REVIEW

Minibrain-related kinase/dual-specificity tyrosine-regulated kinase 1B implication in stem/cancer stem cells biology

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Author contributions: Kokkorakis N and Gaitanou M wrote the manuscript; All authors reviewed, edited and approved the final manuscript.

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Abstract

Dual-specificity tyrosine phosphorylation-regulated kinase 1B (DYRK1B), also known as minibrain-related kinase (MIRK) is one of the best functionally studied members of the DYRK kinase family. DYRKs comprise a family of protein kinases that are emerging modulators of signal transduction pathways, cell proliferation and differentiation, survival, and cell motility. DYRKs were found to participate in several signaling pathways critical for development and cell homeostasis. In this review, we focus on the DYRK1B protein kinase from a functional point of view concerning the signaling pathways through which DYRK1B exerts its cell type-dependent function in a positive or negative manner, in development and human diseases. In particular, we focus on the physiological role of DYRK1B in behavior of stem cells in myogenesis, adipogenesis, spermatogenesis and neurogenesis, as well as in its pathological implication in cancer and metabolic syndrome. Thus, understanding of the molecular mechanisms that regulate signaling pathways is of high importance. Recent studies have identified a close regulatory connection between DYRK1B and the hedgehog (HH) signaling pathway. Here, we aim to bring together what is known about the functional integration and cross-talk between DYRK1B and several signaling pathways, such as HH, RAS and PI3K/mTOR/AKT, as well as how this might affect cellular and molecular processes in development, physiology, and pathology. Thus, this review summarizes the major known functions of DYRK1B kinase, as well as the mechanisms by which DYRK1B exerts its functions in development and human diseases focusing on the homeostasis of stem and cancer stem cells.

Key Words: Dual-specificity tyrosine-regulated kinase 1B; Minibrain-related kinase; Stem cells; Cancer stem cells; Quiescence; Cell proliferation/differentiation; Cell survival; Cancer; Hedgehog

Core Tip: Dual-specificity tyrosine phosphorylation-regulated kinase 1B (DYRK1B), also known as minibrain-related kinase (MIRK) is the well-studied member of the DYRK kinase family. DYRK1B is a key regulator of signaling pathways that control proliferation and differentiation, and is critical for developmental processes and cell homeostasis. In this review, we aim to bring together what is known about the functional integration and cross-talk between DYRK1B and several pathways, such as sonic hedgehog, RAS and PI3K/mTOR/AKT pathways and how this might affect the behavior of stem cells in development and disease, taking into consideration potent therapeutic interventions and approaches.

INTRODUCTION

Stem cells have the potential to self-renew and therefore to perpetuate their lineage, to give rise to progeny capable of differentiating into specialized diverse cell types[1,2] interacting with environmental stimuli in order to maintain the balance between quiescence, proliferation, and restoration[3,4]. All these properties are comprised in the term "stemness". Specific stimuli induce the emergence of new stem cells, as cells that maintain the capacity to re-differentiate and return to an earliest state of development[5-7]. Stem cells can be divided into two broad type categories: embryonic stem cells (ESCs), which are derived from the inner cell mass of the blastocyst and are pluripotent, maintaining the ability to differentiate into any cell type[8-10] and adult (somatic) stem cells being present at niches in adult tissues[11] , which are multipotent, having the ability to differentiate into a limited number of cell lineages and therefore to enable the healing, growth, and replacement of cells that are lost each day of life[10].

Although adult stem cells exhibit regenerating properties when participating in tissue homeostasis, cancer stem cells (CSCs) behave as their malignant counterparts. CSCs were first identified in the acute myeloid leukemia[12,13] and are similar to normal stem cells, which have the ability of self-renewal and differentiation into other cell types[12,14]. CSCs display stemness during cancer progression and through interaction with their environment[15,16]. CSCs constitute a subpopulation of tumor bulk and are considered as the primary tumor-initiating cells. Tumor heterogeneity derived from CSCs and their progeny is considered as a major disadvantage of their roles in cancer therapies. However, scientific evidence from studies investigating the biology of CSCs, will open up new perspectives for the development of novel therapeutic interventions and elimination of cancer recurrence. Further studies have demonstrated that CSCs have a key role in resistance to cancer therapies, such as chemotherapy and radiation therapy, and increased risk of metastatic potential[13,17].

DYRK kinases (for dual-specificity tyrosine-(Y)-phosphorylation-regulated kinases) comprise a family of protein kinases which are key regulators of signal transduction, cell proliferation, survival, and differentiation[18]. DYRKs belong to the CMGC group of proline-directed serine/threonine kinases that are characterized by their ability to phosphorylate tyrosine, serine and threonine amino acid residues[19-21]. To acquire their catalytic activity, DYRKs require phosphorylation at the second tyrosine residue of a conserved YxY motif, located at their activation loop; thus, DYRKs activate themselves during their translation by intramolecular auto-phosphorylation[22]. In particular, a characteristic sequence motif DYRK homology box (known as the DH box), which is located at the N-terminal of the catalytic domain, supports the auto-phosphorylation of the conserved tyrosine during maturation of the catalytic domain[18,21]. DYRK family members have been found in all eukaryotes and constitute an evolutionarily conserved family of protein kinases, which are key players in the regulation of cell cycle and differentiation, regulation of transcription, protein stability and apoptosis, through the phosphorylation of DYRK recognition sites in several target proteins[21,23,24]. There are five members within the mammalian DYRK kinase family: Class I (or DYRK1 group), consisting of DYRK1A and DYRK1B; and Class II (or DYRK2 group), consisting of

DYRK2, DYRK3 and DYRK4[18,21]. Notably, DYRK kinases act as priming kinases, phosphorylating a residue and allowing for additional phosphorylation of a second residue by a subsequent kinase[21].

DYRK1B (also referred to as MIRK; minibrain-related kinase) is closely related to the *DYRK1A* gene, while *minibrain* (*mnb*) is their orthologous gene in Drosophila. The *mnb* gene was named according to the brain phenotype of the mutant flies[21,25]. Disruption of *mnb* causes abnormal arrangement of neuroblasts in the outer proliferation layers of the larval brain, resulting in adult flies with smaller optic lobes and brain hemispheres, suggesting that *mnb* is required for proper proliferation of neuroblasts during larval development and that it plays an essential role during neurogenesis[21,25,26]. These morphological alterations in mutant flies are associated with specific behavioral abnormalities in learning, memory, and visual and olfactory tasks[21,25]. While DYRK1A plays a role in neuronal development[27-34], DYRK1B has a critical role in skeletal muscle differentiation, in spermatogenesis and in cancer *via* its regulatory effects on cell cycle progression and differentiation, cell survival, motility, and transcription[21,23,24]. Recently, we revealed a novel role for DYRK1B in neuronal development, as we will discuss below[35] (Kokkorakis *et al*, 2020 unpublished data).

Here, we will review DYRK1B physiological and pathological roles and its implication in stem/CSC biology, as well as DYRK1B cross-talk with major signaling pathways and the mechanisms by which DYRK1B exerts its function.

DYRK1B EXPRESSION, INTRACELLULAR LOCALIZATION, AND UPSTREAM REGULATION

DYRK1B is normally expressed at high levels in skeletal muscle and testis with increased relative expression in cardiac muscle and brain compared to other normal tissues[24,36]. DYRK1B is overexpressed in various solid tumors and cancer cell lines, where it seems to act as a tumor survival factor[24]. The human DYRK1B gene is located on chromosome 19q13.2, a region often amplified in ovarian and pancreatic cancer[37-42]. DYRK1B is especially highly expressed in colon carcinoma[36,43,44], prostate carcinoma[24,45-47], lung cancer[45], pancreatic ductal adenocarcinomas[37,48-52], rhabdo-myosarcomas[53], osteosarcoma[54], and liposarcoma[55]. Also, overexpression of DYRK1B has been reported in breast cancer[56-58], cervical cancer[59,60], and mela-noma[61,62].

Leder and colleagues[63] have characterized three splicing variants of mouse DYRK1B DYRK1B-p65, DYRK1B-p69 and DYRK1B-p75 with discrete expression patterns and enzymatic activities. DYRK1B-p65 and DYRK1B-p69 display similar expression patterns, where the highest expression of both isoforms has been detected in the murine spleen, lung, brain, bladder, stomach, and testis. In contrast, DYRK1B-p75 was observed specifically in skeletal muscle, as well as in the neuronal cell line GT1-7 and in differentiated adipocyte-like 3T3-L1 but not in non-differentiated 3T3-L1 preadipocyte cells[63].

