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To cite this article: P Fernández-Martínez, C Zahonero & P Sánchez-Gómez (2015) DYRK1A: the double-edged kinase as a protagonist in cell growth and tumorigenesis, *Molecular & Cellular Oncology*, 2:1, e970048, DOI: [10.4161/23723548.2014.970048](https://doi.org/10.4161/23723548.2014.970048)

To link to this article: <https://doi.org/10.4161/23723548.2014.970048>



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Published online: 30 Jan 2015.



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DYRK1A: the double-edged kinase as a protagonist in cell growth and tumorigenesis

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Keywords: cancer, cell proliferation, cell differentiation, DYRK1A, neural progenitors

DYRK1A (dual-specificity tyrosine-regulated kinase 1A) is a kinase with multiple implications for embryonic development, especially in the nervous system where it regulates the balance between proliferation and differentiation of neural progenitors. The *DYRK1A* gene is located in the Down syndrome critical region and may play a significant role in the developmental brain defects, early neurodegeneration, and cancer susceptibility of individuals with this syndrome. *DYRK1A* is also expressed in adults, where it might participate in the regulation of cell cycle, survival, and tumorigenesis, thus representing a potential therapeutic target for certain types of cancer. However, the final readout of *DYRK1A* overexpression or inhibition depends strongly on the cellular context, as it has both tumor suppressor and oncogenic activities. Here, we will discuss the functions and substrates of DYRK1A associated with the control of cell growth and tumorigenesis with a focus on the potential use of DYRK1A inhibitors in cancer therapy.

DYRK1A is the most extensively studied among this family of kinases because its gene maps to human chromosome 21 within the Down syndrome critical region (DSCR).⁷⁻⁹ Moreover, this kinase is overexpressed in the brain of patients with Down syndrome (DS) and many of its known substrates have been linked to neuropathologic traits of this syndrome.^{10,11} In fact, accumulating evidence in experimental models suggests that DYRK1A inhibitors can reverse some of the neurologic alterations associated with its overexpression and therefore could be of use in individuals with DS.¹² These aspects have been extensively reviewed elsewhere. However, there are other less well-known facets of DYRK1A, such as its participation in cancer. Many of its downstream targets are associated with the control of cell growth and survival, especially in the nervous system, where it has been mostly studied, but also in other tissues. Interestingly, in the cancer context, DYRK1A activity might be linked to both oncogenesis and tumor suppression. Here, we will try to summarize all known implications of DYRK1A function in cell growth and cancer with the aim of understanding this dichotomy, which could have clinically relevant implications for the development of therapeutic DYRK1A inhibitors.

Introduction

DYRK (dual-specificity tyrosine-regulated kinase) family members represent a subfamily of protein kinases that have been identified in distantly related organisms such as yeast, *Drosophila*, and human. Seven mammalian Dyrk-related kinases have been identified: DYRK1A, DYRK1B, DYRK1C, DYRK2, DYRK3, DYRK4A, and DYRK4B.^{1,2} The DYRK proteins are dual-specificity protein kinases that autophosphorylate a conserved tyrosine (Y) residue in their own activation loop but phosphorylate their substrates at serine (S) or threonine (T) residues.^{3,4} The Y autophosphorylation occurs during translation and induces kinase activation; however, once the protein is fully translated, kinase activity becomes restricted to S and T residues and no longer depends on Y phosphorylation.^{5,6}

Role of DYRK1A in Embryonic and Adult Neurogenesis

Vertebrate *Dyrk1A* is expressed ubiquitously in a broad spectrum of embryonic tissues at different stages of development but also in some adult tissues, most prevalently in heart, lung, brain, and skeletal muscle.^{7-9,13} In mice, the absence of *Dyrk1A* is lethal at the embryonic stage. Heterozygous animals are viable but have a reduced size at birth that is maintained through adulthood. This reduction is more noticeable in organs such as the brain and liver. Heterozygous mice show decreased neonatal viability, a reduced number of neurons in brain areas, alterations in motor and development, dopaminergic deficiency, and impairment in spatial learning development.^{14,15} Conversely, transgenic mice overexpressing *Dyrk1A* also present a neurodevelopmental delay and motor and cognitive deficits.¹⁶⁻¹⁸ These data reflect the extreme gene dosage sensitivity of this protein and its relevance during neural system (NS) development, where it controls proliferation, neurogenesis, neural differentiation, cell death, and synaptic plasticity.^{19,20}

Drosophila Dyrk1A mutants (*minibrain*, *mnb*^{-/-} flies) develop a smaller adult brain (especially the optic lobes and the central brain hemispheres), which appears to be caused by altered neuroblast

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Submitted: 06/13/2014; Revised: 09/03/2014; Accepted: 09/03/2014
<http://dx.doi.org/10.4161/23723548.2014.970048>

