CK2 Inhibitors and the DYRK Family Protein Kinases

Yoshihiko Miyata

Abstract CK2 is a ubiquitous and pleiotropic Ser-/Thr-targeting acidophilic protein kinase. CK2 plays an important role in the aberrant proliferation of malignant cancer cells. Because of constitutive activity of CK2, its inhibitors have been widely used to analyze the physiological function of CK2 in cellular systems. In addition, CK2 inhibitors are regarded as promising cancer chemotherapeutic candidates. Recently, several commonly used CK2 inhibitors have been shown to suppress DYRK (dual-specificity tyrosine-phosphorylation-regulated protein kinase) family protein kinases. Thus, the results obtained with conventional CK2 inhibitors should be carefully interpreted considering their effects on DYRKs. In this chapter, after an introductory section on CK2 and its inhibitors, the structures and activation mechanism of DYRK family protein kinases are portrayed. DYRK1A is one of the pivotal factors encoded in Down's syndrome critical region on human chromosome 21, and dysregulation of DYRK1A may be a molecular basis of various phenotypes observed in Down's syndrome patients. Substrates, physiological function, binding partners, regulatory mechanisms, and CK2 inhibitor sensitivities of DYRK1A are described in detail. Finally, the biological and clinical importance of CK2 and DYRK1A as therapeutic targets will be discussed.

Keywords CK2 • DYRK1A • Phosphorylation • Protein kinase • Inhibitor • Down's syndrome • TBB • Cancer chemotherapeutics • NFAT • Leukemia

1 CK2 and Its Inhibitors

1.1 Protein Kinase CK2

CK2 (previously misleadingly called as "casein kinase 2") is a ubiquitous serine-/threonine-specific protein kinase [1–6] which was discovered many decades ago as a major responsible enzyme for protein phosphorylation. CK2 is a tetrameric

Y. Miyata (✉)
Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan
e-mail: ymiyata@lif.kyoto-u.ac.jp

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enzyme consisting of two α- and/or α′-catalytic subunits and two accessory β-subunits that dimerize to bring two catalytic subunits together. CK2 preferentially phosphorylates Ser/Thr residues that are followed by a cluster of acidic amino acids; thus, CK2 is categorized in the "acidophilic" protein kinase class. This is a sharp contrast to other classical AGC group "basophilic" protein kinases, including PKA and PKC. Many signaling protein kinases belonging to the CMGC group such as mitogen-activated protein kinases (MAP kinases) and cyclin-dependent kinases (CDKs) specifically phosphorylate Ser/Thr residues followed by a Pro residue; thus, they are categorized as "Pro-directed" protein kinases. CK2 shares a common root with CMGC group protein kinases in the phylogenic tree (Fig. 1), but the amino acid sequence of the catalytic domain of CK2 is only distantly related to other CMGC kinases. CK2 plays essential and pivotal roles in many physiological systems by phosphorylating a wide variety of more than 300 cellular proteins [7]. In fact, phospho-proteomic analyses suggest that CK2 alone may be responsible for more than 20 % of the eukaryotic protein phosphorylation sites [8, 9]. However, even after the long history of CK2 studies, it remains unclear how CK2 activity is regulated in cells. CK2 activity is readily detectable in extracts of cells and tissues without any stimulation. In contrast to many other protein kinases that require activating

