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Invited Mini Review

Regulation of CMGC kinases by hypoxia

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Hypoxia, a widespread occurrence observed in various malignant tumors, results from rapid tumor growth that outpaces the oxygen supply. Tumor hypoxia precipitates several effects on tumor biology; these include activating angiogenesis, intensifying invasiveness, enhancing the survival of tumor cells, suppressing anti-tumor immunity, and fostering resistance to therapy. Aligned with the findings that correlate CMGC kinases with the regulation of Hypoxia-Inducible Factor (HIF), a pivotal modulator, reports also indicate that hypoxia governs the activity of CMGC kinases, including DYRK1 kinases. Prolyl hydroxylation of DYRK1 kinases by PHD1 constitutes a novel mechanism of kinase maturation and activation. This modification "primes" DYRK1 kinases for subsequent tyrosine autophosphorylation, a vital step in their activation cascade. This mechanism adds a layer of intricacy to comprehending the regulation of CMGC kinases, and underscores the complex interplay between distinct post-translational modifications in harmonizing precise kinase activity. Overall, hypoxia assumes a substantial role in cancer progression, influencing diverse aspects of tumor biology that include angiogenesis, invasiveness, cell survival, and resistance to treatment. CMGC kinases are deeply entwined in its regulation. To fathom the molecular mechanisms underpinning hypoxia's impact on cancer cells, comprehending how hypoxia and prolyl hydroxylation govern the activity of CMGC kinases, including DYRK1 kinases, becomes imperative. This insight may pave the way for pioneering therapeutic approaches that target the hypoxic tumor microenvironment and its associated challenges. [BMB Reports 2023; 56(11): 584-593]

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INTRODUCTION

Hypoxia, a condition with reduced oxygen availability, is a crucial stress signal for cell survival and the maintenance of essential cellular functions. This phenomenon holds significant importance across a broad range of biological, physiological, and pathological contexts, including embryonic development, instances of ischemia, and cancer (1-5). Cells respond to hypoxia through the hypoxia-inducible factor (HIF) pathway. HIFs, classified as transcription factors, play a pivotal role in adapting cells to low oxygen conditions (2, 3, 6, 7). In normoxia, a condition with normal oxygen, the HIF pathway is inactivated through the hydroxylation of the HIFα subunit by prolyl-hydroxylase (PHD), which is then followed by ubiquitin-dependent proteasomal degradation facilitated by the von Hippel-Lindau (VHL) ubiquitin ligase complex (8-10). However, hypoxic conditions hamper the activity of PHDs, consequently leading to the stabilization and accumulation of HIFα subunits. These stabilized subunits subsequently translocate to the nucleus, triggering the activation of transcriptional processes that regulate genes responsible for cellular adaptation to hypoxia.

The dynamic interplay between CMGC kinases and hypoxia has been extensively explored in the context of both cell cycle control, and the cellular response to hypoxic stress (11-13). CMGC kinases exert significant influence over cellular signal transduction through the intricate process of reversible protein phosphorylation. This group comprises cyclin-dependent kinases (CDKs), mitogen-activated protein kinases (MAPKs), glycogen synthase kinases (GSKs), CDK-like kinases (CLKs), and Dual-Specificity Tyrosine (Y)-Phosphorylation-Regulated Kinases (DYRKs) (14). These kinases play indispensable roles in a wide range of cellular processes, including cell cycle control, gene expression, and signal transduction. The aberrant regulation of CMGC kinases has been strongly correlated with the initiation and progression of cancer, thus rendering them attractive targets for potential therapeutic interventions. The activation of HIFs under hypoxia can significantly regulate the expression of genes that orchestrate cell cycle regulation, such as cyclins, CDKs, and CDK inhibitors, to subsequently influence the course of cell cycle progression. Moreover, it is noteworthy that CMGC kinases, including CDKs and MAPKs, can themselves be modulated by the signaling pathways initiated by hypoxia (11).

Recent studies have unveiled an additional layer of control

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over HIFα in cancer stem cells, which is initiated by the prolyl hydroxylation of the DYRK1 kinases (DYRK1A and DYRK1B) by PHD1. This discovery reveals a novel mechanism underlying the regulation of HIFα in the context of cancer stem cells (12, 13, 15). Specifically, DYRK1 kinases play a pivotal role in phosphorylating ID2 on Threonine 27 (12, 16). Hypoxia downregulates this phosphorylation through the inactivation of DYRK1 kinases, whose activity is facilitated in normoxia through prolyl hydroxylation by PHD1. Furthermore, ID2 directly interacts with the VHL ubiquitin ligase complex, leading to the displacement of VHL associated Cullin 2. Consequently, this interaction hinders HIF2α ubiquitylation and subsequent degradation. These findings provide valuable insights into the intricate regulatory networks that control cancer stem cells and the HIFα pathway.