Notably, DYRK1B-p65 differs from DYRK1B-p69 by the absence of 40 amino acids within its catalytic domain, resulting in a lack of kinase activity[63]. The amino acid sequence of the DYRK1B kinase domain is 56% identical with the other DYRK family kinases; however, the N- and C-termini are non-conserved[24]. The functional domains in DYRK1B kinase are the DH box, located at the N-terminus of the catalytic domain and supporting auto-phosphorylation[26,64,65], a bipartite nuclear localization signal (NLS) located at the non-conserved N-terminus, 11 canonical kinase subdomains followed by a proline, glutamate, serine, threonine (commonly known as PEST) sequence, considered to act as a degradation signal for rapidly metabolized proteins and as a consensus sequence for mitogen-activated protein kinases' (MAPKs) phosphorylation[24,66].

In agreement, DYRK1B shows a predominant nuclear localization in various cell lines, whereas a major cytosolic staining is observed in adult human muscle fibers, rhabdomyosarcoma, and pancreatic ductal carcinomas[21,43]. Our group has observed the nuclear localization of DYRK1B in all neuronal lineage precursors, as well as in post-mitotic neurons and glial cells of embryonic and postnatal mouse brain and spinal cord (Kokkorakis *et al*, 2020 unpublished data). In mouse neuroblastoma Neuro2A cells, when DYRK1B is transiently expressed alone, it is localized into the nucleus, and when it is co-expressed with its interacting partner, the scaffolding protein RanBPM, DYRK1B is relocated and subsequently degraded in the cytoplasm[35]. It has been proposed that the differential intracellular localization of DYRK1B is associated with its discrete roles, *e.g.*, in the nucleus as a negative regulator of cell cycle progression and in the cytosol as a prosurvival factor[67-69].

DYRK1B kinase is subjected to a high degree of regulatory control at transcriptional, translational and post-translational level, *via* activating and inactivating phosphorylations that result in DYRK1B subcellular relocalization, protein stability, and in its participation in discrete protein-protein interactions[24]. The regulation of DYRK1B expression and activity has been studied in myoblasts and in cancer cell lines. In cultured C2C12 myoblasts, mitogen deprivation increased DYRK1B protein levels through transcriptional mechanisms regulated by small Rho GTPases, RhoA and Cdc42, and by Rac1, but not by MyoD or Myf5[24,70].

Additional studies have shown that DYRK1B is a mitogen-activated protein kinase, down-regulated by activated extracellular signal-regulated kinases (ERKs). It was shown that DYRK1B levels increased 20-fold when ERK activation was blocked by the MEK inhibitor PD98059 in colon carcinoma cell lines. PD98059 inhibitor also activated a DYRK1B promoter construct[70]. Therefore, DYRK1B induction seems to require not only active Rho proteins but also the inhibition of the MEK1-ERK signaling pathway. In accordance, DYRK1B is strongly up-regulated under conditions of mitogen deprivation, *e.g.*, when insulin-like growth factor-1 (IGF-1) is eliminated in a colon carcinoma culture[36], also suggesting that DYRK1B is a stress-activated kinase, negatively regulated by the RAS-MEK-ERK pathway[71] (Figure 1). Moreover, knockdown of DYRK1B by small interfering RNA (commonly referred to as siRNA) performed in human ovarian cancer cell lines, led to up-regulated activation of c-Raf-MEK-ERK1/2 pathway and subsequent changes in cell cycle proteins, such as cyclin D1 and p27^{Kip1}, that are accompanied by increased growth rate and re-entering of cancer cells from the G1/G0 phase into the S phase of the cell cycle[72]. The cell cycle transition could be blocked by MAPK/ERK inhibitor U0126 in a dose-dependent manner, suggesting that DYRK1B and the MAPK/ERK pathway inhibit each other[42,72] (Figure 1).

In another study, it was found that DYRK1B competes with the stress-activated MAPK kinase p38 for their common activator, the MAPK kinase MKK3[71]. DYRK1B is activated *in vivo* by MKK3, while p38 seems to be required for terminal muscle cell differentiation[70]. C2C12 myoblasts expressing a MKK3 dominant negative failed to fuse into myotubes[70]. The lack of MKK3 activity, by the usage of a MKK3 dominant negative, resulted in decreased expression of MyoD and myogenin that are transcriptional targets of DYRK1B and in blocked expression of the late differentiation markers troponin T, myosin heavy chain (commonly known as MHC), and the Cdk inhibitor p21^{Cip1}[70]. In addition, p38 blocks DYRK1B transactivation of the transcription factor HNF1α[24,73]. A possible mechanism concerning the *in vivo* interaction between p38 and DYRK1B was suggested from results of cell cycle synchronization experiments in NIH3T3 cells, where DYRK1B levels fluctuated within the cell cycle, whereas p38 levels remained stable, leading to speculation that endogenous p38 can block DYRK1B function, only when DYRK1B levels are low in S phase and not when DYRK1B levels are elevated during G1/G0 transition[73] (Figure 1).

DYRK1B KINASE FUNCTIONS

DYRK1B is a multifunctional dual-specificity kinase involved in growth arrest, differentiation, and cell survival. The two major functions of DYRK1B are the G1/G0 transition and the subsequent growth arrest in a quiescent state (G0), as well as the maintenance of cell viability[42,69,70]. DYRK1B function is implicated in myogenesis, during which myoblasts are differentiated into skeletal muscle cells[69,70,74]. Moreover, DYRK1B participates in muscle regeneration after injury, promoting the activation of quiescent muscle stem cells, also known as satellite cells[70]. In addition, DYRK1B is involved in fat cell differentiation, from adult mesenchymal stem cells[75] to preadipocytes that differentiate into adipocytes during adipogenesis, and is linked to metabolic syndrome[76]. Furthermore, DYRK1B negatively regulates proliferation of immature male germ cells of the seminiferous epithelium, also called spermatogonial stem cells[77], during spermatogenesis[78].

Recently, our studies revealed a novel role for DYRK1B in neurogenesis[35] (Kokkorakis *et al*, 2020 unpublished data). We have previously demonstrated that DYRK1B overexpression promotes cell cycle exit and neuronal differentiation in mouse neuroblastoma Neuro2A cells by phosphorylating cyclin D1, followed by its cytoplasmic relocation and its subsequent degradation by the 26S proteasome[35]. We have also shown that the negative effect of DYRK1B in Neuro2A proliferation is reversed when DYRK1B is co-expressed with its interacting partner, the scaffolding protein RanBPM that inhibits DYRK1B function by facilitating its proteasomal
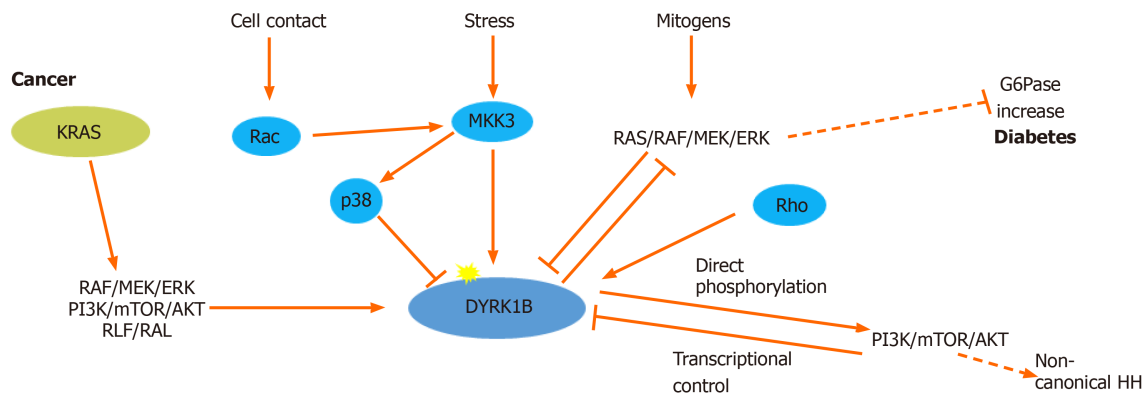
Figure 1 Regulation of dual-specificity tyrosine-regulated kinase 1B expression and activity. Dual-specificity tyrosine-regulated kinase 1B (DYRK1B) expression and activity is regulated at transcriptional, translational and post-translational level. Rho GTPases (RhoA, Cdc42 and Rac1) promote transcriptional up-regulation of DYRK1B, while serum mitogens down-regulate DYRK1B through RAS/RAF/MEK/extracellular signal-regulated kinases (ERK) signaling pathway. Under stress conditions, MKK3 activates DYRK1B and p38, while DYRK1B is physically sequestered and inhibited by p38. In cancer, DYRK1B is involved in a complex crosstalk with hedgehog (HH). Oncogenic mutant RAS (KRAS) initiates the non-canonical HH pathway through the activation of DYRK1B, *via* an unknown mechanism, employing several RAS effectors, such as: RAF/MEK/ERK, PI3K/AKT and RLF/RAL. DYRK1B enhances non-canonical HH signaling by promoting PI3K/mTOR/AKT signaling. Conversely, activated AKT directly inhibits expression of DYRK1B. In metabolic syndrome, which is accompanied by diabetes, DYRK1B is implicated in glucose homeostasis, promoting the expression of the key gluconeogenic enzyme glucose-6-phosphatase (G6pase), through inhibition of the RAS–RAF–MEK pathway. Dashed lines represent indirect mechanisms and yellow stars represent phosphorylations. ERK: Extracellular signal-regulated kinases.

