Updating dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2): molecular basis, functions and role in diseases

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Abstract
Members of the dual-specificity tyrosine-regulated kinase (DYRKs) subfamily possess a distinctive capacity to phosphorylate tyrosine, serine, and threonine residues. Among the DYRK class II members, DYRK2 is considered a unique protein due to its role in disease. According to the post-transcriptional and post-translational modifications, DYRK2 expression greatly differs among human tissues. Regarding its mechanism of action, this kinase performs direct phosphorylation on its substrates or acts as a priming kinase, enabling subsequent substrate phosphorylation by GSK3β. Moreover, DYRK2 acts as a scaffold for the EDVP E3 ligase complex during the G2/M phase of cell cycle. DYRK2 functions such as cell survival, cell development, cell differentiation, proteasome regulation, and microtubules were studied in complete detail in this review. We have also gathered available information from different bioinformatic resources to show DYRK2 interactome, normal and tumoral tissue expression, and recurrent cancer mutations. Then, here we present an innovative approach to clarify DYRK2 functionality and importance. DYRK2 roles in diseases have been studied in detail, highlighting this kinase as a key protein in cancer development. First, DYRK2 regulation of c-Jun, c-Myc, Rpt3, TERT, and katanin p60 reveals the implication of this kinase in cell-cycle-mediated cancer development. Additionally, depletion of this kinase correlated with reduced apoptosis, with consequences on cancer patient response to chemotherapy. Other functions like cancer stem cell formation and epithelial–mesenchymal transition regulation are also controlled by DYRK2. Furthermore, the pharmacological modulation of this protein by different inhibitors (harmine, curcumine, LDN192960, and ID-8) has enabled to clarify DYRK2 functionality.

Keywords DYRK2 · Phosphorylation · Kinase · Cell cycle · Apoptosis · Disease · Cancer

Introduction
Dual-specificity tyrosine-phosphorylation-regulated kinase 2 (DYRK2) is an evolutionarily conserved enzyme belonging to the CMGC family of the eukaryotic kinase that encompasses cyclin-dependent kinases (CDK), mitogen-activated protein kinases (MAPK), glycogen synthase kinase (GSK), and CDK-like kinases (CLKs) [1]. The attribution DYRK (dual-specificity tyrosine (Y) phosphorylation-regulated kinase) highlights the peculiar biochemical properties of this protein kinase family, which possesses the ability to phosphorylate aromatic (tyrosine) as well as aliphatic (serine and threonine) amino acid residues. However, to acquire full catalytic activity, DYRKs require phosphorylation in a conserved “YxY” motif in their activation loop [2–6]. Regarding the homology of the kinase domain, DYRK family is classified into three subfamilies: pre-mRNA Processing Factor 4 kinases (PRP4s), homeodomain-interacting protein kinases (HIPKs), and DYRK kinases [1–3]. Within the DYRK subfamily phylogenetic analysis reveals three groups: Yak group (only in lower eukaryotes: YAK1p in Saccharomyces cerevisiae and YAKA in Dictyostelium discoideum), DYRK1, and DYRK2 groups. Specifically, DYRK1 group encompasses...
mammalian DYRK1A and DYRK1B, minibrain (Mab) in *Drosophila melanogaster* and MBK1 *Caenorhabditis elegans*, whereas DYRK2 group contains several eukaryotic organisms, including mammalian DYRK2, *D. melanogaster* dDYRK2 (Smi35A) and dDYRK3, *C. elegans* MBK-2, and *Schizosaccharomyces pombe* POMP1p [1, 3, 7, 8]. In addition, the atypical class II DYRKs homolog TbDYRK has been described with a singular “FTY” activation loop motif, unlike DYRK kinases in other eukaryotes [9].

In mammals, DYRK subfamily is represented by two classes, class I (DYRK1A and DYRK1B) and class II (DYRK2, DYRK3 and DYRK4) [10]. Apart from the common central kinase domain, there are clear sequence differences between the subclasses defining characteristics involving subcellular localization, substrate specificity, and tissue distribution. Unlike class I DYRKs, which are mainly localized in the nucleus, class II DYRKs are predominantly localized in the cytoplasm. Class I and II subfamily members share a conserved central kinase domain and an adjacent N-terminal DH-box (DYRK homology box) but they possess different extended N- and C- terminal regions [1]. In this review, we intend to summarize in an ordered and detailed way the state of the art of DYRK2 kinase, making a deep review of the published literature as well as the available information in several databases.

**Sequence and structure**

DYRK2 is widely considered to be the most important member among the class II DYRK protein subfamily [2–4, 11]. Database searches have identified different ESTs (expressed sequence tags) in the human genome, mapping this protein to chromosome 12q15 with a 17069 base pair length [12]. Moreover, DYRK2 orthologue genes are present in 99 mammal species, while 13 parologue genes have been identified in other species. According to the Ensembl Genome Browser (January 2020), six different splice variants of DYRK2 have been identified [1], two of which have been isolated. DYRK2-203 transcript codifies 528 amino acids protein, being this sequence considered the most prevalent DYRK2 isoform or isoform 1 (NCBI Reference: NP_003574.1). DYRK2-202 produces a 601 aa sequence encoding a larger N terminus of 73 aa (DYRK2 isoform 2; NCBI Reference: NP_006473.2) (Fig. 1a). Although DYRK2 isoform 1 has been described as the most prevalent, the majority of databases as well as some articles use DYRK2 isoform 2 sequence. Consequently, throughout this review, DYRK2 information will be referred to isoform 2.