The regulation of CMGC kinase activity and its relationship with hypoxia is a complex dynamic process that involves the interplay between signaling pathways, transcriptional regulation, and post-translational modifications. Further research is needed to fully elucidate the molecular mechanisms underlying this regulation and its implications in cellular responses to hypoxic stress. Here, we discuss prolyl hydroxylation of a highly conserved proline in the kinase domain of CMGC kinases including DYRK1s by PHDs. Prolyl hydroxylation precedes and is essential for tyrosine autophosphorylation during translation, which is necessary for kinase activation (13, 17).

INTERPLAY BETWEEN CMGC KINASES, HYPOXIA, AND CELLULAR SIGNAL TRANSDUCTION

Hypoxia sensing and signaling pathways interface with the cell cycle through a variety of mechanisms (1, 18). One of the key players in the hypoxia is the HIF transcription factor family. Oxygen is sensed by 2-oxoglutarate, iron-dependent dioxygenases (2-OGDs), in particular the prolyl hydroxylases (PHD1, PHD2, and PHD3), which hydroxylate specific proline residues in the oxygen-dependent degradation domain (ODD) of HIFα (8, 10, 13, 17-20). Under normal oxygen concentrations, these modifications trigger the binding of the VHL protein complex, which leads to the ubiquitination and degradation of HIFα. However, under hypoxic conditions, PHDs activity is inhibited due to low oxygen affinity, leading to HIFα stabilization (7-10). HIFα forms a heterodimer with HIF1β and translocates to the nucleus; it there acts as a transcription factor, influencing the expression of genes involved in adaptation to hypoxia (1, 7, 8, 12, 21-23). Additionally, the cellular response to hypoxia and the cell cycle are intricately linked. Oxygen is crucial for energy homeostasis and cellular viability, and cells possess complex response mechanisms to restore oxygen balance. The interactions between hypoxia signaling pathways and the cell cycle ensure cell survival, and minimize errors during cell division. This connection is crucial to understand how external and internal stresses, such as hypoxia, impact the regulation of the cell cycle (1). Overall, the interplay between hypoxia sensing, signaling pathways, and the cell cycle constitutes a critical aspect of cellular adaptation to hypoxic conditions, ensuring proper cellular responses and survival in various physiological and pathological contexts (2, 12).

The CMGC kinase group, a subset of protein kinases, plays a crucial role in cellular signal transductions via reversible protein phosphorylation. The intricate relationship between CMGC kinases and hypoxia signaling pathways is significant, due to their involvement in essential cellular processes, and their potential roles in disease pathways, including cancer. The proteomic analysis of CMGC kinases has revealed extensive interactions with other proteins, shedding light on their involvement in various cellular pathways and potential roles in disease (24). These connections suggest that CMGC kinases may play crucial roles in mediating the effects of hypoxia on cellular responses, including cell cycle regulation, transcription, splicing, transport, and translation. Furthermore, the analysis revealed a kinase-kinase subnetwork and candidate substrates for CMGC kinases. CMGC kinases regulate various cellular functions, and are implicated in a various range of human diseases, including cancers. Their dysregulation affects the mechanisms of tumorigenesis and tumor progression, highlighting their role in cancer biology and their potential as therapeutic targets (25). Given the importance of both CMGC kinases and hypoxia signaling pathways in cellular processes and disease, further research in this area could provide insights into novel therapeutic strategies for diseases where both processes are dysregulated. Understanding how these pathways intersect and influence each other can lead to the development of targeted therapies that exploit the connections between CMGC kinases, hypoxia signaling, and disease progression.