decay[35]. In addition, we have demonstrated that the tripartite functional interactions between DYRK1B, RanBPM and the neuronal protein Cend1 (for cell cycle exit and neuronal differentiation 1; also known as BM88) regulate the balance between cellular proliferation and differentiation in Neuro2A cells, suggesting that the three proteins may also play a similar role in cell cycle progression/exit and differentiation of neural stem cells/neural progenitor cells (NSCs/NPCs) during neurogenesis[35,79]. In agreement, we recently found that DYRK1B is expressed during central nervous system (CNS) development and marks all along the neuronal lineage (Kokkorakis *et al*, 2020 unpublished data). We have also found that DYRK1B promotes *in vitro* and *in vivo* cell cycle exit and neuronal differentiation in neuronal precursors, suggesting a role in NSCs' differentiation (Kokkorakis *et al*, 2020 unpublished data).

Notably, excepting its physiological roles, DYRK1B has a significant role in tumorigenesis and cancer progression[43,52,80], as well as in the maintenance of stemness in CSCs[81]. Below, we will describe in detail the known functions of DYRK1B kinase in various tissues and cell lines in health and in disease.

DYRK1B mainly acts as a cell cycle regulator

DYRK1B acts as a G0 checkpoint kinase and its levels are highly increased in G0-arrested non-dividing cells, such as serum-starved NIH3T3 fibroblasts[82] and myoblasts, committing terminal differentiation through cell cycle exit[70]. In cycling myoblasts, DYRK1B protein levels are very low, whereas they are increased at least 10-fold when myoblasts undergo terminal differentiation and are maintained high in the mature muscle cells[70]. Moreover, DYRK1B levels are strongly up-regulated in NIH3T3 fibroblasts that are cell cycle arrested by overexpression of the growth arrest specific 1 GAS1 protein[83]. In agreement, depletion of DYRK1B by RNA interference (RNAi) enables G0-arrested NIH3T3 fibroblasts and C2C12 myoblasts to re-enter the cell cycle, whereas transient overexpression of DYRK1B arrests dividing cells at G0[70,82,84].

Except in fibroblasts and myoblasts, DYRK1B is highly expressed in testis, where it negatively regulates proliferation of immature male germ cells[77]. In addition, DYRK1B is strongly up-regulated in solid tumors and carcinoma cell lines, as mentioned above. Depletion or inhibition of DYRK1B promotes cell cycle re-entry of quiescent cancer cells, indicating that DYRK1B is sufficient to maintain cancer cells in a quiescent state[80]. DYRK1B promotes the maintenance of G0 arrest of differentiating non-transformed myoblasts, NIH3T3 fibroblasts and Mv1Lu epithelial cells by post-translational mechanisms[24]. The regulation of cell cycle by DYRK1B is achieved by the phosphorylation of cell cycle regulators, such as cyclin D isoforms and p27^{Kip1}, leading to their degradation and stability, respectively. Especially, DYRK1B binds to GSK3β, and this kinase complex phosphorylates cyclin D1 at two adjacent conserved ubiquitination sites as follows: DYRK1B at Thr288 and GSK3β at Thr286, respectively, destabilizing and leading cyclin D1 to proteasomal degradation in the cytoplasm[35,84].

Also, DYRK1B stabilizes the cyclin-dependent kinase (CDK) inhibitor p27[Kip1] by phosphorylation at Ser10. Phosphorylation of p27[Kip1] prevents its cytoplasmic translocation and its subsequent proteasomal degradation[23,82,84,85].

The implication of DYRK1B and DYRK1A in the regulation of cell cycle occurs *via* an additional mechanism involving the DREAM complex which consists of MuvB, RB2, E2F4 and DP proteins. Both kinases activate the DREAM complex by phosphorylating LIN52, a subunit of MuvB at the Ser28 residue[86]. The DREAM complex is a major coordinator of the cell cycle and is essential to maintain the quiescent state[87]. LIN52 may be involved in long-term regulation of cell fate[80,88] (Figure 2).

Prosurvival function of DYRK1B in myogenesis and cancer

In a large-scale RNAi screen using HeLa carcinoma cells, DYRK1B and DYRK3 have been identified as prosurvival kinases[59]. In another study, Mercer and colleagues[69] demonstrated that DYRK1B blocks apoptosis through phosphorylation of p21[Cip1], which occurs during the differentiation of C2C12 myoblasts. DYRK1B diminishes the extent of myoblast apoptosis through phosphorylation of p21[Cip1] at the nuclear localization domain, resulting in its cytoplasmic relocation and rendering p21[Cip1] unable to mediate cell cycle arrest[68,69]. The DYRK1B-induced change in p21[Cip1] intracellular localization accompanies myoblast differentiation. Endogenous p21[Cip1] is localized exclusively to the nuclei of proliferating myoblasts, whereas it is relocated at the cytoplasm of post-mitotic multinucleated myotubes and adult human skeletal myofibers. The p21[Cip1] cytoplasmic portion forms a physical complex with the apoptosis signal-regulating kinase 1 (known as ASK1), an upstream activator of the caspase cascade, which blocks apoptosis through the inhibition of caspase 3[67,69]. In agreement, knockdown experiments of endogenous DYRK1B by RNAi in C2C12 myoblasts resulted in decreased myoblast survival by 75%, whereas transient overexpression of DYRK1B increased cell viability[69].

Moreover, depletion of DYRK1B by RNAi or pharmacological inhibition of DYRK1B kinase activity impairs cell survival and induces apoptosis, in many cancer cell lines[39,48,53,58,89]. This is concomitant with the increased intracellular levels of reactive oxygen species (ROS) in pancreatic cancer[49,50,52], ovarian cancer[41,51,89], colon cancer[51], and osteosarcoma[90]. Increased ROS levels that followed DYRK1B depletion are accompanied with DNA damage, as indicated by phosphorylation of histone 2AX (known as H2AX) at Ser139 by ribosomal S6 kinase 2 (known as RSK2)[91], in pancreatic[49,50,52] and ovarian[41,51,80,89] cancers. In addition, DYRK1B up-regulates expression of several antioxidant genes, *e.g.*, ferroxidase and superoxide dismutases 2 and 3 (known as SOD2 and SOD3 respectively) in cancer cell lines[38,49,51,80]. The increased expression of antioxidant genes may be due to the fact that cancer cells maintain higher ROS levels than normal cells and might, thus, be more sensitive to further accumulation of ROS[80,92]. It has been proposed that targeting of the antioxidant mechanisms of cancer cells and the subsequent increase in intracellular cellular ROS levels may be a potential strategy for anticancer therapies[93]. Thus, depletion or pharmacological inhibition of DYRK1B will sensitize cancer cells to chemotherapeutic drugs, such as cisplatin, that increase ROS levels[38,45].

A third mechanism concerning the role of DYRK1B in cell survival, except p21[Cip1] intracellular distribution and regulation of ROS levels, includes the nuclear exclusion and subsequent inactivation of NKX3.1 and the forkhead box O (FOXO) transcription factors, FOXO1 and FOXO3A[72,80,94], mediated by DYRK1B phosphorylation. NKX3.1 and FOXO factors act as tumor suppressors in several cancers by suppressing cell proliferation and promoting apoptosis[95]. DYRK1B abolishment was shown to enhance nuclear translocation of FOXO1 and FOXO3A and increase apoptosis in ovarian cancer cells[72,80] (Figure 2).