Many existing studies in the literature have examined in detail the structure and domains of this protein. In the last few years, much more information on DYRK2 structure based on X-ray crystallography has become available. In 2013, Soundararajan et al. presented DYRK2 protein structure (PDB: 3KL2) [4]. In a major advance in 2018, Banerjee et al. described DYRK2 crystal structure with curcumine (PDB: 5ZTN) [13] and 1 year later with LDN192960 (PDB: 6K0J) [14]. There have also been numerous studies to investigate the functional roles of DYRK2 domains. First, there are two common features shared among all DYRKs members. One is the presence of a distinctive motif called the DYRK-homology box (DH box), which is necessary for the formation of tertiary structure, and the other is the kinase domain (residues 222-535) [1]. Additionally, and like the other DYRK class II members, it presents two NAPA (N-terminal autophosphorylation accessory region) domains (NAP1 and NAP2), which provide a chaperone function, thus enabling DYRKs to catalyze self-phosphorylation in the activation loop tyrosine [3, 4, 6, 15, 16]. The presence of an “activation loop” segment (Tyr380-Thr-Tyr382) where phosphorylation of Tyr382 allows DYRK2 maturation is worth mentioning. Other residues of important relevance are the DYRK2 active site Asp348 and the ATP binding site Lys251. Ultimately, another DYRK2 feature shared with all DYRK members (except DYRK3) is the presence of a nuclear localization signal (NLS) (residues 189-191) enabling DYRK2 translocation into the nucleus (Schematic representation Fig. 1b) [14, 17].

**Expression, regulation, and post-translational modifications**

DYRK2 presents an expression pattern that varies across tissues and which depends on the measured biological level. On the one hand, tissues such as small intestine or heart muscle show a high expression of DYRK2 that correlates well from RNA isoform level to protein level. On the other hand, in lymph nodes or skeletal muscle, the high RNA expression does not correlate with the protein abundance detected through antibody staining, suggesting a tissue-dependent regulation of DYRK2 at protein level (Fig. 2).

A proper understanding of DYRK2 regulatory mechanisms would certainly help to clarify its role in diseases, especially in tumoral progression. At the transcriptional level, it has been described that KLF4 repressed *DYRK2* gene expression in leukemic stem/progenitor cells [18]. Similarly, recent evidence shows that DNMT1 downregulates *DYRK2* gene expression methylating its promoter, thereby increasing the proliferation of human colorectal cancer cells [19, 20]. In the same way, miR-662, miR-208a, miR-338-3p, miR-499, and miR-187-3p directly downregulate *DYRK2* expression in colorectal cancer cells, fibroblasts, human intestinal cancer (HIC) cells, cardiomyocytes, and liver cancer cells, respectively [21–25].
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Like many other kinases, DYRK2 activation requires phosphorylation in an activation segment [26]. However, instead of requiring an upstream kinase, DYRKs catalyze self-phosphorylation to fully activate the kinase. In 2005, Lochhead et al. concluded that autophosphorylation of a crucial tyrosine residue in the activation loop is necessary for a correct kinase functionality. This event is mediated by a transitional intermediate version of DYRK resulting in a full kinase activation and a restricted kinase activity to Thr and Ser residues [3, 6]. Although DYRK self-phosphorylation has been considered as a one-time event, other groups have suggested that phosphorylation in its mature form could contribute to kinase fully activation [27]. In contrast with an increasing number of publications describing the presence of DYRKs active form autophosphorylation [4, 28–31], no information has yet been published addressing this event for DYRK2 (Fig. 1b).

In the case of post-translational modifications (PTMs), DYRK2 is tightly regulated in normal conditions by MDM2 ubiquitin ligase in the nucleus. Because of DNA damage, ATM kinase phosphorylates DYRK2 short isoform in Thr33 and Ser369 (Thr106 and Ser442 in DYRK2 long isoform). Thus enabling DYRK2 disassociation from MDM2 and consequent nuclear stabilization. This event finally leads to higher Ser46 phosphorylation of p53 [17, 32]. In addition, our group described that under hypoxic conditions, SIAH2 polyubiquitinates and degrades DYRK2. As a result, DYRK2-mediated Ser46 phosphorylation of p53.