The activity of CMGC kinases can be influenced by hypoxia through various mechanisms. For example, hypoxia can modulate the expression and activity of specific CMGC kinases involved in cell cycle regulation and signal transduction pathways. The cellular response to hypoxia involves the activation of hypoxia responsive genes, which can affect the activity of CMGC kinases. Furthermore, the interplay between CMGC kinases and hypoxia extends beyond the cell cycle. Investigation of the protein interaction landscape of the human CMGC kinase group has revealed complex interactions and functional enrichment. Additionally, CMGC kinases have been found to target serine/arginine-rich (SR) proteins involved in RNA metabolism, including splicing and mRNA export. This interplay between CMGC kinases and SR proteins has implications for viral replication and disease pathways. Therefore, the regulation of CMGC kinase activity and its interaction with hypoxia is a complex and multifaceted area of research. Understanding these mechanisms is important to unravel the intricate connections between cellular signaling, the cell cycle, and adaptation to stress conditions, such as hypoxia, in various physiological and pathological contexts. From this perspective, we explore the correlations between CMGC kinase group, including DYRK1s, and their association with hypoxia.

CMGC kinase group

Eukaryotic cells exhibit a complexity that arises from the coordinated operation of numerous molecular networks. Key to these networks are the reversible processes of protein phosphorylation and dephosphorylation, which are governed by protein kinases and phosphatases, respectively. Protein kinases, through phosphorylation, play a pivotal role in signal transduction, impacting protein activity, localization, and interactions. These modifications influence diverse cellular functions that include metabolism, transcription, cell cycle progression, apoptosis, and differentiation. Aberrations in protein phosphorylation are implicated in diseases like cancer. Phosphorylation involves transferring a phosphate group from ATP to target proteins at serine, threonine, or tyrosine residues. Protein kinases can be classified into serine/threonine, tyrosine, and dual-specificity kinases, based on substrate preference. The catalytic core shared among eukaryotic protein kinases comprises N- and C-lobes enclosing an ATP-binding cleft. The N-lobe contains β-strands and an α-helix, while the C-lobe houses the substrate binding groove and activation segment. Kinase activation often involves phosphorylation of the activation loop.

Mammalian protein kinases consist of over 500 members divided into families based on structural similarities. The CMGC kinase family, highly conserved among species, regulates essential processes. CMGC kinases have a conserved core, and a unique insert that influences substrate specificity. They are often regulated by tyrosine phosphorylation or substrate priming. Subfamilies within CMGC include CDKs, MAPKs, CLKs, DYRKs and more, with each serving distinct roles (24, 25). The significance of CMGC kinases in cellular processes makes them targets for cancer therapy. FDA-approved inhibitors (Table 1) and ongoing clinical trials highlight their potential. Challenges like resistance and off-target effects call for research into selective inhibitors, combination therapies, and biomarker identification.

The CMGC kinases share a conserved catalytic core structure, and are classified into various subfamilies based on their functional similarities. Activation of CMGC kinases is a complex process involving multiple steps, and phosphorylation events play a key role in this process. Activation of CMGC kinases often requires phosphorylation at specific residues, which can vary, depending on the individual kinase. In the case of dual-specificity tyrosine-phosphorylation-regulated kinases 1A and 1B (DYRK1A and DYRK1B), proline hydroxylation has been identified as a crucial step for their activation (13). The hydroxylation of a conserved proline residue within the CMGC insert of DYRK1 kinase domain by PHD1 initiates a cascade of events that leads to tyrosine autophosphorylation and subsequent activation. This hydroxylation step serves as a unique mechanism of kinase maturation, and is an essential component of catalytic activation (12, 13, 26, 27).

Phosphor-tyrosine is a pivotal player in CMGC kinase activation. In many cases, tyrosine autophosphorylation is essential for achieving full catalytic activity and proper subcellular localization. For example, the homeodomain-interacting protein kinases (HIPKs), members of the CMGC family, rely on tyrosine autophosphorylation in their activation loop to acquire full catalytic activity (28). The autophosphorylation of tyrosine residues in the activation loop is a critical step in the maturation of these kinases, and influences their substrate specificity and cellular functions. Furthermore, proteomic analyses have revealed the extensive protein interaction landscape of the CMGC kinase group (24). The interactions between CMGC kinases and various proteins provide insights into their regulatory mechanisms and potential roles in cellular processes. The identification of kinase-kinase subnetworks and candidate substrates within this interaction landscape offers valuable information for understanding CMGC kinase functions.

In summary, the complex process of activation that CMGC kinases, a diverse family of kinases, undergo often involves phosphorylation events. Proline hydroxylation and tyrosine autophosphorylation play crucial roles in the activation of specific members within this family. The protein interaction landscape further enhances our understanding of CMGC kinase functions and their regulatory networks.

