costa Martins PA, Salic K, Gladka MM, et al. MicroRNA-199b targets the nuclear kinase Dyrk1a in an auto-amplification loop promoting calcineurin/NFAT signalling. *Nat Cell Biol*. 2010;12(12):1220—1227.
85. Hille S, Dierck F, Kühl C, et al. Dyrk1a regulates the cardiomyocyte cell cycle via D-cyclin-dependent Rb/E2f-signalling. *Cardiovasc Res*. 2016;110(3):381—394.
86. Rozen EJ, Roewenstrunk J, Barallobre MJ, et al. DYRK1A kinase positively regulates angiogenic responses in endothelial cells. *Cell Rep*. 2018;23(6):1867—1878.
87. Bhansali RS, Rammohan M, Lee P, et al. DYRK1A regulates B cell acute lymphoblastic leukemia through phosphorylation of FOXO1 and STAT3. *J Clin Invest*. 2021;131(1):e135937.
88. Khor B, Gagnon JD, Goel G, et al. The kinase DYRK1A reciprocally regulates the differentiation of Th17 and regulatory T cells. *Elife*. 2015;4:e05920.
89. Keramati AR, Fathzadeh M, Go GW, et al. A form of the metabolic syndrome associated with mutations in DYRK1B. *N Engl J Med*. 2014;370(20):1909—1919.
90. Mendoza-Caamal EC, Barajas-Olmos F, Mirzaeicheshmeh E, et al. Two novel variants in DYRK1B causative of AOMS3: expanding the clinical spectrum. *Orphanet J Rare Dis*. 2021; 16(1):291.
91. Lee K, Deng X, Friedman E. Mirk protein kinase is a mitogen-activated protein kinase substrate that mediates survival of colon cancer cells. *Cancer Res*. 2000;60(13):3631—3637.

92. Leder S, Weber Y, Altafaj X, et al. Cloning and characterization of DYRK1B, a novel member of the DYRK family of protein kinases. *Biochem Biophys Res Commun*. 1999;254(2):474—479.
93. Mercer SE, Ewton DZ, Deng X, et al. Mirk/Dyrk1B mediates survival during the differentiation of C2C12 myoblasts. *J Biol Chem*. 2005;280(27):25788—25801.
94. Deng X, Ewton DZ, Pawlikowski B, et al. Mirk/dyrk1B is a Rho-induced kinase active in skeletal muscle differentiation. *J Biol Chem*. 2003;278(42):41347—41354.
95. Deng X, Ewton DZ, Mercer SE, et al. Mirk/dyrk1B decreases the nuclear accumulation of class II histone deacetylases during skeletal muscle differentiation. *J Biol Chem*. 2005;280(6):4894—4905.
96. Masuda T, Itoh K, Higashitsuji H, et al. Cold-inducible RNA-binding protein (Cirp) interacts with Dyrk1b/Mirk and promotes proliferation of immature male germ cells in mice. *Proc Natl Acad Sci U S A*. 2012;109(27):10885—10890.
97. Leder S, Czajkowska H, Maenz B, et al. Alternative splicing variants of dual specificity tyrosine phosphorylated and regulated kinase 1B exhibit distinct patterns of expression and functional properties. *Biochem J*. 2003;372(Pt 3):881—888.
98. Bhat N, Narayanan A, Fathzadeh M, et al. *Dyrk1b Is a Key Regulatory Kinase Integrating Fgf, Shh and mTORC1 Signaling in Skeletal Muscle Development and Homeostasis*. bioRxiv; 2020.
99. Mazmanian G, Kovshilovsky M, Yen D, et al. The zebrafish dyrk1b gene is important for endoderm formation. *Genesis*. 2010;48(1):20—30.
100. Raich WB, Moorman C, Lacefield CO, et al. Characterization of Caenorhabditis elegans homologs of the Down syndrome candidate gene DYRK1A. *Genetics*. 2003;163(2):571—580.
101. Yoshida S, Yoshida K. Multiple functions of DYRK2 in cancer and tissue development. *FEBS Lett*. 2019;593(21):2953—2965.
102. Sun W, Jiao S, Tan X, et al. DYRK2 displays muscle fiber type specific function during zebrafish early somitogenesis. *Int J Dev Biol*. 2017;61(6—7):459—463.
103. Tanaka H, Morimura R, Ohshima T. Dpysl2 (CRMP2) and Dpysl3 (CRMP4) phosphorylation by Cdk5 and DYRK2 is required for proper positioning of Rohon-Beard neurons and neural crest cells during neurulation in zebrafish. *Dev Biol*. 2012;370(2):223—236.
104. Morimura R, Nozawa K, Tanaka H, et al. Phosphorylation of Dpsyl2 (CRMP2) and Dpsyl3 (CRMP4) is required for positioning of caudal primary motor neurons in the zebrafish spinal cord. *Dev Neurobiol*. 2013;73(12):911—920.