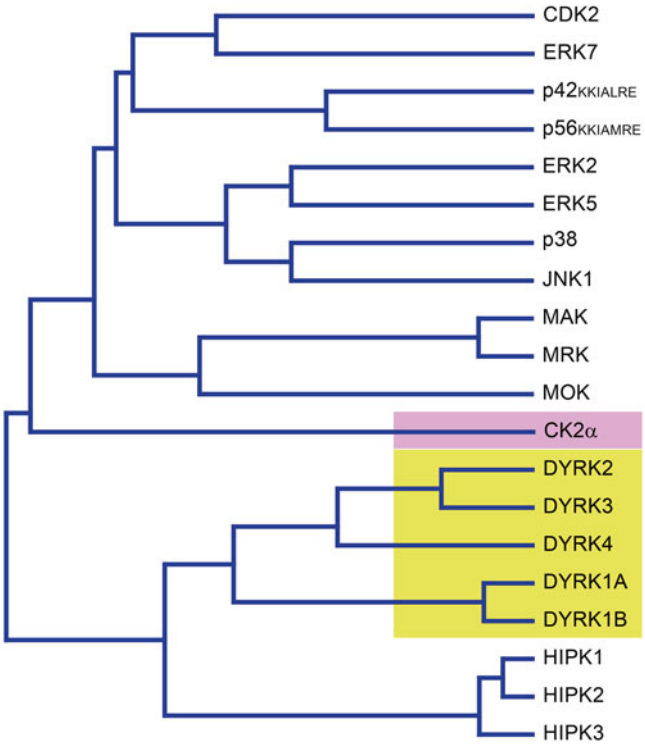

Fig. 1 A phylogenic tree of CMGC group Pro-directed protein kinases and CK2α. Similarity of amino acid sequences of the catalytic domains of indicated kinases was analyzed using the fixed distance scale UMGMA method. CK2 and DYRK family protein kinases are *colored*

low-molecular-weight factors, no pivotal factor that directly activates CK2 in cells is revealed to date. Signaling protein kinases are often positively or negatively controlled by binding to regulatory partner subunits. The β-subunit of CK2 confers substrate specificity in CK2 holoenzyme and CK2β is required for optimal phosphorylation of certain substrates [4, 10, 11]. However, the catalytic CK2α/α′ alone seems to be able to phosphorylate most of CK2 substrates. Many signaling protein kinases are regulated by phosphorylation with upstream "kinase kinases." Several protein kinases, including Cdc2, Src, ERK2, and Akt, have been reported to phosphorylate CK2 [12–15], but none of these kinases seems to be absolutely required for CK2 activity. All the evidence suggests that the regulatory mechanism of CK2 activity is different from that of other well-characterized signaling protein kinases whose activities are responsive to cellular stimulations. Therefore, CK2 has been often suggested to be "constitutively active." On the other hand, CK2 activity is upregulated in highly malignant cancer cells [16, 17]. CK2 activity is intimately related to the cell cycle [18–20] and modulated during the circadian rhythm [21–23]. In addition, CK2 plays an important role in mediating Wnt/β-catenin signaling [24]. These facts altogether indicate that there should be a precise control mechanism for CK2 in cells, despite its constitutive activity when isolated.

1.2 CK2 Inhibitors

CK2 inhibitors have been widely used both in vivo and in vitro to examine the physiological role of CK2. CK2 activity has long been known to be strongly inhibited by polyamines such as heparin, and thus the heparin sensitivity has been regarded as a Merkmal for CK2 [25], but high molecular weights and the highly negatively charged nature of such polyanions limit their applicability to intact cells and bodies. In fact, heparin does not enter into the cells and also it shows many physiological effects on cells other than CK2 inhibition: therefore, it can only be used as a specific CK2 inhibitor in in vitro studies. Several protein kinase inhibitors, including emodin [26], quercetin [27], and apigenin [28, 29] suppress CK2 activity (Table 1); however, they are rather classified as general ATP-competitive ligands that inhibit a large number of protein kinases other than CK2. One of the first clues of a highly specific CK2 inhibitor came from a finding that DRB (5,6-dichloro-1-β-d-ribofuranosylbenzimidazole), an inhibitor of eukaryotic mRNA transcription, inhibits in a parallel manner the activity of CK2 [30]. A screening of derivatives of DRB identified several compounds that showed higher specificity for CK2 among other protein kinases known those days. The most widely used inhibitor for CK2 in the scientific literatures may be 4,5,6,7-tetrabromobenzotriazole (TBB), which was described as a potent and specific inhibitor for CK2 from yeast and mammalian sources in 1995 [31]. As of June 2014, more than 70 publications using TBB as a CK2 inhibitor are included in the PubMed database, and TBB still continues to be used as a specific CK2 inhibitor. TBB strongly inhibits CK2 ($Ki = 120$ nM) but not another ubiquitous acidophilic kinase CK1. This is an advantage of TBB, because some previous CK2 inhibitors such as DRB showed almost comparable inhibition of CK1.

Table 1 Inhibitors for CK2 and DYRK1A. IC_{50} (μM) or % remaining activities collected from literatures are shown

	CK2	DYRK1A
Staurosporine	19.5	0.012
Emodin	0.9–2.0	4.2
Quercetin	19 % at 20 μM	21 % at 10 μM
Apigenin	30	15 % at 10 μM
NBC	0.37	15
dNBC	32	0.6
DRB	6–10	19 % at 10 μM
IQA	0.39	50 % at 10 μM
DMAT	0.15	0.12
DMAT	7 % at 10 μM	5 % at 10 μM
TBB	0.15–1.6	1–4.4
TBB	6 % at 10 μM	4 % at 10 μM
TBBz	10 % at 10 μM	3 % at 10 μM
CX-4945	0.001–0.003	5 % at 0.5 μM for DYRK2
Harmine	>10	0.034
Leucettine L41	0.32	0.012