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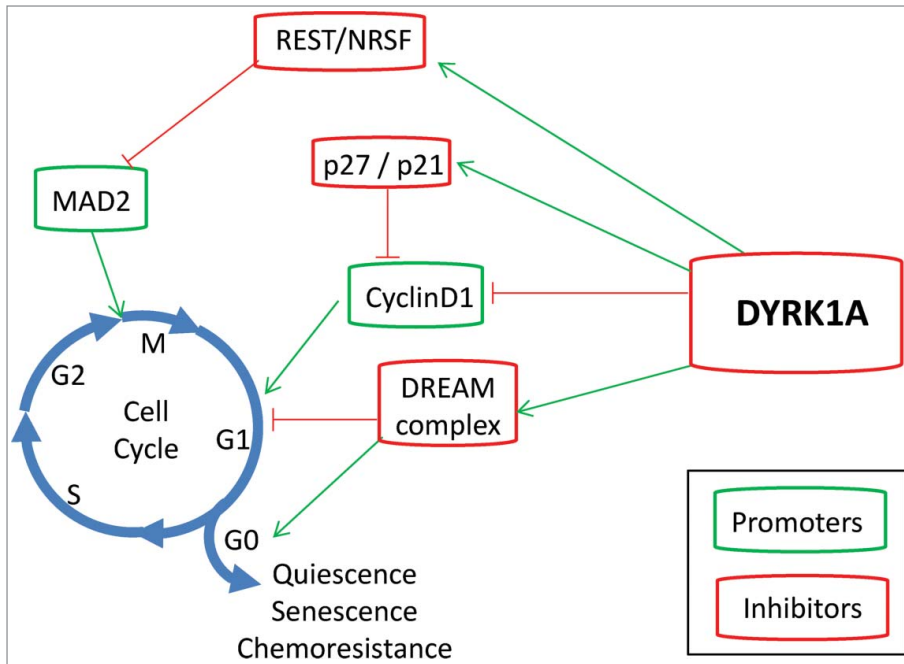


Figure 1. DYRK1A activity blocks cell cycle progression. DYRK1A is considered primarily an inhibitor of proliferation due to its capacity to either block (red lines) cell cycle promoters (green boxes), or activate (green arrows) cell cycle inhibitors (red boxes).

proliferation during postembryonic neurogenesis.²¹ Interestingly, recent evidence suggests that this function is not restricted to the NS as Mnb is also required for normal leg and wing growth control.²² Moreover, truncation of the human *DYRK1A* gene causes microcephaly,²³ further supporting an evolutionary conserved function of this kinase during brain development. There appears to be a dynamic spatiotemporal expression pattern of *Dyrk1A* that is tightly controlled during vertebrate NS development. There is a transient peak of expression of mouse *Dyrk1A* immediately before the transition from proliferating to neurogenic divisions.²⁴ After that, its expression is maintained in neural progenitors (NPs), although at a lower level. Later on, *Dyrk1A* is upregulated in newborn postmitotic neurons and downregulated as the neuron begins to migrate away from the ventricular zone. Once the migrating neuron reaches its target position, *Dyrk1A* is again expressed before the final differentiation and dendrite formation occurs.^{25,26} These changes in *Dyrk1A* expression reinforce the notion that it works as an inhibitor of cell cycle progression. In fact, *in utero* electroporation of *Dyrk1A* in the embryonic mouse neocortex inhibits cell proliferation by inducing the nuclear export and degradation of cyclin D1.²⁷ A more recent study indicates that DyrkA kinase activity is responsible for the stabilization of cellular cyclin D1 and the degradation of p27 (a cyclin-dependent kinase [CDK] inhibitor) in mouse and human cells.²⁸ Other authors have shown that upregulation of *Dyrk1A* induces proliferation arrest of embryonic NPs. Conversely, its loss of function causes overproliferation and cell death in the embryonic chick spinal cord and mouse telencephalon.²⁹ These authors suggest that Dyrk1A is both necessary and sufficient for transcriptional upregulation of the expression of *p27*. Furthermore, Dyrk1A phosphorylates p53 in rat embryonic hippocampal

progenitors H19–7 cells, which leads to a robust induction of *p21*.³⁰ These results support a model in which Dyrk1A impairs cell cycle progression of embryonic progenitors, especially at the transition from G1/G0 to S phase (summarized in Fig. 1). In contrast with these data, *mnb* overexpression promotes organ growth through inhibition of the Salvador-Warts-Hippo (SWH) pathway, also called the Hippo pathway,²² a known inhibitor of proliferation and inducer of apoptosis in flies and mammals.^{31–34} Whether these differences are species- or tissue-specific is not known, but throughout the text we will see more examples of different, and even opposite, readouts of Dyrk1A functions in different contexts. Although there is no clear explanation for this behavior this gene is extremely dosage dependent so one could hypothesize that changes in the level or the duration of Dyrk1A expression could have different consequences. There are even cases in which both downregulation and overexpression of DYRK1A have the same readout.³⁵ Moreover, a recent study using

single-cell image analysis has shown that Dyrk1A mediates a dose-dependent increase in the duration of the G1 phase via direct phosphorylation and subsequent degradation of cyclinD1, directing PC12 cells into a reversible arrested state. In contrast, knockdown or kinase inhibition of Dyrk1A greatly increased cyclinD1 protein levels and split cells into 2 fates, with one subpopulation (with low p21 expression) shortening the G1 phase and the other (with high p21 expression) entering a persistent arrested state that differs from the normal quiescence state in which expression of both proteins is low.³⁶ Thus, both upregulation and downregulation of Dyrk1A levels could lead to cell cycle exit (transient or irreversible, respectively) and have similar consequences on tissue growth.

