Review Article

Post-translational modifications of proteins in gene regulation under hypoxic conditions

Olga S. Safronova[1, 2)]

[1)]Department of Cellular Physiological Chemistry, Tokyo Medical and Dental University, Tokyo, Japan
[2)]Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo, Japan

Hypoxia is a common feature of highly proliferating tissues and tissues with inflammation. The transcriptional response to hypoxia involves activation of signal transduction pathways, which is mainly mediated by post-translational modifications of signaling molecules, transcription factors and histones. Activation of hypoxia responsive transcription factors HIF and NF-κB is a subject of regulation by reversible phosphorylation and acetylation. Moreover, hypoxia affects the balance between protein tyrosine kinases and protein tyrosine phosphatases as well as mitogen-activated protein kinases (MAPK) and mitogen activated kinase phosphatases (MKPs). Activity of both histone acetyltransferases and histone deacetylases and their association with transcription factors is specifically regulated in hypoxic and ischemic conditions. Hypoxic and cancerous switch from mitochondrial oxidative phosphorylation to glycolitic metabolism is regulated by acetylation of enzymes participating in maintaining cellular energy metabolism. This review discusses the current research implicating the regulation of protein post-translational modifications in hypoxic environment. Among the diversity of protein modifications, the regulation of acetylation and phosphorylation will be described in detail with emphasis on how these modifications affect dynamic control of cellular signaling in hypoxia-related physiological responses and pathologies. Finally, the potential of targeting post-translational modifications as therapeutic approach in the treatment of hypoxia-related disorders will be discussed in the conclusion.

Rec.2/4/2013, Acc.6/24/2013, pp203-216

*Correspondence should be addressed to:
Olga Safronova, PhD., Associate Professor Junior, Department of Cellular Physiological Chemistry, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan. Phone: +81-(0)3-5803-5577, Fax: +81-(0)3-5803-0212, E-mail: olga.cell@tmd.ac.jp

Key words acetylation, hypoxia, hypoxia-inducible factor (HIF), nuclear factor- kappa B (NF-κB), phosphorylation

Introduction

Various pathologies, whether generated in response to injury, autoimmunity or infection, although differ in their etiology, are characterized by common changes in metabolic activity referred to as inflammation. At a certain stage of each of these diseases, alteration of blood supply and inflammatory activation can result in limited oxygen availability (hypoxia). Inflammatory disorders with hypoxic component include atherosclerosis, rheumatoid arthritis, colitis, inflammatory bowel disease, cancer, obesity, diabetes mellitus and many others[1, 2]. Hypoxic condition is also important for normal physiological processes such as embryonic development, stem cell maintenance, and for adaptation to exercise and high altitude.

Cellular responses to changes in oxygen tension lead to the activation of a transcriptional program that is under the control of signal transduction pathways. Subsequently, to maintain cellular and tissue homeostasis, rearrangement of global gene expression profile is occurring in response to hypoxia. Hypoxia-inducible factor (HIF) family is considered as a major transcription factor that controls cellular and tissue adaptive responses to hypoxia (Fig.1). In addition to HIF, cellular function at low oxygen levels depends on other transcription factors and cellular pathways, such as nuclear factor-kappa B (NF-κB), cAMP response element binding protein (CREB), p53, Myc family and others[1, 3].

Cellular signals integrated into coordinated program of gene expression through post-translational modifications (PTMs). PTMs regulate many critical events in functional proteomics: they influence and control enzymatic activity, protein confirmation, cellular localization and interaction with other cellular molecules such as proteins, nucleic acids, lipids, and cofactors. Protein modifications are very diverse in nature totaling greater than 200. Among most common modifications are phosphorylation, acetylation, glycosylation, methylation, ubiquitination and sumoylation. In response to a particular signal, modulation of cellular proteome will be perceived as either linear pathway or combinatorial crosstalk of PTMs in the transduction cascades. For example, phosphorylation-based signal, as the first wave of PTMs in response to cellular stimuli, may be converted by the crosstalk to acetylated-based action[4]. Acetylation can also regulate phosphorylation.

In addition to PTMs of transcriptional regulators, chromatin accessibility can be altered by PTMs of histone tails, which include phosphorylation, acetylation, methylation, sumoylation and ubiquitination. Patterns of distinct histone modifications on a given nucleosome form a histone code that permits the assembly of different remodeling complexes and epigenetic states[5]. The activity of histone modifying enzymes, such as histone methyltransferases (HMTs) and histone demethylases (HDMTs), is also influenced in hypoxia by changes in the expression of their coding genes and PTMs. Hypoxia increases G9a HMT levels and activity, targeting histones and non-histone proteins[6]. Among other HMTs that are known to be hypoxia-inducible are Suv39h1, Suv39h2 and PRMT2[7]. Jumonji C (JmjC) domain-containing HDMTs require oxygen for enzymatic activity. Moreover, the majority of these enzymes are hypoxia inducible, and some of them are in-

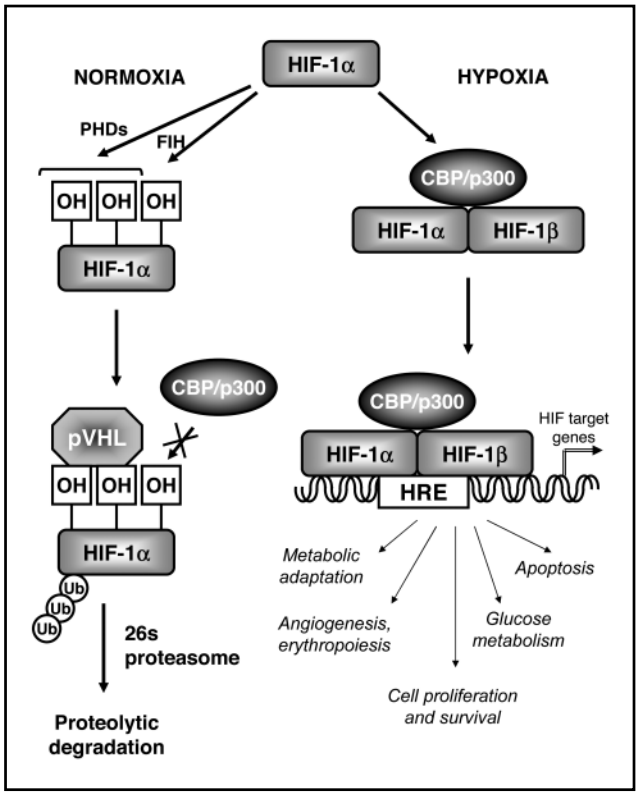

Fig.1 Mechanisms of HIF-1α regulation under normoxic and hypoxic conditions

Under normoxic conditions, HIF-1α is hydroxylated at Pro residues by oxygen-dependent protein hydroxylases (PHDs) followed by binding to von Hippel Lindau protein (pVHL). This event promotes the polyubiquitination of HIF-1α and subsequent 26-S-proteasomal degradation. Factor-inhibiting HIF (FIH) hydroxylates Asn residue in the carboxy-terminal activation domain, which blocks CBP/p300 co-activator recruitment. In hypoxia, HIF hydroxylases are inactive. Stabilized HIF-1α migrates to the nucleus and associates with HIF-1β and the CBP/p300 co-activator. HIF-1 heterodimer activates genes that possess hypoxia-response elements (HRE).

duced in a HIF-1-dependent manner[7, 8]. Sequencing of protein-coding genes in hypoxic renal carcinomas identified somatic truncating mutations in SETD2 histone H3K36 methyltransferase and JARID1C histone H3K4 demethylase[9]. Among the only few studies on changes in histone methylation in hypoxia, our group observed changes in repressive histone and DNA methylation on hypoxia-inhibited MCP-1 gene regulatory region[10]. The existence of histone PTM code have lead to a debate about the existence of a PTM code regulating non-histone proteins, at least in case of some transcription factors[11]. PTMs of transcription factors program their transcriptional activity and can even regulate their ability to function as either an activator or repressor of gene expression.