**Fig. 1** Scheme of DYRK2 genomics, transcripts, protein, and post-translational modifications. **a** Illustrative DYRK2 human expression processing. Exons are presented as rectangles and introns as lines and depending on the DYRK2 transcript, a specific splicing event is shown. Transcript and protein lengths as well as chromosomal location are indicated. Translated and untranslated exons are presented with color and white, respectively. DYRK-202 transcript includes exon 2, thus producing a larger transcript (8912 bp) and a protein of 601 aa (pink). On the other hand, DYRK2-203 transcript does not include this exon, thereby producing a shorter transcript (2209 bp) and a protein of 528 aa (blue). **b** Schematic representation of DYRK2 isoform 2 with described PTMs. Unique sequence motifs are presented in the illustration: two N-terminal autophosphorylation accessory regions (NAPA I and NAPA II), the nuclear localization signal (NLS), DYRK-homology box (DH), the ATP binding pocket, the kinase domain and the active site. DYRK2 is coordinately regulated by phosphorylation and ubiquitination, by upstream modulators altering DYRK2 function, stability, and subcellular localization. The affected residues by those PTMs are represented, as well as the autophosphorylation residue (Tyr382).
is compromised [33]. In a major advance, a high throughput genome-scale screening was performed in 2008, identifying that MAP3K10 directly phosphorylates DYRK2. These phosphorylations on DYRK2 short isoform, Thr308 and Ser376 (Thr381 and Ser449 in DYRK2 long isoform) alter its activity toward GLI, thus modifying the Hedgehog signaling pathway (Fig. 1b) [34]. A recent article on this topic showed that LPS induces DYRK2 nuclear translocation, thereby altering its protein levels [35]. Finally, it has been reported the Cep78 role inhibiting EDD-DYRK2-DDB1 complex, thus compromising CP110 ubiquitination and modifying centriole length and cilia assembly [36]. Apart from the mentioned PTMs directly derived from original works, additional DYRK2 modifications were collected from PhosphoSitePlus [37] and iPTMNet [38] and are included in Supplementary Table 1.

**Mechanisms of action**

DYRK2 exerts its kinase function by phosphorylating Ser/Thr residues, and although at first it was considered a Pro-directed kinase, posterior studies showed that it affects substrates with a diversity of recognition motifs [4]. The
presence of the arginine residue at − 2 position and proline at + 1 turns out to be essential, as reported by Campbell and Proud in 2002 by in vitro experiments [39]. Despite this, a certain degree of variability has been described, sometimes referring to the arginine residue at − 1 position [40]. Taken together, all these data result in a consensus sequence sensitive for DYRK2 phosphorylation: ‘Rx(x)S/TP’ [4]. Nevertheless, it is important to remark that this pattern is not necessarily present in all phosphorylation sites. All the substrates described to date are summarized in Table 1.

Among its phosphorylation activities, DYRK2 has been referred as a priming kinase for GSK3β [41–46]. Glycogen synthase kinase 3 beta (GSK3β) is a Ser/Thr kinase with a wide range of substrates and functions [47, 48]. Although it is not essential, GSK3β usually requires the previous phosphorylation of the substrate to properly exert its activity [49, 50]. In this sense, DYRK2 phosphorylates some substrates that are subsequently recognized by GSK3β, such as CRMP4, c-Myc, c-Jun, SNAI, or elf2Be [43–46] (Table 1). In all those cases, the priming phosphorylation by DYRK2 is followed by a second phosphorylation by GSK3β, leading to the ubiquitination of these substrates and their consequent proteasomal degradation.

Besides the kinase activity, it has been described that DYRK2 also acts as a scaffold protein. In 2009, Maddika et al. described that DYRK2 functions as an intermediate