In addition to directly controlling the cell cycle machinery, it has been suggested that overexpression of Dyrk1A is necessary to induce neural differentiation, although it is not clear whether it is sufficient for this final outcome.^{27,29} Regarding the mechanism, Yang and coworkers have shown that Dyrk1A activity is induced during *in vitro* differentiation of hippocampal progenitor cells, leading to the stimulation of cAMP responsive element binding protein (CREB) transcriptional activity.³⁷ Another group has suggested that Dyrk1A overexpression potentiates nerve growth factor (NGF)-mediated PC12 neuronal differentiation by upregulating the Ras/MAP kinase signaling pathway.³⁸ More recently, it has been proposed that Dyrk1A phosphorylates Notch in the nuclear compartment and is co-expressed with the Notch ligand Delta1 in single NPs. Furthermore, Dyrk1A suppresses Notch signaling and reduces its capacity to sustain transcription in neural cells, counteracting its antidifferentiative actions.^{29,39} Another interesting possibility would be that Dyrk1A influences neuronal differentiation through the

modulation of RE1-silencing transcription factor (REST, also known as neuron-restrictive silencer factor or NRSF). REST is a zinc finger transcription factor that silences a range of neuronal genes in differentiated non-neuronal tissues and NPs.⁴⁰ Furthermore, the dissociation of REST and its co-repressors from the RE1 sites is both necessary and sufficient to trigger the transition from pluripotent embryonic stem cells to NPs, and from these to mature neurons.⁴¹ Moreover, REST is degraded in the G2 phase and this is necessary for the derepression of *Mad2*, an essential component of the spindle assembly checkpoint.⁴² Reduced expression of *REST* has been observed in cultured fetal DS brain cell-derived neurospheres⁴³ as well as in the brains of DS mouse models.^{44,45} By inhibiting *REST* expression, DYRK1A might inhibit the G2-M checkpoint (Fig. 1) as well as promote some of the neural differentiation defects observed in DS.

In contrast to the antiproliferative and prodifferentiative capacity of Dyrk1A during CNS development, we recently suggested that this kinase sustains adult NP self-renewal.⁴⁶ In our experience, Dyrk1A protein is actively distributed during adult NP cell division and the inherited kinase acts as an inhibitor of epidermal growth factor receptor (EGFR) degradation by phosphorylating Sprouty2 (Spry2), modulator of receptor tyrosine kinases (RTK) turnover. Interestingly DYRK1A phosphorylates T75 on Spry2 and impairs its inhibitory activity on extracellular signal-regulated kinase (ERK) signaling downstream of fibroblast growth factor (FGF).⁴⁷ However, Spry2 also phosphorylates and sequesters Cbl, a major effector of EGFR degradation.⁴⁸ The data on adult progenitors suggest that Dyrk1A phosphorylation could be beneficial for the positive function of Spry2 downstream of EGFR but counteractive for the inhibitory function of this protein on FGF signaling (Fig. 3). Therefore, high expression of Dyrk1A should correlate with general activation of RTK function. It would be interesting to test whether phosphorylation by Dyrk1A modulates the binding of Spry2 to regulatory proteins, as it has been described for phosphorylation of other Spry2 residues.⁴⁹ In fact, Dyrk1A overexpression decreases the interaction of Spry2 and growth factor receptor-bound protein 2 (Grb2), an adaptor between RTK and the ERK pathway.⁵⁰ These results suggest that DYRK1A kinase activity could have a positive function in both EGFR and FGFR signaling, at least in the context of the adult CNS. In agreement with this, activation of the AKT pathway, a known transducer of RTK activation, has been observed in several transgenic *Dyrk1A* mouse models, concomitant with an increase in expression of *CCND1* (the gene coding for cyclin D1) in postnatal and adult stages.^{50,51} Together, these data suggest that Dyrk1A might act mainly as a negative regulator of cell cycle during neural development, but also as a positive regulator of cell proliferation in the adult CNS, probably due to differential expression of downstream substrates or regulatory molecules.

Regulation of Quiescence through the DREAM Complex

An important group of proteins that negatively regulate the cell cycle are the retinoblastoma (RB) tumor suppressor protein and the

related family members p107 (RBL1) and p130 (RBL2).⁵² p130 has been shown to accumulate in G0, when it interacts with E2F4 to repress E2F-dependent gene transcription.⁵³⁻⁵⁵ p130 and E2F4 are part of a larger multisubunit protein complex, the mammalian DREAM (dimerization partner, RB-like, E2F, and multivulval class B) complex.^{56,57} In a recent study, phosphorylation of the DREAM component LIN52 at S28 by DYRK1A was shown to regulate complex formation in G0 and E2F repression.⁵⁸ Inhibition of DYRK1A activity or a point mutation in *LIN52* disrupted DREAM assembly and reduced the ability of tumor cells to enter quiescence and the capacity of fibroblasts to undergo Ras-induced senescence⁵⁸ (Fig. 1). These authors have shown that overexpression of DYRK1A (but not a kinase-deficient mutant) can inhibit proliferation and colony formation of a panel of tumor cell lines from different tissues.⁵⁸ Moreover, by phosphorylating DYRK1A, LATS2, a component of the Hippo pathway, could enhance the kinase activity of DYRK1A toward the DREAM subunit LIN52. Intriguingly, the *LATS2* locus is physically linked with *RBI* on 13q, and this region frequently displays loss of heterozygosity in human cancers.⁵⁹ Together, these results provide a possible explanation for the proposed tumor suppressor role of DYRK1A, especially at the tumor initiation stages. However, the same mechanism could have a protective role later on in the response to chemotherapy. In fact, a recent report indicates that inhibition of DYRK1A could enhance imatinib-induced cell death in gastrointestinal tumors by favoring apoptosis at the expense of cell quiescence.⁶⁰

