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REVIEW

DYRK1A: a down syndrome-related dual protein kinase with a versatile role in tumorigenesis

Amina Jamal Laham[1,2] · Maha Saber-Ayad[1,2] · Raafat El-Awady[1,3]

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Abstract

Dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A) is a dual kinase that can phosphorylate its own activation loop on tyrosine residue and phosphorylate its substrates on threonine and serine residues. It is the most studied member of DYRK kinases, because its gene maps to human chromosome 21 within the Down syndrome critical region (DSCR). *DYRK1A* overexpression was found to be responsible for the phenotypic features observed in Down syndrome such as mental retardation, early onset neurodegenerative, and developmental heart defects. Besides its dual activity in phosphorylation, DYRK1A carries the characteristic of duality in tumorigenesis. Many studies indicate its possible role as a tumor suppressor gene; however, others prove its pro-oncogenic activity. In this review, we will focus on its multifaceted role in tumorigenesis by explaining its participation in some cancer hallmarks pathways such as proliferative signaling, transcription, stress, DNA damage repair, apoptosis, and angiogenesis, and finally, we will discuss targeting DYRK1A as a potential strategy for management of cancer and neurodegenerative disorders.

Keywords Carcinogenesis · p53 · RNF169 · 53BP1 · Alzheimer · DNA damage · DNA repair · Cancer therapy

Introduction

Phosphorylation is a critical post-translational modification that regulates many cellular processes such as proliferation, apoptosis, differentiation, homeostasis, and metabolism [8, 9]. This modification is introduced by protein kinases that consume adenosine triphosphate (ATP) to add the Y-phosphate group on protein or lipid substrates [12]. According to the phosphorylated residue, kinases can be classified into tyrosine kinases (TKs) and serine/threonine kinase (STKs) [16]. The addition of Phosphate group induces conformational changes to the protein affecting its function through changing its activity, cellular localization, stability, interaction with other proteins, and DNA [12]. Mutation and dysregulation of kinases are implicated in various human diseases such as cancer, metabolic disorder, neurodegeneration, autoimmunity, and cardiovascular diseases [8, 21, 22].

To date, about 538 protein kinases have been identified in human cells that account for 2% of human genes [8]. These kinases are classified into many groups based on sequence homology of their catalytic domains [9]. CMGC (CDKs, MAPK, GSK, CLK) group which is one of the largest groups including 9 kinase families among them are the cyclin-dependent kinases CDKs, cyclin-dependent kinases like CDKL, mitogen activating protein kinase MAPK, glycogen synthase kinase GSK, cdc2 like kinases CLK, and dual-specificity tyrosine phosphorylation kinase DYRKs [9, 29, 30]. CDKs, MAPK, and GSK are the most studied families in this group. Their involvement in the control of cell cycle, cell fate decision, and metabolism attracts interest in cancer and metabolic disorder research [31, 32]. Although the DYRKs family is evolutionarily conserved from unicellular to multicellular organisms, which indicates their essential role in various cellular processes [18], they are less intensively studied compared to other kinase families.

The dual-specificity tyrosine phosphorylation-regulated kinases (DYRKs) are kinases with dual phosphorylation

✉ Maha Saber-Ayad
msaber@sharjah.ac.ae

✉ Raafat El-Awady
ralawady@sharjah.ac.ae

[1] College of Medicine, University of Sharjah, Sharjah, UAE

[2] Sharjah Institute for Medical Research, University of Sharjah, Sharjah, UAE

[3] College of Pharmacy, University of Sharjah, Sharjah, UAE

activity. They auto-phosphorylate their own activation loop on tyrosine residue motif (YxY) through cis intramolecular mechanism while still attached to the ribosome during translation. However, they phosphorylate their protein substrates on threonine and serine residues [33]. DYRKs are not exclusively serine and threonine kinases. Recent studies showed that DYRKs preserve their ability to phosphorylate tyrosine residues even after maturation, which indicates their capability to further phosphorylate tyrosine residues either on their activation loop or other substrates [34, 35].

Although five members of DYRKs have been identified in humans (Box 1), studies have focused on DYRK1A. Its gene location on Down syndrome critical region on chromosome 21 indicates its fundamental role in Down syndrome phenotypic features, especially in brain developmental disorders. In this review, we will present an overview on *DYRK1A* tissue expression level and its clinical significance, we will pay particular attention to its possible role in various tumorigenic pathways, and how it can serve as a new target in cancer therapy.

Box 1. Classification of DYRKs

According to kinase domain sequences homology, mammalian DYRKs are classified into class 1 (DYRK1A and DYRK1B) and class 2 (DYRK2, DYRK3, and DYRK4) [4] (Table 1). All members share a unique DYRK homology box motif (DH box) that precedes the kinase domain. DH box sequence motif is important to the autophosphorylation event and maturation of the DYRK kinases [5, 6]. For example, the *DYRK1B* DH box mutated sequence causes DYRK1B aggregates with tyrosine dephosphorylated and misfolding of DYRK1B kinase. This variant is found in abdominal obesity-metabolic syndrome 3 that is characterized by high LDH, triglyceride, blood glucose, and abdominal obesity [11]. Class 1 DYRK is characterized by PEST sequence (sequence abundant in proline (P), glutamic acid (E), serine (S), and threonine (T)) located at the C-terminal region [13]. PEST domain is found to be important for protein degradation through anchoring the E3 ubiquitin ligase [15], however, its implication in DYRK 1 degradation is not reported. Outside kinase domain sequences homology, class2 DYRKs share the N-terminal auto-phosphorylation accessory (NAPA) domain [18]. According to sequence alignment, class 2 DYRK NAPA domain is conserved among species and is essential for tyrosine autophosphorylation event but not for the substrate phosphorylation [19]. Both classes contain nuclear localization signals within the N-terminal region which indicates that DYRKs shuttles from cytoplasmic to nuclear compartments (Table 1) [4, 20].

Clinical correlates of DYRK1A:

DYRK1A has a wide spectrum of substrates and is involved in many cellular pathways. As such, it is thought to be involved in many pathological disorders such as neurodegenerative diseases and tumorigenesis. DYRK1A is a dosage-sensitive enzyme, as a consequence, both low and high expression exert significant effects (Fig. 1). Null DYRK1a mice embryos present a severe developmental delay and die around embryonic day 10.5, whereas heterozygous animals show reduction in neuron numbers and deficits in learning capacities [36]. For the first time, Møller et al. described DYRK1A gene truncation in two unrelated patients with microcephaly. Intriguingly, the investigators used cytogenetic analysis and fluorescence in situ hybridization (FISH) to detect the "de novo" translocation [37]. Individuals with

Table 1 Mammalian DYRKs and their characteristics

	Size (amino acids)	Chromosomal location	Cellular localization	Tissue expression	Disease involved in
Class 1					
DYRK1A	763	21q22.13-21q22.2 (21)	Nucleus, cytoplasm (145)	Ubiquitous (145)	Down Syndrome, intellectual disability, neurodegenerative disorders, cancer (21)
DYRK1B	629	19q13.2 (146)	Nucleus (146, 147)	Ubiquitous, predominant in testis and skeletal muscles (146, 147)	Abdominal obesity-metabolic syndrome 3, cancer (16, 148)
Class 2					
DYRK2	601	12q15 (149)	Nucleus, cytoplasm (149)	Ubiquitous (149)	Cancer (150)
DYRK3	588	1q32.1 (151)	Nucleus, cytoplasm (152)	Testis, hematopoietic tissue (153)	Highly expressed in anemia (154,155)
DYRK4	520	12p13.32 (156)	Nucleus, cytoplasm (157,158)	Testis (158)	Renal cancer (159)