While there is an abundance of literature on the role of protein PTMs in general and in hypoxia adaptive responses, it is understandable that it will be impossible to give the full overview of all the modifications of transcriptional regulators in the format of single review paper. A number of comprehensive reviews on the hypoxia-induced changes in histone modifications and chromatin structure have been published in the last years[7, 8, 12]. Hypoxia responsive transcription factors and the principals of their regulation in hypoxic environment have been also reviewed recently[1, 3]. Keeping this in mind, I will focus primarily on the aspects of post-translational phosphorylation and acetylation of non-histone proteins in transcriptional responses to hypoxia, in particular, the recent advances made in the understanding of the role and regulation of phosphatases and deacetylases. Among the majority of hypoxia responsive transcription factors, this review will describe in details only PTMs of HIF and NF-κB, reflecting their important role in hypoxic inflammation. The role of PTMs in cancers and other pathologies with hypoxic component will be also highlighted.

Hypoxia and Phosphoproteome

Protein phosphorylation, which is the most widely studied PTM, dominates the number of experimentally observed PTMs as curated from Swiss-Prot proteome database[13]. It is thought that almost one third of the cellular proteins are phosphorylated. Recently, the emergence of technologies involving high-throughput, system-wide experiments allowed identification and quantification of the global *in vivo* phosphoproteome[14]. Interestingly, individual phospho-sites on the proteins containing multiple phosphorylation sites are regulated with different kinetics, suggesting that they serve as integrating platforms for a variety of incoming signals. Distribution of identified phospho-amino acids determined the low abundance of tyrosine phosphorylation — 1.8% in HeLa and 2.9% in human embryonic stem cells HUES9 — compared to serine and threonine phosphorylation[14, 15]. Turnover in tyrosine phosphorylation occurs faster and from a lower basal level compared to serine/threonine phosphorylation. In addition to protein kinase cascades, numerous phosphorylation sites were identified on transcription factors and other regulatory proteins, suggesting the important role of phosphorylation in coordinating transcription program in response to stressors and differentiation factors[14, 15]. To the author's knowledge, the only global study of phosphorylation under hypoxic conditions was performed on newborn red blood cells targeting only tyrosine phosphorylated soluble proteins[16].

1)Regulation of kinases and phosphatases in hypoxia

Addition or removal of a phosphate is regulated by opposing activities of protein kinases (PK) or protein phosphatases (PP) (Fig.2A). Our recent transcriptome analysis of hypoxia primary response genes (1h of exposure) revealed that, although genes related to protein serine/threonine kinase activity and signaling pathway were almost equally represented among hypoxia down-regulated and up-regulated genes, a majority of protein tyrosine kinase activity related genes were presented among hypoxia down-regulated genes (the Gene Expression Omnibus (GEO) database, accession number GSE41023; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE41023). Certain types of protein tyrosine phosphatases, such as protein tyrosine phosphatases (PTP), receptor type, D, G and K (PTPRD, PTPRG, PTPRK), were presented in hypoxia up-regulated gene group. Previously, hypoxic increase in expression of PTP, receptor type, Z polypeptide 1 (PTPRZ1), which is over-expressed in a number of tumors, including glioblastoma, was shown to be mediated by HIF-2 and ELK1 binding to its promoter region[17]. Among other protein phosphatases that are induced by hypoxia are PP2A and PP1 nuclear targeting subunit (PNUTS)[18, 19]. The hypoxic induction of PP2A enhances glioblastoma cell survival[19] and associates with diastolic dysfunction observed in alveolar hypoxia[20].

In our previous work, we found that hypoxia induces activity of protein kinase CK2 and described different roles of catalytic α and regulatory β subunits of CK2[21]. While CK2β subunits were retained in the cytoplasm upon hy-

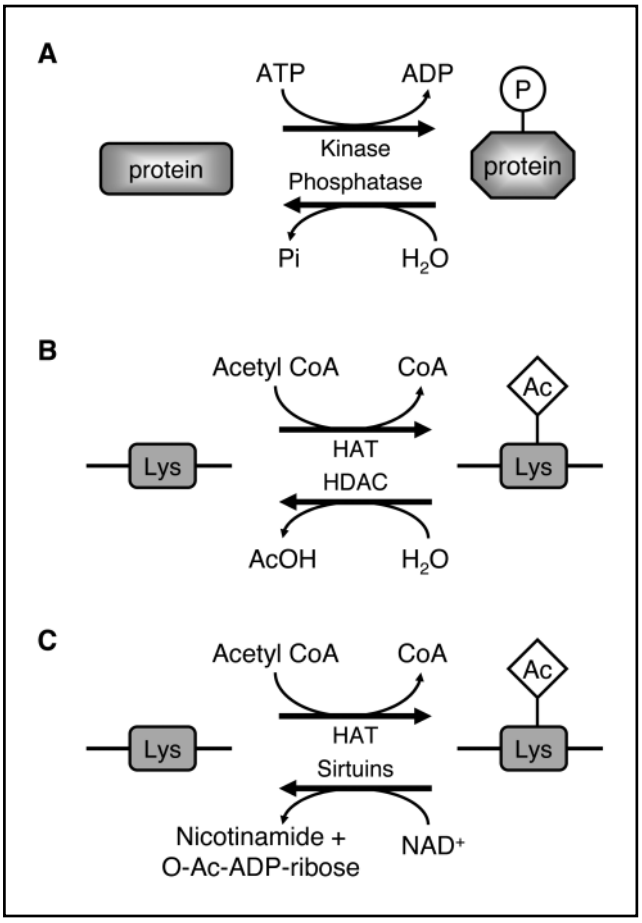

Fig.2 Example of post-translational modifications of proteins
(A) Reversible phosphorylation of proteins is maintained by opposing activities of protein kinases and protein phosphatases. Proteins can be phosphorylated on serine, threonine and tyrosine amino acid side chains. (B, C) Reversible acetylation of proteins. HATs transfer acetyl-groups from acetyl-CoA onto lysines of protein substrates. HDACs reverse the process by protein deacetylation (B). Sirtuins remove acetyl groups in a reaction that requires NAD^+ as the cofactor (C). Letters P and Ac refer to phosphorylation and acetylation, respectively. CoA, coenzyme A; HAT, histone acetyltransferase; HDAC, histone deacetylases; Lys, lysine residue.

poxic treatment, CK2α subunits shuttled to the nucleus, where transcriptional regulators are predominantly localized. CK2 is an important regulator of HIF-1 transcriptional activity via the post-translational phosphorylation of its specific E3-ubiquitin ligase pVHL [22].

Comprehensive studies of the mitogen-activated protein kinase (MAPK) activation in hypoxic cells and animal hypoxia models are actively performed starting from more then 10 years ago and continuing up to the present[23, 24] All three MAPK families — the extracellular signal-regulated protein kinase (ERK), also known as p44 and p42, the c-Jun N-terminal kinase or stress-activated protein kinase (JNK/SAPK) and p38 family — are known to be activated by hypoxia in cell specific manner[25, 26]. MAPK play key roles in a wide range of hypoxia-related physiological processes and human diseases, including embryogenesis, obesity, ischemia, rheumatoid arthritis and cancer[27, 28]. Duration and magnitude of MAPK activation plays a major role in determining the biological outcome of signaling. Although it has been known for many years that mitogen-activated kinase phosphatases (MKP), a distinct subfamily within a larger group of dual-specificity protein phosphatases (DUSP), are a key element of controlling MAPKs, their role in hypoxia responses started being appreciated only recently[27]. Inducible nuclear phosphatase DUSP1/MKP-1 is transcriptionally induced by hypoxia and negatively regulates HIF-1α subunit phosphorylation and interaction with p300 co-activator, thus controlling excessive activation of HIF[29]. DUSP1 is also a key regulator of vascular densities through the regulation of VEGF expression in hypoxic lung[30]. Transcription of nuclear MAPK phosphatase DUSP2 is inhibited while transcription of cytoplasmic DUSP6/MKP-3 is increased in hypoxic cells in a HIF-1-dependent manner[31-33]. Among downstream effects of hypoxic suppression of DUSP2 are ERK-dependent COX-2 overexpression in endometriosis[31] and increase in chemoresistancy and malignancy in human cancer cells[32]. In our microarray transcriptome analysis we observed hypoxic transcriptional down-regulation of DUSP8 (GEO database, GSE41023). Physiological significance of such effect, however, requires further investigation.

