Review Article

iMedPub Journals
www.imedpub.com

Archives in Cancer Research
ISSN 2254-6081

2024
Vol.12 No.4:033

Cross Talk between Epigenetic and HIFs in Mediating Cellular Specificity and Behaviour Modifications in Stressful Conditions

Sami Baccouche*

Preparatory Institute to Engeneiring Study, Sfax, Tunisia

***Corresponding author:** Sami Baccouche, Preparatory Institute to Engeneiring Study, Sfax, Tunisia; Email: sami.baccouche@ipeis.usf.tn

Received: October 18, 2024 Manuscript No. IPACR-24-15287; **Editor assigned:** October 23, 2024, PreQC No. IPACR-24-15287 (PQ); **Reviewed:** November 06, 2024, QC No. IPACR-24-15287; **Revised:** November 13, 2024, Manuscript No. IPACR-24-15287 (R); **Published:** December 11, 2024, Invoice No. J-15287

Citation: Baccouche S (2024) Cross Talk between Epigenetic and HIFs in Mediating Cellular Specificity and Behaviour Modifications in Stressful Conditions. Archives Can Res Vol:12 No:4

Abstract

¢KŜ ăúNŜăăŦdzf Ŏ2yŘλûλ2yã ădzŎK I·ăY IèLJ2ÈλI·Σ wh{ I·yŘ ōλ2f2ΞλŎ ăúNŜăă I·ă ØNJI·f λyŦŜŎûλ2yΣ λΥLJI·Ŏû 2y´ŎŜff ăúNdzŎûdzNŜΣ ăLJŜŎλŦλŎλûè I·yŘ ŎŜffdzfI·NJ ōŜKI·Øλ2dzN℘ ¢KŜ Y´I·i2NJ ăŜyã2NJ ŦI·ŎûΩNă 2Ŧ ădzŎK ăúNŜăă I·NŜ ILCă℘ ¢KŜ ăûI·ōλfăI·ûλ2y´ I·yŘ I·ŎŎdzY´dzfI·ûλ2y´ 2Ŧ ILCăΣ dzyŘŜNJ ăúNŜăă ăûλY´dzfΣ I·NJăŜ ŘλØŜNă ILCă ăλΞyl·fλyΞă ăLJŜŎúNdzYΣ Ŏ2yŘdzŎûλyΞ û2 Ř2ǿy´LJI ØI·fdzŜΣ KλΞK b!5Ь Ŏ2yŎŜyûNJI·ûλ2y´I·yŘ wh{ I·ŎŎdzY´dzfI·ûλ2yT λY´LJI·ŎûλyΞ 2y´ ŜLJλΞŜySûλŎ fI·yŘăŎI·LJŜ I·yŘ Y´ŜŘλI·ûλyΞ ăLJŜŎλŦλŎ ŎŜffdzfI·NJ ōŜKI·Øλ2dzN ăǿλŎK Y´I·yλŦŜăúŜŘ ōè ŎŜff Y´I·fλΞyl·yŎè ă2 ŦI·NJ ûdzY´2dzN λY´Y´dzy2πŜăŎI·LJλyΞ fŜI·ŘλyΞ û2 Y´Sûl·ăûI·ăλă℘ Ly´ úKλă NJŜØλŜǿΣ ǿŜ ŘŜŎ2NJûλŎI·ûŜΣ ûKŜăŜ ŘλØŜNJă ăLJŜŎúNdzY´ 2Ŧ ILCTMʰ ăλΞyl·fλyΞă λyØ2fØλyΞ λy´ úKŜăŜ ŘλŦŦŜNJSyú ûdzY´2dzN LJKŜy2ûèLJŜă℘

Keywords: Hypoxia; Cellular behaviour; Spectrum; Metastasis

Introduction

The vertebrate genome is bundled into a highly organized chromatin structure fundamental for precise gene regulation and maintenance of genome integrity. It is widely believed that dynamic chromatin states, defined as epigenetic landscapes, guide cells through development and impart an epigenetic memory to maintain cell type-specific transcriptional programs.

The epigenetic processes are buffers of genetic variation, pending an epigenetic (or mutational) change of state that leads an identical combination of genes to produce a different developmental outcome [1]. The overall methylation pattern of the genome needs to be maintained for the correct functioning of the cells. In fact, in tumor cells, the DNA methylation pattern is inverted, because CpG islands are sometimes methylated and bulk DNA undergoes hypomethylation, with negative consequences for the cell [2].

Many tumor suppressor genes, whose expression is dependent on the unmethylated state of the CpG islands in their promoters, become downregulated by increased methylation in these regions [3]. The Ten Eleven Translocation protein 1 (TET1) was discovered and shown to be able to modify methylcytosine and potentially erase DNA methylation [4]. In contrast, chromatin decondensation, genomic instability, apoptosis, cance and even mitotic catastrophe can be induced by DNA hypomethylation [5-8].

A critical step in DNA methylation involves DNMTs, the enzymes that catalyze the methylation process. There are three known biologically active DNMTs in mammalian cells: DNMT1, DNMT3a and DNMT3b. Each of these proteins is vital for embryonic development; disabling the corresponding genes in mice causes embryonic or early postnatal death [9].

HIF-1 is the term coined in 1993 by Gregg Semenza for a transcription complex bound to a Hypoxia-Responsive Enhancer (HRE) lying 3' to the erythropoietin gene. Since then, the key components of the HIF-1 system have been identified [10]. In more recent years, the potential role of HIF-1a as regulator of tumor growth, anaerobic glycolysis, antiapoptosis and angiogenesis has been recognized. The potential role of HIF-1a in tumor development was first identified from the observation that it is overexpressed in a broad range of tumor types and is involved in key aspects of tumor development. Independent of anyspecific mechanism, HIF-1a over expression has been associated with an unfavorable prognosis in most cancers, as it activates genes that play a role in promoting cancer metabolism, angiogenesis, invasion, maintenance of stem cell pools, cellular differentiation, genetic instability and metastasis [11].

The HIF family comprises 3 functional nonredundant a subunits, HIF-1a, -2a and -3a which form a heterodimer with the HIF-1b subunit. HIF-1a and HIF-2a are the most studied members of this family and have been thought to be largely overlapping in their proto-oncogenic function. Both HIF-1α and HIF-1β subunit belong to the family of basic helix-loop-helix PAS domain transcription factors. The β subunit is constitutively expressed and is also involved in xenobiotic responses where HIF-1β forms a dimer with the aryl hydrocarbon receptor; an alternative name for HIF-1β is ARNT (for aryl hydrocarbon receptor nuclear translocator [12].

HIF-1α is readily detectable in cells cultured under low oxygen conditions and is virtually undetectable in most cells under standard tissue culture conditions due to rapid proteasomal destruction. In hypoxia, the α subunit dimerises with a β subunit

and translocate to the nucleus. HIF-1α is the basic helix-loop-helix/Per-ARNT-SIM (bHLH-PAS) proteins are a class of transcriptional regulators that commonly occur in living organisms. They play an important role in the regulation of a variety of developmental and physiological events.

Literature Review

HIF-1α is target for posttranslational modifications (Figure 1). These modifications are related to metabolic stress, hypoxia, oxidative stress, pH and oncogenic signaling; making HIF-1α a principal sensor of stressful microenvironment. Importantly, HIFs appear to preferentially bind HREs within regions of permissive chromatin that display histone modifications. In fact, only a small percentage of consensus sequences at so-called "permissive loci" are bound by HIF during hypoxia. Thus, there is mutual effect of epigenetic and HIF-1 on cell adaptation in stressful microenvironment. This review also focuses on different kinds of crosstalk between epigenetic and HIFs.

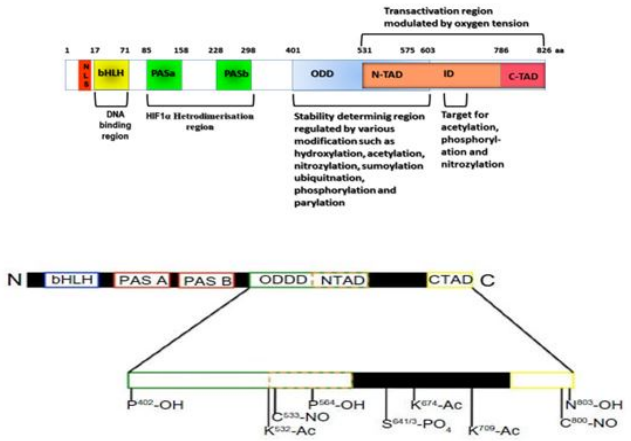

Figure 1: HIF-1α plane structure with postotranslations modifications (PMT) sites.

The HIF-1α subunit has two Transactivation Domains (TAD): NH_2-terminal (N-TAD) and COOH-terminal (C-TAD). These two domains are responsible for HIF-1α transcriptional activity. C-TAD interacts with co-activators such as CBP/p300 to modulate gene transcription of HIF-1α under hypoxia. N-TAD is involved in protein and DNA bindings. The Oxygen-Dependent Degradation Domain (ODDD) overlapping N-TAD in their structures. This ODDD domain is important in mediating O_2 regulation stability. Different types of PMT impacted on HIF-1α stability and on its protein and DNA binding activities. The HIF-1α belongs to bHLH-PAS protein family, because their structures are related to two nuclear proteins found in Drosophila (Per and Sim, PAS) which have basic-helix-loop-helix (bHLH) motif. In general, the PAS motifs are essential to allow heterodimer formation between HIF-1α and HIF-1β subunits and b-HLH is essential for binding to the HRE-DNA sequence on the target genes in the context of permissive chromatin.

HIF-1α stability and activity regulation in normoxia and hypoxia conditions

While hypoxia limits the proliferation of many cell types, some cancer cells, stem/progenitor cells and pulmonary vascular cells continue to grow and divide in low oxygen conditions.

Hypoxia is an important environmental stimulus that causes genetic and metabolic reprogramming in cells to facilitate survival. This programmed response is mediated primarily through stabilization of hypoxia-inducible factor 1α (HIF1α), a transcription factor that coordinates a shift in energy metabolism away from oxidative phosphorylation and toward glycolysis and lactate fermentation through the increased expression of Glucose Transporters (GLUT1), glycolytic enzymes, Lactate Dehydrogenase (LDHA) and pyruvate dehydrogenase kinase.

In fact, in normaxi, HIF-1α is inactivated through hydroxylation, on two conserved prolyl residues (Pro-402 and Pro-564) located at the Oxygen-Dependent Degradation Domain (ODDD) of the protein, by HIF-Prolyl Hydroxylases (HPHs) also referred to as Prolyl Hydroxylase Domain (PHD) proteins form an evolutionarily conserved subfamily of dioxygenases that uses oxygen and 2-oxoglutarate (2-OG) as co-substrates and iron and ascorbate as cofactors. In addition to oxygen-dependent prolyl hydroxylation mediated by PHDs, HIF-1α is subject to oxygen-dependent asparaginyl hydroxylation by factor inhibiting HIF (FIH). FIH is also an iron-dependent dioxygenase and hydroxylates Asn 851 located within the C-terminal transactivation domain of HIF-1a. Hydroxylation at Asn 851 inactivates HIF-1α by preventing its interaction with CBP/p300, an essential coactivator for HIF-dependent transcription (Figure 2) [13].