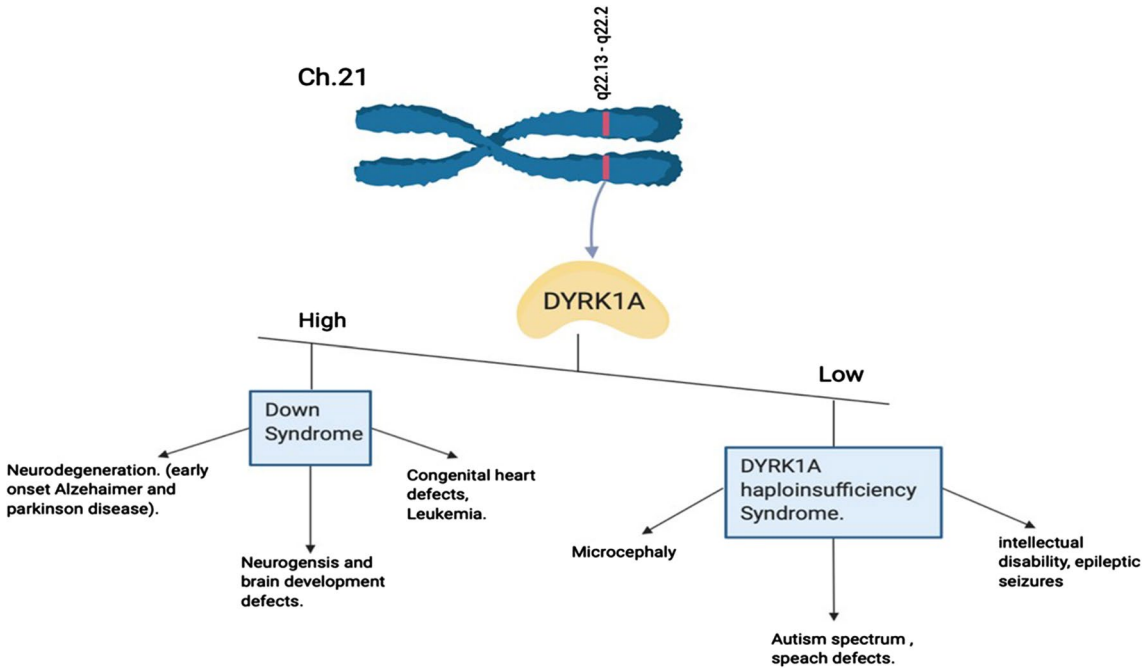

Fig. 1 *DYRK1A* expression level affects neurological development and function. DYRK1A is a dosage-sensitive enzyme; both overexpression and low expression lead to serious consequences during neuronal development. *DYRK1A* overexpression is associated with phenotypic features observed in Down syndrome. However, individuals with heterozygote variant DYRK1A level develop DYRK1A haploinsufficiency syndrome

heterozygous variants of *DYRK1A* develop what is known as *DYRK1A* haploinsufficiency syndrome. This syndrome occurs due to truncation, microdeletion, and missense variant in the *DYRK1A* gene that affects the auto-phosphorylation capacity of DYRK1A, thus impairs its catalytic function. The phenotypic features include microcephaly, speech deficits, distinctive facial appearance, autism spectrum, skeletal, genitourinary defects, and eye abnormalities [38–40]. This concludes that DYRK1A enzyme expression balance is essential for proper neuronal development and function (Fig. 1).

DYRK1A and down syndrome

In 1996, Nobuyoshi Shimizu and his group identified the *DYRK1A* through performing exon trapping experiments to clone the Down syndrome critical region on chromosome 21. Sequencing this clone showed that this region has sequence homology with *mnb* gene in *D. melanogaster, dyrk* in rats, and *yak1* in *S. cerevisiae* [41]. The homology between this clone (known as DYRK1A) and mnb was mainly in the catalytic domain which indicates that these two genes share similar functions [20]. Because *mnb* is highly expressed in the central nervous system of the drosophila and found to be implicated in postembryonic neurogenesis, DYRK1A attracts the attention to have a similar role in Down syndrome. *DYRK1A* gene is located on 21q22.13—21q22.2 chromosomal region (Fig. 1) [42]. In trisomy 21, *DYRK1A* expression is 1.5-fold higher compared to normal individuals [36], which indicates its involvement in various phenotypic features characteristic of Down syndrome.

Generating the *TgDYRK1A* mouse model overexpressing *DYRK1A* showed impairment in neuromotor development, cognitive disability, and defect in memory and spatial learning capabilities [43, 44]. One of the possible explanations of the role of DYRK1A in brain development is that DYRK1A regulates the cell cycle and induces differentiation in neuron cells by inducing G0/G1 arrest [45]. DYRK1A phosphorylates cyclin D which induces its degradation and phosphorylates P27 Kip which leads to its stabilization [45]. Moreover, DYRK1A enhances DREAM complex assembly which inhibits transcription of cell cycle genes in the G0/G1 phase and induces quiescence [46]. A previous study showed that DYRK1A phosphorylates notch transcription factor and attenuates notch signaling in neural cells which induces neuronal cell differentiation [47]. Imbalanced *DYRK1A* expression was found to disrupt the transcriptional regulator neuron-restrictive silence factor REST/NRSF that is responsible for regulating gene expression during neuron cell maturation [48, 49]. Therefore, it was reported that overexpression of DYRK1A might induce premature neuron differentiation in the developing stages [45–48]. Moreover, DYRK1A is involved in dendrite differentiation. DYRK1A is localized from cytoplasm to nucleus during dendrite tree

formation and phosphorylates Dynamin1 and affecting its function, which indicates the involvement of DYRK1A in dendrite differentiation, neurotransmission, and synapsis development [50, 51]. In summary, DYRK1A is a potential pharmacological target to manage neurological symptoms in Down syndrome.

DYRK1A and Alzheimer's disease

In addition to neuronal development disorder and cognitive defect, Down syndrome individuals have a higher risk to develop early onset neurodegenerative diseases such as Alzheimer's disese (AD) [52]. They develop early onset of AD pathology hallmarks such as neurofibrillary degeneration, β- amyloid plaque aggregation, and neuronal loss in their third decade of life [53, 54]. The previous studies have implicated *DYRK1A* overexpression in this risk by different mechanisms [55, 56]:

a) Phosphorylation of microtubule-associated "tau" protein. This phosphorylation reduces the biological activity of tau and promotes its self-aggregation and fibrillation. [53].
b) Distribution of the balance between 3R tau and 4R tau isoforms, through phosphorylating alternative splicing factor (ASF), which promotes 4R Tau isoform formation [57].
c) Accumulation of β- amyloid peptides (Aβ). DYRK1A was found to phosphorylate APP at Thr 668 in mammalian cells. [53].

Inhibiting DYRK1A founds to rescue some symptoms accompany AD in mice models [56]. In summary, targeting DYRK1A might reduce the risk of AD especially in Down syndrome individuals.