2)Phosphorylation of hypoxia responsive transcription factors

Activation of hypoxia responsive transcription factors is also a subject of regulation by phosphorylation. HIF-1, a major oxygen sensing transcription factor, is a phosphorylated protein and its activation depends on several kinase pathways (Fig.3). Activation of both Akt and p38 kinases is involved in hypoxic stabilization and nuclear translocation of HIF-1α [34]. HIF-1α can be directly phosphorylated on Ser641 and Ser643 residues by p42/p44 MAPK increasing thus its nuclear accumulation and transcriptional activity[35]. Raf/MEK/MAPK pathway upregulates the transactivation of HIF-1α through direct phosphorylation of its regulatory/inhibitory domain[36]. Casein kinase 1 (CK1)-dependent phosphorylation of HIF-1α at Ser247 residue impairs its association with

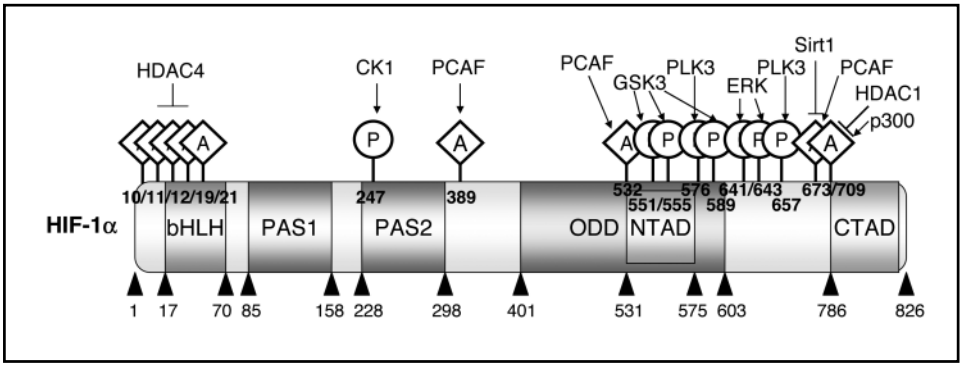

Fig.3 Domain structure and structural location of the post-translational modifications of HIF-1α

Basic structure of the human HIF-1α protein includes: bHLH — basic helix-loop-helix domain involved in DNA binding; PAS1 and PAS2 — Per-Arnt-Sim homology domains 1 and 2 required for heterodimerization with HIF-1β/ARNT; ODD — oxygen-dependent degradation domain that mediates oxygen-regulated stability; NTAD and CTAD — N- and C-terminal transactivation domains required for oxygen-dependent transcriptional activation. Black triangles indicate positions of domains within HIF-1α protein according to the UniProt database, accession number Q16665. Kinases: CK1, casein kinase 1; GSK3, glycogen synthase kinase 3; PLK3, polo-like kinase; ERK, extracellular signal-regulated protein kinase. Letters P and A refer to phosphorylation and acetylation, respectively.

SRNT, thereby diminishing its transcriptional activity[37]. Glycogen synthase kinase 3 (GSK3), a downstream target of Akt, directly phosphorylates the HIF-1α oxygen-dependent degradation domain at Ser551, Thr555 and Ser589 residues, and this mediates HIF-1α proteasomal degradation in a VHL-dependent manner[38]. HIF-1α phosphorylation by polo-like kinase 3 (PLK3) occurs on residues Ser576 and Ser657 and is important for regulating its stability[39]. Activation of PI3K/Act/HIF-1 pathway contributes to hypoxia-induced epithelial-mesenchymal transition in fibroblast-like synoviocytes of rheumatoid arthritis and in chemoresistance in hepatocellular carcinoma[40, 41].

Hypoxic activation of NF-κB is mediated by phosphorylation cascade (Fig.4). In response to hypoxia, IκB kinase (IKK) is induced through a calcium/calmodulin-dependent kinase 2 (CaMK2) in a process dependent on IKK kinase TAK1[42]. Unlike for activation by inflammatory inducers of NF-κB, TAK1-associated proteins TAB1 and TAB2 were not essential for this activation. Activation of IKK leads to phosphorylation of IκB at Tyr42 residue[43] and release of p65 from IκBα without its degradation[42]. This pathway differs from canonical proinflammatory pathway, which mediates NF-κB activation through Ser32 and Ser36 phosphorylation of IκBα with subsequent proteosomal degradation. Among seven reported putative sites of p65 phosphorylation, only Ser276 was reported to be phosphorylated under hypoxic condition via the HIF-1 and ERK1/2 pathway[44].

Hypoxia and Acetylome

Although acetylation has been first discovered and best characterized for histones, comprehensive acetylome study revealed the remarkably ubiquitous and conserved nature of protein acetylation[45]. Acetylated proteins are involved in diverse biological processes such as mRNA processing, chromatin remodeling and regulation of transcription, nuclear transport, cell cycle etc.[45]. Acetylation regulates activity and localization of other posttranslational modifiers. Multiple acetylation sites are present on protein kinases, acetyltransferases and deacetylases, methyltransferases and demethylases, ubiquitin ligases and deubiquitinases[45]. Unlike phosphorylation, which mainly occurs in unstructured regions of proteins, such as hinges and loops[46], acetylation sites are frequently located in regions with ordered secondary structure[45]. In contrast to phosphorylation, no acetylation cascades have been identified yet[47].

Adaptive responses to reduced O_2 availability activate metabolic reprogramming, including HIF-1-mediated transcriptional changes, to promote a switch from mitochondrial oxidative phosphorylation to glycolitic metabolism[48]. Individual reports and global analysis of acetylome indicate an extensive role of acetylation in regulation of cellular energy metabolism. Almost every enzyme in glycolysis, gluconeogenesis, the tricaboxylic acid cycle, fatty acid metabolism, and glycogen metabolism was found to be acetylated in human liver tissue[49]. Pyruvate kinase (PK) is a key glycolitic enzyme which catalyzes a rate-limiting step of glycolysis. Its M2 isoform (PKM2) is highly expressed in cells with high-rate nucleic acid synthesis, such as embryonic and adult stem cells, and re-expressed in cancer cells during tumorigenesis[48]. PKM2 is a direct HIF-1 target gene which participates in a positive feedback loop by interacting directly with HIF-1α subunit to enhance its binding to p300 and further promote

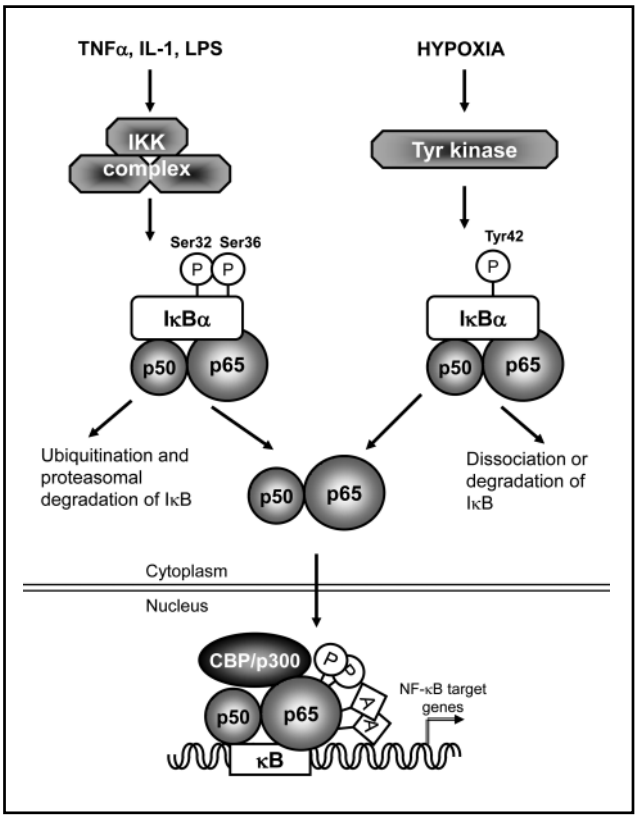

Fig.4 Mechanisms of NF-κB activation by pro-inflammatory mediators and hypoxia

Prototypical NF-κB complex is a heterodimer of p50 and p65 subunits. In inactive state, NF-κB is located in the cytoplasm in a complex with IκB. The canonical pathway is induced by pro-inflammatory mediators such as TNFα, IL-1 and LPS and involves activation of IKK complex. This activation results in phosphorylation of the IκBα protein at Ser32 and Ser36 residues. Hypoxia induces atypical IKK-independent pathway of NF-κB. Activation of tyrosine kinases results in the phosphorylation of IκBα at Tyr42 residue and its subsequent degradation or dissociation from NF-κB heterodimer. Modification of NF-κB subunits by acetylation or phosphorylation determines transcriptional activation or repression effects as well as promoter-specific actions of NF-κB. A, acetylation; IκB, inhibitory protein-κB; IKK, IκB kinase complex, consists of 2 catalytic IKKα and IKKβ subunits and regulatory IKKγ/NEMO subunit. IL-1, interleukin 1; LPS, lipopolysaccharide; P, phosphorylation; TNFα, tumour necrosis factor α.

glycolysis and tumor angiogenesis[50]. PKM2 enzymatic activity is negatively regulated by lysine 305 acetylation[51]. Lysine acetylation is an abundant PTM in the mitochondrion that affects more than 20% of mitochondrial proteins, most of which are involved in energy metabolism and oxidative phosphorylation[52]. Deacetylation of mitochondrial proteins of the electron transport chain primes mitochondria for stress resistance to ischemia[53].