Prolyl substrate specificity hydroxylation occurs on the fourth position on P402 and P564 in human HIF-1α (or at similar positions in HIF-2α) within the so-called Oxygen-Dependent Degradation Domains (ODDs). In cultured cells, all three PHDs contribute to the regulation of both HIF-1a and HIF-2a, by hydroxylation of HIF-1a. The hydroxylation of the HIFa protein causes interaction with the Von HippelLindau (VHL) protein, a component of an E3 ubiquitin ligase complex. This interaction results in the covalent attachment of chains of the small globular protein ubiquitin to lysine residues on HIF-1α. Decoration of HIF with ubiquitin chains earmarks it for degradation by a multiprotease complex called the 26S proteasome. Thus, in the presence of oxygen, once HIF is produced it is hydroxylated, ubiquitinated and degraded. However, in the absence of oxygen the PHDs that use oxygen in the hydroxylation reaction are inactive and consequently HIF reaches a higher steady state level. However, stability does not necessarily mean activity. In fact, once HIF-1α is stabilised, it undergoes acetylation at 709 lysine residue by p300 leading to its activation and enhacing its stabilisation. After p300 was autoacetylated in the same context.

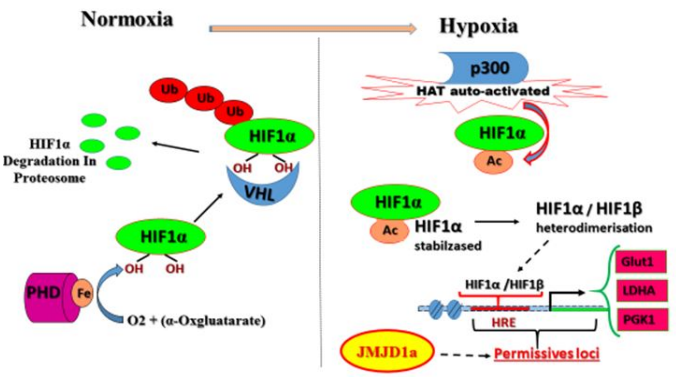

Figure 2: HIF-1α stability and activity regulation in normoxia and hypoxia conditions.

In normoxia, PHD hydroxylates HIF-1α with Fe and α-cetoglutarate as cofactors and leading to its degradation by proteasomes proteins. In hypoxia, HIF-1α escapes from its hydroxylation leading to its stabilisation but its transcriptional activity is achieved by p300 whose HAT activity is auto-activated in hypoxia leading to the acetylation of HIF-1α. HIF-1α acetylated heterodimerisases with HIF-1β; the heterodimer can bind to its target consensus HRE sequences. But only permissive HREs were accessibles, which are in open chromatin that mediated by KDMs such as JMJD1a in this context.

This HIF-1α activation is manifested by heterodimerisation with HIF-1β, DNA binding and transactivation ability. Nevertheless, the heterodimer HIF-1α/HIF-1β DNA binding ability depended on the HRE (Hypoxia Responsive Element) accessibility which related to epigenetic landscape. HRE, upstream of an array of genes that enable the hypoxic response, must be erased from heterochromatin state. In fact, the early hypoxia-responsive genes associated with glycolysis, such as GLUT3, are induced subsequent to the interaction of HIF-1 with lysine (K)-specific demethylase 3A (KDM3A), KDM3A known as Jumonji Domain-containing 1a (JMJD1a) is histone demethylase [14].

The induction of early hypoxia-responsive genes are functionally associated with glycolysis; which encodes glucose transporters (GLUT1 and GLUT3) and glycolytic enzymes PFK-Liver type (PFKL), Aldolase (ALDA), Phosphoglycerate Kinase-1 (PGK1), Enolase (ENOL) and Lactate Dehydrogenase-A (LDHA) (Figure 3).

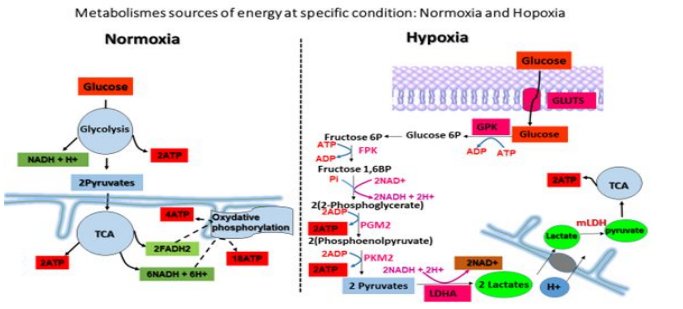

Figure 3: Metabolics sources of energy in normoxia and hypoxia conditions.

The absence of oxidative phosphorylation chain in hypoxia will be recompensed by gylcolysis acceleration to provide maximum of energy for cell adaptation in that stressful condition. In permanent hypoxia, certain epigenetic marks modulator enzymes was upregulated and activated such as KDM3A, which is one of the HIF1-mediated genes, is upregulated, subsequent of HIF-1a accumulation and activation, leading to upregulation of gene associated with glycolysis. As consequence, an acceleration glycolysis, which bring to the metabolites and cofactors accumulation [15]. Such as lactate, NAD+, polyolpathway and AGE which impacts on epigenetic code, so far, on cellular phenotype.

Discussion

Metabolic stress as principal knot of HIF-1α signalings network

As the fields of epigenetics and cellular metabolism-particularly cancer cell metabolism-have developed in recent years, so has the appreciation of the fundamental crosstalk between these processes [16]. Cancer cells undergo fundamental changes in their metabolism to support rapid growth, adapt to limited oxygen and nutrient resources and compete for these supplies with surrounding normal cells. The lack of energy, which can result from the absence of oxidative phosphorylation reactions, is compensated by the high rate of glycolysis overproducing lactate, NAD+, polyolpathway and AGE (Figure 4).

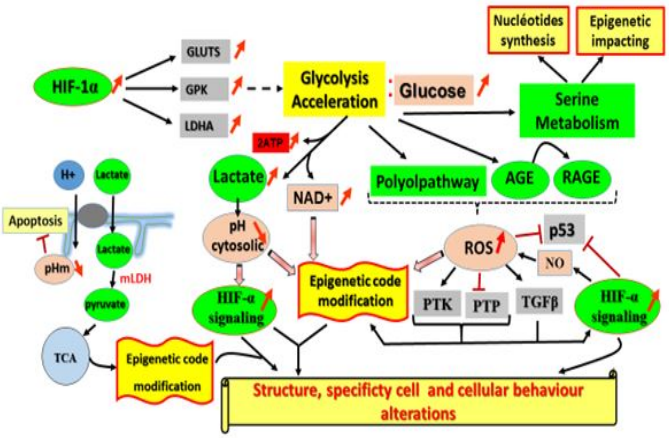

Figure 4: Cross talk between HIF1-α signalling and epigenetic landscape remodelling in mediating structure, specificity cell and cellular behaviour alterations.

HIF dependent glycolysis acceleration impacts on many different cell metabolisms and generates ROS, metabolic substrates and cofactors with high availability; whose, each one can impact on the epigenetic landscape and enhance different types of HIF-1α signalling. Together affects the cell phenotype.

The impact of the accumulation of lactate

One of the metabolic hallmarks of cancer is the activation of glycolysis and lactate production. In order to maintain a

favourable glycolytic rate and therefore glycolytic ATP production, pyruvate is used to oxidize the NADH and is consequently reduced to lactate. Furthermore, lactate itself is used to further advantage by cancer cells. The conversion of pyruvate to lactate regenerates the NAD+ cofactor and contribute to cytosol acidification.

The lactate passively diffuses across the Mitochondrial Outer Membrane (MOM) into the Mitochondrial Intermembrane Space (MIS). An increase in lactate concentrations in the MIS facilitates conversion back into pyruvate catalysed by an isoform of Lactate Dehydrogenase (LDH) located in the mitochondria (mLDH) [17].

Pyruvate is then shuttled across the Mitochondrial Inner Membrane (MIM) into the matrix *via* a mitochondrial Monocarboxylate Transporter (mMCT), where it is oxidized. The two reactions were near of the equilibrium:

$$\text{Lactate} + \text{NAD+} \longleftrightarrow \text{Pyruvate} + \text{NADH+H+}$$

Thus, pyruvate is converted to acetyl-coA, precursor of TCA, leading, in the absence of OXOPHOS, to accumulation of either acetyl-coA, TCA intermediate metabolites and proton H+ intra-mitochondrial.

The acidic pH mitochondrial and apoptosis

Mitochondria play a critical role in apoptosis induction in response to myriad stimuli. These organelles release proteins into the cytosol which trigger caspase activation or perform other functions relevant to apoptosis, including cytochrome c (cyt-c), caspases, AIF and SMAC (Diablo). Emerging evidence suggests that an alteration in cellular pH regulation represents an early event associated with apoptosis induction via the mitochondria-dependent pathway.

Some types of physiologically-relevant apoptotic stimuli appear to induce mitochondrial matrix alkalinization, with attendant hyperpolarization of these organelles. Further, at least under some circumstances, these changes in pH regulation and hyperpolarization can precede cyt-c release, caspase activation and PT pore opening. Genetic and biochemical data also suggest that a change in mitochondrial pH regulation lies close to the mechanism of action of Bcl-2 family proteins [18]. Changes in pH may modulate the functions of Bcl-2 family proteins. For instance, many Bcl-2 family proteins interact with themselves and each other, forming homo- or heterodimers. Dimerization of these proteins *in vitro* is enhanced at lower pH, with optima probably in the pH 4-5 range [19].

Importantly, both hyperpolarization (as measured with cationic dyes) and matrix alkalinization are blocked by Bcl-2, an anti-apoptotic protein that localizes to mitochondria and which is known to prevent cyt-c release (Figure 5).

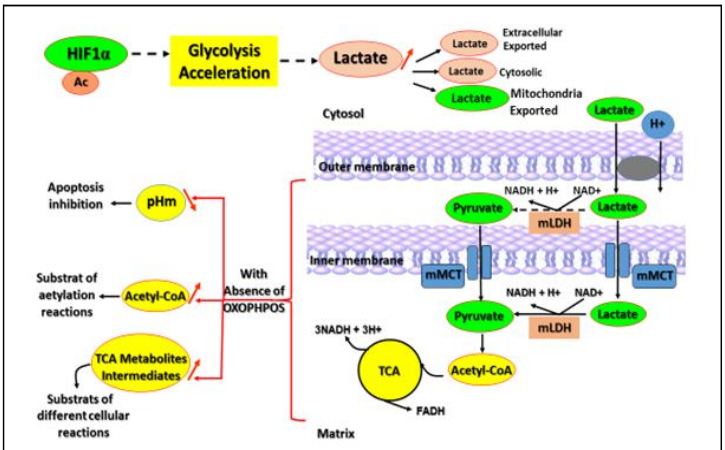

Figure 5: Concomitant of glycolysis acceleration and absence of oxophos, lactae is generated in high availability which will have three destnations: Extracellular export, cytosol or mitochondrial export.