DYRK1A and Parkinson's disease (PD)

DYRK1A has also been reported to be involved in Parkinson's disease (PD), another neurodegenerative disorder, through phosphorylation of α-synuclein that causes aggregation/inclusion formation of α-synuclein (Lewy bodies) which leads to the loss of dopaminergic (DA) neurons that are responsible for voluntary movement and behavioral actions [58, 59]. A Chinese Han population-based study comparing normal vs PD patients revealed that *rs8126696 TT DYRK1A* single-nucleotide polymorphism (SNP) genotype is higher in PD patients than in normal individuals suggesting it as a risk factor for PD development [60]. However, how this SNP affects the *DYRK1A* function and activity and its consequences on neuronal function remains to be elucidated.

DYRK1A and heart diseases

In addition to neurological disorders, individuals with Down syndrome suffer from congenital heart defects [61]. Cardiac specific *DYRK1A* overexpression mice develop dilated cardiomyopathy and heart failure. The molecular explanation of this phenotype is similar to the effects of overexpression of *DYRK1A* in the brain. As in neurological tissues, DYRK1A impedes cardiomyocyte proliferation and induce premature differentiation through phosphorylating cyclin D1, cyclin D2, and cyclin D3, thus inducing G0/G1 arrest in *DYRK1A* overexpressing mice cardiomyocytes [62]. In line with phenotypic features observed in Down syndrome individuals, DYRK1A has been identified as a negative regulator of cardiac hypertrophy which is a cardiac disorder that is rarely observed in Down syndrome [63, 64]. DYRK1A antagonizes the calcineurin/NFATc3 pathway. Calcineurin phosphatase dephosphorylates the NFATc3 transcription factor and localizes it to the nucleus to induce the expression of hypertrophic genes. DYRK1A inhibits this pathway by phosphorylating NFATc3 and reducing the expression of the hypertrophy-associated genes. However, this is only observed in cultured cardiomyocytes. In vivo effects of DYRK1A in impeding hypertrophic response are compensated by pathways other than calcineurin/NFAT pathway [65].

Role of DYRK1A in carcinogenesis

The diseases and the distinct phenotypes observed in Down syndrome serve as a reference to understand how imbalanced expression of DYRK1A can cause adverse consequences. In addition to neurological and heart defects, epidemiological studies reported that Down syndrome patients have a higher risk of developing hematological malignancies but lower risk of developing solid carcinomas [66]. How DYRK1A plays a role in that and how it's involved in tumorigenesis pathways will be further elucidated in the next sections.

The presvious reports highlighted that the role of DYRK1A in cancer is context-dependent; it behaves as a tumor suppressor gene in some types of cancer cells and as an oncogene in others [36, 67]. As many kinases, DYRK1A phosphorylates a wide range of substrates, and leads either to their activation or deactivation, thus indicating the role of DYRK1A in a certain context. In this section, we will focus on the role of DYRK1A kinase in major cellular pathways and how DYRK1A can behave as a tumor suppressor or tumor-promoting kinase (Fig. 2).

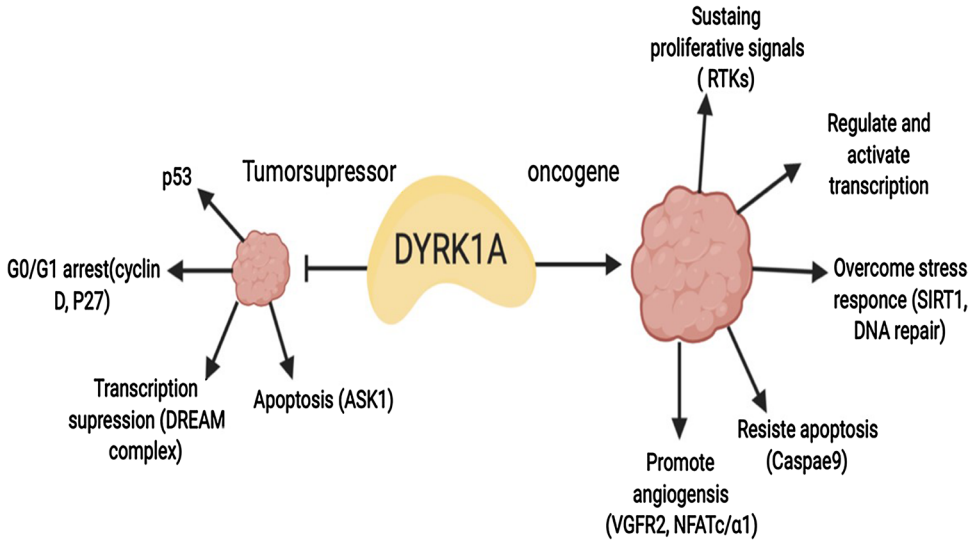

Fig. 2 The duality role of DYRK1A in tumorigenesis: DYRK1A can phosphorylate a plethora of substrates. Based on the type of substrate, DYRK1A can act as a tumor suppressor or tumor-promoting kinase

DYRK1A expression level in cancer

DYRK1A is reported to be overexpressed in some cancer types in comparison to other pathological conditions of the same organ. Comparing *DYRK1A* transcript level in epileptic tissues and glioma showed that in glioma especially oligodendroglioma and glioblastoma multiform, the *DYRK1A* transcript level was higher. This correlates with the expression of DYRK1A in glioma cell lines which showed positive expression of DYRK1A [68]. Moreover, using tissue microarray-based immunohistochemical measurement, *DYRK1A* was found to be overexpressed in 39 out of 40 patient samples of Head and Neck squamous cell carcinoma (HNSCC). Moreover, it was found to be hyperphosphorylated in HNSCC patient samples and its inhibition reduces the tumorigenic ability of HNSCC cell line to form tumor xenograft in mice [69]. Immunohistochemical staining showed that DYRK1A localizes in the nucleus and is strongly expressed in pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma compared to normal pancreatic adjacent tissues. Inhibition of DYRK1A chemically or biologically reduced the ability of pancreatic cancer cell lines to proliferate and to form colonies in vitro and to form a tumor in vivo [70]. In comparison with normal individuals, *DYRK1A* was recently found to be overexpressed in non-small cell lung cancer (NSCLC) tumor samples and to be associated with poor prognosis [71].

As have been mentioned, *DYRK1A* overexpression might play a role in increasing the incidence of hematological malignancies in Down syndrome patients. Children with trisomy 21 have more than tenfold increased risk of developing acute lymphoblastic leukemia and acute megakaryoblastic leukemia [66]. DYRK1A was found to promote tumorigenesis by affecting leukemogenesis through phosphorylating NFATc transcription factors and promoting their cytoplasmic retention [72, 73]. In contradiction to this, DYRK1A in adult's acute myeloid leukemia (AML) was reduced compared to normal controls. DYRK1A was further suppressed in refractory/relapsed AML compared with the newly diagnosed AML patients [74]. Overexpression of *DYRK1A* in leukemia cell lines reduced the proliferation capacity through inducing G0\G1 arrest and inhibition of c-myc transcription factor [74]. Inhibition of certain transcription factors and arresting cell cycle through phosphorylating cyclin D and enhancing P27 Kip function explain the possible role of DYRK1A in inhibiting tumor formation/growth. However, many other pathways indicated its possible involvement in carcinogenesis which will be further explained.