1)Regulation of histone acetyltransferases (HATs) and histone deacetylases (HDACs) in hypoxia

Reversible lysine acetylation is regulated by the counteracting activities of HATs and HDACs (Fig.2B). HATs catalyze the transfer of acetyl groups from Acetyl-CoA to the lysine residues of histone and non-histone proteins. There are three major families of HATs that have been studied extensively: GNATs, p300/CBP (CREB-binding protein) and MYST proteins. Among over 30 HATs identified in mammals, only few have been described to function in hypoxia-related transcription. The most intensively studied HATs are p300 and CBP. Increased expression of p300 mRNA and protein was observed in rat hippocampus and PC12 cells under hypoxic conditions[54]. Hypoxia results in increased phosphorylation levels of p300 and CBP in oxygen-sensitive PC12 and rat carotid body cells[55]. Phosphorylation is essential for acetyltransferase activity of p300/CBP and regulates their interaction with various transcription factors, however direct evidence on the influence of hypoxia on the activity of HATs has not been published yet.

HIF-1α and HIF-2α physically interact with the CH1 domain of p300/CBP via their C-terminal transactivation domain (C-TAD)[56]. CBP/p300 family of coactivators and HDAC inhibitor sensitive pathways together cooperate to mediate greater that 90% of HIF-responsive gene transcription[56]. The interaction of HIF-1 and p300/CBP is negatively regulated by O_2-dependent hydroxylation of Asp-803 in C-TAD by factor inhibiting HIF-1 (FIH-1)[57]. Although p300 and CBP have a high degree of homology, each exhibit different specificities for different HIF-1 target genes[58]. CBP was required for hypoxic induction of vascular endothelial growth factor (VEGF), lactate dehydrogenase-A (LDHA) and phosphoglycerate kinase (PGK), while its homolog p300 enhanced hypoxic induction of VEGF, but was dispensable for the induction of PGK and LDHA[58]. In addition to p300/CBP, the re-

quirement for HAT coactivators, such as PCAF (p300/CBP-associated factor), SPC-1 and SRC-3, has been shown for HIF-1-dependent transcription[58, 59].

Enzymatic removal of acetyl-groups from histones and non-histone proteins is catalyzed by HDACs (Fig.2B). HDACs can be divided into two distinct families: the classical family, zinc-dependent HDACs, and sirtuins, stress-responsive family of nicotinamide adenine dinucleotide (NAD^+)-dependent HDACs. Exposure to hypoxia or serum-deprived hypoxia for 16 hr increases HDAC activity in different cell lines and induces HDAC1, HDAC2 and HDAC3 mRNA and protein expression[60]. Analysis of mRNA levels for HDACs 1 to 11 and co-repressors N-CoR and SMRT in human fetal lung type II cells showed marked induction by 24 hrs hypoxia exposure for all repressors analyzed[61]. Increased HDAC function is also prominent in *in vivo* hypoxic tissues. Human idiopathic pulmonary arterial hypertension lungs exhibited increased expression of HDAC1, HDAC4 and HDAC5 proteins and these observed changes were associated with the pathological vascular remodeling[62]. Lungs and right ventricles from rats exposed to chronic hypoxia exhibited a striking increase in HDAC1 and HDAC5 expression[62]. Our group described the mechanism of HDAC activation in response to hypoxia[21]. We showed that hypoxia induces HDAC1 and HDAC2 activity via protein kinase CK2-dependent post-translational phosphorylation. Interestingly, hypoxic HDAC activation was dependent on catalytic CK2α subunits and did not require formation of CK2 heterotetrameric complex with regulatory CK2β subunits[21]. CK2-induced HDAC activation then favors tumor growth and angiogenesis by mediating pVHL down-regulation and HIF-1α stabilization.

Sirtuins use oxidized NAD^+ as a co-substrate to transfer the acetyl group for deacetylase enzymatic reaction (Fig.2C). Seven sirtuin paralogs (Sirt1-7) have been identified in mammals[47]. Sirt1, Sirt6 and Sirt7 are nuclear proteins with preferential distribution in the nucleoplasm, in heterochromatin and in nucleoli, respectively. Sirt2 is predominantly cytoplasmic. Sirt3, Sirt4 and Sirt5 are mitochondrial proteins and regulators of transcription of mitochondrial genome, of mitochondrial processes and metabolism. Sirtuins' activity is regulated in several ways, however their specific regulation in hypoxic and ischemic conditions is still unclear. Sirtuins are activated in response to a rise in the cellular NAD^+/NADH ratio and, therefore, act as sensors of cellular metabolic and redox state[63]. Hypoxia was reported to reduce NAD^+ to NADH and subsequently decrease Sirt1 activity and mRNA levels[64]. Nevertheless, Sirt1 depletion impaired the ability of endothelial cells to form new vessels in response to ischemic stress, suggesting that its activity is maintained under such conditions[65]. It is possible that NAD^+ levels are kept higher in specific subcellular compartments of hypoxic cell, where the activity of Sirt1 is sufficient to interact with its substrates[63]. Other group demonstrated that, upon hypoxia exposure, HIF-1 and HIF-2 are recruited to the proximal Sirt1 promoter and subsequently increase Sirt1 mRNA and protein expression[66]. Sirt1 activity is also regulated by PTMs. CK2-mediated phosphorylation of Sirt1 increases its substrate-binding affinity and its deacetylase activity[67]. Increase in CK2 enzymatic activity in hypoxic cells was demonstrated previously[21].

2)Acetylation of hypoxia responsive transcription factors

Transcription factors associate with both HATs and HDACs and reversible acetylation of transcription factors itself has been described. Although HDACs are generally found in the transcriptional co-repressor complexes, HDACs are also associated with the activation of HIF-responsive gene expression[56]. HDAC inhibitors, such as trichostatin A, induce proteasomal degradation of HIF-1α via a VHL and ubiquitin-independent mechanism and repress HIF-1α transcriptional activity[68-70]. In agreement with the observations on the effect of HDAC inhibitors, various HDAC isoforms have been described to associate with HIF-1 transcriptional activity. HDAC1, HDAC2, HDAC3, HDAC4, and HDAC6 are associated with increased HIF-1α stability, while HDAC4, HDAC5, HDAC6, HDAC7 result in increased transcriptional activity of HIF-1 via interactions with inhibitory domain, as was described by our previous review and later report[71, 72].

Transcriptional activity and stability of HIF-1α and HIF-2α proteins can be regulated by acetylation of various lysine residues (Fig.3). Acetylation of the first five N-terminal lysines of HIF-1α protein (K10, K11, K12, K19, and K21) disrupts HIF-1α protein level and HIF-1 activity and is specifically regulated by HDAC4[73]. Two lysine residues K532 and K389 within the HIF-1α protein have been identified as acetylation targets of PCAF[59]. Functionally, the acetylation of K532 by arrest defective protein 1 (ARD1) has been linked to HIF transcriptional activity and protein stability[74]. However, several conflicting reports questioned whether this residue is an actual target of ARD1[75]. p300 specifically acetylates HIF-1α at K709, which increases protein stability and HIF-1 activity[76]. This acetylation can be opposed by HDAC1, but not by HDAC3[76].