This figure focuses on that lactate mitochondrial export leading to pH mitochondrial acid, affecting negatively the apoptosis and high level of either acetyl-CoA or TCA metabolites intermediates.

Acetyl-coA and epigenetic

There is a convention, that acetyl-CoA is not only a central intermediate in the oxidation of glucose to produce ATP, but also a precursor for the biosynthesis of numerous metabolites required to build a new cell, such as lipids and sterols.

It's now admitted that, metabolites downstream of acetyl-CoA could be signaling epigenetic modifications. In fact, acetyl-CoA is the principal acetyl donor for acetylation reactions within the cell, essentially, which implicate Histone Acetyl Transferase (HAT) relies on intracellular levels of acetyl-CoA, that stands as a prominent example of the interplay between metabolism and chromatin dynamics [20].

The acetylation of lysine residues in histones represents one of the best-characterized posttranslational modifications, historically implicated as part of the histone-code. Histone acetylation is directly linking to gene activation.

Upon entry into growth, intracellular acetyl-CoA levels increase substantially and consequently induce the Gcn5p/SAGA-catalyzed acetylation of histones at genes important for growth, thereby enabling their rapid transcription and commitment to growth.

The acidic pH cytosolic signalings: Epigenetic impact and sustaining HIF-1α signaling

The glycolytically overproduced lactate is associated with cytosolic acidification. A common feature of hypoxia, as well as the tumor and stem cell microenvironments, is metabolic acidosis [21].

pH and substrate (α-ketoglutarate (α-KG)) availability

α-ketoglutarate (α-KG), an intermediate of the Tricarboxylic Acid (TCA) cycle, is an essential co-substrate for dioxygenases family due to its role in Fe(II) coordination in the catalytic center. In fact, cytosolic acidification moderately elevated 2-hydroxyglutarate (2-HG) in cells and boosting endogenous substrate TCA cycle intermediate α-ketoglutarate (α-KG) levels further stimulated this elevation. pH can independently drive elevated 2-HG levels, pH regulation of 2-HG may have important implications for 2-HG signaling in hypoxia [22]. The L-(S)-enantiomer (L-2-HG) was shown to be generated under hypoxic conditions by Lactate Dehydrogenase (LDH) and Malate Dehydrogenase (MDH) [23].

The most important mechanism for increased L2HG synthesis is likely related to increased substrate (α-KG and NADH) availability, subsequent to accelerated glycolysis and TCA dysfunction [24]. Studies with isolated lactate dehydrogenase-1 and malate dehydrogenase-2 revealed that generation of 2-HG by both enzymes was stimulated severalfold at acidic pH, relative to normal physiologic pH.

α-KG is a weak acid that equilibrates between protonated and deprotonated forms. Low pH drives the equilibrium toward protonation. According to molecular modeling, LDHA prefers the protonated form of α-KG. When the pH was decreased from 7.4 to 6.0, a pH near that in hypoxic cells, the Michaelis-Menten constant (Km; an inverse measure of affinity) of LDHA for α-KG was reduced by about fourfold.

The downstream signaling roles of D-2-HG in cancer biology and of L-2-HG in hypoxia or stem cell biology are thought to be mediated by epigenetic effects, because of competitive inhibition of the α-KG-dependent dioxygenase superfamily of enzymes. This includes the JmjC domain-containing histone demethylases.

The acccumulation of 2-hydroxyglutarate (2-HG) inhibited TET-dependent oxidation of 5 mC into 5 hmC and increased histone methylation marks in several cancers including gliomas and hematological malignancies [25]. D2HG and L2HG are separate and distinct metabolites with unique effects on metabolic regulation. Thus, 2-HG is a potentially important link between metabolism and epigenetic signaling.

Furthermore, because acidic pH is known to stabilize Hypoxia-Inducible Factor (HIF): Through neutralisation function of VHL by triggering its nucleolar sequestration and inhibition of HIF Prolyl Hydroxylases (PHD) by 2-HG, acidosis is involved in HIF signaling feed back loop, that conducts to cell engagement to irreversible malignancy phenotype (Figure 6) [26].

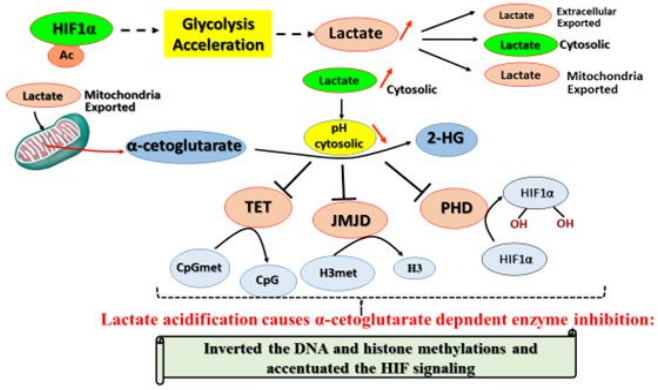

Figure 6: Subsequent to lactate cytosolic availability, glycolysis acceleration dependent, pH cytosolic becames acide leading to 2-HG formation and inhibiting three enzymes dioxygenases: TET, JMJD and PHD, impacting thus on epigenetic landscape and accentuating HIF-1α signalling.

pH can affect directly t he Fe(II/2OG-dependent oxygenase activity

Another source of α-ketoglutarate (α-KG) is the catabolism of glutamine. In fact, with the help of transport systems, extracellular L-glutamine crosses the plasma membrane and is converted into alpha-ketoglutarate (α-KG) through two pathways, namely, the Glutaminase (GLS) I and II pathway. Reversely α-KG can be converted into glutamine by Glutamate Dehydrogenase (GDH) and Glutamine Synthetase (GS) [27]. But, the high rate of glycolysis is associated with seine anabolism which coupled with catabolism of glutamine, leading, thus, to accumulation of α-KG.

Taking in account, with the different sources of α-KG leading to its accumulation, α-KG could be still a substrate of either α-KG-dependent dioxygenase superfamily of enzymes. The inactivation of α-KG-dependent dioxygenase associated with acidic pH can be also due to the directly impact of acidic pH on their catalytic site activity.

In fact, the crystallographic studies on numerous members of the Fe(II)/2OG-dependent oxygenase superfamily have revealed two conserved structural features shared among its members [28]. First, The Fe(II) is ligated by two His residues and (with the exception of the halogenases) a carboxylate from either a Glu or an Asp residue; this metal-binding motif is termed the 2-His-1-carboxylate facial triad [29]. Second, the 2-His-1-carboxylate motif is located within a Double-Stranded-Helix (DSBH) fold, also known as the jelly-roll, cupin or jumonji C fold. Thus, the cytosolic pH acid, in modifying the charges of the facial triad, according to pka of triad elements, alters the

catalytic activity, inactivating directly the α-KG-dependent dioxygenase enzymes.

Effect of pH on redox homeostasis

Enzymes with active-site cysteine residues typically rely on the thiolate (deprotonated) form of the cysteine for activity and reactivity toward substrates (and oxidants) is therefore enhanced by a microenvironment that perturbs the normally high pKa (8.5) of cysteine thiols to a value at or lower than neutral pH [30]. In the case of the disulfide-bond oxidoreductase, the pKa drops as low as 3.5 [31]. Thus, although the vast majority of cysteine residues within cytoplasmic proteins are in the protonated form at physiological pH, the small subset within enzyme catalytic or regulatory sites are largely or fully ionized due to their low pKa values.

Certain redox enzymes (as glutathione peroxidase, GPX) and their substrates, which could be act as non-enzymatic antioxidant (Glutathion, GSH), contain on their catalytic site selenocysteine and cysteine for respectively redox enzyme and antioxidants substrates. These residues play a central role in antioxidant activity. Although gluthation peroxydases (GPX), GPX1, GPX3 and GI-GPX2, are homotetramers, the GPX4 is a monomer with a molecular size smaller than the subunits of other glutathione peroxidases [32].

In GPX, four arginine residues and a lysine residue provide an electrostatic architecture which in each reductive step directs the donor substrate glutathione (GSH) towards the catalytic center in such a way that its sulfhydryl group must react with the selenium moiety. Moreover, co substrate binding mechanisms are unique for the classical type of GPX1 but can not operate in GPX3 and GPX4. Both Glutathione Peroxidase (GPO) and glutathione reductase activities decreased as a function of a decrease in pH from 7.4 to 4.

In fact, of the twenty common amino acids, perhaps the most intriguing and functionally diverse is cysteine, one of the two sulfur-containing amino acids of the set (Figures 7). Unlike methionine, which has its sulfur in a relatively less reactive thioether form, the thiol (or "sulfhydryl") group of cysteine is ionizable, with a negatively-charged thiolate group being generated after deprotonation, boosting its reactivity [33]. Moreover, this thiol/thiolate group is subject to alkylation by electrophiles and oxidation by reactive oxygen and nitrogen species, leading to post-translationally modified forms that can exhibit significantly altered functions.

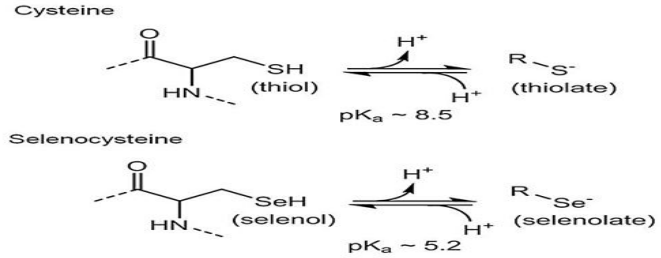

Figure 7: Structures of cysteinyl and selenocysteinyl residues within proteins.

The aminoacyl groups are shown to the left, with dotted lines representing peptide bonds to the next residue on either side. Both protonated (left) and deprotonated (right) forms of these amino acids are depicted with average pK a values (that can vary in particular protein microenvironments).

pH cytosolic and apoptosis

That acidification of the cytosol occurs in mammalian cells undergoing apoptosis. The extent of the change in pH observed varies among reports, but typically represents a drop of 0.3-0.4 pH units.

Moreover, channel formation by poreforming Bcl-2 family proteins in synthetic membranes (including anti-apoptotic Bcl-2, Bcl-XL and pro-apoptotic Bax and Bid) is also markedly enhanced by low pH, again with optima in the pH 4-5 rang pH may influence Bcl-2 family proteins is by affecting their intracellular targeting [34].