DYRK1A sustains proliferative signaling pathways

As is well known in cancer biology, gene amplification or deletion variants that lead to constitutive activation of the receptor tyrosine kinases and their downstream pathways such as MAPK and PI3K/AKT are frequent in many malignancies. Constitutive activation of these pathways leads to uncontrolled cell proliferation, transformation, and invasion [75]. DYRK1A was found to sustain the activation of receptor tyrosine kinase pathways [68, 70, 71]. DYRK1A upregulates Ras/MAP Kinase Signaling and overexpression of DYRK1A sustains ERK activation after Nerve growth factor (NGF) stimulation in PC12 pheochromocytoma cell line. Co-immunoprecipitation experiments showed that DYRK1A interacts with Ras, B-Raf, and MEK1 to facilitate the formation of a Ras/B-Raf/MEK1 multiprotein complex [76]. Moreover, DYRK1A enhances RTK signaling through phosphorylation of Sprouty homolog 2 which is a negative feedback regulator of multiple receptor tyrosine kinases (RTK's) including receptors for fibroblast growth factor

(FGF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF) [77]. DYRK1A phosphorylates Spry2 at Thr75 which leads to its inhibition [68, 70, 77, 78]. Also, studies showed a positive correlation between DYRK1A and C-Met in pancreatic ductal adenocarcinoma samples and between DYRK1A and EGFR expression in glioblastoma and NSCLC patients' samples [68, 70, 71]. In NSCLC, inhibition of DYRK1A enhances the anti-cancer activity of osimertinib (an EGFR inhibitor used to treat EGFR-mutated NSCLC) in EGFR wild-type NSCLC cells [71]. DYRK1A inhibition in combination with RTK-targeted therapy might serve as a new therapeutic approach to sustain the effectiveness of targeting RTK pathways in cancer treatment.

DYRK1A as a transcription regulator

In addition to its effects on receptor tyrosine kinase, DYRK1A was also found to affect the downstream signaling proteins. For example, it can act as a transcription regulator and modulator of gene expression through phosphorylating and activating various transcription factors such as STAT3, GLi1, and CREB transcription factors [79–81]. Activation and overexpression of these transcription factors are implicated in various malignancies [82, 83]. Although DYRK1A has been reported to enhance DREAM complex assembly by phosphorylating LIN52 subunit and induces quiescence [46], the other studies have shown that DYRK1A may function as elongation factor and activate transcription by phosphorylating CTD in RNA polymerase 2 at Ser2 and Ser5 in DYRK1A target genes that participate in the translation, RNA processing, and cell cycle [84]. Moreover, DYRK1A sustains open chromatin structure and activates transcription through phosphorylating histone 3 at Thr45 and Ser57, and prevents heterochromatin protein 1 HP1 from binding and suppressing transcription [85]. In addition to that, it was recently discovered that DYRK1A acts as a kinase and activator for CBP and P300 histone acetyltransferases. Genome-wide ChIP-sequencing revealed the localization of DYRK1A with CBP\P300 at transcription enhancer regions which indicated that DYRK1A phosphorylates CBP\P300 at the transcription start site. Additionally, silencing *DYRK1A* decreased H3K27 acetylation at enhancer regions, and this confirms the positive role of DYRK1A in activating transcription [86]. Collectively, interaction with transcription factors, RNA polymerase, histones, and epigenetic factors elucidates the spacious role of DYRK1A as a transcription regulator.

Role of DYRK1A in stress response

Adaptation to cellular stress is one of the pathways that cancer cells depend on to survive and to resist harsh environmental conditions such as hypoxia, oxidation stress, mechanical stress, metabolic stress, and exposure to genotoxic drugs [87]. *DYRK1A* expression level and activity found to be increased after exposure of cultured cells to toxic stimuli such as etoposide, tumor necrosis factor, hydrogen peroxide, and hyperosmotic pressure [88, 89], which denotes the possible role of DYRK1A in cellular stress pathways. SIRT1 which is a nicotinamide adenosine dinucleotide (NAD)-dependent class 3 histone deacetylase was found to play a major role in cellular stress. Although there are contradictory studies regarding the SIRT1 role in cancer, SIRT1 protects cells from stress by deactivating stress response proteins such as P53, HIC1, NF-κB, and DDR factors [90, 91]. SIRT1 was found to sustain DNA repair which explains another alternative mechanism of protecting cells from genotoxic stress [92].

SIRT1 deacetylates P53 at Lys 383 which is reported to enhance cancer cell survival [93]. DYRK1A acts as a kinase for SIRT1 and activates its deacetylation activity [94]. Activating SIRT1 by DYRK1A promotes the inhibition of P53 which will sustain the survival of cancer cells under stressful conditions (Fig. 2).

DYRK1A and P53

P53 is one of the most frequently mutated proteins in cancer. It is a tumor suppressor transcription factor that induces cell arrest and apoptosis in response to stressful conditions such as DNA damage. Under DNA damage, P53 is phosphorylated at ser15 by ATM (ataxia telangiectasia mutated). This phosphorylation boosts P53 activity in inducing cell cycle arrest to repair the DNA damage or to induce apoptosis in case of repair failure [95].

Although DYRK1A phosphorylates SIRT1 which will attenuate P53 activity, DYRK1A was found to directly phosphorylate P53 at ser 15 and induces its transcription activity in embryonic neuronal cells. Inducing p53 activity reduces neuronal cell proliferation and affects brain development during embryonic stages [96]. However, this adds another contradictory role of DYRK1A in tumorigenesis. It is unknown if these phosphorylation events happen after inducing DNA damage or only during embryonical development. Also, further studies should be done to investigate if this phosphorylation event acts in parallel with ATM or compensate for the ATM deficiency during DNA damage.

Moreover, P53 was found to act in a negative feedback loop in coordinating *DYRK1A* expression during DNA damage. In response to DNA damage, P53 downregulates the *DYRK1A* through inducing the expression of miR-1246 that targets *DYRK1A* mRNA 3′-UTR region [97]. Moreover, activation of p53 was found to induce cellular senescence through the downregulation of DYRK1A and EGFR. Ectopic expression of *DYRK1A* in cancer cells reduces the ability of

P53 to induce senescence [98]. P53 regulates the EGFR-DYRK1A axis through inducing the expression of MDM2, a ubiquitin ligase that found to ubiquitinate DYRK1A and induce its proteasomal degradation [98].

These studies improved our understanding of the link between DYRK1A and P53, and DYRK1A activates p53 and in turn P53 downregulates DYRK1A as negative feedback by inducing its ubiquitination and suppressing its transcription through miR-1246 [97, 98] (Fig. 2).

Role of DYRK1A in DNA damage

Recently DYRK1A was reported to be involved in DNA DSBs repair (Box 2). Based on proteomic interaction studies, recent reports showed that RNF169 interacts with DYRK1A [99–101]. RNF169, which was reported to be phosphorylated at S368, S403, and S688, is recognized as a substrate for DYRK1A [101]. Phosphorylation at S368 and S403 was not essential for RNF169 role in DNA damage; however, these phosphorylated sites were important for the binding of RNF169 to DYRK1A [100]. On the other hand, RNF169 phosphorylation at S688 was essential for the activity of RNF 169 to remove 53BP1 from the DNA damage foci [101]. Moreover, as an interacting partner, DYRK1A was found to interact with RNF169 and localize to DNA damage foci. Localization of DYRK1A on DNA damage foci was dependent on its binding with RNF169. On the other side, Inhibition of DYRK1A reduced RNF169 localization at DNA damage foci, which suggests that these two proteins depend on each other to be recruited to the DNA damage sites [101]. However, it is unknown if phosphorylation of RNF169 by DYRK1A is induced only by DNA damage, because these two proteins were found to interact in the absence of DNA damage which indicates their involvement in other cellular pathways [100, 101].