Table 1  DYRK2 substrates

| Substrate | Phosphosites | Organism | GSK priming | Methodology | Reference |
|-----------|--------------|----------|-------------|-------------|-----------|
| CARHSP1   | Ser30, Ser32, Ser41 | Human |             | In vitro assay; mass spectrometry | [110]     |
| CRMP4     | Ser522       | Human | Yes         | In vitro assay; point mutations | [111]     |
| DCX       | Ser306       | Human |             | Point mutations | [112]     |
| EIF2B5    | Ser544       | Human | Yes         | In vitro assay; mass spectrometry; phospho-specific antibody; point mutations | [45]     |
|           | Ser539 | Rat |             |             |           |
|           | Ser540 | Mouse |             |             |           |
| EIF4EBP1  | Ser65, Ser101 | Human |             | In vitro assay; point mutations; phospho-specific antibody | [113]     |
|           | Ser64 | Rat |             |             |           |
| GLI2      | Ser388, Ser1011 | Human |             | In vitro assay; mass spectrometry; phospho-specific antibody; point mutations | [34]     |
|           | Ser385, Ser1011 | Mouse |             |             |           |
| GYS1      | Ser640       | Human |             | In vitro assay; phospho-specific antibody; point mutations | [114]     |
|           | Ser640 | Rabbit |             |             |           |
| HSF1      | Ser320, Ser326 | Human |             | In vitro assay; mass spectrometry; phospho-specific antibody; point mutations | [64]     |
|           | Ser307, Thr323, Ser363 | Human |             | In vitro assay; mass spectrometry | [115]     |
| H3F3A     | Thr45        | Human |             | In vitro assay; mass spectrometry | [116]     |
| JUN       | Ser243       | Human | Yes         | In vitro assay; phospho-specific antibody; point mutations | [44]     |
| KATNA1    | Ser42, Ser109, Thr133 | Human |             | In vitro assay; phospho-specific antibody; point mutations | [51]     |
| MYC       | Ser62        | Human | Yes         | In vitro assay; phospho-specific antibody; point mutations | [44]     |
| NDEL1     | Ser336       | Mouse | Yes         | Mass spectrometry; phospho-specific antibody | [59]     |
| NFT1      | ?            | Drosophila | Yes | Point mutations | [61]     |
| NOTCH1    | Thr2512      | Human |             | In vitro assay; phospho-specific antibody; point mutations | [62]     |
| TP53      | Ser46        | Human |             | In vitro assay; phospho-specific antibody; point mutations | [32]     |
| RPT3      | Thr25       | Human |             | In vitro assay; phospho-specific antibody; point mutations | [66]     |
| SIAH2     | Ser16, Thr26, Ser28, Ser68, Thr119 | Human |             | In vitro assay; mass spectrometry; phospho-specific antibody; point mutations | [33]     |
| SNAI      | Ser104       | Human | Yes         | In vitro assay; phospho-specific antibody; point mutations | [43]     |
| STAT3     | Ser727       | Human |             | In vitro assay; phospho-specific antibody | [116]     |
|           | Ser727 | Mouse |             | In vitro assay; mass spectrometry | [117]     |
| MAPT/Tau  | Thr212       | Human | Yes         | In vitro assay; mass spectrometry; phospho-specific antibody; point mutations | [45]     |
| TBK1      | Ser527       | Human |             | Phospho-specific antibody; point mutations | [63]     |
| TERT      | Ser457       | Human |             | In vitro assay; phospho-specific antibody; point mutations | [52]     |
adaptor protein between EDD and DDB1 components of the EDVP (EDD-DDB1-VprBP) E3 ligase complex, especially in the G2/M cell cycle phase. Additionally, they showed that DYRK2 also phosphorylates katanin p60, one of its substrates, leading to its ubiquitination and subsequent proteosomal degradation [51]. A similar action was then reported for TERT (telomerase reverse transcriptase). This enzyme is another of the EDVP substrates which has and presents an essential role in telomere maintenance, thus supporting genomic stability [52]. More recently, it has been also reported that DYRK2-dependent phosphorylation of CP110 (Centrosomal Protein of 110 kDa) leads to DYRK2-EDVP E3 ligase complex recognition of the substrate and its consequent proteosomal degradation. The same study reveals a negative regulator of this CP110 degradation event: Cep78 (Centrosomal protein of 78 kDa) [36].

**Biological functions**

One way to predict the functionality of a protein kinase is evaluating its interactome in detail. For this reason, we have reconstructed the DYRK2 interactome using a combination of the physical interactors from the BioGrid database and our list of literature-curated interactors (Fig. 3a). The posterior functional characterization of these data through an over-representation analysis has allowed us to have a global vision of the contribution of DYRK2 to different cell functions, amongst which we can mention “Pathways in cancer”, “Cellular response to stress”, or “ATM pathway” (Fig. 3b). Furthermore, we have performed a detailed study of the available literature to date, where DYRK2 role stands out in:

**Cell survival**: Among the different pathways controlling cell-damage responses, p53 is certainly the most important protein inducing apoptosis-related genes after kinase phosphorylation [53, 54]. In this sense, Taira et al. in 2007 identified DYRK2 as a novel kinase that phosphorylates p53 in Ser46, inducing apoptosis after DNA damage [32]. This work undoubtedly centred the attention of the scientific community in studying DYRK2 role in the apoptosis response. Subsequent articles showed, for example, the ability of ATM to phosphorylate DYRK2 at Thr33 and Ser369 and enable its stabilization after DNA damage. Consequently, this event controls p53-dependent DYRK2 apoptotic response [17]. In 2012, our group proved that under hypoxic conditions DYRK2 expression is compromised due to the ubiquitin ligase SIAH2 activity. Therefore, this event reduces p53 activation and increases chemotherapy resistance [33]. More recently, KLF4 inhibition of DYRK2 has proven to decrease p53 activation promoting leukemic stem/progenitor cells survival and self-renewal [18].

On the other hand, despite these findings of the DYRK2 role as a proapoptotic kinase, other functions of this kinase controlling DNA stability have emerged. That is the case of the DNA double-strand break (DSB) repair process. After genotoxic stress, DYRK2 interacts and colocalizes with RNF8 regulating its ubiquitination activity. Intriguingly, silencing DYRK2 suppresses the mono-ubquitination of γ-H2AX and the foci formation of the p53-binding protein 1 (53BP1), thus failing to repair the DNA damage. This evidence suggests that both proteins cooperate in DSB repair [55].