Sirtuins Sirt1, Sirt3 and Sirt6 are also regulators of HIF protein acetylation and transcriptional activity. Sirt1 binds to HIF-1α and deacetylates it at K674 residue, which is acetylated by PCAF[64]. Such deacetylation inhibits HIF-1α by blocking p300 recruitment and represses HIF-1 target genes. HIF-2α is also deacetylated by Sirt1 during hypoxia at three lysine residues (K385, K685, and K741) within the carboxy terminus[77]. In contrast to HIF-1α, HIF-2α transcriptional activity is enhanced by deacetylation leading to activation of HIF-2α target genes such as VEGFA, superoxide dismutase 2 (SOD2) and erythropoietin (Epo)[77]. Studies in hypoxia preconditioning have reported that Sirt1 down-regulates protein expression of prolyl hydroxylases 2 (PHD2), which leads to the stabilization of HIF-1α[78]. Another sirtuin, Sirt6, behaves as a co-repressor of HIF-1 by deacetylating histone H3 lysine 9 on HIF-1-responsive glycolytic gene promoters[79]. Loss of Sirt6 results in elevated expression of glycolytic genes, increased glucose consumption and glycolysis, and decreased mitochondrial respiration[79]. Switching from the early to the late acute inflammatory responses is supported by metabolic bioenergy switch from increased glycolysis to increased fatty acid oxidation in a process that requires activation of Sirt1 and Sirt6 and subsequent reduction of HIF-1 activity[80]. The mitochondrial deacetylase Sirt3 was reported to be a tumor-suppressor gene[81]. Recently, two independent reports linked anticancer properties of Sirt3 with HIF-1 function[82, 83]. Sirt3 inhibits ROS production, thereby promoting activation of PHDs and destabilization of HIF-1α. Sirt3 deletion appears to promote HIF-1 activation even under normoxic conditions and increases expression of HIF-1 target genes[82, 83].

Biological functions of NF-κB are also regulated by direct acetylation of several NF-κB subunits: p52 and its precursor p100, p50 and p65. Acetylation of the p50 subunit of the classical p65/p50 NF-κB heterodimer at K431, K440 and K441 residues enhances its DNA binding activity and co-recruitment of p300 co-activator[84, 85]. Five acetylation sites have been identified within p65 — lysines K122/K123, K218, K221, K310, K314/K315[85]. Modification of these sites modulates distinct biological responses. Acetylation at K221 enhances DNA binding and, together with K218 acetylation impairs assembly with IκBα[86]. Acetylation of K310 is required for full transcriptional activity of NF-κB[86]. In contrast, K122 and K123 acetylation reduces its ability to bind DNA and facilitates its IκBα-mediated export from the nucleus[87]. The functional relevance of K314 and K315 modifications is not clear yet, however recent observation suggests that acetylation of K314 and possibly K315 might contribute to the repression of certain genes[88]. The p65 is acetylated by p300 on all lysine residue described above[85]. Additionally, dual lysine residues K122/K123 can be targeted for acetylation by PCAF[87]. Acetylated p65 is subsequently deacetylated through a specific interaction with HDAC3[87, 89]. Sirtuins Sirt1 and Sirt2 physically interact with p65 and inhibit expression of a subset of p65 acetylation-dependent target genes by deacetylating p65 at K310[90, 91]. Although hypoxia modulates activity of NF-κB-targeting HATs and HDACs, the impact of hypoxia on NF-κB acetylation is not completely understood. Very limited evidences are mainly referred to ischemia or ischemia-reperfusion. However, although ischemic condition contains hypoxic component, it includes, in addition, other metabolic abnormalities, such as glucose deprivation and defective removal of waste products. Study of ischemia-reperfusion injury on Langendorff isolated hearts revealed that acetylation of p50 at lysine residues is essential for HDAC inhibitor-induced cardioprotection[92]. It was shown that activated NF-κB displayed a high level of p65 K310 acetylation *in vivo* in mice subjected to lethal middle cerebral artery occlusion and *in vitro* in primary cortical neurons exposed to lethal oxygen-glucose deprivation[93].

Conclusions and perspectives

Recent studies clearly demonstrate that hypoxia affects numerous signaling pathways related to inflammatory responses and cancerogenesis. Moreover, the hypoxic microenvironment in various cancers is thought to increase resistance to chemo- and radio-therapies. Expression of hypoxia-responsive genes is subject to tight regulation involving PTMs of both upstream factors and of histone tails surrounding regulatory and coding regions of gene targets.

Creation of Swiss-Prot protein database led to a striking discovery that the number of PTMs far exceeded the number of mutations identified[13]. Much remains to be learned about the role of disregulation of PTMs in etiogenesis and progression of a variety of diseases, including inflammatory conditions and different types of cancers. Analysis of the complete protein kinase gene family detected only a very few or no somatic mutations in primary cancers, indicating that changes in enzymatic activity, which are primarily regulated by PTMs, are more often the cause of disease progression[94]. Certain inhibitors of PTMs have been already introduced into clinical oncology. The effectiveness of inhibi-

tors of HDACs and specific tyrosine kinases has been proven in the treatment of various malignancies, such as acute and chronic myeloid leukemias, B- and T-cell lymphomas, colorectal and pancreatic cancers and many others[95, 96]. Progress in understanding the function of broader spectrum of regulators of PTMs has provided insights into which protein tyrosine phosphatases and protein kinase CK2 might be novel potential therapeutic targets in human cancer[97, 98] Therefore, considerable interest in understanding how PTMs signaling pathways enhance tumor cell survival under hypoxia might lead to the development of more effective alternative therapy to target these resistant cell subpopulations.

In addition, inhibitors of PTMs are valuable tools in pharmacological studies on inflammation. Anti-inflammatory properties of HDAC inhibitors, such as reduction in cytokine production, are being studied in models of a broad range of diseases not related to cancer[99]. Distinct from their use in oncology, reduction of inflammation requires doses considerably lower than the higher concentrations of HDAC inhibitors that are required for tumor cells death. The potential of HDAC inhibition has been proposed as therapeutic strategy in pulmonary arterial hypertension[62].

A disbalance between specific kinase-mediated phosphorylation and corresponding phosphatases is considered to be involved in the etiology of chronic inflammatory and immunologic diseases such as allergy and rheumatoid arthritis; neural diseases such as Alzheimer and Parkinson disease; and diabetes mellitus[100, 101]. Since the first kinase inhibitor, imatilib mesilate (Novartis), came to market in 2001, the research moved toward the opportunities and challenges of kinase and phosphatase inhibitors as a promising therapeutic approach in inflammatory disease[100, 101]. Even though inhibitors specific for the individual enzyme molecules involved in PTMs are difficult to develop, studies in cell cultures and animal models have revealed a promising therapeutic option and great clinical impact.

Acknowlegements and Sources of funding

I thank Professor Ikuo Morita for continuous support and encouragement. Work in our laboratory is supported by the Japan Society for the Promotion of Science (JSPS) and funding of Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases.