Documented Roles of DYRK1A in Cancer

Epidemiologic studies suggest that individuals with DS have an increased risk of acute megakaryoblastic leukemia (AMLK) and acute lymphoblastic leukemia (ALL) in combination with acquired mutations of the transcription factor globin transcription factor 1 (*GATA1*).⁶¹⁻⁶⁴ These *GATA1* mutations are localized in exon 2 and provoke the expression of a shorter isoform named GATA1s.⁶¹ However, non-DS individuals with germline *GATA1* mutations similar to those seen in DS-AMLK have no predisposition to leukemia.⁶³ Moreover, trisomy for the DSCR that includes *DYRK1A* markedly increases the proliferation of megakaryocytes and is sufficient to cooperate with *GATA1* mutations in this mouse model, reinforcing the oncogenic function of DYRK1A in this cell type.⁶⁵ *GATA1* is essential for erythroid and megakaryocytic development. *GATA1s* retains some of the functions of the wild-type protein although it does not repress oncogenic *MYC* and the proproliferative *E2F*.^{66,67} This could explain its oncogenic action but might also lead to a positive feedback loop as E2F stimulates *DYRK1A* transcription, further increasing DYRK1A expression.⁶⁸

In contrast to the increased risk of leukemia, individuals with DS show a considerably reduced incidence of most solid tumors.^{69,70} It has been suggested that overexpression of *DYRK1A*, in cooperation with *DSCR1* (another gene located in the DS critical region) could diminish angiogenesis through attenuation of vascular endothelial growth factor (VEGF)-calcineurin- nuclear factor of activated T cells (NFAT) signaling in

endothelial cells.^{71,72} NFAT proteins are transcription factors that are activated as a result of a calcium flux released from endoplasmic reticulum stores and from the activation of channels in the plasma membrane. This increase in calcium provokes dephosphorylation of NFAT by the phosphatase calcineurin and its translocation to the nucleus, where it cooperates with other factors and coactivators to promote gene transcription.⁷³ Since the discovery of NFAT proteins 2 decades ago as promoters of interleukin (IL)-2 during the activation of T cells,⁷⁴ it has become increasingly clear that these proteins are not only expressed in immune cells but are also overexpressed in human solid tumors and hematologic malignancies. In general, activation of the NFAT pathway is considered to be a cancer-promoting event by enhancing proliferation and survival (in T cell lymphoid malignancies), by enhancing metastatic dissemination (in epithelial cancers), and by promoting angiogenesis.^{73,75} Phosphorylation of NFAT by DYRK1A^{71,76} primes it for subsequent phosphorylation by casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK3), which drives the inactivation and nuclear export of NFAT.⁷⁷⁻⁷⁹ As *VEGF* is one of the known NFAT targets, *DYRK1A* overexpression could lead to decreased angiogenesis, thus contributing to the lower incidence of adult solid tumors in DS individuals.⁷² However, it is important to keep in mind that DS is a multifactorial syndrome and the overexpression of other genes could be responsible for the reduced cancer predisposition. Paradoxically, it has been proposed that *DYRK1A* also contributes to megakaryocytic malignancies through the inhibition of NFAT, although the possibility that other substrates of the kinase are implicated in the oncogenic response has not been excluded.⁶⁵ Therefore, it seems that while *DYRK1A* and NFAT suppress the growth of epithelial and lymphoid tumors in the adult, they could act as megakaryocytic oncogenes in children. This led us to hypothesize that *DYRK1A* has opposite functions during normal development and carcinogenesis of the NS and blood cells.

These context-dependent functions of NFAT have similarities with other well-known substrates of *DYRK1A* and reinforce the notion that this kinase can have different, and even antagonistic functions, depending on the cellular context or even the cancer stage. This idea has already been suggested by others, at least in the DS context.⁸⁰ **Figure 2** summarizes some of the *DYRK1A* substrates, focusing on their pro- or antitumoral roles. For example, NOTCH function is inhibited by *DYRK1A* phosphorylation, as previously mentioned.^{29,39} Hyperactivation of the NOTCH pathway has classically been viewed as oncogenic in several cancers (solid tumors and blood cancers) although recent studies have revealed tumor suppressor roles for NOTCH signaling in myeloid malignancies.⁸¹ Therefore, *DYRK1A* inhibition in NOTCH-related cancers could have positive or negative consequences for tumor growth and survival. A similar scenario might exist for the protein REST, which has antitumor properties in epithelial cancers but can function as an oncogene for neural tumors.⁴⁰ REST was first identified as a cancer-related gene a decade ago from studies in medulloblastoma (MD), a childhood tumor of the cerebellum,^{82,83} and was later also characterized as an oncogene in neuroblastoma (NB), the most common

extracranial solid tumor in children⁸⁴ and glioblastoma (GBM), a deadly adult brain tumor.^{85,86} These data suggest that REST controls the maintenance of embryonic and adult NPs and, when deregulated, can be implicated in neural tumors. As we previously mentioned, *REST* appears to be downregulated in DS brains. However, more recent observations indicate that REST can activate *DYRK1A* transcription through a NRSE site in the human *DYRK1A* promoter region. Moreover, *REST* and *Dyrk1A* are coordinately expressed during neural development, and *DYRK1A* imbalance can destabilize REST protein expression and reduce its transcriptional activity.³⁵ Therefore, it seems that in this case the regulation is even more complicated: *DYRK1A* can work as a positive or a negative modulator of REST expression/activity, which in turn could have oncogenic or tumor suppressor activity depending on the tissue.