Note that the data were obtained in different systems with different ATP concentrations and different kinase sources

Recently, because many more kinases are available to examine the effects and specificities of protein kinase inhibitors, it is now apparent that the specificities of some of conventional CK2 inhibitors seem to be inadequate for cell biological and clinical purposes. Notably, many of the commonly used CK2 inhibitors, including DRB, TBB, TBBz (4,5,6,7-tetrabromo-1H-benzimidazole), IQA ([5-oxo-5,6-dihydro-indolo(1,2-a)quinazolin-7-yl]acetic acid), and DMAT (2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole), were revealed to inhibit DYRK family protein kinases as well (Table 1). Therefore, the results obtained with these conventional CK2 inhibitors should be carefully reinterpreted considering their effects on DYRK family protein kinases. In the following sections, a concise description on DYRK family protein kinases will be presented to be of help for those who are interested in and investigating CK2.

2 DYRK Family Protein Kinases

2.1 Structures of DYRK Family Protein Kinases

DYRK (dual-specificity tyrosine-phosphorylation-regulated protein kinase) family protein kinases belong to the CMGC protein kinase group that also includes MAP kinases and CDKs (Fig. 1). In mammalian species, the DYRK family consists of

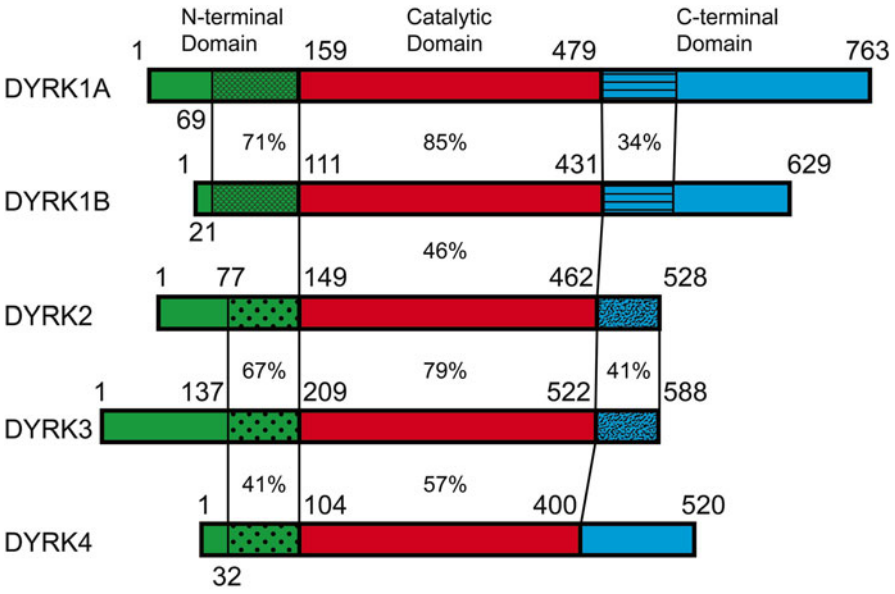

Fig. 2 A schematic illustration of the DYRK family protein kinase structures. Amino acid identities in the N-terminal, kinase catalytic, and C-terminal domains of the five DYRK members with amino acid numbers are shown

five members, including DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4. According to the amino acid sequence similarity, DYRK1A and DYRK1B are categorized into "type 1" DYRKs, whereas DYRK2, DYRK3, and DYRK4 are "type 2" DYRKs. All DYRK family protein kinases share a homologous protein kinase catalytic domain in the center of their amino acid sequences (Fig. 2). The amino acid identity in the kinase domain is highest between DYRK1A and DYRK1B (85 %) and then between DYRK2 and DYRK3 (79 %). On the other hand, the amino acid identities between type 1 and type 2 DYRKs in the catalytic domain do not exceed 50 % (Fig. 2). All DYRKs possess characteristic N-terminal and C-terminal domains. DYRK1A and DYRK1B share two regions in their N- and C-terminal domains with amino acid identities of 71 % and 34 %, respectively. On the other hand, all the type 2 DYRKs share in their N-terminal domain a homologous region that is not observed in type 1 DYRKs. Finally, DYRK2 and DYRK3 have in common a similar C-terminal domain with 41 % amino acid identity (Fig. 2). DYRK1A is ubiquitously expressed in many species in multiple tissues and rich in testis and heart [32]. On the other hand, other DYRKs expressed mostly in testis [32]. DYRK1A has been most intensively studied so far; therefore, this chapter focuses mostly on DYRK1A. The detailed description of other members of DYRKs can be obtained in several review articles [33–36].