After cytosolic acidification induced by ischemia, Bax is translocated to mitochondria. There is strong linking between acidic milieu and Bax translocation. Changes in pH affect the conformation of Bax and a change in conformation facilitates Bax translocation to the mitochondria. In this regard, both positively and negatively charged residues contribute to the pH dependence of Bax conformation. It is plausible that similar to the effect of an alkaline pH, a considerable acidic shift in the pH could also induce a conformational change (dimerization/ multimerization) in Bax, thus making it more amenable for membrane insertion, where it can form homo- or heterodimers with other Bcl-2 family members. The resultant conformational change in Bax can result in channel formation that could mediate egress of proteins, such as cytochrome c, from the mitochondrial intermembrane space.

But the acidic pH mitochondria constitute a constraint to Bax induced apoptosis in inhibiting caspase-c release [35].

NAD+ relationships

NAD+ and its redox counterpart, NADH, are key metabolites influencing a large constellation of metabolic reactions. Nicotinamide Adenine Dinucleotide (NAD) is a co-enzyme that mediates redox reactions in various metabolic pathways, including glycolysis, Tricarboxylic Acid (TCA) cycle, oxidative phosphorylation and serine biosynthesis [36].

Continuous replenishment of NAD promotes the proliferation and survival of fast-dividing cancer cells because elevated NAD levels enhance glycolysis *via* Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) and Lactate Dehydrogenase (LDH) that require NAD as a co-enzyme PHGDH, a rate-limiting enzyme of the serine biosynthesis pathway, also uses NAD as a co-enzyme and the intracellular level of NAD is considered to be an important regulator for serine biosynthesis in cancer cells. Furthermore, NAD serves as a substrate for Poly (ADP-Ribose) Polymerase (PARP) and sirtuins (NAD-dependent deacetylases) and mediates poly-ADP-ribosylation and deacetylation, respectively [36].

Therefore, the dynamic NAD+ and its metabolites levels, in response to diverse cellular stress and physiological stimuli, rewire biological processes *via* post-synthesis modification of fundamental biomolecules, including DNA, RNA and proteins (Figure 8).

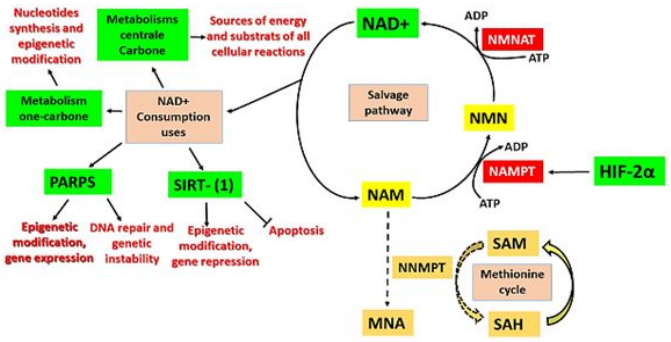

Figure 8: NAD+ consumption uses and its salvage pathway. **Note:** SAM: S-Adenosylmethionine; NA: Nicotinic Acid; NAM: Nicotinamide; NMN: Nicotinamide Mononucleotide; NAMN: Nicotinic Acid Mononucleotide; NAD: Nicotinamide Adenine Dinucleotide; NAMPT: Nicotinamide Phosphoribosyl Transferase; NMNAT: Nicotinamide Mononucleotide Adenylyl Transferase; PARP: Poly (ADP-Ribose) Polymerase

The NAD+-dependent enzymatic activities: PARPS and sirtuins

NAD+ and its reduced form NADH function as acceptor and donor molecules of two electrons and one proton. In addition to its function in redox reactions, NAD+ serves as an ADP ribose donor for enzymes regulating important biological processes such as transcriptional regulation, calcium signaling and DNA repair [37]. These enzymes include PARPs, the deacetylase family of sirtuins and synthetases generating the second messenger cyclic ADP-ribose.

The ADP-ribosylation of proteins, a post-translational modification, has been implicated in many physiological processes, including gene transcription, protein degradation, cell proliferation and differentiation, DNA damage and repair, aging, inflammation, cell death, hoste virus interactions and metabolism [38]. The reaction is catalyzed by ADP-Ribosyltransferases (ARTs) that include most members of the Poly-ADP-Ribose Polymerase (PARP) family of proteins as well as some members of the sirtuin family [39]. Although PARPs and sirtuins differ substantially in their protein structure, they both use NAD+ as a substrate. Unlike PARPs, the majority of sirtuins use NAD+ for deacetylation and not ADP ribosylation.

Nad+/PARP1

Regulation of PARP1 and its role in transcription is an important mediator of extra- and intracellular stress signals. The cellular outcome depends on the level of PARP1 activation, which related to rate of NAD+ and can range from activation of the DNA damage repair machinery to histone modification, chromatin remodeling oncogenic transcription and genome instability.

NAD+/PARP and epigenetic

The global regulatory aspects of epigenetic events are largely unknown. PARylation and PARP1 are recently emerging as multi-level regulatory effectors that modulate the topology of chromatin by orchestrating very different processes. PARPs are involved in the regulation of epigenetic modifications of histones and DNA, also, in the global organization of chromatin domains in the nucleus. NAD+ deficiency can promote the DNA methylation, resulting in gene silencing. The NAD+-consuming enzymes, PARPs, are associated with the regulation of DNA modification. As a consequence, the high cellular NAD+ content enhances the PARP1-catalyzed ADP-ribosylation and the following DNA hypomethylation. Inhibition of the PARPs-mediated ADP-ribosylation causes a chromatin compaction DNA hypermethylation. PARPs use NAD+ as a source of ADP-ribose moieties to synthesize proteinbound polymers of variable size (from 2 to more than 200 residues) and structural complexity (linear or branched) [40].

Polymers present on PARP-1 interact noncovalently with DNA methyltransferase 1 (Dnmt1), preventing its enzymatic activity. In the absence of PARylated PARP-1, Dnmt1 is free to methylate DNA; if, in contrast, high levels of PARylated PARP-1 persist, Dnmt1 will be stably inhibited, preventing DNA methylation [41]. Histones also serve as acceptors of ADP-ribose upon DNA damage to initiate DNA repair. The ADP-ribosylation of histones by PARP-1 induces the dissociation of nucleosomes, leading to the decompaction of chromatin. Furthermore, PARP-1-mediated PARylation of KDM5B prevents the demethylation of H3K4me3, rendering the exclusion of H1 and the opening of chromatin. The decompensation of chromatin structure, therefore, allows the loading of the transcriptional machinery and facilitates gene transcription. Therefore, the NAD+-dependent enzymatic activity of PARP-1 is a crucial regulator of gene expression.

PARP1 and HIF-1

Poly (ADP-Ribose) Polymerase 1 (PARP1) regulates accessibility of chromatin, also, alters functions of transcriptional activators and repressors and has been directly implicated in transcriptional activation. PARP-1 activation is needed to fine-tune HIF-1α signaling. PARP-1 activation leads to HIF-1α parylation at its C-terminal domain. This PMT is necessary for its optimal stability and activity, enlarging its spectre target genes [42]. HIF-1α forms a PARylated complex with PARP1 and both HIF-1α and PARP1 are present at promoter regions of HIF-1α downstream targets, leading to accumulation of positive histone marks at these regions. Complex formation, PARylation and binding of PARP1 and HIF-1α at promoter regions of HIF-1α downstream targets can all be attenuated by PARP1 inhibition, subsequently leading to build up repressive histone marks and loss of positive histone marks [43].

Sirtuins as regulator of gene expression

The post-translational modifications of histones including deacetylation, is regulated by NAD+-dependent enzymes, sirtuins. Sirtuins, also known as NAD+-dependent HDACs. The sirtuin deactylation reaction require NAD+ as a cofactor for their

enzymatic activity and involves the removal of an acetyl group from target substrates *via* the conversion of NAD+ to nicotinamide and O-acetyl-ADP-ribose [44].

Mammalian sirtuins are NAD+-dependent deacetylases with a huge range of roles in transcription regulation, energy metabolism modulation, cell survival, DNA repair, inflammation and circadian rhythm regulation SIRT1 which is the most extensively studied sirtuin is found in the nucleus and cytosol and along with histone deacetylation also modulates transcription factors, such as p53, SIRT1 physically interacts with and deacetylates p53 and represses p53 dependent apoptosis in response to DNA damage, while a dominant negative SIRT1 mutant increases cell sensitivity to stress. SIRT1 deacetylation of p53 antagonizes PML/p53-induced cellular senescence. Moreover, The reduced intracellular NAD+ concentration limits the deacetylase activity of SIRT1, resulting in elevated H4K16Ac and gene expression [45].

The ROS metabolites beget a weight impact on cell phenotype

ROS (Reactive Oxygen Species) are an intricate part of normal cellular physiology. In excess, however, ROS can damage all three major classes of macromolecules and compromise cell viability.

Feedback between ROS synthesis and HIF signalling

Accelerated glycolysis and ROS production: Through HIF-1α-glycolysis acceleration inducing, different derivative metabolisms can arise such as polyol and AGE (Aadvanced Glycation End-products) pathways releasing harmful reactive oxygen species. The cell metabolism of excess of glucose can product metabolic intermediates promoting unfavourable biochemical consequences. Such metabolic intermediates: (1) sorbitol/polyol and (2) hexosamine pathways; (3) augmented intracellular formation of AGEs and expression of the Receptor for AGE (RAGE). These products are usually sources of intracellular Reactive Oxygen Species (ROS) [46].

Feedback effect between NO synthesis and HIF-1α

Nitric Oxide (NO) is a pleiotropic molecule involved in neurotransmission and vascular homeostasis [47]. NO radical is generated during the oxidation of L-arginine to L-citrulline by at least three different isoforms of the enzyme Nitric Oxide Synthase (NOS).

However, NO generated by the inducible form of NO synthase (iNOS) has been implicated in many pathophysiological states. Hypoxia causes an increase in iNOS expression and that HIF-1 is essential for the hypoxic regulation of iNOS gene expression. HIF-1 or a closely related nuclear factor binds to the HIF-1 consensus sequence of the iNOS promoter [48].

In otherwise, accumulation of NO, by feedback, affect the stability and the level expression of HIF-1α. NO-evoked HIF-1 induction as a heretofore unappreciated inflammatory response in association with massive NO formation. In fact, NO transfer reactions between protein and peptide cysteines have been proposed to represent regulated signaling processes. Extensive biochemical and genetic data-including both mutational analyses of cysteine (Cys) residues in over 30 proteins that are targets of NO and creation of plants and mice deficient in S-Nitrosothiol (SNO) metabolism-have led to the current understanding that most actions of NOSs are in fact conveyed by S-nitrosylation, the modification of protein Cys thiols by NO [49].