Survival- or Apoptosis-Related Substrates of *DYRK1A*

In contrast to the dual (pro- or antitumoral) function of some of the genes mentioned above, there is a list of prosurvival or antiapoptotic proteins that are activated upon *DYRK1A* phosphorylation and could be important for carcinogenesis in different tissues (**Fig. 3**). Signal transducer and activator of transcription (STAT) is a latent transcription factor that transmits signals generated primarily by cell surface receptors into the nucleus. STAT3 is transiently activated in normal cells but is constitutively activated in a wide variety of hematologic malignancies (leukemia, lymphomas, and multiple myelomas) and solid tumors (such as head and neck, breast, lung, gastric, hepatocellular, colorectal, brain, and prostate cancers).⁸⁷ There is strong evidence suggesting that aberrant STAT3 signaling promotes initiation and progression of cancer by either inhibiting apoptosis or inducing proliferation, angiogenesis, invasion, and metastasis.⁸⁸ Phosphorylation of STAT3 at position Y705 by Janus kinase (JAK) induces its dimerization and nuclear translocation.⁸⁹ However, the STAT3 molecule contains a second phosphorylation site, S727, within its C terminus that can be phosphorylated by *DYRK1A*⁹⁰ and is necessary to achieve maximal transcriptional activity.⁸⁹ In fact, recent data suggest that phosphorylation at S727 plays a principal role in regulation of cell survival activity and nuclear translocation of STAT3.⁹¹ Nevertheless, inhibition of *DYRK1A* has not been explored as a way to modulate the growth of STAT3-related tumors. GLI1, a major effector of Sonic hedgehog (SHH) signaling, is another oncogenic transcription factor whose nuclear translocation and function seems to be mediated by *DYRK1A* phosphorylation, most likely through the retention of GLI1 in the nucleus.⁹² SHH-GLI signaling regulates tumor growth and survival, as well as metastasis, in a number of tumors.⁹³ However, although STAT3 is a *bona fide* *DYRK1A* substrate, the relevance of GLI1 phosphorylation by the kinase and the possible applications of *DYRK1A* inhibitors in SHH-dependent tumors is still under debate.

DYRK1A function has been linked to negative regulation of the intrinsic apoptotic pathway through the phosphorylation of