2.2 Activation Mechanism of DYRK1A

Many CMGC group protein kinases contain a phosphorylation site in the activation loop region between the protein kinase subdomains VII and VIII. Phosphorylation of the activation loop by an upstream kinase is essential for the optimal activity of these kinases. For example, conventional MAP kinases, including ERK1/2, JNK|SAPK, and p38, encode a TXY motif in the activation loop, and phosphorylation of both the Thr and Tyr residues in the motif by upstream activating kinases, MAP kinase kinases (MAPK kinase, or MEK), activates the MAP kinases. DYRK family protein kinases contain a YXY motif in the corresponding region of the activation loop. Instead of being phosphorylated by an activating upstream kinase, DYRK1A phosphorylates the Tyr in the motif by a *cis*-autophosphorylation mechanism [37]. This autophosphorylation is required for the full activity of DYRK1A; a replacement of Tyr in the region by a non-phosphorylatable amino acid significantly obstructs the protein kinase activity of DYRK1A. In addition, it was proposed that the autophosphorylation of DYRKs occurs during the polypeptide translation and plays an important role in switching the kinase from the autophosphorylating Tyr-kinase into a Ser/Thr kinase toward exogenous substrates [38]. A recent analysis modified the scheme and it is proposed that DYRK1A is in a structural equilibrium between the Tyr-autophosphorylating kinase and the Ser-/Thr-targeting kinase and that the Tyr-autophosphorylation stabilizes the conformation suitable for the Ser-/Thr-targeting kinase activity in the equilibrium [39]. The involvement of molecular chaperones has been reported in the maturation process of GSK3β [40]. Similarly, molecular chaperones may play a role in the conformational regulation of DYRKs.

Just like CK2, it seems that DYRK1A does not require a cofactor, upstream activating kinases, or an associating subunit for its activity, and it is "constitutively active" after translation and Tyr-autophosphorylation. A possibility cannot be excluded, however, that phosphorylation of DYRK1A by other kinases modulates its activity. In addition, there should be binding partners of DYRK1A that control the activity, stability, and localization of DYRK1A in cells (see Sect. 4).

2.3 Substrates for DYRK1A

Substrate specificities of DYRKs have been determined using peptide-based in vitro assays [32, 41, 42]. Although there may be slight specificity differences between the members, DYRKs in common preferentially phosphorylate Ser/Thr residues followed by a Pro. Therefore, DYRKs can be categorized into the Pro-directed protein kinases as many other CMGC group kinases. In addition, DYRK1A efficiently phosphorylates Ser/Thr residues with Arg at the –3 position and Pro at the –2 position, resulting in the optimal recognition sequence of RPX[ST]P [41]. This is strikingly different from the phosphorylation consensus sequence of CK2, [ST]XX[DEpSpTpY]; therefore, most of CK2 phosphorylation sites in proteins might

not be phosphorylated by DYRK1A, except in rare cases that a sequence fulfills the optimal substrate consensus for both CK2 and DYRK1A.

The number of reported DYRK1A substrates has been rapidly increasing in the last decade. The list of DYRK1A substrates includes eIF2B [43], microtubule-binding protein tau [43–46], splicing factor SF3b1|SAP155 [47], caspase 9 [48, 49], glycogen synthase [50], CRY2 [51], cyclin L2 [52], DSCR1|RCAN1 [53], and several transcription factors, including STAT3 [37, 54], CREB [55], FKHR [56], Gli1 [57], and NFAT [58, 59]. The best characterized DYRK1A substrate, NFAT (nuclear factor of activated T cells), locates in the cytosol when phosphorylated and hence transcriptionally inactive. After dephosphorylation by a Ca^{2+}-activated phosphatase calcineurin, NFAT translocates to the nucleus and cooperates with multiple transcription factors to regulate the target gene expression. Nuclear NFAT protein is phosphorylated by several protein kinases including DYRK1A and translocates back to the cytosol [58, 59]. Therefore, DYRK1A is a negative regulator of NFAT-dependent signaling process.