Conflict of interest

None

References

1) Safronova O, Morita I: Transcriptome remodeling in hypoxic inflammation. J Dent Res. 2010; 89: 430-444.
2) Konisti S, Kiriakidis S, Paleolog EM: Hypoxia—a key regulator of angiogenesis and inflammation in rheumatoid arthritis. Nat Rev Rheumatol. 2012; 8: 153-162.
3) Kenneth NS, Rocha S: Regulation of gene expression by hypoxia. Biochem J. 2008; 414: 19-29.
4) Yang XJ, Seto E: Lysine acetylation: codified crosstalk with other posttranslational modifications. Mol Cell. 2008; 31: 449-461.
5) Ruthenburg AJ, Li H, Patel DJ, Allis CD: Multivalent engagement of chromatin modifications by linked binding modules. Nat Rev Mol Cell Biol. 2007; 8: 983-994.
6) Lee JS, Kim Y, Bhin J, Shin HJ, Nam HJ, Lee SH, Yoon JB, Binda O, Gozani O, Hwang D, Baek SH: Hypoxia-induced methylation of a pontin chromatin remodeling factor. Proc Natl Acad Sci U S A. 2011; 108: 13510-13515.
7) Melvin A, Rocha S: Chromatin as an oxygen sensor and active player in the hypoxia response. Cell Signal. 2012; 24: 35-43.
8) Mimura I, Tanaka T, Wada Y, Kodama T, Nangaku M: Pathophysiological response to hypoxia - from the molecular mechanisms of malady to drug discovery: epigenetic regulation of the hypoxic response via hypoxia-inducible factor and histone modifying enzymes. J Pharmacol Sci. 2011; 115: 453-458.
9) Dalgliesh GL, Furge K, Greenman C, Chen L, Bignell G, Butler A, Davies H, Edkins S, Hardy C, Latimer C, Teague J, Andrews J, Barthorpe S, Beare D, Buck G, Campbell PJ, Forbes S, Jia M, Jones D, Knott H, Kok CY, Lau KW, Leroy C, Lin ML, McBride DJ, Maddison M, Maguire S, McLay K, Menzies A, Mironenko T, Mulderrig L, Mudie L, O'Meara S, Pleasance E, Rajasingham A, Shepherd R, Smith R, Stebbings L, Stephens P, Tang G, Tarpey PS, Turrell K, Dykema KJ, Khoo SK, Petillo D, Wondergem B, Anema J, Kahnoski RJ, Teh BT, Stratton MR, Futreal PA: Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature. 2010; 463: 360-363.
10) Aoi Y, Nakahama K, Morita I, Safronova O: The involvement of DNA and histone methylation in the repression of IL-1beta-induced MCP-1 production by hypoxia. Biochem Biophys Res Commun. 2011; 414: 252-258.

11) Benayoun BA, Veitia RA: A post-translational modification code for transcription factors: sorting through a sea of signals. Trends Cell Biol. 2009; 19: 189-197.
12) Johnson AB, Barton MC: Hypoxia-induced and stress-specific changes in chromatin structure and function. Mutat Res. 2007; 618: 149-162.
13) Khoury GA, Baliban RC, Floudas CA: Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. Sci Rep. 2011; 1.
14) Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M: Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell. 2006; 127: 635-648.
15) Rigbolt KT, Prokhorova TA, Akimov V, Henningsen J, Johansen PT, Kratchmarova I, Kassem M, Mann M, Olsen JV, Blagoev B: System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation. Sci Signal. 2011; 4: rs3.
16) Marzocchi B, Ciccoli L, Tani C, Leoncini S, Rossi V, Bini L, Perrone S, Buonocore G: Hypoxia-induced post-translational changes in red blood cell protein map of newborns. Pediatr Res. 2005; 58: 660-665.
17) Wang V, Davis DA, Veeranna RP, Haque M, Yarchoan R: Characterization of the activation of protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1) by hypoxia inducible factor-2 alpha. PLoS One. 2010; 5: e9641.
18) Lee SJ, Lim CJ, Min JK, Lee JK, Kim YM, Lee JY, Won MH, Kwon YG: Protein phosphatase 1 nuclear targeting subunit is a hypoxia inducible gene: its role in post-translational modification of p53 and MDM2. Cell Death Differ. 2007; 14: 1106-1116.
19) Hofstetter CP, Burkhardt JK, Shin BJ, Gursel DB, Mubita L, Gorrepati R, Brennan C, Holland EC, Boockvar JA: Protein phosphatase 2A mediates dormancy of glioblastoma multiforme-derived tumor stem-like cells during hypoxia. PLoS One. 2012; 7: e30059.
20) Larsen KO, Lygren B, Sjaastad I, Krobert KA, Arnkvaern K, Florholmen G, Larsen AK, Levy FO, Tasken K, Skjonsberg OH, Christensen G: Diastolic dysfunction in alveolar hypoxia: a role for interleukin-18-mediated increase in protein phosphatase 2A. Cardiovasc Res. 2008; 80: 47-54.
21) Pluemsampant S, Safronova OS, Nakahama K, Morita I: Protein kinase CK2 is a key activator of histone deacetylase in hypoxia-associated tumors. Int J Cancer. 2008; 122: 333-341.
22) Ampofo E, Kietzmann T, Zimmer A, Jakupovic M, Montenarh M, Gotz C: Phosphorylation of the von Hippel-Lindau protein (VHL) by protein kinase CK2 reduces its protein stability and affects p53 and HIF-1alpha mediated transcription. Int J Biochem Cell Biol. 2010; 42: 1729-1735.
23) Minet E, Michel G, Mottet D, Raes M, Michiels C: Transduction pathways involved in Hypoxia-Inducible Factor-1 phosphorylation and activation. Free Radic Biol Med. 2001; 31: 847-855.
24) Maulik D, Ashraf QM, Mishra OP, Delivoria-Papadopoulos M: Activation of p38 mitogen-activated protein kinase (p38 MAPK), extracellular signal-regulated kinase (ERK) and c-jun N-terminal kinase (JNK) during hypoxia in cerebral cortical nuclei of guinea pig fetus at term: role of nitric oxide. Neurosci Lett. 2008; 439: 94-99.
25) Chae HJ, Kim SC, Han KS, Chae SW, An NH, Kim HM, Kim HH, Lee ZH, Kim HR: Hypoxia induces apoptosis by caspase activation accompanying cytochrome C release from mitochondria in MC3T3E1 osteoblasts. p38 MAPK is related in hypoxia-induced apoptosis. Immunopharmacol Immunotoxicol. 2001; 23: 133-152.
26) Le YJ, Corry PM: Hypoxia-induced bFGF gene expression is mediated through the JNK signal transduction pathway. Mol Cell Biochem. 1999; 202: 1-8.
27) Caunt CJ, Keyse SM: Dual-specificity MAP kinase phosphatases (MKPs): Shaping the outcome of MAP kinase signalling. FEBS J. 2012; 280：489-504.
28) Lawrence MC, Jivan A, Shao C, Duan L, Goad D, Zaganjor E, Osborne J, McGlynn K, Stippec S, Earnest S, Chen W, Cobb MH: The roles of MAPKs in disease. Cell Res. 2008; 18: 436-442.
29) Liu C, Shi Y, Du Y, Ning X, Liu N, Huang D, Liang J, Xue Y, Fan D: Dual-specificity phosphatase DUSP1 protects overactivation of hypoxia-inducible factor 1 through inactivating ERK MAPK. Exp Cell Res. 2005; 309: 410-418.
30) Shields KM, Panzhinskiy E, Burns N, Zawada WM, Das M: Mitogen-activated protein kinase phosphatase-1 is a key regulator of hypoxia-induced vascular endothelial growth factor expression and vessel density in lung. Am J Pathol. 2011; 178: 98-109.
31) Wu MH, Lin SC, Hsiao KY, Tsai SJ: Hypoxia-inhibited