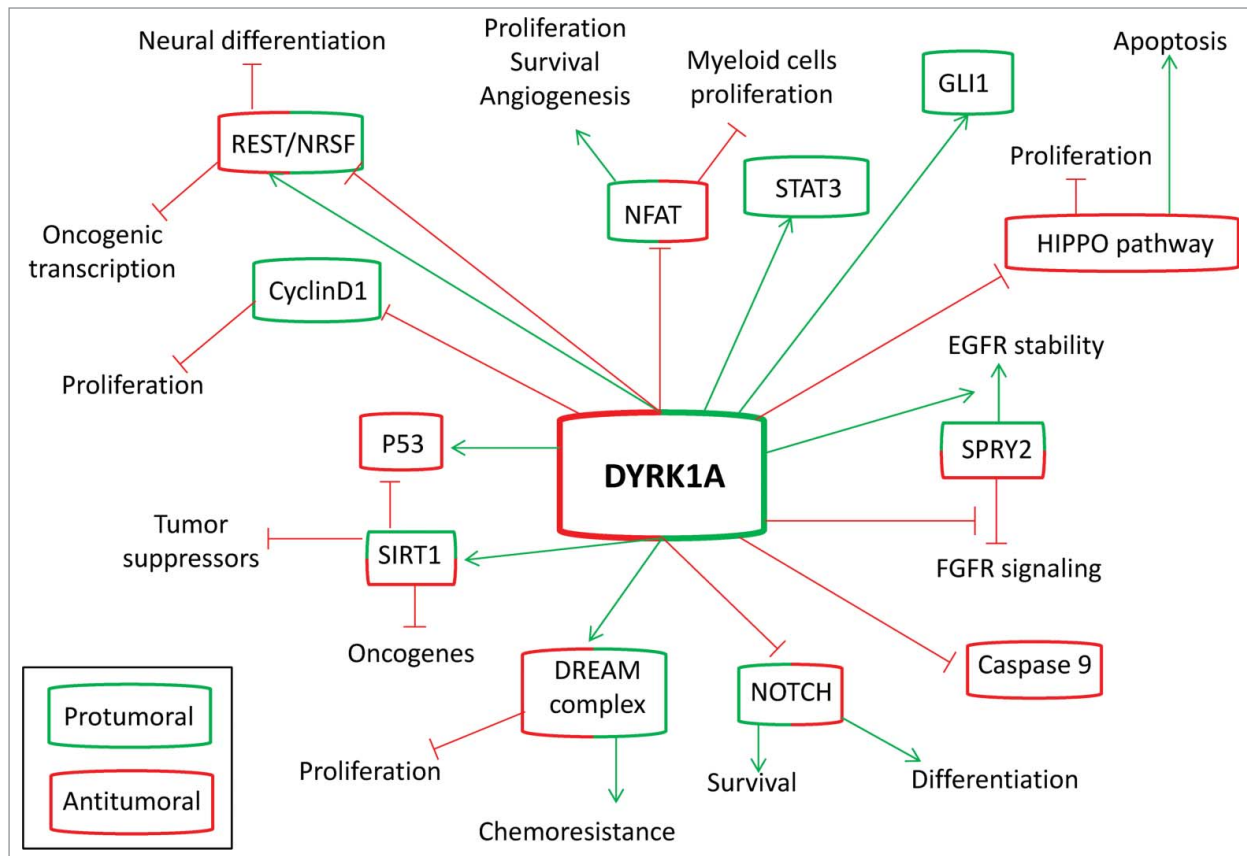


Figure 2. Double-edged regulation of tumorigenesis by DYRK1A. DYRK1A has been associated with protumoral activity (green boxes) by activating (green arrows) known oncogenic proteins (red boxes) or by inhibiting (red lines) tumor suppressors (red boxes). However, an antitumoral capacity of DYRK1A has been also described through its activation of tumor suppressors or its inhibition of oncogenic proteins. To add complexity to DYRK1A function, some of the known substrates of this kinase can have both oncogenic and tumor suppressor activities (green and red boxes), depending on the cellular context and the developmental stage.

caspase-9.^{94,95} Caspase-9 is activated by a variety of apoptotic stimuli that trigger the release of cytochrome c from mitochondria. Once in the cytosol, cytochrome c induces the oligomerization of apoptotic protease activating factor 1 (Apaf-1) and the subsequent recruitment of procaspase-9. These events lead to activation of procaspase-9 by autocatalytic processing and consequently to the activation of effector caspase-3 and caspase-7.⁹⁶ Activation of caspase-9 is inhibited by phosphorylation at T125, which is catalyzed, among others, by Dyrk1A.^{94,95} This is important during the development of retina progenitors,⁹⁵ and in the response of cells to hyperosmotic stress.⁹⁷ Therefore, the inhibition of DYRK1A would lead to caspase-9 induction and could be exploited to enhance the apoptotic response of cancer cells to chemotherapy. In addition, DYRK1A might promote cell survival through phosphorylation and activation of sirtuin 1 (SIRT1; also known as silent mating type information regulation 2 homolog or NAD (+)-dependent protein deacetylase), which participates in the stress response and cellular metabolism.⁹⁸ It has been shown that DYRK1A and DYRK3 directly phosphorylate SIRT1 at T522, promoting deacetylation of p53, and, more importantly, that the knockdown of endogenous DYRK1A and DYRK3 sensitizes cells to DNA damage-induced cell death.⁹⁹

These data, together with the impairment of quiescence mediated by inhibition of the DREAM complex and inhibition of the HIPPO pathway (which induces apoptosis) by DYRK1A, suggest that DYRK1A could be a good therapeutic target to increase cell death in response to both chemo- and radiotherapy in different tumors, as has been suggested by others.¹⁰⁰

Despite these prosurvival functions of DYRK1A, and excluding its well-known link to DS-related leukemia, it was not until recently that our group described a clear association of this kinase with tumor growth. Our results suggest that *DYRK1A* is highly expressed in a subset of GBMs, where it correlates with the expression and genetic amplification of *EGFR*. In parallel to events in normal NPs, downregulation of *DYRK1A* leads to an increase in EGFR degradation and therefore to inhibition of the self-renewal capacity of GBM cells.¹⁰¹ Furthermore, overexpression of SPRY2 was able to compensate for the EGFR degradation promoted by DYRK1A inhibition and rescue the self-renewal effect. On top of this, we have shown that genetic or pharmacologic blockade of DYRK1A severely impaired tumor growth *in vivo*, thus opening the door to the use of DYRK1A inhibitors in glioma therapy, at least for EGFR-dependent tumors.¹⁰¹ *EGFR* is a well-known oncogene in a variety of tumors and inhibitors of

its tyrosine kinase activity are being tested in many of these, including GBM, although the results have not always been always optimal.¹⁰² Impaired endocytic downregulation of this receptor is frequently associated with cancer. Indeed, dominant-negative forms of *CBL* have been identified as oncogenes in human myeloid neoplasms¹⁰³ and *SPRY2* has tumor-promoting activity in colon cancer.¹⁰⁴ Therefore, the induction of EGFR degradation mediated by inhibition of DYRK1A could represent an alternative strategy for EGFR-related cancers such as colon, lung, and head and neck tumors.

To summarize this section, there is not a simple answer to the question of whether DYRK1A functions as an oncogene or a tumor suppressor (Fig. 2). In addition, it is important to note that the relevance of some of the targets described above has not been confirmed in a tumor context, or even in cancer cell lines. Moreover, in the most conceivable situations several targets would act in a combinatorial way. Therefore, the final response of a cell to DYRK1A overexpression or inhibition probably depends on the molecular and cellular context of each cancer.