DYRK1B maintains quiescence in CSCs

In cancer tumors, there is a subpopulation of cells that possess stemness features. These CSCs, also known as the tumor-initiating cells, are cells capable of maintaining themselves *via* self-renewal and restoration[96]. CSCs are responsible for tumor growth and metastasis, making them a prime target for efficient therapeutic interventions. However, CSCs are extremely resistant to current therapeutic approaches, implicating them as the main reason for cancer recurrence[97]. Stem cells from various cancers have been reported to be often quiescent[98]. The relative resistance of CSCs to chemo- and radio-therapies, both targeting dividing cells, is due to the ability of some CSCs to remain in a non-dividing quiescent state (G0)[42,97,99]. A portion of pancreatic cancer cells out of the cell cycle may be post-mitotic, while other pancreatic cancer cells out of cell cycle seem to be in a quiescent, reversible G0 state, thus remaining resistant to drugs
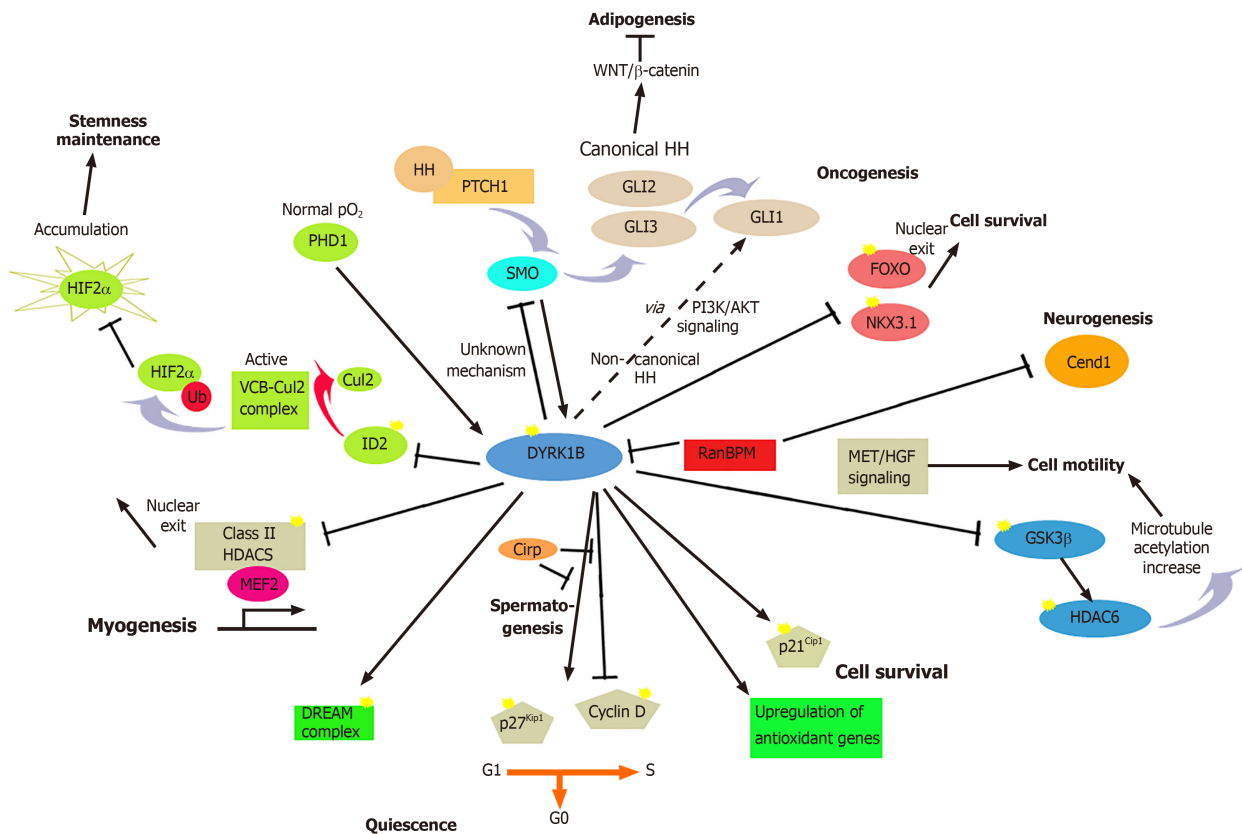
Figure 2 Summary of the major known functions of dual-specificity tyrosine-regulated kinase 1B in development and disease. Dual-specificity tyrosine-regulated kinase 1B (DYRK1B) plays a critical role in many biological processes in development and human diseases, regulating cell cycle progression/exit, differentiation, transcription, and cell survival and motility. DYRK1B facilitates growth arrest and promotes quiescence (G0) by stabilizing cyclin-dependent kinase inhibitor p27^{Kip1} and destabilizing cyclin D1, *via* phosphorylation. The anti-proliferative function of DYRK1B occurs in myogenesis, spermatogenesis, neurogenesis, and cancer. Moreover, DYRK1B maintains quiescence by stabilization of the DREAM complex *via* phosphorylation of LIN52, a subunit of MuvB in the complex. In oncogenesis, DYRK1B induces degradation of the tumor suppressor NKX3.1, while reducing the activity of tumor suppressors and apoptotic promoters of the forkhead box O (FOXO) family, resulting in survival of cancer cells and enhancement of oncogenic GLI1 activity. The prosurvival function of DYRK1B in myogenesis and cancer is mediated through phosphorylation of p21^{Cip1}, DYRK1B counteracts oxidative stress by reducing intracellular levels of oxygen reactive species (ROS) through the up-regulation of antioxidant genes. DYRK1B modulates stemness of cancer stem cells. In normoxia, oxygen-sensing prolyl-hydroxylase (PHD1) activates DYRK1B, which inactivates ID2, makes it unable to displace the Cul2 component from the VCB-Cul2 ubiquitin ligase complex, which remains active, promoting HIF2α degradation. In hypoxia, PHD1 and DYRK1B are inactivated, leading to activated ID2 and resulting in HIF2α accumulation that facilitates cancer stem cell maintenance. DYRK1B is involved in a complex cross-talk with hedgehog (HH). DYRK1B inhibits canonical HH signaling initiated by Smoothened (SMO), while it promotes non-canonical HH signaling by promoting PI3K-AKT-mediated stability of the GLI1 transcription factor. In metabolic syndrome, DYRK1B inhibits sonic hedgehog (SHH) and Wnt signaling, enhancing adipogenesis. In spermatogenesis, DYRK1B interacts with cold-inducible RNA-binding protein (Cirp), resulting in destabilization of p27^{Kip1} and cyclin D1 stabilization that promote cell cycle progression of undifferentiated spermatogonia. In myogenesis, DYRK1B inactivates Class II histone deacetylases (HDACs), resulting in myogenic regulatory factor (MEF) 2-dependent transcription of myogenic genes. In neurogenesis, tripartite functional interactions between DYRK1B, RanBPM and the neuronal protein Cend1 regulate the balance between cellular proliferation and differentiation. Increased levels of DYRK1B block cell motility through interaction with the adaptor protein RanBPM and Met/HGF signaling. DYRK1B modifies indirectly the microtubules, through phosphorylation of GSK3β and subsequent inactivation of HDAC6, leading to increase of microtubules acetylation. Dashed lines represent indirect mechanisms and yellow stars represent phosphorylations. ERK: Extracellular signal-regulated kinases.

and able to repopulate the tumor[11]. DYRK1B kinase could be of clinical relevance, since it is included among the factors which allow the survival of quiescent CSCs *in vivo*[98]. As we have mentioned above, DYRK1B is highly expressed in several cancers and it was found to be amplified or hyperactive in ovarian and pancreatic cancers[18,37,40]. Experimental data suggest that increased levels of DYRK1B are related to tumor development and poor outcome[43]. Also, it is known that the orthologous DYRK1A kinase is implicated in cancer, with a partially similar role as that of DYRK1B[100-109]. DYRK1B has been shown to confer on CSCs the ability to remain in a quiescent state, in such a way that when exposed to therapeutic agents/drugs (chemotherapy) or radiation (radiotherapy), making them chemo- and radio-resistant by controlling the balance between quiescence and apoptosis[36,53,56,106,110]. Thus, the wake-up of quiescent CSCs may be achieved using DYRK1B pharmacological inhibitors, which could serve as potent drugs in cancer therapy[43,80]. Moreover, DYRK1B may be used as a diagnostic marker and survival factor for various types of human cancer[43].