Cyclin-dependent kinases (CDKs)

CDKs are a class of protein kinases that play pivotal roles in the regulation of cell cycle progression and gene transcription, with their activity dependent on the interaction with cyclin subunits. They are crucial components of cellular processes that include cell cycle control, transcriptional regulation, and cell differentiation. CDKs are divided into multiple subfamilies that include cell cycle related subfamilies (Cdk1, Cdk4, Cdk5) and transcriptional subfamilies (Cdk7, Cdk8, Cdk9, Cdk11, Cdk20), each associated with specific functions and regulation (29, 30). The interaction between CDKs and their cyclin partners facilitates their activation and drives the progression of various phases of the cell cycle, ensuring accurate DNA replication and cell division (30-32). CDKs are both responsible for regulating cell cycle transitions, and display significant involvement in transcriptional regulation, allowing cells to respond to external and internal cues. Dysregulation of CDKs has been linked to various diseases, particularly cancer, making them important targets for therapeutic interventions (30, 32). Thus, CDK inhibitors have shown promise in clinical trials as potential cancer treatments, demonstrating the potential therapeutic value of targeting these kinases (29, 32-37).

The relationship between CDKs and hypoxia involves intricate interactions that in response to reduced oxygen levels, influence cell cycle control. Cells under hypoxic conditions can exhibit alterations in cell cycle progression, such as arrest at specific points, like G1/S. This arrest response can vary among different cell types, reflecting the diverse ways cells adapt to hypoxic stress (1, 11). Furthermore, hypoxia-induced cell cycle arrest has been associated with changes in the expression of cell cycle kinase inhibitors and HIF1α. HIF1α is central to the cellular response to hypoxia, and its stability and activity can be influenced by the cell cycle, and vice versa (1,

Table 1. FDA-approved CMGC kinase inhibitors

Active ingredient	Synonyms	Drug	Company	PubMED CID	Formula	Primary targets	Year approved	FDA granted approval
Abemaciclib	LY2835219	Verzenio	Eli Lilly	46220502	$C_{27}H_{32}F_2N_8$	CDK4/6	2017	The treatment of patients with HR-positive and HER2-negative advanced or metastatic breast cancer that has progressed after unsuccessful endocrine therapy
Binimetinib	MEK162	Mektovi	Array Bio-Pharma	10288191	$C_{17}H_{15}BrF_2N_4O_3$	MEK1/2	2018	The treatment of patients with unresectable or metastatic *BRAF*$^{V600E/K}$ melanoma in combination with encorafenib
Cobimetinib	GDC-0973	Cotellic	Genen-tech	16222096	$C_{21}H_{21}F_3IN_3O_2$	MEK1/2	2015	The treatment of patients with unresectable or metastatic *BRAF*$^{V600E/K}$ melanoma in combination with vemurafenib
Dabrafenib	GSK2118436	Tafinlar	GSK	44462760	$C_{23}H_{20}F_3N_5O_2S_2$	BRAF	2013	The treatment of patients with *BRAF*$^{V600E/K}$ melanoma, *BRAF*^{V600E} NSCLC, *BRAF*^{V600E} anaplastic thyroid cancer (in combination with Mekinist)
Encorafenib	LGX818	Braftovi	Array Bio-Pharma	50922675	$C_{22}H_{27}ClFN_7O_4S$	BRAF	2018	The treatment of patients with unresectable or metastatic *BRAF*$^{V600E/K}$ melanoma in combination with binimetinib
Palbociclib	PD0332991	Ibrance	Pfizer	5330286	$C_{24}H_{29}N_7O_2$	CDK4/6	2015	The treatment of patients with HR-positive and HER2-negative advanced or metastatic breast cancer
Ribociclib	LEE011	Kisqali	Novartis	44631912	$C_{23}H_{30}N_8O$	CDK4/6	2017	The treatment of patients with HR-positive, HER2-negative locally advanced or metastatic breast cancer in combination with an aromatase inhibitor or fulvestrant as initial endocrine-based therapy, or in women who have received prior endocrine therapy
Selumetinib	AZD6244	Koselugo	Astra-Zeneca	10127622	$C_{17}H_{15}BrClFN_4O_3$	MEK1/2	2020	The treatment of patients with Neurofibromatosis type 1 (NF-1) in a limited age group
Trametinib	GSK1120212	Mekinist	GSK	11707110	$C_{26}H_{23}FIN_5O_4$	MEK1/2	2013	The treatment of patients with unresectable or metastatic *BRAF*$^{V600E/K}$ melanoma, and patients with metastatic *BRAF*^{V600E} NSCLC or anaplastic thyroid cancer
Trilaciclib	G1T28	Cosela	G1 Thera-peutics	68029831	$C_{24}H_{30}N_8O$	CDK4/6	2021	To reduce the incidence of chemotherapy induced myelosuppression in patients prior to receiving platinum and etoposide-containing or topotecan-containing chemotherapy regimens for extensive-stage small cell lung cancer
Vemurafenib	PLX4032	Zelboraf	Genen-tech	42611257	$C_{23}H_{18}ClF_2N_3O_3S$	BRAF	2011/ 2017	The treatment of patients with unresectable or metastatic *BRAF*^{V600E} melanoma/ The treatment of adult patients with Erdheim-Chester Disease whose cancer cells present *BRAF*^{V600E}