105. Imawari Y, Mimoto R, Hirooka S, et al. Downregulation of dual-specificity tyrosine-regulated kinase 2 promotes tumor cell proliferation and invasion by enhancing cyclin-dependent kinase 14 expression in breast cancer. *Cancer Sci*. 2018;109(2):363—372.
106. Yokoyama-Mashima S, Yogosawa S, Kanegae Y, et al. Forced expression of DYRK2 exerts anti-tumor effects via apoptotic induction in liver cancer. *Cancer Lett*. 2019;451:100—109.
107. Ito D, Yogosawa S, Mimoto R, et al. Dual-specificity tyrosine-regulated kinase 2 is a suppressor and potential prognostic marker for liver metastasis of colorectal cancer. *Cancer Sci*. 2017;108(8):1565—1573.
108. Kumamoto T, Yamada K, Yoshida S, et al. Impairment of DYRK2 by DNMT1-mediated transcription augments carcinogenesis in human colorectal cancer. *Int J Oncol*. 2020;56(6):1529—1539.
109. Yoshida S, Aoki K, Fujiwara K, et al. The novel ciliogenesis regulator DYRK2 governs Hedgehog signaling during mouse embryogenesis. *Elife*. 2020;9:e57381.
110. Yogosawa S, Ohkido M, Horii T, et al. Mice lacking DYRK2 exhibit congenital malformations with lung hypoplasia and altered Foxf1 expression gradient. *Commun Biol*. 2021;4(1):1204.
111. Mo R, Freer AM, Zinyk DL, et al. Specific and redundant functions of Gli2 and Gli3 zinc finger genes in skeletal patterning and development. *Development*. 1997;124(1):113—123.
112. Niewiadomski P, Niedziółka SM, Ł Markiewicz, et al. Gli proteins: regulation in development and cancer. *Cells*. 2019;8(2):147.
113. Huangfu D, Liu A, Rakeman AS, et al. Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature*. 2003;426(6962):83—87.
114. Wen X, Lai CK, Evangelista M, et al. Kinetics of hedgehog-dependent full-length Gli3 accumulation in primary cilia and subsequent degradation. *Mol Cell Biol*. 2010;30(8):1910—1922.
115. Chen MH, Wilson CW, Li YJ, et al. Cilium-independent regulation of Gli protein function by Sufu in Hedgehog signaling is evolutionarily conserved. *Genes Dev*. 2009;23(16):1910—1928.
116. Kim J, Kato M, Beachy PA. Gli2 trafficking links Hedgehog-dependent activation of Smoothened in the primary cilium to transcriptional activation in the nucleus. *Proc Natl Acad Sci U S A*. 2009;106(51):21666—21671.
117. Tukachinsky H, Lopez LV, Salic A. A mechanism for vertebrate Hedgehog signaling: recruitment to cilia and dissociation of SuFu-Gli protein complexes. *J Cell Biol*. 2010;191(2):415—428.
118. He M, Subramanian R, Bangs F, et al. The kinesin-4 protein Kif7 regulates mammalian Hedgehog signalling by organizing the cilium tip compartment. *Nat Cell Biol*. 2014;16(7):663—672.
119. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol Cell Biol*. 2017;18(9):533—547.
120. Wemmer KA, Marshall WF. Flagellar length control in chlamydomonas–paradigm for organelle size regulation. *Int Rev Cytol*. 2007;260:175—212.
121. Reiter JF, Blacque OE, Leroux MR. The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep*. 2012;13(7):608—618.
122. Handa A, Voss U, Hammarsjö A, et al. Skeletal ciliopathies: a pattern recognition approach. *Jpn J Radiol*. 2020;38(3):193—206.
123. Hammarsjö A, Pettersson M, Chitayat D, et al. High diagnostic yield in skeletal ciliopathies using massively parallel genome sequencing, structural variant screening and RNA analyses. *J Hum Genet*. 2021;66(10):995—1008.
124. Bogacheva O, Bogachev O, Menon M, et al. DYRK3 dual-specificity kinase attenuates erythropoiesis during anemia. *J Biol Chem*. 2008;283(52):36665—36675.
125. Sacher F, Möller C, Bone W, et al. The expression of the testis-specific Dyrk4 kinase is highly restricted to step 8 spermatids but is not required for male fertility in mice. *Mol Cell Endocrinol*. 2007;267(1—2):80—88.
126. Lord KA, Creasy CL, King AG, et al. REDK, a novel human regulatory erythroid kinase. *Blood*. 2000;95(9):2838—2846.
127. Geiger JN, Knudsen GT, Panek L, et al. mDYRK3 kinase is expressed selectively in late erythroid progenitor cells and attenuates colony-forming unit-erythroid development. *Blood*. 2001;97(4):901—910.
128. Zhang D, Li K, Erickson-Miller CL, et al. DYRK gene structure and erythroid-restricted features of DYRK3 gene expression. *Genomics*. 2005;85(1):117—130.