2.4 Physiological Function of DYRK1A

Among the five members of DYRKs, DYRK1A has attracted most extensive attention, not only because of its ubiquitous expression but also because of its pivotal role in pleiotropic phenotypes observed in Down's syndrome caused by chromosome 21 trisomy. The analysis of partial trisomy cases suggested that Down's syndrome critical region (DSCR) in chromosome 21 between 21q22.1 and 21q22.3 is responsible for Down's syndrome caused by the trisomy. DSCR includes 33 genes, and DYRK1A is one of them [60, 61]. In fact, DYRK1A is overexpressed in Down's syndrome patients [62, 63], and the analyses of mouse models suggested that overexpression of DYRK1A is responsible for at least a part of pleiotropic phenotypes observed in Down's syndrome patients [64–67], although other genes in chromosome 21 DSCR should also play a role. *Minibrain* is a mutant of *Drosophila* that exhibits a marked brain size reduction in the optic lobes and central hemispheres [68]. The responsible gene *mnb* was identified, which encodes a fruit fly homologue of DYRK1A [68]. These results indicate that DYRK1A plays an important role in developmental and functional regulation of neuronal cells from insects to human. In addition, triplication of DYRK1A is necessary and sufficient in model mice to cause the structural and functional retinal alterations that are also observed in Down's syndrome children [69]. Gene knockout mice of the major DYRK1A substrate NFAT share various phenotypic alterations in facial structure, social interaction, motor function, and cardiac morphogenesis, with patients as well as model mice of Down's syndrome [59]. Altogether, DYRK1A is one of the pivotal factors encoded in DSCR and regulates many physiological functions of cells, and dysregulation of DYRK1A leading to NFAT deactivation by hyper-phosphorylation may be a molecular basis of various phenotypes observed in Down's syndrome patients. The functional importance of phosphorylation of other DYRK1A substrates in Down's syndrome awaits further experimental evidence.

3 Effect of CK2 Inhibitors on DYRKs (See Table 1)

The human genome encodes more than 500 protein kinases, and recently the effect of conventional CK2 inhibitors has been tested on many more kinases than before. While most of CK2 inhibitors have no impact on most of protein kinases examined, DYRK family protein kinases are often strongly suppressed by conventional CK2 inhibitors (Table 1). This is rather an unexpected result because the amino acid sequences of DYRKs are only distantly related to that of CK2 (Fig. 1).

IQA is an effective and selective CK2 inhibitor ($Ki = 170$ nM), and the structural details of the binding of IQA to CK2 were described by analyzing the CK2-IQA complex [70]. Examination of the effect of 10 μM IQA on a panel of 44 protein kinases indicated that most of the kinases other than CK2 (90 % inhibition) in the panel were not affected by IQA, except only one kinase, DYRK1A (50 % inhibition). Examination of 34 protein kinases for the effect of TBB and its derivatives resulted in a conclusion that TBB and 2-dimethylamino TBB (with better efficiency in CK2 inhibition) significantly inhibited DYRK1A, but not other kinases [71]. Pagano et al. examined a panel of 70 protein kinases for their sensitivity toward several conventional CK2 inhibitors [72]. While most of protein kinases tested were insensitive to 10 μM DMAT (93 % inhibition of CK2), it strongly inhibited the activity of DYRK1A (95 % inhibition), DYRK2 (94 % inhibition), and DYRK3 (97 % inhibition). Several other protein kinases, including PKD1, PIM1/2/3, and HIPK2/3, were also inhibited by DMAT. The effect of TBB on the same expanded set of 70 protein kinases was also examined, and DYRK and PIM kinases were strongly inhibited [72]. Several other newly developed TBB derivatives also significantly inhibited DYRK1A, whereas there are some compounds that showed only a modest inhibition of DYRK1A remaining sufficient efficacy in CK2 inhibition. Specific inhibition of CK2 without touching DYRK1A activity may be achieved if carefully selected compounds are used with appropriate concentrations.

Leucettines were originally developed as specific CLK and DYRK inhibitors, and leucettine L41 effectively suppressed all the members of DYRKs (IC_{50} for DYRK1A = 10 nM), while many other Pro-directed kinases, including CDKs and ERKs, were not affected [73]. However, the activity of CK2 was strongly suppressed by leucettine L41 (Table 1). Binding proteins for immobilized leucettine L41 were analyzed, and both DYRK1A and CK2 were found to specifically bind to the inhibitor [73]. These results further implicate the significant overlap of the binding and inhibiting spectrum of inhibitors for CK2 and DYRK1A.