Importantly, endogenous formation of NO in RCC4 cells *via* inducible NO synthase elicited S-nitrosation of HIF-1 alpha leading to its stabilisation. All 15 free thiol groups found in human HIF-1 alpha are subjected to S-nitrosation, as the ractive Cys 800 [49].

NO can also inhibit PHD activity through nitrosylation of cysteine residues or by binding the catalytic iron. The ability of NO to bind the iron center of PHD appears to be affected by the concentration of 2-OG, because inhibition is only seen when 2-OG is unbound, indicating the metabolic status of the cell can alter the effects of NO on HIF-1α stability [50]. Nitrosylation of Cys162 in VHL prevents it from ubiquitinating hydroxylated HIF-1α (Figure 9).

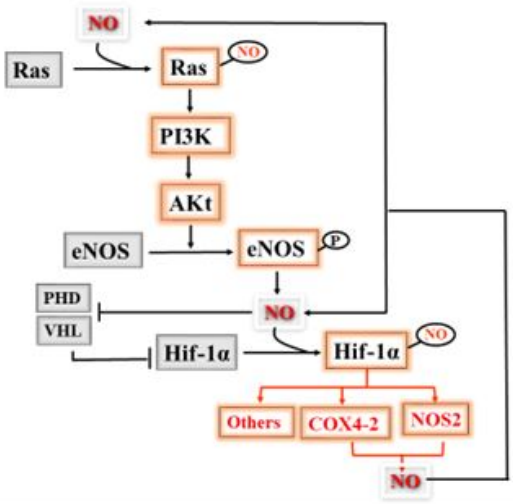

Figure 9: NO is both upstream and downstream of PI3K/Akt and HIF-1 signaling and can affect many proteins and pathways within the cell, including Ras and HIF-1α.

NO can also stabilize HIF-1α *via* the PI3K/Akt signaling pathway. S-nitrosylation of Ras-Cys118 increases its activity, resulting in active PI3K/Akt signalling. PI3K/Akt signaling then increases HIF-1α expression and also leads to phosphorylation and activation of eNOS.

Cysteine residues particularly redox-sensitive and proteins potentially redox-regulated

Regulatory thiol modifications involve one or more cysteines, whose reactivity is largely determined by the cysteine's structural environment and its pKa value. Most cytoplasmic protein thiols have pKa values of greater than 8.0, which render the thiol groups predominantly protonated and largely nonreactive at intracellular pH. Thiol groups of redox-sensitive cysteines, on the other hand, have characteristically much lower pKa values, ranging from as low as ~3.5 in thiol transferase to

5.1-5.6 in protein tyrosine phosphatases. Under physiological pH conditions, these thiols are therefore present as deprotonated, highly reactive thiolate anions (RS-). The low pKa values of redox-sensitive cysteines arise primarily from stabilizing charge-charge interactions between the thiolate anion and neighboring positively charged or aromatic side chains thiolate anions, in contrast to their protonated counterparts, are highly susceptible to oxidation by ROS and RNS and can undergo a diverse spectrum of oxidative modifications. These include sulfenic (SOH), sulfinic (SO_2H) and sulfonic (SO_3H) acids, disulfide bonds (PrSSPr) or nitrosothiols (SNO).

Crosslinking of subunits of the same or different proteins can have a big effect on signaling proteins. For example, the signalling kinase PKGIα is activated upon dimerization which is enforced when a disulfide bond is formed between the same Cys residue (Cys42 and Cys42') on two different subunits (implying the oxidation of Cys42 in one subunit to sulfenic acid, then condensation with another subunit as the other Cys42' thiol group approaches).

Extracellular matrix integrity and composition is also under redox control. For some if not all matrix metalloproteinases (enzymes involved in the degradation of extracellular matrix components), activation occurs through sulfinic acid generation at Cys100 in the propeptide, also *via* initial oxidation to snitrosocysteine and/or cys sulfenic acid (e.g., in rodent and human MMP-9 and MMP-7).

Thus, the details and effects of redox modification, are unique to each protein and critical to understand at a detailed molecular level.

ROS lead to disul ide bond-mediated PHD inactivation through its homo-dimerization

Oxidizing conditions induce disulfide bond formation in many cytosolic proteins, which can affect their biological function. Although, several reports have shown that oxidizing ferrous iron inactivates PHD2 under oxidative stress. Interestingly, 1.5 µM of T-hydro significantly decreased HIF-1α P402 and P564 hydroxylation by approximately 50% in RCC4 cells, suggesting that oxidative stress could stabilize and activates HIF-1α by modulating its hydroxylation status. GIbok and coworkers showed that disulfide bond-mediated PHD2 dimerization and inactivation result in the activation of HIF-1α in normoxia in response to oxidative stress, which is similar to other redox sensitive cytosolic proteins such as NEMO and receptor protein-tyrosine phosphatase α (RPTPα). Oxidative stress leads to disulfide bond-mediated PHD2 homo-dimerization. Disulfide bond-mediated PHD2 dimerization ceased with reducing agent, β-ME or DTT regulating its enzymatic activity. Cysteine residues in the catalytic DSBH region appeared to be responsible for the oxidative dimerization of PHD. Particularly, the mutations at Cys326 led to a near complete loss of the observed oxidative dimerization. PHD inactivation mediated by intermolecular disulfide bond between the Cys326 drives HIF-1α activation.

ROS cause activation of PT K (a s Src an d Ya p and inhibition of PTP

Redox signalling is currently broadly recognised as part of the mitotic apparatus elicited by Growth Factors (GFs), cytokines and integrins. Redox regulation of PTPs leads to transient enzymatic inhibition, while oxidation of PTKs or GTPases allows for upregulation of their enzymatic activity.

ROS induce PTPs Inactivation

In fact, enzymes with low pKa cysteine residues at their active sites tend to be more susceptible to oxidation. Given their obligate role in catalysis, modification of such active-site cysteines inhibits the enzymes that possess them. The most well-known example of regulatory oxidation occurring at the active site of cell signaling proteins is in the case of PTPs (Protein Tyrosine Phosphatases), which interfere with PTK signaling. PTP proteins possess a low pKa cysteine (pKa 4-6.5) that attacks the phosphorylated protein substrate to dephosphorylate it and generate a cysteinyl phosphate intermediate within the PTP enzyme that is then hydrolyzed. Under oxydatif stress, disulfide bond formation with a resolving cysteine located nearby or within the catalytic site, sometimes referred to as a backdoor cysteine, has been observed in several PTPs, including the low-molecular-weight PTPs, cell cycle Cdc phosphatases, SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase 2; PTP 1 1), Phosphatase and Tensin homolog (PTEN).

Protein Tyrosine Phosphatases (PTPs) contain multiple Cys residues that play a paramount role regulating signaling pathways. The formation of a disulfide bridge between the catalytic Cys and a backdoor Cys residue located within the catalytic pocket is a structural feature that can finely control the redox mechanism of PTPs.

PTEN, defined as tumor suppressor protein, also contains back-door cysteine residues that engage with the catalytic cysteine residues to generate disulfides during oxidative inactivation of the enzymes. These back-door cysteine's reside 5.7 A˚-10 A˚ away from the catalytic cysteines in the crystal structures of the native enzymes (Figure 10).

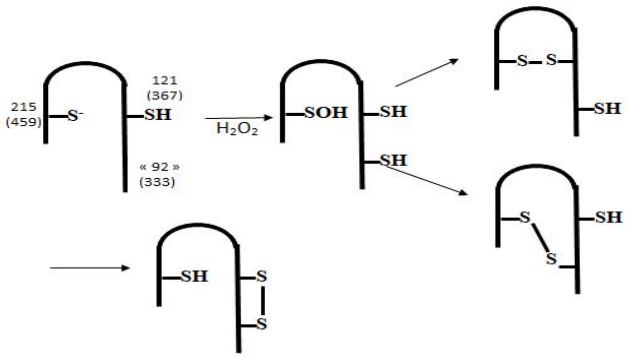

Figure 10: Chematic mechanism for oxidative inactivation of SHP-1 and SHP-2.

Due to the propensity for backdoor and distal cysteine residues to engage with the active-site cysteine after oxidative inactivation, differences in the structures of the oxidatively inactivated PTPs may stem, to a large degree, from differences in the number and location of cysteine residues surrounding the active site of the enzymes. PTPs with key cysteine residues in structurally similar locations may be expected to share similar mechanisms of oxidative inactivation. (The first number is PTP1B numbering and the second number is SHP-2 numbering).

ROS induce constitutive PTK activation

During redox regulation of PTKs, similar to what happens for PTPs, PTK cysteine oxidation may take place, although this event has opposing enzymatic consequences leading to PTK activation by inducing a conformational change necessary for their activation. ROS production caused the dimerization and activation of the RTK Ret.

This event is mediated by the formation of a disulfide bond between the Cys720 residues of each monomer, leading dimerized receptors to autophosphorylation resulting in their activation. This cysteine residue is highly conserved in various nonreceptor PTKs, including Abl, Src and Lck, suggesting that it might also play a role in the activation of these enzymes. Next to direct PTK oxidation, as PTKs themselves are often tyrosine phosphorylated proteins and their activity increased owing to phosphorylation, redox inhibition of PTPs indirectly leads to persistent activation of PTKs. Both these regulatory event are relevant for Src redox regulation.

ROS induce constitutive Src activation

Among intracellular PTKs, the Src tyrosine kinase and some of the members of its family have been reported as redox regulated proteins.

It has been reported that Src tyrosine kinase undergoes oxidation/activation in response to the formation of an S-S bond between Cys245 and Cys487, respectively located in the SH_2 and in the kinase domain of the Src molecule (Figure 11).

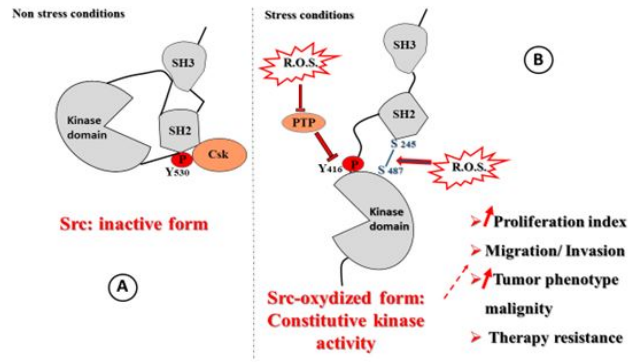

Figure 11: ROS induce Src-oxydezed form with constitutive kinase activity.

ROS induce Cys-245-Cys487 disulfud bond promoting the release of Src tyrosine kinase (Csk) from the inhibitory tyrosine 530 residue of Src. This is followed by phosphorylation of the tyrosine 419 residue in the activation loop of the Src kinase domain. Therefore Src-oxydized form acquired a constitutive kinase activity

Consequently, negative PTP oxidized/inhibited and activation loop Tyr hyperphosphorylated extend the Src-mediated cell proliferation to functional regulation of cytoskeletal rearrangement and the acquirement of a spread cell shape for anchorage dependent cells.