**Cell development and differentiation**: Accumulating studies have revealed that DYRK2 has important roles in cell development and differentiation in several species. Regarding *D. melanogaster*, it has been described that the orthologue DmDyrk2 is critical for nervous system development. Specifically, DmDyrk2 expression is triggered in late larva state when the eye is patterned in the third antennal segment. This structure is responsible for smell and proper visual system development [56]. In the light of these observations, some authors have suggested a possible cross talk between DmDYRK2 and the Hedgehog signaling pathway [40]. In the case of *C. elegans*, several studies have found that the DYRK2 orthologous gene MBK-2 is essential for embryo viability. On the one hand, MBK-2 plays an important role in cytokinesis in the early embryo due to its regulation of maternal protein degradation [57]. Similarly, OMA-1 and OMA-2 MBK-2-mediated phosphorylation is necessary to convert this protein into key regulators of 1-cell embryo development [42]. In Zebrafish (*Danio rerio*), DYRK2/CDK5 phosphorylation of Dpyssl2 and Dpyssl3 (Dihydropyrimidinase-related protein 2 and 3) contributes to properly position Rohon–Beard neurons and neural crest cells in neural tube formation [41]. Additionally, there is a positive correlation between DYRK2 and MyoD (myoblast determination protein 1) during the early stages of embryo development, suggesting that fast-twitch muscle differentiation depends on this regulation [58]. Similarly, in mammals, DYRK2 emerged as responsible for sequential phosphorylation of NDEL1 modulating F-actin dynamics, thus altering both axonal and dendritic neurites development.
Thr25 phosphorylation kinase controlling G1/S transition.

Cations in 26S proteasome complex as the primary Rpt3

ous cancers. In 2016, Guo et al. revealed the DYRK2 impli-
regulation and the development and progression of numer-

cellular proteins, with enormous importance in cell cycle
protein complex in eukaryotes responsible for degrading

catenin signaling pathway [65].

Heat Shock Factor 1 (HSF1) [64]. In 2007, Imawari et al.
regulate the proteotoxic-stress response pathway regulating
response [63]. DYRK2 has also been reported to negatively
shown to take part in the IFN-β-mediated cellular antiviral

Hes1 and Hes5, but also in cell migration and invasiveness
[62]. Additionally, DYRK2 negative regulation of eIF2Be in cardiac myocytes results in
a reduced cardiomyocyte cell growth playing a physiological role in cardiomyocyte hypertrophy [68].

DYRK2 and disease

Due to the wide range of biological functions of this pro-
tein, DYRK2 role in the development of human diseases is
an increasingly important area. Although during the last 2
decades, an extensive work in this sense has been developed
[35, 65, 69, 70], there are still remarkable controversies.

Cancer: DYRK2 expression in cancer may vary widely
depending on the tissue as well as on the expression at the
RNA or protein level. Likewise, there is a large difference
of expression among the cell lines normally used in cancer
research. Data collected from different resources are sum-
marized in Fig. 4. In the same way, the bibliography includes
many works which analyze DYRK2 expression in human
tumor tissue compared to adjacent healthy tissue at differ-
ent levels (Table 2). Although in most studies, a decrease in
the expression is described (both at protein and RNA level)
in tumor tissues compared to normal tissue, in some cases,
such as ovarian cancer, it shows the opposite pattern. Also,
nowadays there are certain pathologies where the avail-
able studies are not yet conclusive. This happens especially
in breast, lung, and esophageal cancer, where more com-
plete and detailed studies should be performed to clarify
these differences. Likewise, and although to date, different
DYRK2 mutations in tumors have been described (Fig. S1),

[59]. Moreover, it has been proposed that this event leads
to fibroblast proliferation and myofibroblast differentiation
via NFAT phosphorylation [23]. Finally, a recent article
found an orthologue DYRK2 gene in Trypanosoma brucei
(TbDYRK2) with a transcriptional function in the regulation
of this parasite developmental pathway [60].

Gene transcription: DYRK protein kinase family has been
considered a master regulator of gene expression programs
[3]. In 2006, DYRK1A and DYRK2 were first identified as
novels regulators of the transcription factor NFAT. DYRK2
phosphorylates the conserved serine-proline repeat 3 (SP-
3) motif of the NFAT regulatory domain priming further
phosphorylation by GSK3β and CK1 [61]. As discussed
above, DYRK2 phosphorylation of p53 in Ser46 triggers
the activation of the apoptosis-related pathway. Besides that,
TUNEL assays physiologically suggested an increase of the
apoptosis, those effects were also visualized in the p53AIP1
expression increase [32]. Next, Varjosalo et al. demon-
strated that DYRK2 phosphorylates Gli2 and inhibits Sonic
hedgehog (Shh) signaling, thereby resulting in a GLI protein
expression reduction [34]. Similarly, it has been described
as a priming kinase of c-Jun and c-Myc [44]. Very recently,
our group revealed the DYRK2 implication in NOTCH1
signaling pathway mediating NOTCH1-IC degradation.
NOTCH1-IC regulation mediated by DYRK2 reveals an
increase not only in NOTCH1 downstream genes, such as
Hes1 and Hes5, but also in cell migration and invasiveness
[62]. Additionally, DYRK2 negative regulation of TBK1 has
shown to take part in the IFN-β-mediated cellular antiviral
response [63]. DYRK2 has also been reported to negatively
regulate the proteotoxic-stress response pathway regulating
Heat Shock Factor 1 (HSF1) [64]. In 2007, Imawari et al.
reported DYRK2 regulation of CDK14 through the Wnt/β-
catenin signaling pathway [65].

Proteasomal regulation: The 26S proteasome is a crucial
protein complex in eukaryotes responsible for degrading
cellular proteins, with enormous importance in cell cycle
regulation and the development and progression of numer-
ous cancers. In 2016, Guo et al. revealed the DYRK2 impli-
cations in 26S proteasome complex as the primary Rpt3
Thr25 phosphorylation kinase controlling G1/S transition.
DYRK2 inactivation and consequent loss of phosphorylation
are enough to reduce protein activity, slowdown cell prolif-
eration, and potentiate bortezomib anti-growth effect [13,
14, 66].