All of these analyses suggested that CK2 and DYRKs may share a common structural factor in the ATP binding pocket, which may accept a similar array of ATP-analogous competitive inhibitors. The amino acid sequence alignment of the kinase catalytic domains of CK2α and DYRK1A is shown in Fig. 3. Both of the sequences have in common many characteristic amino acid motifs conserved in most of Ser/Thr kinases. In addition, there are several conserved amino acids between CK2α and DYRK1A, but it remains unclear which of them rule the sensitivity to shared inhibitors for both kinases.

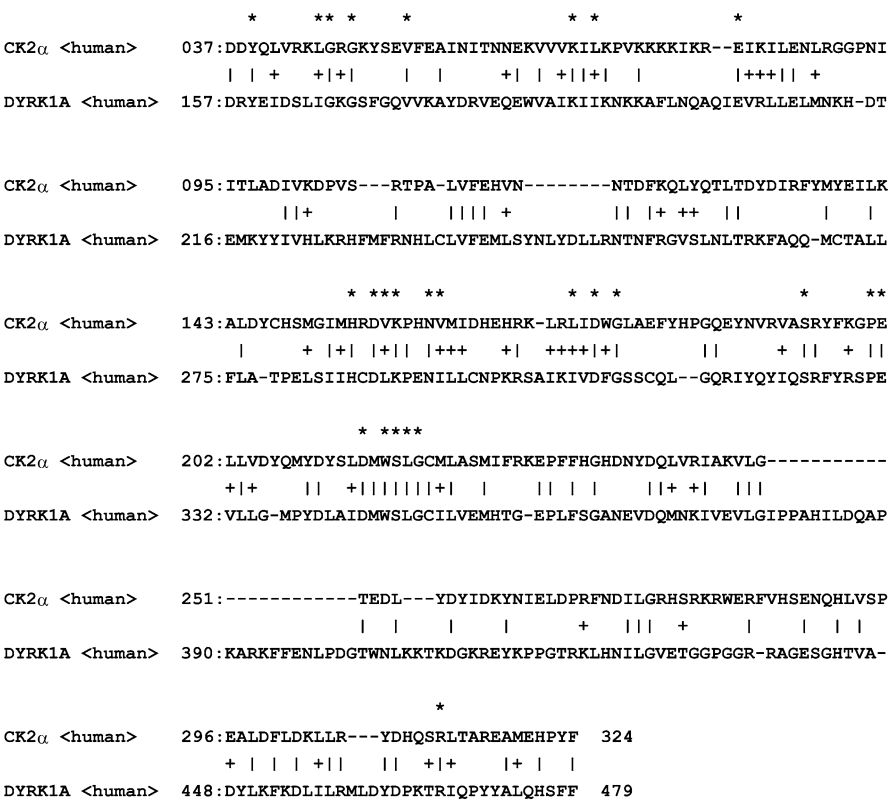

Fig. 3 Alignment of amino acid sequences of the kinase catalytic domains of CK2α and DYRK1A. Identical amino acids are shown by "|" and similar amino acids are shown by "+." Amino acids conserved in most of Ser/Thr kinases are indicated by "*"

4 Functional Regulation of DYRK1A by Cellular Binding Partners

4.1 WDR68

To unveil the physiological function and regulation, the identification of cellular binding partners for DYRK1A should be of potential importance. Several studies revealed that a WD40-repeat protein WDR68 is a major binding partner for DYRK1A and DYRK1B, but not for class 2 DYRKs [50, 74–76]. The amino acid sequence of WDR68 is extremely conserved among species from plant to human, and all known mammalian WDR68 sequences encode exactly the same 342 amino acids. WDR68 is essential for cell proliferation and cell survival in mammalian cultured cells [75]. WDR68 is one of the components of a ubiquitin-conjugating

enzyme DDB1-CUL4 complex [77], thus also called as DDB1-CUL4-associated factor 7 (DCAF7). The analysis of its amino acid sequence revealed that WDR68 contains five WD40-repeats; however, our computational structural analysis indicates that WDR68 forms a seven-bladed β-propeller ring [78]. All of these facts suggest that WDR68 plays a fundamental biological role in cells possibly by facilitating protein–protein interactions. Cellular localization of WDR68 is ubiquitous both in the cytoplasm and nucleus [75]. The cellular localization of WDR68 seems to be critical for its proper function, since mislocalization of WDR68 resulted in the developmental malformation of craniofacial structure in zebrafish [79]. Overexpression of DYRK1A induced nuclear accumulation of WDR68 [75]. Moreover, the molecular chaperone TRiC|CCT was essential for the DYRK1A-binding and nuclear accumulation of WDR68 [78]. The balance between cytoplasmic and nuclear WDR68 distribution may be precisely controlled, and DYRK1A should be a pivotal factor for the normal distribution and function of WDR68 in cells. The dysregulation of cellular localization of WDR68 by overexpressed DYRK1A might be a part of the molecular mechanism underlining the pleiotropic pathological alterations observed in Down's syndrome patients.