11, 12). Conversely, hypoxia-induced pathways, particularly the HIF system, can impact the cell cycle by altering the expression of cell cycle regulators. The interplay between CDKs and hypoxia is a complex mechanism that ensures cells respond appropriately to reduced oxygen levels, while maintaining proper cell cycle progression and genetic fidelity.

Mitogen-activated protein kinases (MAPKs)

MAPKs are serine/threonine-specific protein kinases that respond to extracellular signals, and govern essential cellular processes that include proliferation, gene expression, differentiation, mitosis, cell survival, apoptosis, stress responses, and the regulation of CDKs through transcriptional control. Extensive research has been conducted over the past few decades, to unravel the intricacies of MAPKs, delving into their substrates, functions, and their roles in both health and cancer (14, 38). In mammals, well-characterized MAPK pathways include MAPK/ERK and MAPK/JNK. These pathways are implicated in controlling biological responses, such as cell division, invasion, proliferation, differentiation, and metastasis in various physiological processes and diseases, including cancer. These enzymes phosphorylate substrate proteins on conserved Serine-Proline (SP) and Threonine-Proline (TP) motifs, regulating a diverse array of cell processes. MAPKs are categorized into three major groups in mammals: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38 mitogen-activated protein kinases (p38s). ERKs are typically activated by growth factors and mitogens, while JNKs and p38s are activated by cellular stresses and inflammatory cytokines (39-42).

Interestingly, the activation of MAPKs and their association with hypoxia have been studied in relation to pathological conditions like hypoxic/ischemic nephropathy, cancer, and other disorders (38, 43, 44). Despite HIFα not being a direct substrate of MAPK and not requiring phosphorylation for HIF-p300/CBP interactions, MAPK signaling enhances HIFα transactivation through p300/CBP in tumors. This is further evidenced by the increased activity of ERK and p38 kinases in hypoxic cells compared to normoxic ones. The hypoxia-induced up-regulation of uPAR expression, possibly mediated by the ERK and p38 kinase pathways, contributes to tumor cell invasion. Moreover, in mild hypoxia (5% O_2), the pluripotency-promoting PI3K/AKT signaling pathway is weakened, likely due to reduced levels of reactive oxygen species (ROS). This dampening effect on PI3K/AKT signaling due to lower ROS levels is significant. Notably, ROS production, which typically activate PI3K/AKT via HIFα, are inhibited by MEK1/2-ERK1/2, creating a negative feedback loop from MAPK to FGFR1 and PI3K/AKT. These findings offer valuable insights into the regulation of cancer stem cell signaling, considering the oncogenic transformation potential of the PI3K/AKT pathway (43-48).

Glycogen synthase kinases (GSKs)

GSKs are highly conserved serine/threonine protein kinases that are found in both animals and plants. GSKs play a crucial role in various cellular processes, including glycogen synthesis, cell proliferation, differentiation, apoptosis, neuronal function, oncogenesis, and development. One of the well-studied isoforms, GSK3β, has attracted significant attention, due to its intricate involvement in intracellular signaling cascades. GSK3 can modulate HIF1α levels. Inhibition or depletion of GSK3 leads to an increase in HIF1α levels, while overexpression of GSK3β reduces HIF1α levels (49). Studies have demonstrated that GSK3 activity can be modulated in response to hypoxia. Under normoxia, GSK3 phosphorylates HIF1α, marking it for degradation via the ubiquitin-proteasome pathway. However, during hypoxia, reduced GSK3 activity leads to decreased HIF1α phosphorylation, preventing its degradation, and allowing it to translocate to the nucleus. Once in the nucleus, HIF1α forms a transcriptional complex with HIF1β, promoting the expression of various genes involved in adaptive responses to hypoxia, such as angiogenesis, glucose metabolism, and erythropoiesis.