Box 2. 53BP1 and RNF169 in DNA DSBs

Induction of DNA damage is one of the fundamental therapeutic approaches in treating cancer. Radiation and many genotoxic FDA approved drugs work mainly by inducing DNA damage through alkylating DNA bases, inducing intra/inter-strand cross-links, DNA- protein cross-link and DNA double-strand breaks (DSBs) [1, 2]. Cells respond to these genotoxic agents by activating DNA damage response and repair machinery [3]. According to the type and the time of damage cells activate different repair pathways such as base excision repair, nucleotide excision repair and in response to double-strand breaks, cells either activate the homologous recombination (HRR) or the nonhomologous end rejoining (NHEJs) repair pathways [7].

HRR is activated during the S and G2 phases of the cell cycle after DSBs and inter-strand cross-links lesions that impede replication and cause replication fork arrest and collapse [10].

HRR pathway depends on homologous recombination through inducing 5′end resection by MRN/CtIP and searching and invading adjacent complementary strand on sister chromatid through RAD51, thus repairing the damage by error-free mechanism [14]. On the other hand, NHEJs can be activated in any phase of the cell cycle [17]. NHEJ repair pathway is an error-prone mechanism of repair because it does not require any end processing or invasion of the strand, it directly ligates the two ends of the DSBs [23]. The preference of the cell to select HRR or NHEJs pathways depends on many factors such as the availability of sister chromatids and the repair proteins that are recruited to the DSBs site [24]. One of the early responses to the DSBs is the binding of 53BP1 to the H2A ubiquitin chains added by RNF8/RNF168 E3 ubiquitin ligase in response to DSBs. Binding of 53BP1 favors nonhomologous end-joining repair by preventing end resection, thus antagonizing the homologous repair pathway [17, 25]. A newly identified competitor of 53BP1 that enhances HRR repair is the E3 ubiquitin ligase RNF169 [26, 27]. The main function of RNF169 is not ubiquitination. Depending on its MIU2 domain, it recognizes the H2A 13/15Ub chain added by its paralog RNF168 in response to DNA damage [27]. This interaction is thought to impede 53BP1 from binding and enhancing end resection, thus RNF169 promotes the HRR pathway [26, 28]. Recently it was found that RNF169, by promoting end resection, can promote single-strand annealing and supporting alternative nonhomologous end-joining repair pathways which are highly mutagenic [26].

Treating U2-OS cell lines with the DYRK1A inhibitor harmine increases 53BP localization to DNA damage foci [100]. Moreover, overexpressing kinase active DYRK1A in U2-OS and HeLa cell lines further decreases 53bp1 foci formation after irradiation. This is in line with the role of RNF169 in depleting 53BP1, and as a binding partner for RNF169, DYRK1A kinase activation reduces 53bp1 foci formation after DSB and induces HR repair pathway by RNF169 dependent mechanism [100, 101].

However, in addition to the interaction with RNF169, this dual kinase acquires duality in DSB repair choice. As *DYRK1A* expression dosage effects neuronal development; *DYRK1A* expression level was found to affect DSB repair pathway choice. Overexpression of kinase active DYRK1A depletes 53BP1. However, the complete loss of DYRK1A decreased 53BP1 foci formation after irradiation. Moreover, *DYRK1A* knockout (KO) HeLa cell line was found to have reduced expression of 53BP1 even before inducing damage, which might explain that

DYRK1A is important for 53Bp1 basal line-level expression and stability. Moreover, in *DYRK1A*-depleted cells, overexpressing RNF169 does not rescue the reduction of 53BP1 which proves that DYRK1A might be involved in the NHEJ repair pathway through a mechanism independent of RNF169 [100].

DYRK1A depletion reduced RNF169 and 53BP1 localization to the DNA damage sites, which adds more complexity to the DYRK1A role in DNA damage response. Moreover, there is no effect of *DYRK1A* knock out on BRCA1 (stimulate HR repair pathway) localization to DNA damage sites [100]. The overall role of DYRK1A in DNA damage response is unknown, but a balance in DYRK1A activity and expression might be a new factor that determines the choice of DSB repair pathway (Fig. 3).

Moreover, it remains to be determined if DYRK1A is involved in the initial DNA damage response, although it activates SIRT1 which is found to affect the DRR pathway [90, 92], further studies are needed to unravel the link between DYRK1A, SIRT1, and DNA damage response. The involvement of DYRK1A in DNA damage repair might show/reveal the potential role of DYRK1A in tumorigenesis. On the other hand, as constitutive activation of DNA repair might lead to genotoxic drug resistance, thus combining DYRK1A inhibition with chemotherapeutic drugs that cause DNA damage might provide a new therapeutic approach to overcome chemotherapy and radiotherapy resistance (Fig. 4).

DYRK1A and apoptosis

DYRK1A overexpression in Down syndrome patients was found to affect neuronal cell proliferation and differentiation. However, reduced expression of *DYRK1A* also causes microcephaly and reduction in neuronal cell numbers. Apoptosis is a programmed cell death that has an important role during development. In copy-variant *DYRK1A*+\− mice, programmed cell death was highly active in dopaminergic neurons during the developmental stage. Overexpressing *DYRK1A* attenuated apoptosis and aid in dopaminergic neurons' survival [102]. Moreover, comparing the eyes morphologies in *Dyrk1A*+/− mice and mice with triplicate DYRK1A showed that *Dyrk1A*+/− had smaller eyes and thinner retina compared to *DYRK1A* overexpressing mice. DYRK1A affects retinal cells number during development through resisting apoptosis [103]. Both studies report that DYRK1A acts as an inhibitory kinase for caspase 9, thus phosphorylating it at Thr125 leading to inhibition of the intrinsic apoptosis pathway [102, 103]. However, this occurs not only during development, but DYRK1A was also found to phosphorylate caspase 9 under hyperosmotic stress in mammalian cells [89]. This phosphorylation event was found to inhibit caspase 9 auto-processing and sequestering it in the nucleus (Fig. 5A) [104]. This role of DYRK1A in apoptosis reflected another role of DYRK1A in tumorigenesis as resisting apoptosis is one of the major hallmarks of cancer, and inhibiting DYRK1A chemically or biologically was reported to activate apoptosis in many cancer cells [69, 104].