dual-specificity phosphatase-2 expression in endometriotic cells regulates cyclooxygenase-2 expression. J Pathol. 2011; 225: 390-400.
32) Lin SC, Chien CW, Lee JC, Yeh YC, Hsu KF, Lai YY, Tsai SJ: Suppression of dual-specificity phosphatase-2 by hypoxia increases chemoresistance and malignancy in human cancer cells. J Clin Invest. 2011; 121: 1905-1916.
33) Bermudez O, Jouandin P, Rottier J, Bourcier C, Pages G, Gimond C: Post-transcriptional regulation of the DUSP6/MKP-3 phosphatase by MEK/ERK signaling and hypoxia. J Cell Physiol. 2011; 226: 276-284.
34) Kanichai M, Ferguson D, Prendergast PJ, Campbell VA: Hypoxia promotes chondrogenesis in rat mesenchymal stem cells: a role for AKT and hypoxia-inducible factor (HIF)-1alpha. J Cell Physiol. 2008; 216: 708-715.
35) Mylonis I, Chachami G, Samiotaki M, Panayotou G, Paraskeva E, Kalousi A, Georgatsou E, Bonanou S, Simos G: Identification of MAPK phosphorylation sites and their role in the localization and activity of hypoxia-inducible factor-1alpha. J Biol Chem. 2006; 281: 33095-33106.
36) Sodhi A, Montaner S, Miyazaki H, Gutkind JS: MAPK and Akt act cooperatively but independently on hypoxia inducible factor-1alpha in rasV12 upregulation of VEGF. Biochem Biophys Res Commun. 2001; 287: 292-300.
37) Kalousi A, Mylonis I, Politou AS, Chachami G, Paraskeva E, Simos G: Casein kinase 1 regulates human hypoxia-inducible factor HIF-1. J Cell Sci. 2010; 123: 2976-2986.
38) Flugel D, Gorlach A, Michiels C, Kietzmann T: Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner. Mol Cell Biol. 2007; 27: 3253-3265.
39) Xu D, Yao Y, Lu L, Costa M, Dai W: Plk3 functions as an essential component of the hypoxia regulatory pathway by direct phosphorylation of HIF-1alpha. J Biol Chem. 2010; 285: 38944-38950.
40) Li GQ, Zhang Y, Liu D, Qian YY, Zhang H, Guo SY, Sunagawa M, Hisamitsu T, Liu YQ: PI3 kinase/Akt/HIF-1alpha pathway is associated with hypoxia-induced epithelial-mesenchymal transition in fibroblast-like synoviocytes of rheumatoid arthritis. Mol Cell Biochem. 2012; 372: 221-231.
41) Jiao M, Nan KJ: Activation of PI3 kinase/Akt/HIF-1alpha pathway contributes to hypoxia-induced epithelial-mesenchymal transition and chemoresistance in hepatocellular carcinoma. Int J Oncol. 2012; 40 :461-468.
42) Culver C, Sundqvist A, Mudie S, Melvin A, Xirodimas D, Rocha S: Mechanism of hypoxia-induced NF-kappaB. Mol Cell Biol. 2010; 30: 4901-4921.
43) Koong AC, Chen EY, Giaccia AJ: Hypoxia causes the activation of nuclear factor kappa B through the phosphorylation of I kappa B alpha on tyrosine residues. Cancer Res. 1994; 54: 1425-1430.
44) Scortegagna M, Cataisson C, Martin RJ, Hicklin DJ, Schreiber RD, Yuspa SH, Arbeit JM: HIF-1alpha regulates epithelial inflammation by cell autonomous NF kappaB activation and paracrine stromal remodeling. Blood. 2008; 111: 3343-3354.
45) Choudhary C, Kumar C, Gnad F, Nielsen ML, Rehman M, Walther TC, Olsen JV, Mann M: Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009; 325: 834-840.
46) Gnad F, Ren S, Cox J, Olsen JV, Macek B, Oroshi M, Mann M: PHOSIDA (phosphorylation site database): management, structural and evolutionary investigation, and prediction of phosphosites. Genome Biol. 2007; 8: R250.
47) Spange S, Wagner T, Heinzel T, Kramer OH: Acetylation of non-histone proteins modulates cellular signalling at multiple levels. Int J Biochem Cell Biol. 2009; 41: 185-98.
48) Porporato PE, Dhup S, Dadhich RK, Copetti T, Sonveaux P: Anticancer targets in the glycolytic metabolism of tumors: a comprehensive review. Front Pharmacol. 2011; 2 :49.
49) Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L, Zeng Y, Li H, Li Y, Shi J, An W, Hancock SM, He F, Qin L, Chin J, Yang P, Chen X, Lei Q, Xiong Y, Guan KL: Regulation of cellular metabolism by protein lysine acetylation. Science. 2010; 327: 1000-1004.
50) Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R, Cole RN, Pandey A, Semenza GL: Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell. 2011; 145: 732-744.
51) Lv L, Li D, Zhao D, Lin R, Chu Y, Zhang H, Zha Z, Liu Y, Li Z, Xu Y, Wang G, Huang Y, Xiong Y, Guan KL, Lei QY: Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell. 2011; 42: 719-730.

52) Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Cheng T, Kho Y, Xiao H, Xiao L, Grishin NV, White M, Yang XJ, Zhao Y: Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. Mol Cell. 2006; 23: 607-618.
53) Shinmura K, Tamaki K, Sano M, Nakashima-Kamimura N, Wolf AM, Amo T, Ohta S, Katsumata Y, Fukuda K, Ishiwata K, Suematsu M, Adachi T: Caloric restriction primes mitochondria for ischemic stress by deacetylating specific mitochondrial proteins of the electron transport chain. Circ Res. 2011; 109: 396-406.
54) Tan XL, Zhai Y, Gao WX, Fan YM, Liu FY, Huang QY, Gao YQ: p300 expression is induced by oxygen deficiency and protects neuron cells from damage. Brain Res. 2009; 1254: 1-9.
55) Zakrzewska A, Schnell PO, Striet JB, Hui A, Robbins JR, Petrovic M, Conforti L, Gozal D, Wathelet MG, Czyzyk-Krzeska MF: Hypoxia-activated metabolic pathway stimulates phosphorylation of p300 and CBP in oxygen-sensitive cells. J Neurochem. 2005; 94: 1288-1296.
56) Kasper LH, Boussouar F, Boyd K, Xu W, Biesen M, Rehg J, Baudino TA, Cleveland JL, Brindle PK: Two transactivation mechanisms cooperate for the bulk of HIF-1-responsive gene expression. EMBO J. 2005; 24: 3846-3858.
57) Semenza GL: Physiology meets biophysics: visualizing the interaction of hypoxia-inducible factor 1 alpha with p300 and CBP. Proc Natl Acad Sci U S A. 2002; 99: 11570-11572.
58) Wang F, Zhang R, Wu X, Hankinson O: Roles of coactivators in hypoxic induction of the erythropoietin gene. PLoS One. 2010; 5: e10002.
59) Xenaki G, Ontikatze T, Rajendran R, Stratford IJ, Dive C, Krstic-Demonacos M, Demonacos C: PCAF is an HIF-1alpha cofactor that regulates p53 transcriptional activity in hypoxia. Oncogene. 2008; 27: 5785-5796.
60) Kim MS, Kwon HJ, Lee YM, Baek JH, Jang JE, Lee SW, Moon EJ, Kim HS, Lee SK, Chung HY, Kim CW, Kim KW: Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med. 2001; 7: 437-443.
61) Islam KN, Mendelson CR: Permissive effects of oxygen on cyclic AMP and interleukin-1 stimulation of surfactant protein A gene expression are mediated by epigenetic mechanisms. Mol Cell Biol. 2006; 26: 2901-2912.
62) Zhao L, Chen CN, Hajji N, Oliver E, Cotroneo E, Wharton J, Wang D, Li M, McKinsey TA, Stenmark KR, Wilkins MR: Histone deacetylation inhibition in pulmonary hypertension: therapeutic potential of valproic acid and suberoylanilide hydroxamic acid. Circulation. 2012; 126: 455-467.
63) Guarani V, Potente M: SIRT1 - a metabolic sensor that controls blood vessel growth. Curr Opin Pharmacol. 2010; 10: 139-145.
64) Lim JH, Lee YM, Chun YS, Chen J, Kim JE, Park JW: Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1alpha. Mol Cell. 2010; 38: 864-878.
65) Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, Dequiedt F, Haendeler J, Mione M, Dejana E, Alt FW, Zeiher AM, Dimmeler S: SIRT1 controls endothelial angiogenic functions during vascular growth. Genes Dev. 2007; 21: 2644-2658.
66) Chen R, Dioum EM, Hogg RT, Gerard RD, Garcia JA: Hypoxia increases sirtuin 1 expression in a hypoxia-inducible factor-dependent manner. J Biol Chem. 2011; 286: 13869-13878.
67) Kang H, Jung JW, Kim MK, Chung JH: CK2 is the regulator of SIRT1 substrate-binding affinity, deacetylase activity and cellular response to DNA-damage. PLoS One. 2009; 4: e6611.
68) Qian DZ, Kachhap SK, Collis SJ, Verheul HM, Carducci MA, Atadja P, Pili R: Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 alpha. Cancer Res. 2006; 66: 8814-8821.
69) Fath DM, Kong X, Liang D, Lin Z, Chou A, Jiang Y, Fang J, Caro J, Sang N: Histone deacetylase inhibitors repress the transactivation potential of hypoxia-inducible factors independently of direct acetylation of HIF-alpha. J Biol Chem. 2006; 281: 13612-13619.
70) Kong X, Lin Z, Liang D, Fath D, Sang N, Caro J: Histone deacetylase inhibitors induce VHL and ubiquitin-independent proteasomal degradation of hypoxia-inducible factor 1alpha. Mol Cell Biol. 2006; 26: 2019-2028.
71) Chang CC, Lin BR, Chen ST, Hsieh TH, Li YJ, Kuo MY: HDAC2 promotes cell migration/invasion abilities through HIF-1alpha stabilization in human oral squamous cell carcinoma. J Oral Pathol Med. 2011; 40: 567-575.
72) Safronova O, Pluemsampant S, Nakahama K, Morita I:

Regulation of chemokine gene expression by hypoxia via cooperative activation of NF-kappaB and histone deacetylase. Int J Biochem Cell Biol. 2009; 41: 2270-2280.