This reciprocal relationship between GSKs and hypoxia highlights the intricate interplay between cellular signaling pathways and environmental cues. For comprehensive understanding of cell physiology and the development of potential therapeutic interventions for conditions like ischemic diseases and cancer, it is essential to elucidate the precise molecular mechanisms governing the activity modulation of GSKs under hypoxia.

CDC-like kinases (CLKs)

CLKs are a family of serine-threonine protein kinases that play significant roles in various cellular processes, including splicing regulation and alternative splicing. They belong to the dual-specificity protein kinase family, and are involved in phosphorylating splicing factors, particularly SR proteins (SRSF1-12), to control pre-mRNA splicing. The CLK family consists of four evolutionarily conserved homologous proteins: CLK1, CLK2, CLK3, and CLK4. Their kinase domain is characterized by an "EHLAMMERILG" motif, and is located at the C-terminus of each family member. This domain phosphorylates serine, threonine, and tyrosine residues of substrates. Functionally, CLKs control pre-mRNA splicing by phosphorylating the serine/arginine (Ser-Arg)-rich domain of splicing factors. CLKs recognize a universal consensus R-x-x-S/T sequence in downstream substrates, and mediate the alternative splicing processes crucial for generating diverse protein isoforms, which isoforms in turn impact cell growth and survival. This phosphorylation-driven regulation of alternative splicing is vital to ensure the proper functioning of the cellular machinery (50).

While the specific interactions between CLKs and hypoxia are not directly mentioned or explicitly discussed, CLKs could plausibly play a role in modulating splicing events under hypoxic conditions. For example, notable alterations in the alternative splicing of genes linked to cancer are observed in prostate cancer cells exposed to hypoxic conditions. Specifically, the expression of certain splice factors and their kinases,

notably Cdc-like splice factor kinases CLK1 and CLK3, increases under hypoxia. This heightened expression of splicing regulators during hypoxia is believed to aid cells in adapting by altering the splicing patterns of crucial cancer-related genes (51). Since alternative splicing is a key regulatory mechanism in cellular adaptation to changing environments, including hypoxia, further research might uncover potential links between CLKs and hypoxia-induced splicing changes.

Dual-specificity tyrosine (Y)-phosphorylation-regulated kinases (DYRKs)

DYRK1s, including DYRK1A and DYRK1B, are members of the DYRK kinase family, which belongs to the CMGC group of protein kinases. The DYRK family of kinases is evolutionarily conserved, and acts to inhibit proliferation and activate cellular quiescence programs (15, 33, 52-56). These data are consistent with the tumor suppressor activity of DYRK1 in glioblastoma, which is primarily executed by suppressing ID2 and HIF2α driven glioma stemness (12). In addition, dysregulation of DYRK kinases has been linked to several human diseases, including Down syndrome, Alzheimer's disease, and cancer (13, 15, 57). The DYRK kinase family is composed of five members, including DYRK1A, DYRK1B, DYRK2, DYRK3, and DYRK4. Of the DYRK family, DYRK1A and DYRK1B are the most extensively studied members (14, 15, 58). Structurally, compared to other protein kinases, DYRK1 kinases display unique characteristics. They possess a conserved kinase domain and a regulatory N-terminal domain that contains a PEST sequence, nuclear localization signal, and potential phosphorylation sites (52). DYRK1A autophosphorylation sites have been identified and characterized, shedding light on the mechanisms of kinase activation and substrate recognition (59, 60).

DYRK1A has been implicated in several biological processes and diseases. During postembryonic development, it plays a role in neurogenesis. In humans, DYRK1A is associated with Down syndrome, and has been mapped to the Down syndrome "critical region" on chromosome 21 in humans (21q22.13) (15, 52). Haploinsufficiency of DYRK1A can lead to a recognizable syndrome characterized by microcephaly, intellectual disability, speech impairment, and distinct facial features. Additionally, DYRK1A has been studied as a therapeutic target to improve cognitive deficits in Down syndrome.