DYRK1B regulates the maintenance of CSCs under hypoxia or normoxia

The stemness capacity of stem cells depends on the balance of complex signals in their microenvironment[111]. It has been suggested[111,112] that stem cells are localized in a microenvironment of low oxygen, indicating that hypoxia may be a critical factor for stem cell maintenance and that low oxygen tension in cell culture has positive effects on the survival and self-renewal of stem cells. Furthermore, a hypoxic microenvironment assists in maintaining the multipotency of ESCs[113], the undifferentiated state of hematopoietic, mesenchymal and NSCs, and in the regulation of proliferation and cell-fate commitment[111].

Recent advances in cancer research have indicated that mechanisms maintaining CSCs are crucial to tumor progression[112]. Hypoxia is the most critical factor for the maintenance of stemness, as well as the enhanced expression/activation of hypoxia-inducible factors (HIFs), which frequently occurs in cancer cells during cancer progression. The enhanced expression and activation of HIFs is associated with the acquisition of cancer cells with a more malignant behavior, treatment resistance and poor outcome for cancer patients. HIF1α and HIF2α are transcription factors that act as key mediators of the adaptation of CSCs to oxygen and nutrient deprivation, during cancer progression, under normoxic and hypoxic conditions[81]. Especially, the HIF2α transcription factor is required for maintenance of CSCs.

Another protein that participates and promotes the cancer hallmarks, including CSC state, is the ID2 protein. However, the pathways that are engaged by ID2 or drive HIF2α accumulation in CSCs still remain unclear[81]. DYRK1B modulates stemness of CSCs through a mechanism taking place under normoxia or hypoxia conditions. In normoxia, oxygen-sensing prolyl-hydroxylase (PHD1) activates DYRK1B, which inactivates the ID2 protein by phosphorylation at Thr27[81], making it unable to displace the VHL-associated protein cullin-2 (Cul2) component from the VCB-Cul2 ubiquitin ligase complex, which remains active and capable of promoting HIF2α degradation[81] (Figure 2). In contrast, in hypoxia conditions, PHD1 and DYRK1B are inactivated, leading to activated ID2[81]. Then, the activated ID2 binds to the VHL ubiquitin ligase complex, displacing the Cul2 and subsequently impairing HIF2α ubiquitination and degradation. Thus, HIF2α stabilization facilitates CSC maintenance and increases the aggressiveness of human hypoxic brain tumors[81]. In glioblastoma cell lines, under hypoxia conditions, ID2 positively modulates HIF2α activity and, conversely, in normoxia conditions, the elevated expression of DYRK1A/1B phosphorylates ID2, promoting HIF2α destabilization, inhibition of tumor growth, loss of glioma stemness and a more favorable prognosis for patients with glioblastoma[81] (Figure 2).

MOLECULAR MECHANISMS OF DYRK1B FUNCTION IN DEVELOPMENT AND HUMAN DISEASES

As we have discussed above, DYRKs comprise a family of kinases which are key regulators of signal transduction, cell proliferation, survival, and differentiation[18]. In particular, DYRK1B is a multifunctional dual-specificity kinase involved in cell cycle progression, differentiation and cell viability[42,69,70], and plays key roles in a variety of physiological developmental processes during myogenesis[69,70,74], spermatogenesis[77], neurogenesis[35] and cell motility[55], as well as in as in human diseases, such as cancer[24,36,39,41,50] and metabolic syndrome[76].

Identification of molecular mechanisms that regulate signaling pathways, through which DYRK1B exerts aspects of its function, in a positive or negative manner, in development and human diseases, is therefore of great interest. Many studies have identified a close regulatory link between DYRK1B and the hedgehog (HH)/GLI signaling pathway, which is essential during development, stem cell maintenance and cell differentiation, and also plays a crucial role in development of many malignancies[114-121]. Below, we will discuss in detail the functional integration and cross-talk between DYRK1B and the HH/GLI with other signaling pathways, such as RAS and PI3K/mTOR/AKT, and how this might affect cellular and molecular processes in development, physiology and pathology, focusing on the homeostasis of stem and CSCs, respectively.

DYRK1B implication in HH/GLI signaling in cancer

Elucidation of molecular mechanisms that determine the characteristics of malignancies of CSCs is of great importance. An essential signaling pathway during

mammalian embryonic development, involved in proper tissue patterning, stem cell maintenance and cell differentiation, is the HH/GLI signaling pathway, which also plays a crucial role in tumorigenesis, in development of many pediatric and adult malignancies, such as those of pancreas, lung, prostate, brain, and skin[114-121]. The 'canonical' HH signaling cascade is initiated in the target cell by the HH ligand binding to the Patched receptor (PTCH1, 2), which is located at the primary cilium, functioning as an antenna-like cell compartment and relieving the repression of Smoothened (SMO) transmembrane protein, a member of the G protein-coupled receptor superfamily. Subsequently, SMO enters the primary cilium and initiates signaling by activating the zinc finger transcription factors, GLI2/3, which are released from Suppressor of Fused (SUFU), in order to translocate into the nucleus and initiate the transcription of HH/GLI target genes, including the *GLI1* oncogene. In the absence of HH ligand, the PTCH represses HH signaling by preventing SMO translocation to the primary cilium, resulting in the inactivation of the GLI effectors[122-128] (Figure 2).

Transcriptional feedback loops take place in HH signaling in order to fine-tune the entire system. Additional modulation of HH signaling is achieved by several kinases, such as PKA, PKC, GRK2, MEK, ERK, AKT, S6K, and GSK3β[129-138]. Cancer cells often take advantage of these mechanisms in non-canonical modes of signaling, such as HH ligand/receptor-independent activation of GLI transcription factors[18,118,135,139,140]. The mammalian DYRK1A, DYRK1B and DYRK2 participate in the regulation of HH signaling. Notably, DYRK1A possesses an activating function on GLI1, promoting GLI1 nuclear translocation[141,142] *via* its direct phosphorylation at Ser102/104/130/132 residues located at NLS[143] and at Ser408[144]. Moreover, DYRK1A exerts a negative function by inducing GLI1 degradation through an indirect mechanism that engages the actin cytoskeleton and its regulators[143]. The dual role of DYRK1A in the regulation of HH signaling is probably due to its interactions with different sets of protein partners that have opposing effects[138]. On the other hand, DYRK2 has been shown to negatively affect the HH pathway by directly phosphorylating GLI2 at two conserved serine residues, Ser385 and Ser1011, inducing its proteasomal decay[138,145].

DYRK1B inhibits canonical HH signaling / cross-talk between RAS and HH signaling: DYRK1B has a complicated role in modulation of the HH pathway. A cell-autonomous synergism between RAS and GLI oncogenes during tumor formation in the pancreas, lung and colon carcinomas has been reported[114-116,118]. DYRK1B can inhibit HH signaling by blocking GLI2, which mainly functions as an activator, and by promoting GLI3R formation, which mainly functions as a repressor[119]. Specifically, Lauth and colleagues[119], studying the cross-talk between RAS and HH signaling, found that oncogenic mutant RAS (KRAS) acts as: (1) an inducer of sonic hedgehog (SHH) expression; (2) a potent inhibitor of the canonical (HH-PTCH-SMO-initiated) HH pathway; and (3) a regulator of the non-canonical (TGFβ-initiated) HH pathway *via* activation of DYRK1B. Consequently, mutant KRAS induces signaling to neighboring cells (paracrine effect), while at the same time inhibits HH signaling into the cells (autocrine effect), thereby initiating the non-canonical HH pathway by increasing DYRK1B expression through an unknown mechanism. Increased DYRK1B blocks the SMO-induced cascade but is ineffective in cells lacking SUFU, mimicking the effect of mutated KRAS (Figure 2). Lauth *et al*[119] suggest that mutant KRAS blocks signaling upstream of SUFU through DYRK1B or that the SMO inhibition is actually SUFU-dependent. Moreover, DYRK1B is not as effective as KRAS in the inhibition of HH signaling, suggesting that RAS also activates inhibitory unknown effectors in addition to DYRK1B.

The inhibitory mechanisms initiated by KRAS may be complex, as several RAS effectors, such as RAF/MEK/ERK, PI3K/AKT and RLF/RAL, are required for full inhibition (Figure 1). Remarkably, DYRK1B kinase can be activated by several RAS effectors, such as Tiam/Rac1, MEK, PI3 kinase and possibly RAL-A[37,119]. The biological significance of the RAS-mediated HH inhibition in cancer cells, with the participation of DYRK1B, could explain why high GLI1 levels are detrimental to normal but not to CSCs in the brain[119,146]. Blocking autocrine in favor of paracrine HH signaling may enhance the survival of early CSCs. In addition, the presence of RAS-DYRK1B-HH regulatory network has an important impact in developmental disorders caused by aberrant RAS signaling, such as Noonan, cardio-facio-cutaneous and Costello syndromes[147]. To summarize, the mutant RAS (*i.e.* KRAS) is a cell-autonomous negative regulator of the HH pathway, participating also in the paracrine HH signaling in lung and pancreatic cancers that have accumulated KRAS mutations. Thus, the mechanism of shift from autocrine towards paracrine signaling mechanisms involving the RAS effector kinase DYRK1B remains to be elucidated[119].