DYRK1A Inhibitors and their Possible Use in Cancer Therapeutics

In recent years there has been increased interest in inhibition of DYRK1A activity for the treatment of the mental impairment associated with several neurodegenerative diseases (including Alzheimer's Disease) and DS.^{12,105} The fact that this kinase is extremely dosage dependent increases its therapeutic potential as a target. The goal then would be not to inhibit DYRK1A completely but rather to a level comparable with that of healthy conditions, which could reduce the potential side effects of targeting this kinase. Most DYRK1A inhibitors are ATP competitors and act by binding within the DYRK1A kinase domain (direct competitors) or by preventing the functionality of the ATP binding site (indirect competitors).⁶ They can be classified based on their structure (see¹⁰⁰ for a comprehensive review) or based on their origin: i.e., natural compounds and their derivatives, synthetic inhibitors, and broad spectrum kinase inhibitors that show activity against DYRK1A. Table 1 reviews the most potent DYRK1A inhibitors and their possible implications in cancer. Many of them can inhibit other members of the DYRK family, as well as structurally related kinases such as CDKs (important regulators of cell cycle progression) and CDK-like kinases (CLKs), which participate in pre-mRNA splicing.¹⁰⁶ Different modifications of these compounds have been envisioned in order to increase specificity and avoid side effects.

Among DYRK1A inhibitors, the β -carboline alkaloid known as harmine is currently the most selective and effective, although it also blocks other DYRKs, especially DYRK1B. It functions as an ATP-competitive inhibitor to specifically block the S/T kinase activity.^{6,107} Harmine is isolated from divergent plant species, including the South American vine *Banisteriopsis caapi* and the mideastern shrub *Peganum harmala*. It is a component of ayahuasca, a hallucinogenic brew of plant extracts that has been used for centuries in healing and to cure illnesses, including some types of

cancer. However, several active principles could be involved in this action and there is no direct evidence that targeting DYRK1A is the relevant anticancer action of ayahuasca.¹⁰⁸ Harmine has been shown to inhibit neovessel formation *in vitro* and *in vivo* through the regulation of several angiogenic factors and inflammatory cytokines.¹⁰⁹ Moreover, harmine and related β -carboniles display cytostatic and/or cytotoxic activity toward cancer cells, including leukemia, colon, liver, gastric, and glioma cells.^{65,101,110-114} However, harmine has long been known to be a potent inhibitor of monoamine oxidase-A¹¹⁵ and to have hallucinogenic properties as a result of its affinity for the tryptamine and serotonin receptor binding sites.¹¹⁶ These properties seriously limit the *in vivo* therapeutic applications of this compound. Nonetheless, molecular docking analysis showed that harmine has many degrees of freedom in the ATP-binding pocket of DYRK1A and this could be exploited to more selectively inhibit the kinase.¹¹⁷ Glioma cells are also sensitive *in vitro* to INDY, a benzothiazol derivative.¹⁰¹ The *in vitro* biological activity of INDY has been confirmed to involve blockage of Tau phosphorylation and restoration of NFAT activity, and it can rescue the developmental defects of Dyrk1A overexpressing tadpoles *in vivo*.^{105,118} However, nothing is currently known about the pharmacokinetic properties of this compound in whole animals or humans, which hampers its therapeutic use. Another 2 synthetic compounds, lamerallins and meriolins, have shown some anticancer and proapoptotic effects in cancer cells and mouse glioma models, although other kinases (especially CDKs) could be implicated in this activity.¹¹⁹⁻¹²³

Epigallocatechin gallate (EGCG) is a polyphenol and a major catechin component of green tea that shows selective inhibition of DYRK1A compared to other structurally and functionally related kinases by functioning as a noncompetitive inhibitor of ATP.¹²⁴ Interestingly, a diet rich in green tea clearly improved the brain structure defects and cognitive impairments of DYRK1A-overexpressing mice.⁵¹ EGCG has been shown to have anticancer activity by inhibiting topoisomerases I/II, the antiapoptotic enzyme Bcl-xl, and cancer promoting proteases. Moreover, it has additional health properties as it shows potent antibacterial and antiviral activity and elicits antioxidative and anti-inflammatory capacity by suppressing the nitric oxide synthase pathway.¹⁰⁵ Although it seems clear that the anticancer activities of green tea and EGCG are not limited to DYRK1A inhibition, their safety for human consumption make them good candidates for use in DYRK1A-related tumors and neurodegenerative diseases.

Most of the inhibitors mentioned in Table 1 have the capacity to block other DYRK proteins, especially the closely related DYRK1B (also known as MIRK). MIRK has been characterized as a negative regulator of cell proliferation that modulates the protein stability of several cell cycle-related molecules.^{125,126} However, upregulation of MIRK expression and/or constitutive activation of this kinase has been observed in several different types of cancer, where it has been associated with the survival of tumor cells in response to stress.¹²⁷ For example, depletion of Mirk has been associated with apoptosis in lung cancer¹²⁸ and also in pancreatic and ovarian cancer cell lines, where it seems to

Table 1. DYRK1A inhibitors. The table shows the IC₅₀ of different classes of DYRK1A inhibitors against DYRK1A, other DYRK proteins, and different kinases (only targets with submicromolar IC₅₀ values are included). Cancer-related studies performed with those inhibitors are also indicated