DYRK1B, also known as mini brain-related kinase (Mirk), plays a crucial role in various biological processes, such as growth control, differentiation, and cell survival. In particular, DYRK1B is highly expressed in skeletal muscle, and has been extensively studied in the context of myogenesis, where it regulates motility, transcription, cell cycle progression, and cell survival (61). Studies have also linked DYRK1B to muscle differentiation and its regulatory effects on growth arrest, differentiation, and cell survival. The multifunctional nature of DYRK1B makes it a key player in controlling cellular processes, and a potential target for therapeutic interventions in diseases such as cancer and muscle-related disorders. In addition, DYRK1B is expressed at high levels in some solid tumors, and has been shown to be associated with tumor survival, particularly in rhabdomyosarcoma and pancreatic ductal adenocarcinoma, in which DYRK1B appears to act as an oncogene. However, in contrast to these few examples, DYRK1B, together with DYRK1A, strongly suppresses glioblastoma multiforme, which is a malignant brain tumor (12, 13). This finding is supported by multiple studies that have reported that both DYRK1A and DYRK1B inhibit proliferation and activate cellular quiescence (15, 33, 52-56). Whether these conflicting results or functional differences are simply due to differences in cancer types, or are related to other genes or genomic alterations, requires further study and discussion.

The regulation of the activity of CMGC kinases including DYRK1A and DYRK1B by hypoxia is not well characterized. However, it can provide information that hypoxia is potentially involved in regulating the activity of DYRK1s. Previous research has been investigated the role of prolyl hydroxylation in the activation of protein kinases (13). They demonstrate that proline hydroxylation primes protein kinases for autophosphorylation and activation, and that loss of prolyl hydroxylation or PHD inhibition affects kinase function. Specifically, they show that the hydroxylation of proline 332 on the DYRK1B and proline 380 on the DYRK1A regulate VHL activity, and that inhibition of PHD enzymes leads to the decreased hydroxylation of proline residues and impaired kinase activation. They also identify a conserved proline hydroxylation motif in the activation loop of many protein kinases, suggesting that this mechanism may be widespread in kinase regulation. Overall, this study provides new insights into the role of proline hydroxylation in kinase activation, and highlights the potential therapeutic implications of targeting PHD enzymes for the treatment of diseases that involve dysregulated kinase activity (12, 13).

REGULATORY MECHANISM OF THE CATALYTIC ACTIVITY OF CMGC KINASES, INCLUDING DYRK1, BY PROLYL HYDROXYLATION AND AUTOPHOSPHORYLATION

Prolyl hydroxylation is a post-translational modification that plays a significant role in the activation of certain protein kinases. This modification involves the addition of a hydroxyl group to specific proline residues in target proteins, and is catalyzed by PHDs (8, 17, 19, 62, 63). One fascinating aspect of prolyl hydroxylation is its role in priming protein kinases, especially DYRK1A and DYRK1B, for autophosphorylation and subsequent activation. This process has been observed in CMGC kinases that include DYRKs, MAPK/p38, GSK3, HIPK, and CDKs (13). Prolyl hydroxylation by PHD1 is a crucial step in activating DYRK1 kinases. The prolyl hydroxylation occurs at a conserved proline residue in the CMGC insert of the DYRK1 kinase domain. Prolyl hydroxylation is essential for initiating a cascade of events that ultimately lead to the activation

of DYRK1 kinases. Notably, this prolyl hydroxylation precedes tyrosine autophosphorylation, which is necessary for the full activation of DYRK1 kinases and potentially other CMGC kinases, including DYRK3, DYRK4, p38, and GSK3β. Mutation of the proline residue that is targeted for hydroxylation prevents proper tyrosine autophosphorylation and folding of DYRK1s, resulting in a kinase that lacks functional activity, and is unable to preserve the function of the VHL ubiquitin ligase tumor suppressor (13). CMGC kinases, including DYRK1A and DYRK1B, substrates of PHDs, contain highly conserved motifs for hydroxylated proline, such as the L/xGxP, where x represents any amino acid. The hydroxylated proline motif in CMGC kinases is suggestive of the crucial function of this proline for the catalytic activity of CMGC kinases, which are located approximately 60 amino acids away from the activation loop autophosphorylation site, especially phosphor-tyrosine (13).

The chaperone complex, especially HSP90-CDC37, is particularly important for the maturation of protein kinases, as it plays a significant role in ensuring their proper conformation and activity (13, 64-66). One key aspect of kinase maturation is the autophosphorylation of specific residues, such as tyrosine, which is essential for their full catalytic activity. For example, the autophosphorylation of tyrosine residue in the activation loop of the catalytic domain is crucial for the activation of DYRK1s, a family of CMGC kinases. This autophosphorylation step occurs during the folding of the kinase, and it is catalyzed by a distinct folding intermediate that differs from the mature conformation following prolyl hydroxylation (13, 26, 27, 66-68). From this perspective, prolyl hydroxylation-autophosphorylation of the kinase and chaperone-mediated activation of the CMGC kinases would emerge as important targets for cancer therapy (13, 26, 69, 70).