Another study suggested a possible role of DYRK1A as a proapoptotic kinase. Under stressful stimuli, DYRK1A was

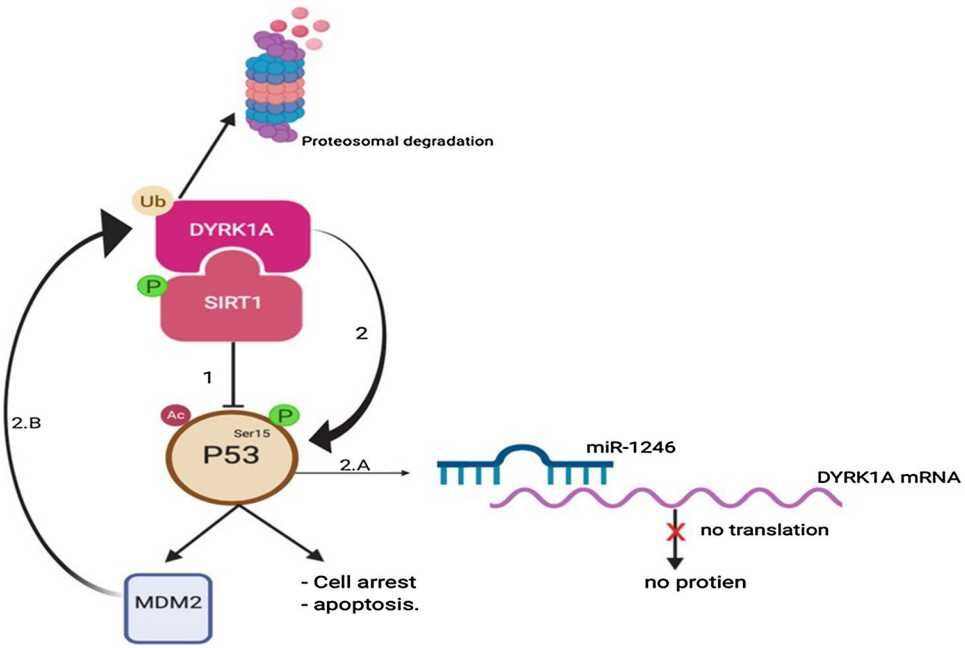

Fig. 3 Crosstalk between DYRK1A and p53: (1) DYRK1A phosphorylates SIRT1 which, in turn, deacetylates P53 and inhibits its transcriptional activity. (2) DYRK1A directly phosphorylates P53 at ser 15 and activates it. (2.A) P53 regulates *DYRK1A* expression through activating the transcription of miR-1246 that causes degradation of *DYRK1A* mRNA. (2.B) P53 inhibits the DYRK1A at the protein level by indirectly activating MDM2 transcription which, in turn, ubiquitinates DYRK1A and causes its proteasomal degradation

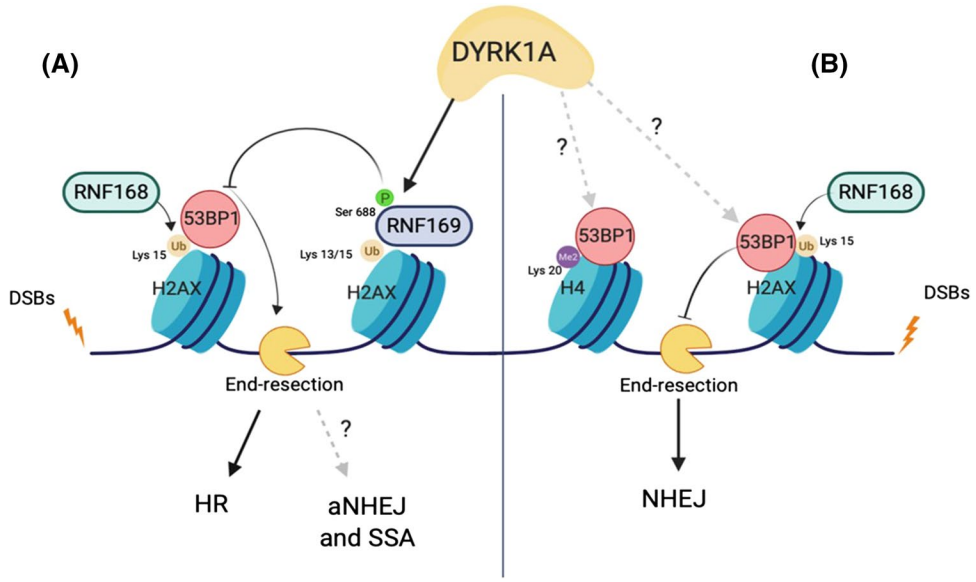

Fig. 4 DYRK1A has a dual role in DSBs repair choice: (**a**) DYRK1A phosphorylates RNF169 at Ser688 which is important to impede 53BP1 from Ub Lys 15 at H2AX on the DNA damage site. Removal of 53BP1 will allow end resection of the DNA strand which will further sustain the repair via homologous recombination (HR). Still to be determined if DYRK1A through activation of RNF169, has a role in other end resection-dependent repair pathways such as alternative nonhomologous end-joining (aNHEJ) and single-strand annealing (SSA). (**b**) DYRK1A by undefined mechanism modulates the level of 53BP1 at the DNA damage foci. Still to be determined if DYRK1A regulates 53BP1 level by induction of epigenetic modifications at Ub Lys 15 at H2AX or Me Lys20 at H4, which explain its potential role in nonhomologues end-joining repair (NHEJ) [26, 99–101]

found to sustain the c-Jun N-terminal kinase (JNKs) pathway through increasing c-jun phosphorylation. However, DYRK1A do not interact directly with JNK, and DYRK1A was found to phosphorylate and activates apoptosis signal-regulating kinase 1 (ASK1) which is the upstream activator of JNK and P38α MAP kinases in response to cellular stress [88]. ASK1 induces the intrinsic apoptosis pathway [105], and interaction with DYRK1A might explain a proapoptotic role of DYRK1A. Moreover, ASK1 was found to be upregulated in neurodegenerative diseases and, thus, has a positive correlation with DYRK1A [106]. In addition, ASK1 was found to be upregulated in melanoma and gastric cancers and has an important role in cell survival and inflammation, and DYRK1A as a kinase might activate ASK1 in other pathways than apoptosis (Fig. 5a) [107]. Collectively, further studies are needed to explore the possible role of DYRK1A in apoptosis and how it can serve as ASK1 activation factor in pathways other than apoptosis in cancers.

DYRK1A and angiogenesis

Angiogenesis, the formation of new blood vessels from pre-existing ones, is one of the major hallmarks of tumorigenesis. As in normal tissues, cancer cells demand nourishment and oxygen to survive and invade adjacent tissues, so tumors depend highly on angiogenesis as a way to metastases and spreading [108]. In healthy status, angiogenesis is a highly regulated process, the balance between pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and anti-angiogenic factors such as endostatin and thrombospondin ensures the proper formation of blood vessels during developmental stages or in other physiological conditions such as wound healing and female menstrual cycle. However, in tumorigenesis, the hypoxia and extreme need for nutrient shifts this balance toward pro-angiogenic signaling by upregulating the expression of VEGFs or their receptors such as vascular endothelial growth factor receptors (VGEFR) [109], and because of that VEGF and VEGFR became important therapeutic targets in various cancer types [110].

In Down syndrome, incidences of vascular malformation such as pulmonary vein stenosis, umbilico-portal venous system defects, vertebral, and right subclavian artery deformity in Down syndrome children were reported [111, 112]. This indicates that certain genes in chromosome 21 are responsible for proper vasculature formation. The previous studies showed that Down syndrome critical region expresses anti-angiogenesis inhibitors such as Down syndrome candidate region 1 gene (DSCR-1) and endostatin precursor collagen 18a which explains the reduced angiogenesis tumor incidence and increased vascular abnormalities in Down syndrome [113, 114].