73) Geng H, Harvey CT, Pittsenbarger J, Liu Q, Beer TM, Xue C, Qian DZ: HDAC4 protein regulates HIF1alpha protein lysine acetylation and cancer cell response to hypoxia. J Biol Chem. 2011; 286: 38095-38102.

74) Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, Yoo MA, Song EJ, Lee KJ, Kim KW: Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell. 2002; 111: 709-720.

75) Bilton R, Trottier E, Pouyssegur J, Brahimi-Horn MC: ARDent about acetylation and deacetylation in hypoxia signalling. Trends Cell Biol. 2006; 16: 616-621.

76) Geng H, Liu Q, Xue C, David LL, Beer TM, Thomas GV, Dai MS, Qian DZ: HIF1alpha protein stability is increased by acetylation at lysine 709. J Biol Chem. 2012; 287: 35496-35505.

77) Dioum EM, Chen R, Alexander MS, Zhang Q, Hogg RT, Gerard RD, Garcia JA: Regulation of hypoxia-inducible factor 2alpha signaling by the stress-responsive deacetylase sirtuin 1. Science. 2009; 324: 1289-1293.

78) Rane S, He M, Sayed D, Vashistha H, Malhotra A, Sadoshima J, Vatner DE, Vatner SF, Abdellatif M: Downregulation of miR-199a derepresses hypoxia-inducible factor-1alpha and Sirtuin 1 and recapitulates hypoxia preconditioning in cardiac myocytes. Circ Res. 2009;104(7):879-86.

79) Zhong L, D'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, Guimaraes A, Marinelli B, Wikstrom JD, Nir T, Clish CB, Vaitheesvaran B, Iliopoulos O, Kurland I, Dor Y, Weissleder R, Shirihai OS, Ellisen LW, Espinosa JM, Mostoslavsky R: The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1alpha. Cell. 2010; 140: 280-293.

80) Liu TF, Vachharajani VT, Yoza BK, McCall CE: NAD+-dependent sirtuin 1 and 6 proteins coordinate a switch from glucose to fatty acid oxidation during the acute inflammatory response. J Biol Chem. 2012; 287: 25758-25769.

81) Kim HS, Patel K, Muldoon-Jacobs K, Bisht KS, Aykin-Burns N, Pennington JD, van der Meer R, Nguyen P, Savage J, Owens KM, Vassilopoulos A, Ozden O, Park SH, Singh KK, Abdulkadir SA, Spitz DR, Deng CX, Gius D: SIRT3 is a mitochondria-localized tumor suppressor required for maintenance of mitochondrial integrity and metabolism during stress. Cancer Cell. 2010; 17: 41-52.

82) Finley LW, Carracedo A, Lee J, Souza A, Egia A, Zhang J, Teruya-Feldstein J, Moreira PI, Cardoso SM, Clish CB, Pandolfi PP, Haigis MC: SIRT3 opposes reprogramming of cancer cell metabolism through HIF1alpha destabilization. Cancer Cell. 2011; 19: 416-428.

83) Bell EL, Emerling BM, Ricoult SJ, Guarente L: SirT3 suppresses hypoxia inducible factor 1alpha and tumor growth by inhibiting mitochondrial ROS production. Oncogene. 2011; 30: 2986-2996.

84) Furia B, Deng L, Wu K, Baylor S, Kehn K, Li H, Donnelly R, Coleman T, Kashanchi F: Enhancement of nuclear factor-kappa B acetylation by coactivator p300 and HIV-1 Tat proteins. J Biol Chem. 2002; 277: 4973-4980.

85) Calao M, Burny A, Quivy V, Dekoninck A, Van Lint C: A pervasive role of histone acetyltransferases and deacetylases in an NF-kappaB-signaling code. Trends Biochem Sci. 2008; 33: 339-349.

86) Chen LF, Mu Y, Greene WC: Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J. 2002; 21: 6539-6548.

87) Kiernan R, Bres V, Ng RW, Coudart MP, El Messaoudi S, Sardet C, Jin DY, Emiliani S, Benkirane M: Post-activation turn-off of NF-kappa B-dependent transcription is regulated by acetylation of p65. J Biol Chem. 2003; 278: 2758-2766.

88) Rothgiesser KM, Fey M, Hottiger MO: Acetylation of p65 at lysine 314 is important for late NF-kappaB-dependent gene expression. BMC Genomics. 2010; 11: 22.

89) Chen L, Fischle W, Verdin E, Greene WC: Duration of nuclear NF-kappaB action regulated by reversible acetylation. Science. 2001; 293: 1653-1657.

90) Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW: Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. 2004; 23: 2369-2380.

91) Rothgiesser KM, Erener S, Waibel S, Luscher B, Hottiger MO: SIRT2 regulates NF-kappaB dependent gene expression through deacetylation of p65 Lys310. J Cell Sci. 2010; 123: 4251-4258.

92) Zhang LX, Zhao Y, Cheng G, Guo TL, Chin YE, Liu PY, Zhao TC: Targeted deletion of NF-kappaB p50 diminishes the cardioprotection of histone deacetylase inhi-

bition. Am J Physiol Heart Circ Physiol. 2010; 298: H2154-H2163.

93) Lanzillotta A, Sarnico I, Ingrassia R, Boroni F, Branca C, Benarese M, Faraco G, Blasi F, Chiarugi A, Spano P, Pizzi M: The acetylation of RelA in Lys310 dictates the NF-kappaB-dependent response in post-ischemic injury. Cell Death Dis. 2010; 1: e96.

94) Stephens P, Edkins S, Davies H, Greenman C, Cox C, Hunter C, Bignell G, Teague J, Smith R, Stevens C, O'Meara S, Parker A, Tarpey P, Avis T, Barthorpe A, Brackenbury L, Buck G, Butler A, Clements J, Cole J, Dicks E, Edwards K, Forbes S, Gorton M, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jones D, Kosmidou V, Laman R, Lugg R, Menzies A, Perry J, Petty R, Raine K, Shepherd R, Small A, Solomon H, Stephens Y, Tofts C, Varian J, Webb A, West S, Widaa S, Yates A, Brasseur F, Cooper CS, Flanagan AM, Green A, Knowles M, Leung SY, Looijenga LH, Malkowicz B, Pierotti MA, Teh B, Yuen ST, Nicholson AG, Lakhani S, Easton DF, Weber BL, Stratton MR, Futreal PA, Wooster R: A screen of the complete protein kinase gene family identifies diverse patterns of somatic mutations in human breast cancer. Nat Genet. 2005; 37: 590-592.

95) Hartmann JT, Haap M, Kopp HG, Lipp HP: Tyrosine kinase inhibitors - a review on pharmacology, metabolism and side effects. Curr Drug Metab. 2009; 10: 470-481.

96) Bolden JE, Peart MJ, Johnstone RW: Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006; 5: 769-784.

97) Cozza G, Meggio F, Moro S: The dark side of protein kinase CK2 inhibition. Curr Med Chem. 2011; 18: 2867-2884.

98) Julien SG, Dube N, Hardy S, Tremblay ML: Inside the human cancer tyrosine phosphatome. Nat Rev Cancer. 2011; 11: 35-49.

99) Dinarello CA, Fossati G, Mascagni P: Histone deacetylase inhibitors for treating a spectrum of diseases not related to cancer. Mol Med. 2011; 17: 333-352.

100) Heneberg P: Use of protein tyrosine phosphatase inhibitors as promising targeted therapeutic drugs. Curr Med Chem. 2009; 16: 706-733.

101) Rokosz LL, Beasley JR, Carroll CD, Lin T, Zhao J, Appell KC, Webb ML: Kinase inhibitors as drugs for chronic inflammatory and immunological diseases: progress and challenges. Expert Opin Ther Targets. 2008; 12: 883-903.