Src activation and epigenetic

The activation of Src occurs as a result of disruption of the intrinsic negative regulatory processes that normally suppress Src activity. Src was also shown to phosphorylate and increase the activity of HDAC3. Beside the membrane cytoplasmic function, Src has been described in other subcellular compartments, as the nucleus.

Src may activate a transcrptional repressor to associate with chromatin and/or alter its subcellular localisation. Inhibition of Src prevented gene silencing mediated by Kruppel-Like Factor 16 (KLF16), a transcription factor with domains that regulate acetylases.

ROS induce constitutive Yap1 activation

Upon activation by increased levels of reactive oxygen species, Yap1 rapidly redistributes to the nucleus where it regulates the expression of up to 70 genes involved in induced cell proliferation, Epithelium Mesenchymatous Transition (EMT) and cell migration.

In the active oxidized form, a Nuclear Export Signal (NES) in the carboxy-terminal cysteine-rich domain is masked by disulfide-bond-mediated interactions with a conserved amino-terminal alpha-helix. The oxidized form of Yap 1 contains two disulfid bonds between C303-C598 and C310-C629. Point mutations that weaken the hydrophobic interactions between the N-terminal alpha-helix and the C-terminal NES-containing domain abolished redox-regulated changes in subcellular localization of Yap1. Upon reduction of the disulphide bonds, Yap1 undergoes a change to an unstructured conformation that exposes the NES and allows redistribution to the cytoplasm (Figure 12).

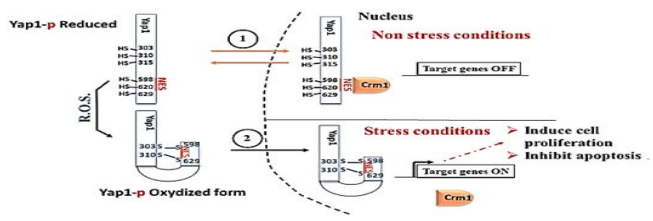

Figure 12: ROS induce Yap1-p oxidized form with constitutive transcriptional activity.

Upon exposure to ROS: Triggered by this interdomain disulfide bond formation, Yap1p undergoes further conformational changes that apparently mask the NES and disrupt the Yap1p-Crm1 interaction: Formation of the Cys303-Cys598 disulfide bond seems to initiate the activation of Yap1p by directly causing conformational changes that bury the NES. Formation of the second inter-domain disulfide bond between Cys310 and Cys629 appears to further increase Yap1p's transcriptional activity.

ROS impact on epigenetic code

ROS affect TET protein activity: TET proteins contain a carboxyl-terminal core catalytic domain that comprises a conserved cysteine-rich domain and a Double Stranded β-helix domain (DSBH). Within the DSBH domain, there are key catalytic residues that interact with Fe(II) and 2OG. Upon cofactor binding, molecular oxygen oxidizes Fe(II) in the catalytic pocket, thereby inducing the oxidative decarboxylation of 2OG and substrate oxidation. TET proteins also have an additional domain that potentially regulates their chromatin targeting. At the amino-terminal region, TET1 and TET3 have a DNA-binding domain called the CXXC domain, which is composed of two Cys4-type zinc finger motifs.

The crystal structure of the TET2 catalytic core domain revealed that two subdomains of the Cys-rich domain wrap around the DSBH domain on which DNA is located. Interestingly, two out of three zinc fingers, coordinated by several residues from the Cys-rich and DSBH domains, bring the two domains into close proximity to facilitate the formation of a compact globular structure, creating a unique structure for DNA substrate recognition. The inserted Cys-rich domain in the catalytic region of TET proteins is likely to chelate two or more Zn^{2+} ions *via* nine conserved Cys residues and one His residue and has been postulated to be part of a DNA-binding surface that might help in target recognition.

Although all Tet family members contain a conserved C-terminal catalytic domain, only Tet1 and Tet3 contain the CXXC domain, a potential DNA binding module characterized by two CXXCXXC repeats. The CXXC domains, found in other proteins such as DNMT1, MLL and CFP1, have been shown to specifically bind to unmethylated CpG dinucleotides and participate in gene transcription regulation.

As mentioned above, redox regulation affects thiol posttranslational modification-altering molecule activity. In fact, in stressful condition, iterative of ROS production, the thiol group within these different domains of TET proteins could be undergone oxidative modifications such what occur at PTP, PTK as Src or either Yap proteins have strongly tendency to form a diverse spectrum of oxidative modifications. These include sulfenic (SOH), sulfinic (SO_2H) and sulfonic (SO_3H) acids, disulfide bonds (PrSSPr) or nitrosothiols (SNO). Such modifications can alter automatically the TET protein activities such as TET-DNA-binding ability and α-KG-dependent dioxygenase activity.

ROS impact on structural function of p53

The p53 tumor suppressor is a transcription factor. In response to various types of genotoxic stresses, p53 transactivates a number of genes by binding to specific DNA sequences, thereby targeting cell cycle arrest, damaged DNA repair, differentiation or apoptosis as the cell fates.

The structure of the p53 core DNA-binding domain (residues 94-312) that binds directly to the DNA sequence has been resolved by x-ray crystallography.

P53 is biologically active as a homotetramer. It has a modular domain structure, consisting of folded DNA-binding and tetramerization domains, flanked by intrinsically disordered regions at both the amino- and carboxy-termini. The structure of the DNA-binding core domain (residues 94-292) consists of a central immunoglobulin-like β-sandwich scaffold and additional structural elements that form the DNA-binding surface which include a loop-sheet-helix motif and two large loops (L2 and L3). The architecture of the L2/L3 region is stabilized by a zinc ion, which is tetrahedrally coordinated by Cys176, His179, Cys238, and Cys242. p53 itself is redox active due to the presence of cysteines (Cys) that contain redox sensitive thiol groups (-SH) (Figure 13).

In fact, in human p53, there are two clusters of cysteines in the DNA-binding domain, which are essential to the specific binding of p53 to its consensus sequence, Cys 176, 238 and 242, along with histidine 179, consist of a binding site for Zn^{2+}. Mutation of these Zn^{2+}-ligands diminishes the sequence-specific DNA binding of p53, Cys 124, 135, 141, 182 and 277 are located in the loop-sheet-helix region of the proximal DNA-binding domain of p53. They constitute a structural platform for redox modulation. Theoretically, there are multiple possible structures of oxidized thiol groups in proteins, including sulphenic acid (-SOH), disulfide (-S-S-), sulphenamide ($-SNR_1R_2$), sulphinic acid ($-SO_2H$) and sulphonic acid ($-SO_3H$).

GSH was found to be attached to either Cys124 or 141 and to 182 of p53 *via* disulfide bond after oxidant treatment, decreasing the DNA-binding activity of p53, which could be reversed by antioxidants.

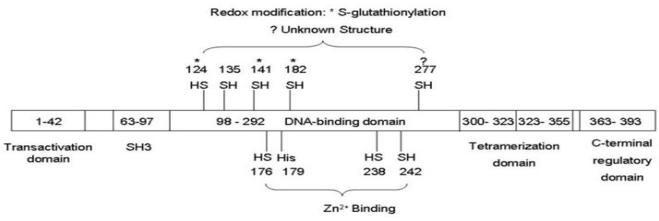

Figure 13: Cartography of cysteine residues on p53 proteine. DNA-binding domain contain 8 cysteine residues where 4 involved in Zn^{2+} interaction.

Thus, there are different effects of redox modifications of Cys residue in p53 depending on its structure p53 belonging. If Cys

residue belongs to the first groups (involving in Zn^{2+} binding), these modifications inhibit completely the transcriptional activity of p53. But if it belongs to the second group these modifications alter relatively differentially this activity.

Resultant effect of these cross talks

An array of genes involved in critical cell viability processes are the product of the resultant effect of epigenetic code and HIF-1α signaling. In this part, we summarize some of these resultant effects.

JMJDs inhibition induces INK4-ARF related genes repression

The INK4a-ARF locus encodes the tumor suppressors, p16INK4a and p14ARF, which block the cell cycle. p16INK4a inhibits cell cycle-dependent kinase 4/6 (CDK4/6), thus keeping Rb in its unphosphorylated active form, whereas p14ARF (p19ARF in mice) blocks the degradation of p53 by inhibiting the p53-specific ubiquitin ligase MDM2.

Many studies have investigated the transcription factors that regulate the expression of the INK4a-ARF locus, mediated the recruitment of the Polycomb group (PcG) to the INK4a-ARF locus. A subunit of the PcG complex, enhancer of zeste homolog 2 (EZH2), has H3K27 methyl transferase activity, which increases the repressive histone mark H3K27me3 at the INK4a-ARF locus.

H3K27me3 and H3K9me3 on the promoter and enhancer region are generally associated with the transcriptional inactivation, whereas H3K4me3 and the acetylation of H3K27 are associated with the transcriptional activation. In growing mammalian cells, the INK4A-ARF locus is silenced by the repressive histone mark H3K27me3. Ras/Raf signaling has been studied to activate, p16INK4a and p14ARF encoded in INK4a locus by reducing a repressive histone mark H3K27me3 by two synergistic mechanisms; by downregulating EZH2 and by inducing JMJD3.

However, hypoxia decreased the catalytic activity of JMJD3 without changing the recruitment of JMJD3 to the promoter region of INK4a suggested that hypoxic inhibition of JMJD3 activity maintains high levels of H3K27me3 on the INK4a locus. As type of α-ketoglutarate-dependent dioxygenases, hypoxia indeed changes the histone methylation of the endogenous JMJD3 target, the INK4a gene, leading to changes in gene expression promoting cell proliferation and dedifferentiation.

VHL gene repressor tumor

Many genes modified by promoter hypermethylation have classic tumor-suppressor function. Examples are the VHL gene in renal cancer. The von Hippel-Lindau (VHL) tumor suppressor is inactivated in the majority of sporadic clear-cell Renal Carcinomas (RCC), with VHL-deficient RCC cells exhibiting constitutive HIF-1α and/or HIF-2α activity irrespective of oxygen availability. In this context epigenetic signaling enlarges the impact of HIF-1α signaling.

HIF-dependent adhesion, migration and invasion

E-cadherine: The suppression of E-cadherin expression is regarded as one of the main molecular events responsible for dysfunction in cell-cell adhesion. Most tumors have abnormal cellular architecture and loss of tissue integrity can lead to local invasion. Thus, loss of function of E-cadherin tumor suppressor protein correlates with increased invasiveness and metastasis of tumors. Loss of epithelial adhesion and polarity causing mesenchymal cell morphology occurs during mesoderm formation. As far as normal adult epithelial tissue structure and integrity is concerned, E-cadherin is also involved in its maintenance and homeostasis. As already mentioned, its function lies primarily in the formation of adherens junctions.