	Compound name (Chemical class)	IC ₅₀ (nM)		Evidence related to cancer therapy	Refs.
		DYRK1A	Other targets		
Natural inhibitors	Harmine (β-carboline)	22–400	DYRK1B (166–300), DYRK2 (900–1,900), DYRK3 (800–1000), DYRK4 (80,000), MAO-A (5), CLK1 (27)	Angiogenesis inhibition; Cytotoxic activity in tumor cell lines <i>in vitro</i> (glioma, leukemia, colon, gastric, liver), and <i>in vivo</i> (glioma)	101,107,111–115,117
	EGCG (Polyphenol)	40–330	Vimentin (3), COMT (70)	Clinical trials on cancer: urothelial, bladder, prostate, myeloma, breast, lung	124,132,133
	Staurosporine (glycosylated indolocarbazole)	20	Aurora A (7.2), Aurora B (20), Chk1 (1), Ftl3 (3), HGK (1), Ikkb (0.5), Jak2 (1), KDR (10), SYK (4)		134
Synthetic inhibitors	Lamerallins (Chromenoindole)	40–5,000	CDK5 (720->10,000), GSK3 (310->10,000), CDK1/CyclinB, PIM1, CK1 (70–8,000)	Apoptosis induction and multidrug resistance phenotype reversion	119–121,135
	INDY (Benzothiazol)	200	DYRK1B (240)	Antiproliferative in gliomas	101,118
	Meriolins (Pyrimidinylindol/azaindol)	30	CDK1 (7–170), CDK2 (3–18), CDK5 (3–170), CDK9 (5.6–18), GSK3 (21–400), CK1 (50–200)	Antiproliferative and proapoptotic effects in tumor cells and glioma xenografts	122,123
	Variolin B (Pyrimidinylindol/azaindol)	80	CDK1 (60), CDK2 (80) CDK5 (90) CDK9 (26) GSK3 (70) CK1 (5)		122
	Meridianins (Pyrimidinylindol/azaindol)	34–900	CDK5 (680->10,000), CK1 (490->10,000), CLK1 (30–70)		136,137
	KHCB19 (dichloroindol)	55	CLK1 (20), CLK3 (500)		138
	Imidazolone (Leucettamine)	70–1,000	CLK1 (15–71), GSK3(21–38)		139
Promiscuous kinase inhibitors	Purlavanol A (Purine)	300	CDK2 (30–100), PAK4 (100), SRC (<100), CDK2/CyclinA (100)		107,124
	A-443654 (Pyridine)	<10	DYRK2, DYRK3 (<100), ERK8, RSK1, RSK2, PKBα, PKBβ, S6K1, PKA, ROCK2, PRK2, PKCa,PKD1,MSK1, SmMLCK, SK3b, CDK2-CyclinA, PIM1,2,3,MST2,HIPK2 (<100)	Initially identified as a potent Akt inhibitor for use in anticancer research field	140
	TBB, TBI, DMAT, TDI (Tetrahalo-bicycles)	100–10,000	DYRK2 (300–35,000), DYRK3 (2,000–5,000), CK2 (100–600), PIM1 (100–1,000), PIM2 (200–4,000), PIM3 (70–1,000)		117,141–143

modulate the levels of reactive oxygen species (ROS).^{129–131} Therefore, the effect of some DYRK1A inhibitors in cancer could also be due to MIRK inhibition, especially if they are associated with changes in the response of tumor cells to hypoxia, nutrient deprivation, and/or cytotoxic stimuli.

Conclusions and Future Perspectives

The results of several *in vitro* and *in vivo* studies link DYRK1A activity with cell cycle exit, oncogene-induced senescence, and cell differentiation, especially in the embryonic NS. This notion is reinforced by the nature of many known DYRK1A substrates, and also by the decreased cancer susceptibility of individuals with DS. Therefore one would expect this gene to behave as a tumor suppressor, at least for the initial cancer stages. However, recent evidence indicates that DYRK1A can induce clonogenic and prosurvival properties in certain types of cell or in certain developmental conditions, and that this kinase can be considered as an oncogene for at least 2 types of cancer: myeloid

leukemias and gliomas. As a proof of principle, harmine, a potent DYRK1A inhibitor, can block cell growth and tumorigenesis in those tumors. Moreover, DYRK1A might confer chemo- and radioresistance capacities in several tumors by controlling the balance between quiescence and apoptosis. We propose that newly identified DYRK1A inhibitors, which are being developed primarily for their use in mental diseases, should also be considered as anticancer agents, at least for AMKL and GBM. Moreover, considering the relevance of DYRK1A in the NS, it would be important to test the efficacy of such inhibitors in other neural tumors such as oligodendrogliomas, which express high levels of DYRK1A, and MB, given the important cerebellar expression of this kinase. In such brain tumors, the regulation of EGFR turnover by DYRK1A might participate in the oncogenic action of this kinase, in addition to modulation of REST, which is highly expressed in cerebellar NPs, and SHH and/or NOTCH signaling, which are associated with NS development. Special attention should be given to secondary hits of DYRK1A inhibitors and to the fact that DYRK1A blockade could affect several pathways. However, in the case of cancer some promiscuity may be

acceptable and might even represent an advantage, further encouraging the preclinical and clinical analysis of such compounds in cancer therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Funding

This study was supported by grants from Ministerio de Economía y Competitividad, Fondo de Investigación Sanitaria, PI12/00775 and from Ministerio de Economía y Competitividad, Red Temática de Investigación Cooperativa en Cáncer (RD12/0036/0027) to PSG.

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