Recent studies have explored the positive role of DYRK1A in angiogenesis. As in the case of sustaining EGFR signaling,

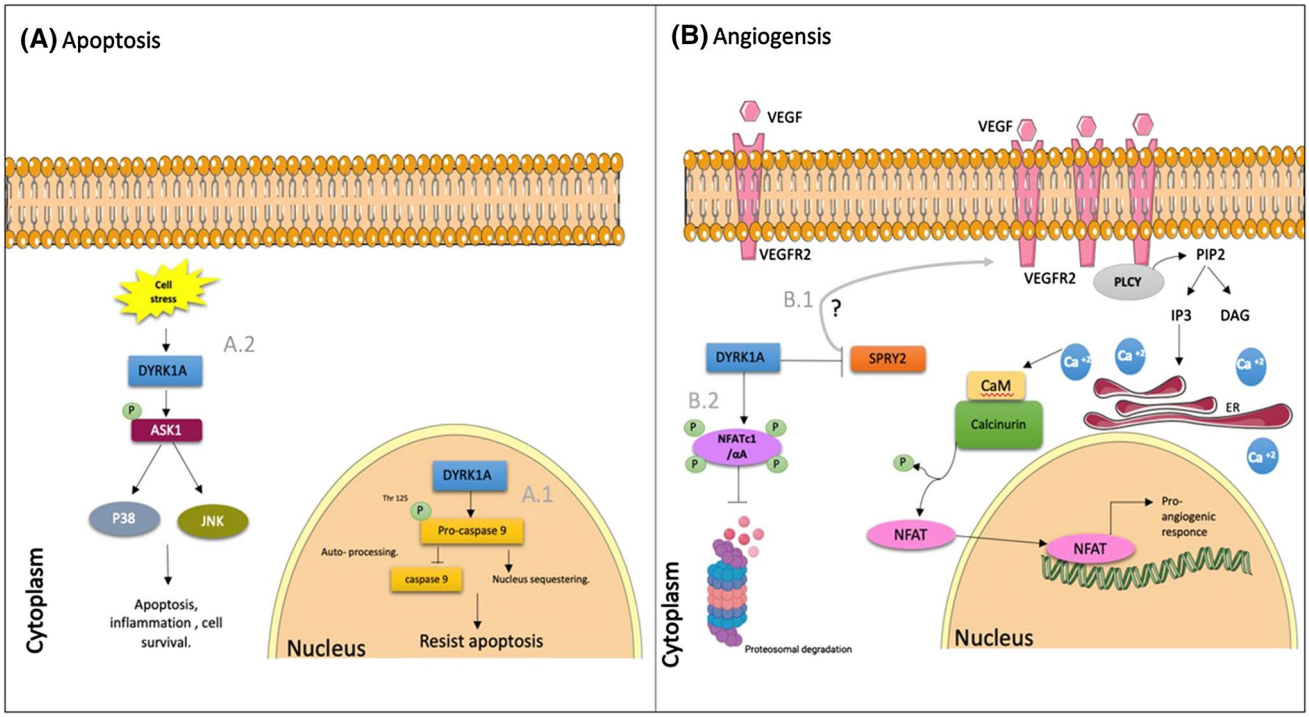

Fig. 5 DYRK1A role in (**a**) Apoptosis and (**b**) Angiogenesis: (A.1) DYRK1A phosphorylates procaspase 9 at Thr125 in the nucleus which prevents its auto-processing, and localization into the cytoplasm, this will lead to apoptosis resistance. (A.2) Under cellular stress, DYRK1A phosphorylate ASK1 which will lead to its activation and phosphorylation JNK and P38 resulting in apoptosis, inflammation, or cell survival. (B.1) DYRK1A sustains VEGFR2 accumulation and signaling which increases the intracellular calcium level that activates calmodulin/calcineurin, and this dephosphorylates NFAT transcription factor and activates pro-angiogenic gene expression. It is still to be determined if DYRK1A sustains VEGFR2 accumulation through inhibiting SPRY2. (B.2) Another possible role of DYRK1A in angiogenesis is the phosphorylation of NFATc1/αA at multiple sites and inhibition of its proteasomal degradation

DYRK1A was found to sustain the accumulation VEGFR2 receptor, and as a consequence, this leads to the upregulation of intracellular calcium and calcineurin/NFAT signaling, a well-known proangiogenic pathway (Fig. 5b). Although DYRK1A was found to suppress some NFAT transcription factors isoforms such as NFATc2, NFATc3, and NFATc4 in the nucleus, in the case of angiogenesis, DYRK1A upregulates NFAT transcription factors by an indirect mechanism in the cytoplasm [115]. Moreover, DYRK1A was found to stabilize NFATc1/alpha A isoform which is the short conversion of NFATc1 that is induced after TCR activation and it is the only isoform from NFAT transcription factors that are reported to have an oncogenic role (Fig. 5b) [116, 117]. It is still not completely determined if DYRK1A sustain NFATc1/αA in endothelial cells as part of enhancing angiogenesis.

Furthermore, inducing mutant *DYRK1A* in zebrafish embryos showed increased cerebral hemorrhage and vascular abnormality. Treatment with calcium chelating agents rescued the vascular permeability and defects which supports the involvement of DYRK1A in the calcium signaling pathway to regulate angiogenesis [118].

All these studies focused on DYRK1A role in developmental angiogenesis, further studies are needed to explore the role of DYRK1A in tumor angiogenesis, because this might serve as a potential therapeutic approach to resensitizes cancers that develop resistance toward anti-angiogenic targeted therapy.

DYRK1A as a target for the management of neurodegenerative disorders and cancer

Pharmacological inhibition of DYRK1A

Natural DYRK1A inhibitors

Several natural-based DYRK1A inhibitors have been discovered [reviewed in [119, 120]]. Harmine belongs to β-carboline family derived from vine *Ban- isteriopsis caapi* and rue *Peganum harmala* [119]. Harmine is an ATP-competitive inhibitor, inhibiting DYRK1A in higher potency than other kinases [121]. It has been shown to reduce Tau phosphorylation in vitro and to enhance the memory performance of APP/PS1 mice [122]. Moreover, harmine exerts anti-cancer effects through DYRK1A inhibition in neuroblastoma, head and neck cancer, pancreatic cancer, and non-small cell lung carcinoma cell lines [69, 123]. Although harmine is a good experimental chemical to target neurodegenerative disease and cancer, it has an off-target, multiple side effects, and cytotoxicity that impedes its clinical usage.

Another much safer natural non-ATP-competitive DYRK1A inhibitor is the epigallocatechin gallate (EGCG), a polyphenol extracted from green tea [124]. Adding EGCG to the mice diets rescues the cognitive defects, and improves the synaptic plasticity and brain morphology in Down syndrome mouse model Ts65Dn and transgenic *DYRK1A* overexpressing mice [57, 125]. EGCG was found to have preventive effects. Treating *DYRK1A* overexpressing mice with EGCG at the gestation period only improves the recognition memory and improves neuronal function after birth [126]. Clinical trials have been conducted on EGCG as a potential treatment for AD with Down syndrome. In a study by De la Torre in 2013, 29 adults with Down syndrome were given 9 mg/kg/day. The effects of EGCG were checked after 1 and 3 months of treatment and after 3 months of treatment discontinuation. There was a significant improvement in social performance and in episodic, working, and visual memories compared with the control group. However, this improvement disappeared after EGCG discontinuation [125]. In 2014, another phase II clinical trial for EGCG treatment have been conducted. EGCG treatment with cognitive training was reported to improve memory, adaptive behavior, and executive function skills compared with placebo-cognitive training only [127]. FontUp® is a new nutritional formula with a chocolate taste that contains the effective EGCG extract dose, which is found to be safe in vivo and targeting DYRK1A [128]. FontUp® is currently undergoing clinical trial for Down syndrome individuals (clinical trial identifier: NCT03624556). In addition, EGCG was reported to induce anti-cancer effects in various cancer cell lines and can be used as adjuvant therapy with chemo- and radiotherapy [129, 130]. EGCG has several targets other than DYRK1A, and no study indicated that EGCG preventive and anti-cancer effects are induced through DYRK1A inhibition specifically.