DYRK1B enhances non-canonical HH signaling: DYRK1B has been shown to increase GLI1 activity, whereas DYRK1B inhibition down-regulates GLI1 expression[121]. Gruber and colleagues[121], using pancreatic adenocarcinoma (PANC-1) and Ewing sarcoma cell lines, identified DYRK1B as a critical positive regulator of HH/GLI signaling downstream of SMO (Figure 2). In the DAOY human medulloblastoma cells and mouse embryonic fibroblasts (MEFs) deficient in either PTCH or SUFU, the RNAi knockdown of DYRK1B or its pharmacological inhibition (*e.g.*, with harmine or DYRKi), resulted in remarkable repression of HH signaling and GLI1 expression. Notably, DYRKi inhibitor impairs SMO-dependent and SMO-independent oncogenic GLI activity. These results support the usage of DYRK1B inhibitors for the treatment of HH/GLI-associated cancers, instead of SMO inhibitors which have failed to be efficient for cancer therapy[121]. Furthermore, the addition of the proteasome inhibitor bortezomib reversed the negative effect of DYRK1B inhibition, suggesting that DYRK1B prevents activated GLI1 and GLI2 forms from proteasomal degradation[23,121]. The detailed mechanism of GLI1 and GLI2 protein stabilization by DYRK1B remains unclear, and whether stabilization of GLI1 and GLI2 involves direct phosphorylation by DYRK1B or depends on alternative indirect unknown mechanisms, will be further investigated.

DYRK1B regulates HH signaling/cross-talk between PI3K/mTOR/AKT and HH signaling both in positive and negative manners: Interaction of DYRK1B with the mammalian HH/GLI pathway has dual and opposing effects. On one hand, the ectopic expression of DYRK1B in NIH3T3 cells blocks canonical SMO-initiated signaling, *via* an elusive mechanism; on the other hand, overexpressed DYRK1B enhances the protein stability of GLI1, by preventing its proteasomal degradation. Stabilization of GLI1 is most likely executed through the AKT pathway[137,138], which is activated by DYRK1B. AKT subsequently phosphorylates and protects GLI transcription factors from degradation[130,137,148] (Figures 1 and 2). The exact mechanism of AKT stimulation by DYRK1B is currently unknown[137]. In addition, because DYRK1B activates the PI3K/mTOR/AKT pathway, the DYRK1B-HH/ GLI pathway is subjected to pronounced feedback control[137] (Figures 1 and 2). In agreement, stimulation of the HH pathway by SMO activation increases DYRK1B protein levels, by unknown post-transcriptional mechanisms, suggesting the ability of HH signaling to stimulate AKT phosphorylation[137] (Figure 2). In accordance, pharmacological inhibition of the PI3K/AKT/mTOR pathway, performed in pancreatic cancer cell lines (Panc1, AsPc1, SU86.86, CAPAN2 and BxPc3), resulted in up-regulation of DYRK1B kinase and conversely when AKT signaling was activated the DYRK1B mRNA levels were reduced[51].

The pharmacological inhibition of DYRK1B results in initial up-regulation of HH, followed by down-regulation of AKT phosphorylation, reducing GLI stabilization due to the fact that the PI3K/AKT/mTOR pathway is itself subjected to strong negative feedback regulation[137]. It has been shown that short-term inhibition of DYRK1B by siRNA resulted in an enhancement of HH signaling, whereas long-term blockade of DYRK1B function by short-hairpin RNA resulted in suppression of GLI1 levels in Panc1 cells[137]. Furthermore, the involvement of DYRK1B mutations in metabolic syndrome concerning PI3K signaling is intriguing[76,149,150], as we will mention below. Summarizing, the crosstalk between DYRK1B and HH signaling has to be further elucidated, since the DYRK1B function related to the HH pathway is dependent on DYRK1B expression levels and canonical/non-canonical HH signaling.

DYRK1B regulates cell motility

DYRK1B kinase exerts its function in the regulation of cell motility *via* two discrete mechanisms: HH-induced microtubule (MT) acetylation and Met/ hepatocyte growth factor (HGF) signaling. DYRK1B, through HH signaling, facilitates MT-dependent processes, such as intracellular mitochondrial transport, mesenchymal cell polarization, and directed cell migration. HH signaling affects MT acetylation in mammalian cells[151]. Using the NI3T3, HeLa and MEF cell lines, it has been shown that HH pathway activity increases the levels of the MT-associated acetylation *via* a mechanism in which DYRK1B participates[151]. GSK3β is inhibited by its phosphorylation at Ser9 by DYRK1B, resulting in suppression of HDAC6 enzyme activity. The inhibition of HDAC6, that represents a major tubulin deacetylase, subsequently increases the levels of acetylated MTs. In summary, intercellular communication *via* HH signals can regulate the MT cytoskeleton and contribute to MT-dependent processes by altering the level of tubulin acetylation through DYRK1B activation[151]. This could explain the reason that pancreatic cancer cells overexpressing

DYRK1B have shown resistance to the MT-depolymerizing agent Nocodazole[48].

Except regulation of HH-induced microtubule acetylation, DYRK1B has also been shown to act as an inhibitor of cell motility through its interactions with the Met/HGF signaling pathway. In Mv1Lu mink lung epithelial cells, DYRK1B overexpression inhibited the migration of cells in wound experiments and their invasion through specific polycarbonate filters[152]. Furthermore, the ability of DYRK1B to inhibit Mv1Lu cell migration was attenuated when cells were exposed to HGF or to elevated levels of transiently expressed RanBPM. RanBPM inhibited the kinase activity of DYRK1B. In addition, RanBPM and HGF inhibited the function of DYRK1B as a transcriptional co-activator. These findings suggest that DYRK1B plays a role in modulating cell migration through opposing the action of RanBPM, which is a Met signaling cascade adaptor protein. Met plays an important role in tumor cell invasion and cell migration. RanBPM has been reported to bind to the tyrosine kinase domain of the HGF receptor Met, enhance Met downstream signaling, and enhance HGF-induced A704 kidney carcinoma cell invasion, as a study has demonstrated[153]. Moreover, *DYRK1B* was found to be one of the four most promigratory genes in the highly motile SKOV3 tumor cells by an RNAi screen of > 5200 genes[154]. It is possible that motility induced by oncogenic KRAS was due to DYRK1B activity[37]. In contrast, another study has shown that inhibition of DYRK1B suppresses the proliferation and migration of liposarcoma cells, indicating a positive role for DYRK1B in cell motility[55]. In particular, DYRK1B targeting in liposarcoma cells, with small molecule inhibitor AZ191 or RNAi-mediated knockdown, results in reduction of proliferation, as well as in suppression of cell motility, induction of apoptosis, and sensitization of liposarcoma cells to chemotherapy drugs, indicating that DYRK1B could play a significant role in liposarcoma cell growth and proliferation motility[55].

DYRK1B in metabolic syndrome

Except cancer, very little is known about the implication of DYRK1B in human diseases. Nevertheless, DYRK1B was found to be implicated in a rare autosomal-dominant form of metabolic syndrome, called abdominal obesity metabolic syndrome (or AOMS3) with its two missense mutations H90P and R102C. Affected individuals develop early-onset central obesity, diabetes, coronary artery disease, and hypertension[76]. The overexpression of DYRK1B-H90P or DYRK1B-R102C mutations in HepG2 hepatoma cells resulted in increased induction of glucose-6-phosphatase (G6Pase), a gluconeogenic enzyme, in a dose-dependent manner[76]. In addition, the DYRK1B-R102C mutation enhances the effect of DYRK1B on the adipogenic differentiation of 3T3-L1 preadipocytes[76], as we will discuss below.