HIF and EMT: Local tumour invasion represents the first step of the metastatic cascade of carcinomas and requires profound changes in the cell adhesion and migration properties of tumour cells that are reminiscent of developmental Epithelial-Mesenchymal Transition (EMT). EMT is thought to be a dynamic and transient process and as such is a manifestation of epithelial cell plasticity during tumour progression. The SNAI1 plays a more prominent role for in the induction of EMT in primary tumours.

SNAIL-1 expression in tumor interface

The transcriptional inhibitor snail is a critical regulator for Epithelial-Mesenchymal Transition (EMT). Although low oxygen induces snail transcription, thereby stimulating EMT, a direct role of HIF-1α in this process. Hypoxia induces the expression of snail *via* HIF-1α. *In silico* analysis identified a potential Hypoxia-Response Element (HRE) close to the minimal promoter of the human and mouse genome of the snail gene.

HIF binds to a HRE cis-acting element in the promoter region of the snail gene that activates its transcription and subsequently suppress the expression of E-cadherin and promotes migration. The snail family members snail (Sna1) and slug (Sna2) are essential for triggering Epithelial-to-Mesenchymal Transitions (EMTs) during embryonic development and tumor progression.

The products of both genes are transcriptional repressors that are able to bind and inhibit E-cadherin promoter activity. Snail-induced E-cadherin depletion is necessary for early phases of embryonic development, as mice deficient in Sna1 expression fail to downregulate E-cadherin levels and to complete gastrulation. Repression of E-cadherin transcription is also particularly relevant during the late steps of epithelial tumorigenesis, as a causal relationship between loss of expression of this protein and the invasive properties of some tumors has been established.

HIF-1α and cell motility

Microenvironmental stimulus, hypoxia, can activate a critical signal transduction pathway, independent of genomic alterations, to drive cancer progression.

HIFs activate transcription of the Rho family member RHOA and Rho kinase 1 (ROCK1) genes, leading to cytoskeletal changes that underlie the invasive cancer cell phenotype. ROCK1 is a kinase that regulates myosin light-chain activity, leading to actin-myosin contraction, which is the basis for cell movement.

HIF- and integrin α5β3: HIF-dependent adhesion, migration and invasion

Hypoxia specifically induces integrin α5β3 overexpression in HIF-1 dependent manner ulting in increased adhesion and migration. At the same time paxillin and FAK were overexpressed under HIF-1 induction. HIF signaling pathway are important in cell motility and adhesion in a number of biological contexts. Together, arising an amplified FAK transducing integerin signalling which promotes the activation of multi-tyrosine kinase signalings. The hyperphosphorylation of FAK, corresponding to the phosphorylation sites between YP397 et YP925, recruits other signaling pathways than Src and p300/cas tranducting signals, such as PI3/AKT signaling. Ampliphying, thus the out-come of integrinβ3 signaling leading to cross-talk with other protein kinases tranducting proliferation signal such as MAPK. Together conducts to a highly mitototic index and at the same time enhance the alteration of ECM. (Extra-Cellular Matrix) leading to the EMT (Epithelial Mesenchymal Transition) phenotype and providing cell invasion and migration.

Epigenetic and HIF in Immunoescape roles

Tumor cells use different mechanisms to evade immune surveillance. Among these mechanisms, tumor cells express immune checkpoint inhibitor ligands and promote $CD8^+$ T cell exhaustion, thus leading to the suppression of the antitumor immune response. PD-1 (CD279), Programmed Cell Death-1 Receptor, is an immune checkpoint inhibitor that is expressed mainly on the surface of immune effector cells, like on activated T cells, NK but also on the surface of B lymphocytes, macrophages, Dendritic Cells (DCs) and monocytes.

The level of expression of PD-1 on immune cells is low, but increases after antigen stimulation. PD-1 expression is induced on both activated $CD8^+$, Tfh and Treg cells localized in the tumor microenvironment and on activated B cells and NK cells. PD1 is also a marker of T cell activation and exhaustion.

PD-1 has two ligands: PD-L1 (B7-H1) and PD-L2 (B7-DC). Contrary to PD-1, PD-L1 is expressed at the basal level on many cell types, such as $CD4^+$ and $CD8^+$ T lymphocytes, tumor cells, CAFs and Tumor-Associated Macrophages (TAMs). Its expression can also be increased in macrophages, DCs and some activated T cells and B cells under inflammatory conditions. Independently of PD-1, PD-L1 and PD-L2 regulate several pathways in cancer cells, such as proliferation, survival, migration and motility.

Thus, PD-Ls may act as a pro-tumorigenic factor, per se. Because of the wide expression of PD-1 and PD-Ls on many cell types, their interactions and mechanisms leading to immune tolerance are exceedingly complex. However, the widely studied mechanism is the interaction of PD-L1 on tumor cells with PD-1 on $CD8^+$ T cells. The PD-1/PD-L1 pathway can be modulated by various signals in cancer cells and plays a role in maintaining immune tolerance.

In fact, hypoxia increased the expression of PD-L1 on macrophages, myeloid-derived suppressor cells, dendritic cells and tumor cells. PD-L1 up-regulation under hypoxia was dependent on hypoxia-inducible factor-1α (HIF-1α) but not HIF-2α. Chromatin immunoprecipitation and luciferase reporter assay revealed direct binding of HIF-1α to a transcriptionally active Hypoxia-Response Element (HRE) in the PD-L1 proximal promoter. The upregulation of HIF leads to PD-L1 expression leading to immunoescaping and enlarging te champ of metastasis. This upregulation can be results of activated EHZ. The high expression of Programmed Death Ligand 1 (PD-L1) is an important factor that promotes immune escape of major cell carcinomas, thus aggravates chemotherapy resistance and poor prognosis.

Enhancer of Zeste homolog2 (EZH2), an epigenetic regulatory molecule with histonemethyltransferase activity, promotes the formation of an immunosuppressive microenvironment. EZH2 was upregulated in lung cancer tissues and positively correlated with PD-L1 levels and poor prognosis. Further, shRNA-expressing lentivirus mediated EZH2 knockdown suppressed boththe mRNA and protein expression level of PD-L1, thus delaying lung cancer progression *in vivo* by enhancing anti-tumor immune responses. Moreover, the regulatory effect of EZH2 on PD-L1 depended on HIF-1a. It has been shown that, EZH2 promotes HIF-1α expression, which was correlated with PD-L1 levels in hypoxic environments. Interestingly, EZH2 silencing alleviated the effects of hypoxia on PD-L1 expression, indicating that EZH2 is upstream of HIF-1α-induced PD-L1 expression. Our results show that EZH2 regulates PD-L1 through HIF-1α upregulation.

HIF-1α Mxi-1-induction represses c-Myc target genes (as p53 a putative c-Myc target gene)

Mxi-1, Max interactor 1, is a transcriptional target of the HIF-1α. Mxi1 contains a bHLH-Zip motif that is similar to that found in Myc family proteins. Mxi1 interacts specifically with Max to form heterodimers that efficiently bind to the Myc-Max consensus recognition site.

c-Myc protein levels decreased during hypoxia. Analysis of downstream transcriptional targets of c-Myc during hypoxia revealed that genes regulated by c-Myc, such as Ornithine Decarboxylase (ODC), were downregulated during hypoxia. S Wu and coworkers showed that overexpression of Mxi1 does in fact inhibit ODC gene expression in a dose-dependent manner both *in vivo* and *in vitro*. And that alterations in the levels of Max-associated proteins such as Mxi1 can modulate critical levels of functional Myc/Max protein complexes. This can alter transcriptional transactivation of Myc-regulated targets.

Mxi1 inhibits the transcriptional activity of MYC by competing for Max, another basic helix-loop-helix protein that binds to Myc and is required for its function. The antagonistic actions of Mxi-l on Myc activity that appears to be mediated in part through the recruitment of a putative transcriptional repressor. Highly conserved (z-helical) "repression" domain of Mxil associates with a homolog of the yeast transcriptional repressor SIN3.

¢KŃ2dzƎK Ŏ2ƛƳƳdzy2LJŃŜŎƛLJƛúƗ·úƛ2y ăúdzŘƛŜă ƛy ƳƗ·ƳƳƗ·fƛƗ·y ŎŜftăΣ aÈƛf Ɨ·yŘ Ƴ2dzăŜ {ƛyo óŃ2{ƛyoú ǿŜŃŜ ăK2ǿy´úž ōŜ LJƗ·ŃŭŤ 2ŦƗ· úŜŃyƗ·ŃÈ Ŏ2ƳLJtŜÈ úKƗ·ú Ɨ·tă2 ƛyŎfdzŘŜŘ aƗ·È¢

Mxi1 protein not only competes with Myc for dimerization to Max and binding to Myc/Max consensus sites but also recruit powerful repressors of gene expression. The human Sin3 protein was identified as a corepressor that interacts with the E-box-binding repressor complex Mad-Max.

Mxi1-Max heterodimers indirectly inhibit Myc function in two ways: First, by sequestering Max, thus preventing the formation of Myc-Max heterodimers and second, by competing with Myc-Max heterodimers for binding to target sites.

TP53 is a c-Myc putative target gene. Infact, TP53 gene posses in its first intron a Myc/Max binding sites and p53 was found overexpressed after oncogenic c-Myc expression.

Other works confirmed that the p53 promoter contains a conserved recognition sequence for the basic-Helixloop-Helix (HLH)-containing proteins of the Myc/MyoD family of transcriptional regulators. As members of this type of DNA-binding proteins, c-Myc/Max heterodimers indeed trans-activate the p53 promoter and lead to an increased expression of p53. Which suggest, that TP53 gene can be a putative Mxi-1 repressed target gene under HIF-1 induction (Figure 14).

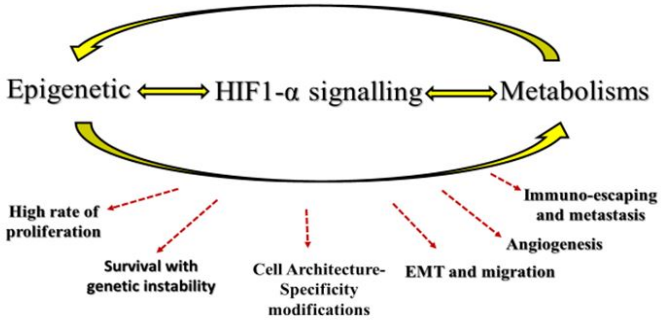

Figure 14: Results of cross-talks between HIF-1α and epigenetic on cell phenotypes (cell structure, specificity and cellular behaviour). Metabolisms are the mojor knot of these cross-talks network.