Considering the broad range of role of activation loop transphosphorylation in protein kinase activation and the widespread occurrence of proline hydroxylation in mammalian proteins, it is plausible that the translational prolyl hydroxylation of CMGC kinases is a potentially common mechanism for protein kinase activation (13).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

DYRK1 is a protein kinase that belongs to the CMGC group of kinases. Two paralogous genes encode the mammalian class 1 DYRKs: DYRK1A and DYRK1B. These kinases play important roles in cell cycle control, and although they have different patterns of expression and divergent sequences in their C-terminal domains, have similar functions (66). The activation of DYRK1A and DYRK1B involves prolyl hydroxylation by prolyl hydroxylase PHD1. Prolyl hydroxylation of DYRK1 initiates a cascade of events that lead to the release of molecular constraints on the VHL ubiquitin ligase tumor suppressor function. The hydroxylation of a highly conserved proline residue in the CMGC insert of the DYRK1 kinase domain by PHD1 precedes tyrosine autophosphorylation. This prolyl hydroxylation event is essential for catalytic activation, and mutation of the hydroxylation acceptor proline prevents tyrosine autophosphorylation and the proper folding of DYRK1 (13). Although the direct regulation of DYRK1 and CMGC kinases by hypoxia has not been sufficiently addressed, it is worth noting that hypoxia can have broad effects on cellular signaling pathways and protein kinases. HIFα is a key transcription factor that is stabilized and activated under hypoxic conditions; it regulates the expression of numerous genes linked with physiology and disease pathogenesis involved in oxygen homeostasis, angiogenesis, metabolism, and cell survival (3, 6). It is possible that hypoxia may indirectly modulate the activity of DYRK1 and CMGC kinases through the regulation of downstream signaling pathways, or through the modulation of other proteins or factors involved in their activation or regulation (Fig. 1). However, further research to elucidate the specific mechanisms and effects of hypoxia on the regulation of DYRK1 and CMGC kinases would be required. Furthermore, the US FDA has recently approved 72 small molecule inhibitors for protein kinases. Among them, 11 inhibitors are designed to specifically target CMGC kinases (Table 1). These results underscore the significance of regulating CMGC kinase activity in future disease treatments, such as cancer, and highlight the necessity for the development of agonists and antagonists to modulate

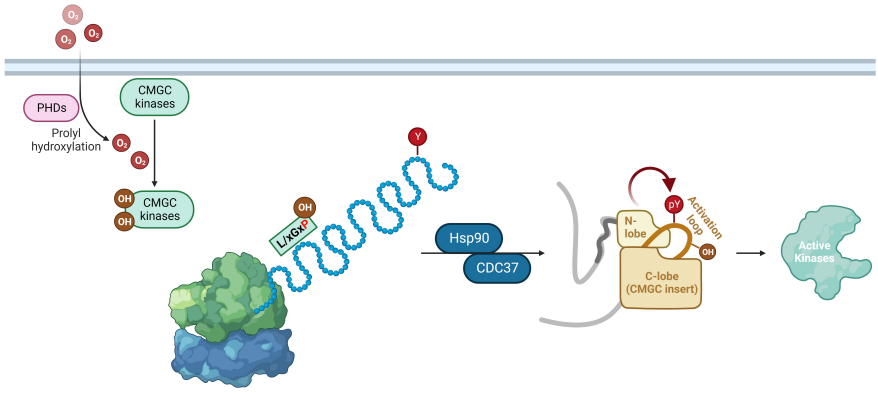

Fig. 1. Regulation of CMGC kinases by prolyl hydroxylation. The activation of DYRK1s, a group of CMGC kinases, critically relies on the autophosphorylation of a tyrosine residue within the activation loop of the catalytic domain. This essential autophosphorylation event takes place during the kinase's folding process and is facilitated by a unique folding intermediate. Notably, this folding intermediate differs from the mature conformation that results from prolyl hydroxylation by PHDs.

kinase activity. In summary, while the direct regulation of DYRK1 and CMGC kinases by hypoxia is not explicitly mentioned in the provided information, DYRK1 kinases require prolyl hydroxylation for their activation and function. Hypoxia can have broad effects on cellular signaling pathways, and might indirectly modulate the activity of DYRK1 and CMGC kinases through the regulation of downstream signaling pathways or other associated factors. Further research is needed to fully understand the specific mechanisms underlying the regulation of these kinases by hypoxia.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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