4.2 14-3-3

The 14-3-3 proteins are a family of regulatory molecules and participate in a wide range of cellular processes through binding to hundreds of structurally and functionally diverse proteins [80, 81]. 14-3-3 proteins recognize a sequence motif containing phospho-Ser/Thr in target proteins. 14-3-3 proteins were identified as binding partners of DYRK1A by a yeast two-hybrid screening [82, 83]. In yeast, a DYRK1A homologue Yak1p and a 14-3-3 homologue Bmh1/2p have been also shown to be associated together [84, 85]. 14-3-3 binds to either the N-terminal region of DYRK1A in a phosphorylation-independent manner or to the PEST domain near the C-terminal region after autophosphorylation of DYRK1A [82, 83]. The binding of 14-3-3 modestly increased DYRK1A kinase activity in vitro, and the inhibition of the 14-3-3 binding to DYRK1A by a small peptide decreased DYRK1A kinase activity. These results indicate that the 14-3-3 binding facilitates the protein kinase activity of DYRK1A.

4.3 REST|NRSF

REST (RE1-silencing transcription factor)|NRSF (neuron-restrictive silencer factor) plays a pivotal role in neuronal differentiation process by modulating transcription of its target genes by binding to a specific DNA element, the repressor element 1 (RE1)|neuron-restrictive silencer element (NRSE) [86, 87]. Target proteins for REST, including ion channels, synaptic proteins, and neurotransmitter receptors, have fundamental functions in neuronal cells. REST acts as a repressor of neuronal

differentiation and activates proliferation. REST is induced during normal aging in human neurons, but is lost in Alzheimer's disease patients [88]. REST levels are closely correlated with cognitive preservation during aging, suggesting an important role of REST in neuroprotection [88]. DYRK1A overexpression reduces REST protein levels by facilitating its ubiquitination and degradation [89]. DYRK1A interacts with a SWI/SNF complex that is known to bind to REST [89]. REST stability is regulated by phosphorylation-dependent ubiquitination by an E3 ligase; however, it remains unclear if DYRK1A directly phosphorylates REST. Altogether, the DYRK1A function in neural cell differentiation may in part be ascribed to its REST level regulation. On the other hand, REST can activate DYRK1A transcription via a RE1|NRSE site in the human DYRK1A promoter [90], suggesting a negative feedback loop mechanism that precisely controls the expression levels of REST and DYRK1A. Dysregulation of the DYRK1A–REST combination may result in developmental as well as functional defects of neural system observed in Down's syndrome patients.

5 CK2 and DYRK1A as Therapeutic Targets

5.1 CK2 Inhibitors as Cancer Chemotherapeutic Agents

Early observations suggested that CK2 could be activated by growth factors such as epidermal growth factor and insulin-like growth factor [91–93]; however, recent analyses indicated that this might not generally be the case [94, 95]. On the other hand, CK2 activity is recognized to be higher in rapidly proliferating cells [16, 17], and exogenous overexpression of CK2 in transgenic mice is tumorigenic [96]. CK2 is thus implicated to play an important role in supporting the malignant growth of cancer cells and tumors. There are many known CK2 substrates that are involved in cell growth and proliferation; however, the detailed molecular mechanism of CK2-mediated cell proliferation is not yet fully revealed. CK2-dependent phosphorylation of Cdc37, a kinase-targeting co-chaperone for Hsp90, is essential for the folding and function of many Cdc37/Hsp90 client signaling kinases, including Cdk4 and Raf1 [97–100]. Therefore, phosphorylation of Cdc37 alone might significantly contribute to the important role of CK2 in cell growth and proliferation. CK2 is regarded as a promising molecular target for cancer chemotherapy, and many new-generation CK2 inhibitors have been recently developed. CX-4945 [Silmitasertib®] (5-((3-chlorophenyl)amino)benzo[c][2,6]naphthyridine-8-carboxylic acid) is a potent ($Ki < 1$ nM) and orally available ATP-competitive inhibitor with unprecedented specificity for CK2α and CK2α′ catalytic subunits [101, 102]. CX-4945 has antiproliferative activity in multiple cancer cell lines, shows antitumor efficacy in mouse models, and is under clinical trials for cancer chemotherapy [103, 104]. In phase I clinical trials, CX-4945 induced stable disease in 20 % of patients with different solid tumors, having promising pharmacodynamic and safety profiles. A combinatorial treatment with CK2 inhibitors and other chemotherapeutic agents might be also a valid therapeutic option.