Conclusion

¢KŜ ăúƗ·ōƛfăƗ·úƛ2y Ɨ·yŘ Ɨ·ŎŎdzƳdzfƗ·úƛ2y´2Ŧ ILCăΣ dzyŘŜŃ ăúŃŜăă ăúƛƳdzfƛΣ úŃƛƎƎŜŃă ŘƛƬŦŜŃŜyú ƳŜúƗ·ō2fƛăƳăŸ DfèŎ2fèăƛă Ɨ·ŎŎŜfŜŃƗ·úƛ2yΣ ƳŜúƗ·ō2fƛăƳ 2yŜ ŎƗ·ŃŎ2yŜΣ fƗ·ŎúƗ·ûŜ Ɨ·ŎŎdzƳdzfƗ·úƛ2y´ Ɨ·yŘ ¢/!T Ɨ·ŃƛăƛyƎ ŘƛØŜŃă ILCă ăƛƎyƗ·tƛyƎă ăLJŜŎúŃdzƳΣ úKƗ·ú Ŏ2yŘdzŎú û2 Ř2ǿy´LJI ØƗ·fdzŜΣ KƛƎK b!5b Ŏ2yŎŜyúŃƗ·úƛ2y´ Ɨ·yŘ wh{ Ɨ·ŎŎdzƳdzfƗ·úƛ2yT ƛƳLJƗ·ŎúƛyƎ 2y´ ŜLJƛŜŷŜúƛŎ fƗ·yŘăŎƗ·LJŜT ǿKƛŎKΣ ƛy´ ŃŜúdzŃy£ ădzăúƗ·ƛyã úKŜ ILCπMʰ ăƛƎyƗ·tƛyƎ LJƗ·úKǿƗ·èă¢ !ff 2Ŧ úKŜăŜ ŎŃ2ăă úƗ·f¶ă ƳŜŘƛƗ·ú ăLJŜŎƛƬƛŎ ŎŜftdzfƗ·Ń ōŜKƗ·Ø2dzŃ ăǿƛŎK ƳƗ·yƛŦŜăúŜŘ ōè ŎŜff ƳƗ·fƛƎyƗ·yŎè ă2 ŦƗ·Ń údzƳ2dzŃ ƛƳƳdzy2πŜăŎƗ·LJƛyƎ fŜƗ·ŘƛyƎ û2 ƳŜúƗ·ăúƗ·ăƛă¢

References

1. Bird A (2007) Perceptions of epigenetics. Nature 447: 396-398.
2. Bird A (2002) DNA methylation patterns and epigenetic memory. Genes Dev 16: 6-21.
3. Herman JG, Baylin SB (2003) Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 349: 2042-2054.
4. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, et al. (2009) Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 324: 930-935.
5. Bird AP, Wolffe AP (1999) Methylation-induced repression-belts, braces and chromatin. Cell 99: 451-454.
6. Eden A, Gaudet F, Waghmare A, Jaenisch R (2003) Chromosomal instability and tumors promoted by DNA hypomethylation. Science 300: 455.
7. Jackson-Grusby L, Beard C, Possemato R, Tudor M, Fambrough D, et al. (2001) Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. Nat Genet 27: 31-39.
8. Chen T, Hevi S, Gay F, Tsujimoto N, He T, et al. (2007) Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. Nat Genet 39: 391-396.
9. Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247-257.
10. Wang GL, Semenza GL (1993) General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. Proc Natl Acad Sci U S A 90: 4304-4308.
11. Cancer Genome Atlas Network (2012) Comprehensive molecular portraits of human breast tumours. Nature 490: 61-70.
12. Semenza GL (2003) Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3: 721-732.
13. Mahon PC, Hirota K, Semenza GL (2001) FIH-1: A novel protein that interacts with HIF-1α and VHL to mediate repression of HIF-1 transcriptional activity. Genes Dev 15: 2675-2686.
14. Mimura I, Nangaku M, Kanki Y, Tsutsumi S, Inoue T, et al. (2012) Dynamic change of chromatin conformation in response to hypoxia enhances the expression of GLUT3 (SLC2A3) by cooperative interaction of hypoxia-inducible factor 1 and KDM3A. Mol Cell Biol 32: 3018-3032.
15. Warburg O, Wind F, Negelein E (1927) The metabolism of tumors in the body. J Gen Physiol 8: 519.
16. Gut P, Verdin E (2013) The nexus of chromatin regulation and intermediary metabolism. Nature 502: 489-498.
17. Lemire J, Mailloux RJ, Appanna VD (2008) Mitochondrial lactate dehydrogenase is involved in oxidative-energy metabolism in human astrocytoma cells (CCF-STTG1). PloS One 3: e1550.
18. Shimizu S, Eguchi Y, Kamiike W, Funahashi Y, Mignon A, et al. (1998) Bcl-2 prevents apoptotic mitochondrial dysfunction by regulating proton flux. Proc Natl Acad Sci U S A 95: 1455-1459.
19. Xie Z, Schendel S, Matsuyama S, Reed JC (1998) Acidic pH promotes dimerization of Bcl-2 family proteins. Biochemistry 37: 6410-6418.
20. Cai L, Sutter BM, Li B, Tu BP (2011) Acetyl-CoA induces cell growth and proliferation by promoting the acetylation of histones at growth genes. Mol Cell 42: 426-437.
21. Phypers B, Pierce JT (2006) Lactate physiology in health and disease. Anaesth Critical Care Pain 6: 128-132.

22. Intlekofer AM, Wang B, Liu H, Shah H, Carmona-Fontaine C, et al. (2017) L-2-Hydroxyglutarate production arises from noncanonical enzyme function at acidic pH. Nat Chem Biol 13: 494-500.

23. Nadtochiy SM, Schafer X, Fu D, Nehrke K, Munger J, et al. (2016) Acidic pH is a metabolic switch for 2-hydroxyglutarate generation and signaling. J Biol Chem 291: 20188-20197.

24. Nadtochiy SM, Schafer X, Fu D, Nehrke K, Munger J, et al. (2016) Acidic pH is a metabolic switch for 2-hydroxyglutarate generation and signaling. J Biol Chem 291: 20188-20197.

25. Xu W, Yang H, Liu Y, Yang Y, Wang P, et al. (2011) Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of α-ketoglutarate-dependent dioxygenases. Cancer Cell 19: 17-30.

26. Mekhail K, Gunaratnam L, Bonicalzi ME, Lee S (2004) HIF activation by pH-dependent nucleolar sequestration of VHL. Nat Cell Biol 6: 642-647.

27. Xiao D, Zeng L, Yao K, Kong X, Wu G, et al. (2016) The glutamine-Alpha-Ketoglutarate (AKG) metabolism and its nutritional implications. Amino Acids 48: 2067-2080.

28. Aik WS, Chowdhury R, Clifton IJ, Hopkinson RJ, Leissing T, et al. (2015) Introduction to structural studies on 2-oxoglutarate-dependent oxygenases and related enzymes. The Royal Society of Chemistry, Cambridge, UK, pp. 59-94.

29. Hegg EL, Jr LQ (1997) The 2-His-1-carboxylate facial triad-an emerging structural motif in mononuclear non-heme iron (II) enzymes. Eur J Biochem 250: 625-629.

30. Winterbourn CC, Hampton MB (2008) Thiol chemistry and specificity in redox signaling. Free Radic Biol Med 45: 549-561.

31. Nelson JW, Creighton TE (1994) Reactivity and ionization of the active site cysteine residues of DsbA, a protein required for disulfide bond formation *in vivo*. Biochemistry 33: 5974-5983.

32. Ikebuchi M, Kashiwagi A, Asahina T, Tanaka Y, Takagi Y, et al. (1993) Effect of medium pH on glutathione redox cycle in cultured human umbilical vein endothelial cells. Metabolism 42: 1121-1126.

33. Poole LB (2015) The basics of thiols and cysteines in redox biology and chemistry. Free Radic Biol Med 80: 148-157.

34. Afani M, Cohn JA, Karpinich NO, Rothman RJ, Russo MA, et al. (2002) Regulation of intracellular pH mediates bax activation in HeLa cells treated with staurosporine or tumor necrosis factor-α. J Biol Chem 277: 49569-49576.

35. Shimizu S, Eguchi Y, Kamiike W, Funahashi Y, Mignon A, et al. (1988) Bcl-2 prevents apoptotic mitochondrial dysfunction by regulating proton flux. Proc Natl Acad Sci U S A 95: 1455-1459.

36. Yaku K, Okabe K, Nakagawa T (2018) NAD metabolism: Implications in aging and longevity. Ageing Res Rev 47: 1-7.

37. Stromland O, Niere M, Nikiforov AA, VanLinden MR, Heiland I, et al. (2019) Keeping the balance in NAD metabolism. Biochem Soc Trans 47: 119-130.

38. Gibson BA, Kraus WL (2012) New insights into the molecular and cellular functions of poly (ADP-ribose) and PARPs. Nat Rev Mol Cell Biol 13: 411-424.

39. Liszt G, Ford E, Kurtev M, Guarente L (2005) Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase. J Biol Chem 280: 21313-21320.

40. Kim MY, Mauro S, Gevry N, Lis JT, Kraus WL (2004) NAD+ dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. Cell 119: 803-814.

41. Gupte R, Liu Z, Kraus WL (2017) PARPs and ADP-ribosylation: Recent advances linking molecular functions to biological outcomes. Genes Dev 31: 101-126.

42. Marti JM, Garcia-Diaz A, Delgado-Bellido D, O'Valle F, Gonzalez-Flores A, et al. (2021) Selective modulation by PARP-1 of HIF-1α-recruitment to chromatin during hypoxia is required for tumor adaptation to hypoxic conditions. Redox Biol 41: 101885.

43. Elser M, Borsig L, Hassa PO, Erener S, Messner S, et al. (2008) Poly (ADP-ribose) polymerase 1 promotes tumor cell survival by coactivating hypoxia-inducible factor-1-dependent gene expression. Mol Cancer Res 6: 282-290.

44. Haigis MC, Sinclair DA (2010) Mammalian sirtuins: Biological insights and disease relevance. Annu Rev Pathol 5: 253-295.

45. Luo J, Nikolaev AY, Imai SI, Chen D, Su F, et al. (2001) Negative control of p53 by Sir2α promotes cell survival under stress. Cell 107: 137-148.

46. Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. Circ Res 107: 1058-1070.

47. Lowenstein CJ, Snyder SH (1992) Nitric oxide, a novel biologic messenger. Cell 70: 705-707.

48. Jung F, Palmer LA, Zhou N, Johns RA (2000) Hypoxic regulation of inducible nitric oxide synthase *via* hypoxia inducible factor-1 in cardiac myocytes. Circ Res 86: 319-325.

49. Gaston B, Singel D, Doctor A, Stamler JS (2006) S-nitrosothiol signaling in respiratory biology. Am J Respir Crit Care Med 173: 1186-1193.

50. Chowdhury R, Flashman E, Mecinovic J, Kramer HB, Kessler BM, et al. (2011) Studies on the reaction of nitric oxide with the hypoxia-inducible factor prolyl hydroxylase domain 2 (EGLN1). J Mol Biol 410: 268-279.