Microtubule formation: As we pointed out before in this
review, DYRK2 is part of the EDD–DDB1–VPRBP (EDVP)
E3 ligase complex. This complex has been described to
degrade katanin p60 due to the main action of DYRK2 by
recognizing and phosphorylating katanin p60, thus control-
ling mitotic transition. Silencing either EDD or DYRK2
leads to defective mitotic progression [51]. Likewise,
EDVP–DYRK2 complex controls the centrosome homeo-
asis (considered the major microtubule-organizing center
in most eukaryotic cells) through a regulation mechanism
mediated by Cep78 protein [36].

Other functions: Finally, to conclude this section, the lit-
erature has identified other areas where DYRK2 function is
involved. Using a kinome-wide siRNA screen, DYRK2 was
reported to negatively regulate the human pregnane X recep-
tor (hPXR). Moreover, this observation was further validated
by in vitro kinase and ubiquitination studies. It has been
previously described that hPXR is part of the human liver
detoxification system. Therefore, DYRK2 regulation of this
system is a plausible idea [67]. On the other hand, DYRK2
negative regulation of elf2Be in cardiac myocytes results in
a reduced cardiomyocyte cell growth playing a physiological
role in cardiomyocyte hypertrophy [68].

Fig. 4 DYRK2 expression in cancer. a DYRK2 RNA expression
across TCGA cohorts. Data were obtained with FireBrowseR [107].
The boxplot represents the DYRK2 transcripts per million (TPM)
distributions for the tumor tissues of each cohort and when available,
for matching normal tissues. b DYRK2 RNA expression on cell lines
from the Cancer Cell Line Encyclopedia (https://portals.broadinsti-
tute.org/ccle) [108] grouped by cell line origin (X-axis). c DYRK2
protein staining levels from the Human Protein Atlas pathology data
(https://www.proteinatlas.org/about/download) [103]. For each cancer
type (X-axis), the point size indicates the number of samples with a
particular antibody staining level (Y-axis).
additional studies are needed to evaluate its incidence and effects.

As described above, DYRK2 modulates functions as important in cancer development as apoptosis, cell proliferation, or cell growth, among others. For that reason, it is not surprising that most of the DYRK2 knowledge is related to this pathology. Below we describe the DYRK2 role on the control of these functions.

**Cell cycle:** First, DYRK2 ability to affect G2 phase through the degradation of essential phase transition proteins was described. As discussed above, katanin p60 and TERT are phosphorylated and degraded by the EDVP–DYRK2 complex at G2/M transition [51, 52]. Later in 2012, Taira et al. described DYRK2 ability to degrade c-Jun and c-Myc, two transcription factors essential in the cell cycle control, by sequential phosphorylation together with GSK3β. They observed higher levels of the unphosphorylated forms after DYRK2 downregulation, followed by a shortened G1 phase and higher levels of breast cancer invasiveness [44]. The authors support that G1 phase prolongation leads to a delay in the expression of other key proteins such as Cyclin E [71]. Tumor growth was potentiated after DYRK2 silencing in xenograft models, thereby revealing the clear implications of this event in cancer development. In line with these results and supporting the importance of DYRK2 phosphorylation in G1/S transition, the ability to regulate 26S proteasome complex through Rpt3 Thr25 phosphorylation was described [66]. In this case and contrary to the previous evidence, loss of DYRK2 attenuated cell growth. Moreover, the same group later described how the inhibition of DYRK2 activity mediated by curcumin and LDN192960 reduces cell proliferation and induces cell death, thus compromising triple-negative breast cancer (TNBC) [13] and multiple myeloma (MM) growth [14]. These differences between both observations need to be clarified in the future and could be explained because of the use of different cell lines and the studied cancer subtype.

**Apoptosis:** As mentioned above, among its several functions, it has been reported that this kinase is a regulator of the apoptotic response. Different works sustain this role and the first of them showed that DYRK2 regulates p53-dependent apoptosis in response to DNA damage [32, 44]. This, together with the findings that DYRK2 is stabilized in the apoptotic response to DNA damage as a result of ATM-dependent Thr33 and Ser369 phosphorylation, reinforces the idea of DYRK2 as a proapoptotic protein. Other works are in line with this idea, for example, a recently published study showed that KLF4 protein inhibits DYRK2, reducing apoptosis and inducing survival of leukemic and stem progenitor cells [18]. Additionally, it has been demonstrated that DYRK2 suppression by knockout or inhibition with curcumin or LDN192960 leads to an increase of apoptosis in triple-negative breast cancer cells and xenografts due to a reduction of the 26S proteasome activity [13, 14].