5.2 *Down's Syndrome, Leukemia, and DYRK1A Inhibitors*

Involvement of DYRK1A in tumorigenesis is rather complex. Adult Down's syndrome individuals overexpressing DYRK1A show reduced tendency of most of malignant solid tumors of epithelial origin [105, 106]. This suggests that DYRK1A may have a tumor-suppressive function, but the molecular basis for this observation is not yet completely understood. It should be pointed out that DYRK family protein kinases have been proposed to play a role in promoting cell apoptosis [36], which is intimately involved in the exclusion of cancer cells in the body. On the contrary, children with Down's syndrome have a markedly increased risk of developing both acute megakaryoblastic leukemia and acute lymphoblastic leukemia as compared with children who do not have Down's syndrome [105, 106]. Therefore, DYRK1A could be proleukemic in children and antitumorigenic in adults.

The molecular mechanism behind this paradox is only partially figured out. In general, activation of the NFAT pathway is considered cancer promoting through several mechanisms [107]. The NFAT family proteins were originally discovered in T cells and shown to facilitate T cell activation and proliferation. In addition, NFAT pathway can enhance angiogenesis by activating transcription of VEGF (vascular endothelial growth factor). As describe in Sects. 2.3 and 2.4, increased expression of DYRK1A suppresses NFAT function by the phosphorylation-dependent nuclear export of NFAT. DYRK1A, in cooperation with another protein DSCR1|RCAN1 encoded in DSCR, suppresses VEGF-dependent endothelial cell proliferation [108]. The suppression of tumor angiogenesis by the DYRK1A-provoked NFAT inhibition may explain the lower rate of epithelial cancers in Down's syndrome adults. Angiogenesis is critical for the growth and expansion of cancer cells in solid tumor, whereas most of leukemic cells in blood do not rely on angiogenesis for proliferation. This could be one of the reasons why leukemia is increased, while solid tumors are decreased in Down's syndrome patients. Obviously, contributions of other DYRK1A substrates and other proteins encoded in chromosome 21 DSCR cannot be ruled out in the trisomy-related tumor suppression.

In any case, cell biological analyses with specific DYRK1A inhibitors may shed new light on the molecular basis of Down's syndrome and tumorigenesis as well. In addition, DYRK1A inhibition may have a therapeutic benefit. Recently, several specific DYRK1A inhibitors have been identified [109]. Harmine (7-methoxy-1-methyl-9H-pyrido[3,4-b]indole), originally isolated from South American vine, was reported to suppress the activity of DYRK1A [110], though it also shows a potent inhibitory effect on monoamine oxidase A [111]. Malinge et al. suggested that DYRK1A inhibitors may be clinically useful in the context of Down's syndrome-related acute megakaryoblastic leukemia by demonstrating that harmine inhibited the growth of megakaryoblastic leukemic cell lines with trisomy 21 [112]. Since some of conventional CK2 inhibitors have potent inhibitory activity on DYRK1A (see Sect. 3), one may consider the CK2 inhibitors as lead compounds for the development of DYRK1A-specific inhibitors that have no impact on CK2 activity.

Down's syndrome is caused by congenital alteration by the chromosome trisomy, and the therapeutic aim should be the modulation of DYRK1A from 1.5-fold increase back to the normal level [111]. This should be quite challenging having only a narrow therapeutic range for the DYRK1A inhibitors, but certainly worth the further investigation.

6 Conclusion

CK2 is a ubiquitous and constitutively active protein kinase implicated in the malignant proliferation of cancer cells. Low-molecular-weight specific inhibitors for CK2 have been developed both for biological and clinical applications. Recent studies revealed that many conventional CK2 inhibitors including DMAT and TBB also suppress the activity of DYRK family protein kinases; therefore, the alterations in tissues and cells observed with these drugs should not be ascribed solely to CK2. New-generation highly specific CK2 inhibitors such as CX-4945 have been recently developed and tested for clinical applications as cancer chemotherapeutic agents. DYRK1A plays a pivotal role in Down's syndrome, and DYRK1A-dependent phosphorylation of NFAT is a key event that causes various phenotypes and also low incidence of solid tumors in Down's syndrome patients. Specific inhibitors for DYRK1A may also have biological and clinical importance.

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