Cell-based assays have shown that the mutant alleles behave as gain-of-function variants of DYRK1B[76], although the kinase activity of DYRK1B-R102C mutant was found to be reduced in *in vitro* assays, while the kinase activity of DYRK1B-H90P mutation has not yet been studied[155]. It has been shown that H90P and R102C mutations are located at the N-terminal of DYRK1B catalytic domain[26] and, thus, are unlikely to be directly involved in substrate recognition and catalysis[65]. There is evidence that these mutations affect the DH box, not directly by interfering with the conformation of the catalytic domain, but by interfering with the HSP90 chaperone/CDC379 co-chaperone-mediated maturation of DYRK1B kinase by tyrosine auto-phosphorylation. This perturbs the conformational thermodynamic stability of the catalytic domain, which renders the kinase susceptible to misfolding and resulting in its intracellular aggregation[65]. These findings, described above, point to a role for DYRK1B in adipogenesis and glucose homeostasis, providing a link between DYRK1B altered function and an inherited form of the metabolic syndrome. In agreement, the overexpression of DYRK1B homologues, DYRK1A and MNB in mice and Drosophila, respectively, leads to an increase in food uptake and body weight, and, conversely, their deficiencies are associated with loss of body weight[76,156].

DYRK1B signaling in adipogenic transformation and glucose homeostasis: DYRK1B plays a central role in signaling pathways disrupted in metabolic syndrome, which is another example of cross-talk between DYRK1B and HH, which occurs during differentiation of mesenchymal stem cells into adipocytes[76]. The HH pathway has an inhibitory function on adipocytic differentiation, involving redirecting cellular fate towards the osteogenic lineage[18,157-159]. In contrast, DYRK1B favors differentiation into adipocytes[76]. As we mentioned above, DYRK1B inhibits SHH signaling[119] and its expression is increased dramatically during adipogenic differentiation[63]. Also, it has been demonstrated that inhibition of the SHH pathway results in decreased expression of Wnt proteins[160,161], which are negative regulators of adipogenesis[162].

In a study by Keramati and colleagues[76], elucidation of the mechanism of metabolic

syndrome was achieved by examining the effects of DYRK1B, DYRK1B-R102C and knockdown of DYRK1B during adipogenic differentiation of 3T3-L1 preadipocyte cells, using an adipogenic medium containing the Wnt inhibitor IBMX. This approach revealed that DYRK1B protein inhibits SHH through the reduction of the GLI2 effector and the subsequent reduction of Wnt signaling, resulting in enhanced adipogenesis[76] (Figure 2). Moreover, the DYRK1B-R102C mutation exhibits a maximally strong effect, compared to DYRK1B, in the inhibition of SHH pathway *via* GLI2 effector. Additionally, DYRK1B-R102C mutation suppresses Wnt signaling, revealing its gain-of-function properties[76]. Notably, DYRK1B is a nutrient-sensing protein that inhibits the RAS–RAF–MEK pathway[42], which is responsible for the regulation of glucose uptake and glycolysis[163]. It is known that activation of the MEK pathway results in decreased expression of the *G6Pase* gene and lowers glucose output[164] (Figure 1).

DYRK1B in myogenesis

DYRK1B has strong expression in differentiated skeletal muscle, indicating a physiological role in muscle development and function. DYRK1B is expressed at low levels in most tissues, including dividing myoblasts, while is increased dramatically, at least 10-fold, when myoblasts undergo terminal differentiation and is maintained at elevated levels in mature muscle cells[18,70]. Overexpression of DYRK1B facilitated myoblasts to fuse more rapidly when placed in differentiation medium. DYRK1B favors myoblast fusion and the subsequent expression of differentiation markers, such as myogenin, troponin T and muscle myosin heavy chain; while, its depletion, by siRNA, prevents myoblast fusion into myotubes and inhibits the induction of differentiation markers[70].

Moreover, it is known that induction of DYRK1B within the initial 24 h of myogenic differentiation, enables the transcription of myogenin, which is a myogenic regulatory factor (MRF), through indirect activation of another MRF, the MEF2[74]. More specifically, DYRK1B relieves MEF2 from HDAC5, HDAC7 and MITR (the MEF2-interacting transcriptional repressor), in a dose- and kinase-dependent manner, by phosphorylating the class II HDACs (HDAC5 and HDAC7), at a conserved serine residue located at the nuclear localization sequence, resulting in decreased nuclear accumulation of Class II HDACs and leading them to exit the nucleus[74] (Figure 2).

The ability of DYRK1B to activate myogenin transcription facilitates myoblast differentiation. In addition, DYRK1B promotes myoblast differentiation by mediating cell cycle arrest of proliferating myoblasts and by increasing their survival during differentiation[74]. Also, DYRK1B has been shown to increase the survival of rhabdomyosarcoma cells[18,53,69]. The induction of DYRK1B under stress conditions suggests that DYRK1B could play a role in response to cellular injury. Skeletal muscle regeneration after injury is achieved by the activation of quiescent muscle stem cells (satellite cells), which enter the cell cycle and then differentiate and fuse with uninjured muscle fibers, in order to repair the damage[165]. DYRK1B is expressed at low levels in muscle stem cells and its expression is increased when quiescent muscle stem cells are activated to re-enter the cell cycle[70]. DYRK1B seems to be unimportant for muscle embryonic development, because DYRK1B knockout mice were viable for 18 d after conception, a crucial period for the development of skeletal muscle[63]. Thus, DYRK1B seems to function more during the repair of normal skeletal muscle[53]. Studies of DYRK1B in differentiating C2C12 myoblasts strongly suggest that DYRK1B functions as a survival factor, particularly during skeletal muscle regeneration[53].

DYRK1B has anti-apoptotic functions in both differentiating myoblasts and muscle-related cancer cells, as mentioned above. The anti-apoptotic properties of DYRK1B are also observed in skeletal myoblasts, where DYRK1B is most abundant. A large portion (20%-30%) of cycling myoblasts are not able to differentiate and undergo apoptosis when deprived of mitogens. Depletion of DYRK1B by RNAi blocked myoblast survival and increased the activation of caspase-3[69]. Moreover, overexpression of DYRK1B eliminated apoptosis during muscle differentiation, whereas overexpression of a DYRK1B dominant negative showed no anti-apoptotic activity[69]. Muscle satellite cells constitute a self-renewing pool of stem cells in adult muscle, where they function in tissue growth and repair. Disruption of regulatory control between proliferation and differentiation of these cells results in tumor formation[166]. Notably, although the precise cause of rhabdomyosarcoma is unknown, it has been suggested that cancer arises in 'satellite' stem cells[75]. It is likely that DYRK1B also facilitates the survival of CSCs of rhabdomyosarcoma, thereby rendering DYRK1B as a novel therapeutic target in rhabdomyosarcoma[53].

DYRK1B signaling in myogenesis: SHH regulates the cell fate of adult muscle satellite cells in mammals, promoting proliferation of satellite cells and of C2C12

myoblasts and preventing their differentiation into multinucleated myotubes[166]. DYRK1B seems to have the opposite effect compared to SHH signaling, in muscle stem cells (satellite cells) as well as in C2C12 progenitors[18]. It remains elusive if the influence of SHH or DYRK1B takes place at the same developmental stage. Experimental data suggest a primarily antagonistic relationship between these two pathways[18]. On one hand, DYRK1B dampens SMO-induced HH signaling but, on the other hand, it promotes stability of the GLI1 effector *via* DYRK1B-induced stimulation of the PI3K-AKT pathway[119,121,130,137,167] (Figure 2).

In agreement, other members of the DYRK/MNB/HIPK kinases family have been shown to regulate the transition from growth to differentiation, *e.g.*, the related kinase, Yak1, acts as a growth attenuator in response to stresses and nutrient conditions in yeast[168] and the YakA kinase regulates stress responses in *Dictyostelium discoideum* in response to nutrient starvation[169]. In this line, DYRK1B induction complements the observations in myoblast differentiation, as response to growth factor deprivation, as previously described.

DYRK1B in spermatogenesis

DYRK1B is also highly expressed, excepting skeletal muscle, in testis where it negatively regulates proliferation of immature male germ cells by an indirect mechanism[77]. In adult mouse, spermatogenesis is maintained by germ-line stem cells that undergo mitosis and self-renew or differentiate into committed spermatogonia, which called undifferentiated spermatogonia[170]. Undifferentiated spermatogonia or spermatogonial stem cells (SSCs)[77,170] constitute less than 1% of total testicular cells and differentiate into differentiating spermatogonia, which will finally undergo meiosis[171,172].