### Table 2: Expression of DYRK2 in human tumor tissue compared with normal

| Cancer type                        | Expression | Analyzed level | Role /function | References |
|------------------------------------|------------|----------------|----------------|------------|
| Colorectal cancer                  | Low        | RNA/Protein    | Tumor suppressor | [19]      |
|                                    | Low        | RNA/Protein    | Tumor suppressor | [22]      |
|                                    | Low        | Protein        | Tumor suppressor | [44]      |
|                                    | Low        | RNA/Protein    | Tumor suppressor | [77]      |
| Breast cancer                      | Low        | Protein        | Tumor suppressor | [43]      |
| Triple-negative breast cancer      | High       | RNA/Protein    | Oncogenic       | [14]      |
| Hepatocellular carcinoma           | Low        | Protein        | Tumor suppressor | [118]     |
|                                    | Low        | Protein        | Tumor suppressor | [80]      |
| Lung cancer                        | High       | RNA/Protein    | Oncogenic       | [69]      |
|                                    | Low        | Protein        | Tumor suppressor | [119]     |
| Esophageal cancer                  | Low        | Protein        | Tumor suppressor | [44]      |
|                                    | High       | RNA/Protein    | Oncogenic       | [69]      |
| Chronic myeloid leukemia           | Low        | RNA            | Tumor suppressor | [18]      |
| Ovarian cancer                     | High       | Protein        | Oncogenic       | [73]      |
| Glioma                             | Low        | Protein        | Tumor suppressor | [70]      |
| Kidney cancer                      | Low        | Protein        | Tumor suppressor | [44]      |
| Anus cancer                        | Low        | Protein        | Tumor suppressor | [44]      |
| Prostate cancer                    | Low        | Protein        | Tumor suppressor | [44]      |

The table lists the different studies where DYRK2 levels in tumor have been analyzed as compared to healthy tissue. We show the expression level, the analyzed level (protein or RNA) as well as the proposed function in carcinogenesis.
that DYRK2 negatively regulates invasion and metastatic antitumoral role [22]. Nevertheless, not all works state migration and invasion, thus reinforcing potential DYRK2 lator of DYRK2 expression of CRC cells, modulating their regulatory mechanisms and implications of DYRK2 in cancer development and aggressiveness is the ability of the cells to migrate and invade different niches. In connection with this, it is remarkable to mention the role of DYRK2 in the control of epithelial to mesenchymal transition (EMT) and cell motility. In this sense, it has been described that DYRK2 is able to phosphorylate SNAIL as a priming kinase for GSK3β, inducing its degradation with the subsequent suppression of the EMT [72]. This DYRK2-dependent process leads to a reduction of the metastatic potential in human breast and ovarian cancer cell lines and xenografts [43, 73, 74]. In a recent study, Ryu et al. demonstrated that SNAIL phosphorylation can be avoided by the action of p38, then promoting EMT and metastasis in xenograft models of ovarian cancer [73]. In addition, DYRK2 has been reported to be implicated in the suppression of EMT in glioma since it is associated with decreased expression of Vimentin and SNAIL and stabilization of E-cadherin levels [70]. Additionally, in 2013, Mrugala et al. showed that DYRK2 protein levels are significantly reduced in high-grade glioma tissue compared to low-grade glioma tissue, suggesting its relevance in metastatic process and invasiveness potential [75]. In the case of colorectal cancer (CRC), it has been shown that DYRK2 compromises metastasis in CRC cell lines and xenografts and this also correlates with a better survival rate [76, 77]. Together with its role in EMT, DYRK2 also regulates several substrates related to cell motility and metastatic potential. NOTCH1 protein levels are increased in DYRK2 knockout human breast cancer cell lines, leading to a rise of the invasion and migration potential of these cells compared to the wild type [62]. Using DYRK2-depleted breast cancer cell lines, it has been described that this kinase mediates the invasion potential of these cells via CDK4 regulation [65]. Additionally, miR-622 has been reported to be a regulator of DYRK2 expression of CRC cells, modulating their migration and invasion, thus reinforcing potential DYRK2 antitumoral role [22]. Nevertheless, not all works state that DYRK2 negatively regulates invasion and metastatic potential of cancer cells. For example, it has been reported that DYRK2 inhibition by LDN192960 affects the tumorigenic potential of triple-negative breast cancer cells due to DYRK2 activity on 26S proteasome [13, 14].

**Cancer stem cell (CSC) formation:** To date there are several studies relating DYRK2 expression with this relevant cell population. First, Mimoto et al. proved that DYRK2 negatively regulates breast cancer stemness formation targeting KLF4 expression. In this work, the authors observed that DYRK2 inversely correlates with CD44+/CD24− subpopulation and KLF4 expression. Consequently, DYRK2 down-regulation leads to a sphere-forming ability, thus indicating self-renewal capacity [78, 79]. In the same sense and as we have previously mentioned, several studies have described DYRK2 ability to regulate c-Myc and SNAIL levels, both considered key stemness proteins [44]. Likewise, further efforts have been done to elucidate DYRK2 role in chronic myeloid leukemia (CML) stem/progenitor cells. In 2019, Park et al. demonstrated that SIAH2 ubiquitin E3 ligase inhibition using vitamin K3 produces the stabilization of DYRK2, which inhibits self-renewal via c-Myc depletion and p53 activation [18].