Synthetic DYRK1A inhibitors

Off-target effects are one of the problems limiting the usefulness of natural DYRK1A inhibitors. Therefore, efforts have been made to synthesize specific DYRK1A inhibitors.

A benzothiazole derivative INDY has been reported to be an ATP-competitive DYRK1A inhibitor. It was shown to rescue the phenotypic features associated with *DYRK1A* overexpression, such as reducing tau phosphorylation and activation of NFAT signaling. Moreover, pro-INDY was utilized to rescue the brain malformation associated with *DYRK1A* overexpression in Xenopus embryos. In contrast, INDY can inhibit DYRK1B with an IC50 of 0.23 μM [131].

Diaryl-azaindole inhibitors of DYRK1A (DANDY) are 3,5-diaryl-7-azaindoles derived from 3,5-diaryl-1H-pyrrolo[2,3-b]pyridines. DANDY 28 is the most potent ATP-competitive DYRK1A inhibitor of this series with IC50 = 3 nM [132]. These compounds contain polar hydroxy groups which might impede blood–brain barrier penetration. Series of F-DANDY derivatives were developed by adding fluorine atoms replacing the hydroxy groups. Of them, F-DANDY derivative 5a compounds were found to rescue the memory and cognitive defects in Ts65Dn mice [133]. Further studies should be done to report the efficacy of F-DANDY in cancer cells overexpressing *DYRK1A*.

Another DYRK1A inhibitor, DYR291, has been synthesized by the hybrid structure of harmine, inadazole compound D15, and benzothiazole derivative INDY. This benzimidazole compound DYR291 was found to improve memory and learning defects in 3xTg-AD mice. However, it did not affect the NFT formation level [55]. Chronic administration of DYR291 into 3xTg-AD mice at an early age before developing AD prevented the development of AD hallmarks such as tau pathology, accumulation of β- amyloid peptides, and neurofibrillary tangles (NFTs) aggregates [134]. Accordingly, DYRK1A inhibition could be used as prevention therapy for AD.

Silmitasertib (CX-4945) a CK2 kinase inhibitor with clinically tested and proven safety, recently found to be an ATP-competitive inhibitor for DYRK1A. It has a higher inhibitory potency than harmine and INDY. When administered orally, it was found to reduce tau phosphorylation in the hippocampus of *DYRK1A* overexpressed mice. Moreover, it rescued the neurological defects in *minibrian* overexpressing *D. melanogaster* [135]. Silmitasertib reported having anti-cancer effects through targeting CK2, not DYRK1A [136]. In 2017, the FDA granted Silmitasertib an orphan drug status to treat cholangiocarcinoma [137]. This suggests that

Silmitasertib could be a good choice for targeting DYRK1A in neurodegenerative, Down syndrome, and cancer.

Targeting DYRK1A folding and proteolysis

The majority of DYRK1A inhibitors are ATP-competitive inhibitors. These inhibitors have limited selectivity toward one kinase, which makes their clinical utilization challenging [119]. As aforementioned, DYRK1A possesses an auto-phosphorylation ability during the translational process. This auto-phosphorylation is important to its maturation and activity [138].

FINDY, a rhodamine derivative compound, found to inhibit DYRK1A auto-phosphorylation at ser 27 which interferes with the folding intermediate and prevents ATP incorporation and eventually leads to DYRK1A degradation. This means that FINDY does not inhibit mature DYRK1A, but it inhibits DYRK1A during its synthesis. Testing it in vivo was found to rescue developmental defects in Xenopus laevis embryos [139].

Another way to reduce the effects of DYRK1A is to target its proteolysis. Decreased full-length DYRK1A and increased truncated DYRK1A was recently observed in the hippocampus of AD patients [140]. In-vitro study showed that truncation of DYRK1A increases its affinity to STAT3α which is involved in the transcription of pro-inflammatory cytokines. leucettine L41 a natural alkaloid extracted from *Leucetta microraphis*, reported to normalize DYRK1A activity and improve recognition memory in Down Syndrome mouse models [141]. A recent study reported a novel mechanism of action of L41, which is inhibiting proteolysis of DYRK1A in APP/PS1 mice. L41 reduces STAT3α phosphorylation and improves microglia recruitment to amyloid plaques and rescue synaptic and memory deficits in APP/PS1 mice [56]. Therefore, targeting DYRK1A folding or preventing its proteolysis might reduce the risk of AD and neuronal developmental defects associated with *DYRK1A* overexpression.

Targeting DYRK1A biologically through miRNA

miRNAs are short non-coding RNAs regulating gene expression through targeting mRNA of a specific gene and inducing its degradation or activation [142, 143]. Many miRNA species have been identified to be implicated in various human diseases. Thus, targeting miRNA or enhancing their formation becomes one of the future therapeutic approaches, and these therapeutics are in phase 1 and 2 clinical trials [144].

The previous studies have identified miRNA that targets *DYRK1A* mRNA. As aforementioned, miR-1246 targets DYRK1A mRNA through the P53 transcription factor. Enhancing miR-1246 expression could be a possible strategy to treat p53 null tumors with overexpressed *DYRK1A*.

Another miRNA found to downregulate *DYRK1A* mRNA is miR-199b [145, 146]. Enhancing miR-199b could inhibit DYRK1A in neurodegeneration and tumors overexpressing *DYRK1A*.

Recently, miR-204-5p was also reported to be upregulated in PD patients' samples. In dopaminergic cells, miR-204-5p upregulates phospo-tau and phospho-α-synuclein. In addition, miR-204-5p through upregulating *DYRK1A* induces apoptosis via activation of JNK pathway which leads to the loss of dopaminergic neurons [147]. In cancers, miR-204-5p was found to act as a tumor suppressor through apoptosis activation [148, 149], thus inhibiting miR-204-5p could help only in neurodegeneration disorders that involve DYRK1A.

Concluding remarks and future directions

The role of DYRK1A in neurological developmental defects and neurodegeneration is well known. The balance of its expression is quite important because over- or down-expression can lead to serious neurological related syndromes. Thus, targeting DYRK1A can serve as a single approach to manage Down syndrome and neurogenerative disease. However, its role in tumorigenesis is still to be elucidated; its tumor suppression or activation is context-dependent. As many kinases, DYRK1A phosphorylates a wide range of substrates, leading either to their activation or deactivation, thus supporting the assumption that the role of DYRK1A in many pathways is context-dependent.

This broad role of DYRK1A in the major players of cancer makes it a good pharmacological target, especially in combination with chemo- and radiotherapy, RTKs (approved to synergize with osimertinib), DNA damaging agents, and angiogenesis-targeted therapy. As a developmental essential kinase, inhibiting DYRK1A kinase might not introduce the same phenotypic features associated with its imbalance expression. It is also crucial to investigate other physiological effects that could emerge accompany DYRK1A inhibition, especially in cancer patients.

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