Cold-inducible RNA-binding protein (Cirp) is a cold-shock protein identified in mammals. It is induced in response only to mild hypothermia and is also induced by cellular stress, such as ultraviolet irradiation and hypoxia[173-175]. In response to stress, Cirp migrates from the nucleus to the cytoplasm and affects expression of its target mRNAs[176,177]. Cirp is expressed in the murine germ cells and its expression levels vary depending on the stage of differentiation[178]. When mouse testis is exposed to heat stress, expression of Cirp is decreased, in response to the heat-induced testicular damage. Cirp-knockout (cirp$^{-/-}$) mice did not show gross abnormality or defect in fertility but did show significantly reduced number of undifferentiated spermatogonia and exhibited delayed recovery of spermatogenesis after treatment with busulfan, a cytotoxic agent[77]. It was found that Cirp accelerates cell cycle progression from G0 to G1 as well as from G1 to S phase in cultured MEFs. Notably, in undifferentiated spermatogonia, Cirp and DYRK1B co-localized in the nucleus. The interaction between Cirp and DYRK1B is required to fully maintain the undifferentiated spermatogonia in mice, by promoting their proliferation[77]. In particular, direct binding of Cirp to DYRK1B prevents DYRK1B binding to p27^{Kip1}, resulting in decreased phosphorylation and destabilization of p27^{Kip1} (Figure 2). In contrast, Cirp did not affect DYRK1B binding to cyclin D1 but inhibited phosphorylation of cyclin D1 by DYRK1B, resulting in cyclin D1 stabilization (Figure 2). In the spermatogonial GC-1spg cell line, suppression of Cirp expression resulted in increased levels of p27^{Kip1} and decreased levels of cyclin D1. Consistent changes in the protein levels of p27^{Kip1} and cyclin D1, as well as the percentage of cells in G0 phase, were observed in undifferentiated spermatogonia of cirp$^{-/-}$ mice[77].

Those findings demonstrated a physiological function of the mammalian cold-shock protein Cirp that explains partly why testis should be kept cool. Cirp fine-tunes cell-cycle progression/exit in undifferentiated spermatogonia, fibroblasts, and cancer cells[179], by suppressing DYRK1B and modulating the protein levels of cell cycle regulators p27^{Kip1} and cyclin D1. Moreover, Cirp suppresses growth signals indirectly through discrete protein–protein and protein-RNA interactions, which depend on multiple factors, including cell types, stress, and conditions of cells[77].

DYRK1B in neurogenesis

During development of the CNS, coordinated regulation of cell cycle progression/exit and differentiation of NSCs/NPCs is essential for the proper formation and function of the nervous system[180-183]. During CNS development, NSCs undergo symmetric and asymmetric divisions and finally exit cell cycle and subsequently differentiate to obtain discrete neuronal identities[182,184,185]. A number of studies have shown that key regulators of cell cycle progression can influence neural cell fate and differentiation program and reverse cell fate determinants and differentiation-inducing factors can regulate cell cycle progression[186-189].

Many studies have shown that DYRK1A has an important role in neurogenesis. Hyperactivity or increased gene dosage (*e.g.*, in trisomy 21) of DYRK1A has been linked with abnormal brain development, neurodegeneration[105], cognitive disabilities and early onset Alzheimer's disease in individuals with Down syndrome[29,32,34,105,190]. Additional studies have shown that DYRK1A mutations, resulting in loss-of-function, are responsible for intellectual disabilities accompanied by microcephaly, epilepsy and autism, which are generally referred to as autosomal dominant mental retardation 7 syndrome (known as MRD7)[22,33,34,191,192].

Although the role of DYRK1A in neurogenesis is well documented, the function of its closely related kinase, DYRK1B, in CNS development remains elusive. It is difficult to figure out the functional diversity between DYRK1A and DYRK1B, since the two molecules have been studied in discrete systems. Most of the studies of DYRK1A have been performed in models of neurogenesis, whereas most of the studies of DYRK1B have been performed in models of myogenesis[69,70,74] and cancers[24,36,39,41,50].

We have recently demonstrated that DYRK1B is expressed in the adult mouse brain and in cultured primary cortical neurons and we have first studied DYRK1B function in mouse neuroblastoma Neuro2A cells, a suitable model for studies of neuronal development. We found that DYRK1B overexpression in Neuro2A cells promotes cell cycle exit and neuronal differentiation, by promoting Cyclin D1 cytoplasmic relocation and its proteasomal degradation by 26S proteasome[35,79]. It is worthy of note that transient overexpression of DYRK1B in Neuro2A cells also promotes neuronal differentiation, as indicated by the increase of the mean neurite length by 2-fold and by the expression of the neuronal marker, βIII-tubulin, when Neuro2A cells were subjected to differentiation using retinoic acid[35]. Further, in Neuro2A cells, DYRK1B-dependent down-regulation of Cyclin D1 was reversed following DYRK1B interaction with the scaffolding protein RanBPM. Interestingly, binding of RanBPM to DYRK1B stabilized Cyclin D1 in the nucleus and increased 5-bromo-2'-deoxyuridine (commonly known as BrdU) incorporation, which was used as a measure of cellular proliferation. Moreover, we have found that RanBPM facilitated DYRK1B proteasomal turnover[35].

In addition, we have demonstrated that the tripartite functional interactions between DYRK1B, RanBPM and the neuronal protein Cend1 (termed for cell cycle exit and neuronal differentiation 1; also known as BM88) regulate the balance between cellular proliferation and differentiation in Neuro2A cells, suggesting that the three proteins may also play a similar role in cell cycle progression/exit and differentiation of NSCs/NPCs during neurogenesis[35,79] (Figure 2). This is in agreement with the fact that both RanBPM and DYRK1B are expressed in neuronal precursors in parallel with Cend1[35,79]. Recently, we have found that DYRK1B is expressed in embryonic chick and mouse brain and spinal cord, and is highly expressed by cycling NSCs, while DYRK1B expression marks all along the neuronal lineage, suggesting thus DYRK1B implication in proliferation and differentiation of NSCs (Kokkorakis *et al*, 2020 unpublished data).

CONCLUSION

Stem cells exist in most tissues of the body at all stages of development, from early stages of embryogenesis all the way throughout adult life, with significant roles in patterning during embryogenesis and differentiation of all body tissues. In the adult life, mesenchymal stem cells have roles as repository cells that have the capacity to enable healing, growth and replacement of cells that are lost due to aging or trauma[10]. CSCs are the mutated equivalents of normal stem cells that share similar characteristics with them, especially the capacity to give rise to all cell types that residue in a particular cancer. CSCs are thus tumorigenic, in contrast to other non-tumorigenic cancer cells. CSCs may generate tumors through the stem cell capacity of self-renewal and differentiation into multiple cell types. CSCs persist in tumors as a distinct population and cause recurrence and metastasis by giving rise to new tumors[16].

Multiple factors that regulate the physical environments within stem cell niches can significantly influence cell fate decisions. Among these factors, DYRK kinases comprise a family of protein kinases that are highly evolutionarily conserved, from yeast to humans. DYRK kinases are emerging modulators of signal transduction, cell proliferation, survival, and differentiation[18] and act as inducers of cell cycle exit and quiescence, as well as promote cell viability through their anti-apoptotic functions[42]. The studies described herein have revealed that DYRK1B kinase is a multifunctional protein implicating several signaling pathways in development and in human diseases, regulating cell functions, such as cell proliferation, differentiation, cell

viability, motility and transcription. The mechanism under which DYRK1B exerts its function each time seems to be cell type- and context-dependent. DYRK1B is involved in cancer, metabolic syndrome, glucose homeostasis, survival, myogenesis, spermatogenesis, adipogenesis and neurogenesis, as we have recently shown[35] (Kokkorakis *et al*, 2020 unpublished data). Several of the studies described above have shown that DYRK1B is involved in various signaling pathways, such as HH, RAS and PI3K/mTOR/AKT, while HH comprises the major signaling pathway in which DYRK1B participates. DYRK1B seems to be not required for HH signaling, but it seems to act as a modulator involved in many HH-driven cascades during embryonic development.

DYRK1B has an important role in stem cell biology as is essential for the regulation of the balance between proliferation and differentiation of stem cells during myogenesis, spermatogenesis, and neurogenesis. In human cancers, DYRK1B is crucial for maintaining CSCs in a quiescence state, rendering them resistant to cancer chemo- and radio-therapies by controlling the balance between quiescence and apoptosis. Moreover, DYRK1B regulates the maintenance of CSCs under hypoxia by phosphorylation of the ID2 protein, resulting in the maintenance of stemness. Re-entering of quiescent CSCs into cell cycle may be achieved using DYRK1B pharmacological inhibitors, which may serve as valuable drugs in cancer therapy[43,80]. Moreover, DYRK1B may be used as a diagnostic marker for various types of human cancer[43].

ACKNOWLEDGEMENTS

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