**Cell survival:** Studies conducted in different cancer patients show that DYRK2 correlates with the survival rate according to the specific cancer subtype. For example, there is a positive correlation of DYRK2 expression with patient survival in hepatocellular, non-small cell lung, pulmonary adenocarcinoma, colorectal and bladder cancers, and non-Hodgkin’s lymphoma [76, 77, 80–84]. On the contrary, other results have revealed that DYRK2 expression in neuroblastoma inversely correlates with patient survival [85]. In contrast to the previously observed positive relation in breast cancer [43, 86], Guo et al. observed an inverse correlation between DYRK2 expression and survival in the same cancer subtype [66]. Additionally, DYRK2 depleted and parental multiple myeloma cell lines such as MPC11 and 5TGM1 have been used in xenograft models, proving a negative relation with mice survival [14]. In the same line, this pro-survival function is related to higher chemotherapy response. It has been revealed that higher DYRK2 expression tumors are more susceptible to being treated using chemotherapy. Some well-known examples are bladder cancer, hepatocellular cancer, breast cancer, and non-small cell lung cancer [43, 80, 81, 83].

**Other diseases:** Together with the efforts to elucidate the regulatory mechanisms and implications of DYRK2 in cancer, the role of this kinase in other pathologies has been studied. For example, DYRK2 has been described as an essential kinase for virus-triggered IFN-β negative induction and the cellular antiviral response [63]. Similarly, in HIV-1 infection, DYRK2 orchestrates its effects on the regulation of microtubule dynamics during the virus replication cycle. HIV-1 accessory viral protein R (Vpr) possesses an
Pharmacological inhibition of DYRK2

To date, different molecules have demonstrated their ability to inhibit DYRK2 activity. Although the majority shows an effect on other members of the DYRK family or even on other kinases, some of these chemicals have shown enough specificity to help to elucidate DYRK2 functionality both in vitro and in vivo.

Harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole): Tricyclic β-carboline alkaloid isolated from Peganum harmala L., this molecule is a high-affinity inhibitor of monoamine oxidase A (MAO-A) [88, 89] and probably the most widely used to study the chemical inhibition of DYRK family. Able to inhibit all the DYRK family, it is an ATP-competitive inhibitor with a potent activity against DYRK1A with an IC₅₀ of 0.08 mM. Regarding DYRK2, it shows an IC₅₀ of 0.8 mM [90, 91]. As a cell permeable inhibitor, it is commonly used to study DYRK family functionality [92]. It inhibits cell proliferation, migration, and invasion in several human cancer cell lines [85, 93].

Curcumin (diferuloylmethane): Active component of the perennial herb Curcuma longa, it presents multiple biological activities through different mechanisms of actions [94]. It occupies the ATP-binding pocket of DYRK2, inhibiting its activity in vitro with an IC₅₀ of 5 nM, as well as pThr25 RPT3 phosphorylation in HEK293T cells stably transfected with Flag-DYRK2 in a dose-dependent manner, with maximum effects at 3–10 μM concentrations. It shows the ability to inhibit other members of the DYRK family, but to a lesser extent (DYRK1A, IC₅₀: 190 nM; DYRK3, IC₅₀: 20 nM). It has shown its ability to sensitize triple-negative breast cancer and multiple myeloma TNBC cell lines through the partial inhibition of the proteasome activity [13].

LDN192960 (3-(2,7-dimethoxyacridin-9-yl)sulfanlypropan-1-amine:dihydrochloride): Initially developed as an inhibitor of Haspin [95, 96], it occupies the ATP-binding pocket of DYRK2, inhibiting its activity in vitro with an IC₅₀ of 13 nM, as well as Thr25 RPT3 phosphorylation in HEK-293T cells stably transfected with Flag-DYRK2 in a dose-dependent manner, with maximum effects at concentrations between 1–10 μM. It also inhibits other DYRK family members (DYRK1A, IC₅₀: 122 nM; DYRK3, IC₅₀: < 3 nM). Like curcumin, different approximations have demonstrated its ability to inhibit neoplastic progression in triple-negative breast cancer and multiple myeloma cell lines [14].

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Conclusions and perspectives

Although, during the last 20 years, new insights have been provided into DYRK2 biological function and its role in some diseases such as cancer, there is still little work on this kinase despite the relevance of some of its substrates. Its key role in the control of cell processes as important as those described in this revision shows the need of enlarging the knowledge of many biological, functional, and regulating aspects of this protein. On the one hand, it is remarkable that the small number of regulating mechanisms and stimuli able to modify the expression and activity of this kinase that we know to date. In this sense, an effort should be made to describe new post-translational modifications able to alter DYRK2 functionality, since the number and types described so far are surprisingly low. Similarly, new DYRK2 substrates should be described, thus opening the way to the discovery of their role in disease.
of new functions. These two aspects would no doubt help to clarify its role in the control of the cell cycle and apoptosis, which have not been clarified yet. On the other hand, it would be relevant to clarify the role of this kinase in tumor control and development. Even though the available literature to date mainly describes it as a tumor suppressor, this could not be the case in all tumor subtypes. This needs to be explained, above all in breast and lung cancer. Finally, and despite the existence of several molecules able to inhibit DYRK2 activity, it would be a challenge to research new and more specific molecules, which may allow us to pharmacologically modify the relevant functionality of this kinase in those required diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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