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Epigenome modifiers and metabolic rewiring: New frontiers in therapeutics

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ABSTRACT

In the last decade numerous publications highlighted the connection between metabolism and epigenetics in different physiological and pathological conditions. The availability of metabolites for cells represents indeed a crucial factor, which is able to condition cell fate and development, differentiation and proliferation partially trough epigenetic control. This tight link provides novel therapeutic possibilities to treat many pathological conditions induced by epigenetic alterations, by manipulating metabolic pathways producing metabolites that work also as epigenetic modifiers. This review will explore specifically the relevance of epigenetics and metabolism in the onset of metabolic disorders and cancer, highlighting potential epigenetic-based pharmacological approaches for the treatment of these disorders trough a rewiring of cellular metabolism. We will also report recent studies on stem cells, demonstrating how epigenetic setting is influenced by metabolism and how these processes affect cell pluripotency and differentiation capacity. These findings suggest a big pharmacological potential, as the modulation of epigenetics and metabolism in stem cells may represent a new tool for regenerative medicine, offering a plethora of novel possibilities for the treatment of severe pathological conditions.

Abbreviations: 1-MNA, 1-methylnicotinamide; 2-DG-P, 2-deoxy-D-glucose-6-phosphate; 2-DG, 2-deoxyglucose; 2HG, R(−)-2-hydroxyglutarate; 5-Aza, 5-aza-2′-deoxycytidine; 5-MTHF, 5-methyltetrahydrofolate; 5hmC, 5-hydroxymethylcitosine; 5mC, 5-methylcytosine; AceCS1, acetyl-CoA synthetase short-chain family 1; AcH4, histone H4 acetylated; ACLY, ATP-citrate lyase; AHCY, s-adenosylhomocysteine hydrolase; APO, apolipoprotein; BMI, body mass index; BPTES, bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide; CD36, cluster of differentiation 36; ChREBP, carbohydrate-responsive element-binding protein; CPT1, carnitine palmitoyltransferase; CR, calorie restriction; DNMT, DNA methyltransferases; DZNep, 3-deazaneplanocin A; EPL, early primitive ectoderm-like; ES, embryonic stem; EWAS, epigenome-wide association studies; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; FASN, fatty acid synthase; FGF21, fibroblast growth factor 21; FH, fumarate hydratase; FTO, fat mass and obesity associated; GISTs, gastrointestinal stromal tumors; GLS, glutaminase; GNMT, Glycine N-methyltransferase; GWAS, genome-wide association studies; H3K18ac, histone H3 lysine 18 acetylated; H3K27, histone H3 lysine 27; H3K27ac, histone H3 lysine 27 acetylated; H3K27me3, histone H3 lysine 27 trimethylated; H3K36, histone H3 lysine 36; H3K36me3, histone H3 lysine 36 trimethylated; H3K4, histone H3 lysine 4; H3K4me3, histone H3 lysine 4 trimethylated; H3K79, histone H3 lysine 79; H3K9, histone H3 lysine 9; H3K9ac, histone H3 lysine 9 acetylated; H3K9me2/3, histone H3 lysine 9 dimethylated/trimethylated; H3K9me3, histone H3 lysine 9 trimethylated; H4K12ac, histone H4 lysine 12 acetylated; H4K16, histone H4 lysine 16; H4K20, histone H4 lysine 20; H4K20me3, histone H4 lysine 20 trimethylated; HAT-GCN5, histone acetyltransferase GCN5; HAT, histone acetyltransferases; HCC, hepatocellular carcinoma; HDAC, histone deacetylases; HK, hexokinase; HMT, histone methyltransferases; IDH, isocitrate dehydrogenase; IGF2, insulin-like growth factor 2; JHDM, jumonji-C domain containing histone demethylases; JHDM1A/KDM2A, lysine demethylase 2A; Jmjd1a, lysine (K)-specific demethylase 3A; JMJD2A/KDM4A, lysine demethylase 4A; JMJD2C/KDM4C, lysine demethylase 4C; KDM7A, lysine demethylase 7A; Kras, Kirsten rat sarcoma viral oncogene homolog; *L2HGDH*, L-2HG dehydrogenase; LAT1, solute carrier family 7 member 5; LAT4, solute carrier family 43 member 2; LSD, lysine-specific histone demethylases; MAT2A/MAT2B, methionine adenosyltransferases 2a/2b; MeCP2, Methyl CpG-binding protein 2; mESCs, mouse embryonic stem cells; mGWAS, metabomics genome-wide association studies; MTR, 5-methyltetrahydrofolate-homocysteine methyltransferase; MYC, myelocytomatosis oncogene; NAFLD, non-alcoholic fatty liver disease; NANOG, homeobox protein NANOG; NASH, non-alcoholic steatohepatitis; NNMT, nicotinamide N-methyl-transferase; Osn, Oct4/Sox2/Nanog; PC, phosphatidylcholine; PEMT, Phosphatidylethanolamine *N*-methyltransferase; PFK1, phosphofructokinase 1; PFKBF3, 6-phosphofructo kinase/2,6-fructose bisphosphatase 3; PGDH, 3-phosphoglycerate dehydrogenase; PPARα, peroxisome proliferator-activated receptor α; PPARγ, peroxisome proliferator-activated receptor γ; Psat1, phosphoserine aminotransferase 1; PSC, primed pluripotent stem cells; RASSF1, Ras association family member 1; ROS, reactive oxygen species; RXRα, retinoid X receptor α; SAH hydrolase, S-adenosylhomocysteine hydrolase; SAH, S-adenosyl homocysteine; SAMe, S-adenosyl methionine; SDH, succinate dehydrogenase; SDHb, succinate dehydrogenase subunit b; SIRT1, sirtuin 1; SNP, single nucleotide polymorphism; SOCS2, suppressor of cytokine signaling 2; SQLE, squalene epoxidase; SREBF2, sterol regulatory element-binding transcription factor 2; STARD, steroidogenic acute regulatory protein related lipid transfer domain; T2DM, type 2 diabetes mellitus; TCA, tricarboxylic acid; TDH, threonine dehydrogenase; TET, ten-eleven Translocation; UCP1, uncoupling protein 1; VE-cadherin, vascular endothelial- cadherin; α-KG, α-ketoglutarate.

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1. Introduction

All organisms engage a permanent communication with the surrounding environment. To survive to changing environmental conditions molecular systems sensing surrounding milieu have developed to provide proper adaptive responses. Epigenetic mechanisms of gene regulation play a pivotal role in this adaptation. The classical definition of "epigenetics" comprises mechanisms that affect the genome function without changing the DNA sequence, i.e. DNA methylation, histone post-translational modifications and non-coding RNA mediated mechanisms. From this perspective, chromatin itself can be viewed as an "interface" between the environment, the genome and the underlying cellular functions (Benayoun, Pollina, & Brunet, 2015). It follows that environmental cues not only induce rapid changes in cellular function but also that these changes are incorporated in an "epigenetic memory" (Nieborak & Schneider, 2018) that can be transferred to subsequent generations. This represents a good example of transgenerational inheritance of epigenetic modifications. The observation that epigenome modifiers need metabolic intermediates or cofactors for their enzyme reactions provides a strong link between metabolism and epigenetics (Fig. 1). Any alteration of metabolism that results in variation of metabolite levels could, in fact, differentially regulate the activity of enzymes modifying the epigenome, thus driving chromatin remodelling (Lu & Thompson, 2012).

Here we will discuss examples of the mechanisms whereby activity of epigenetic enzymes is influenced by intermediate metabolites.

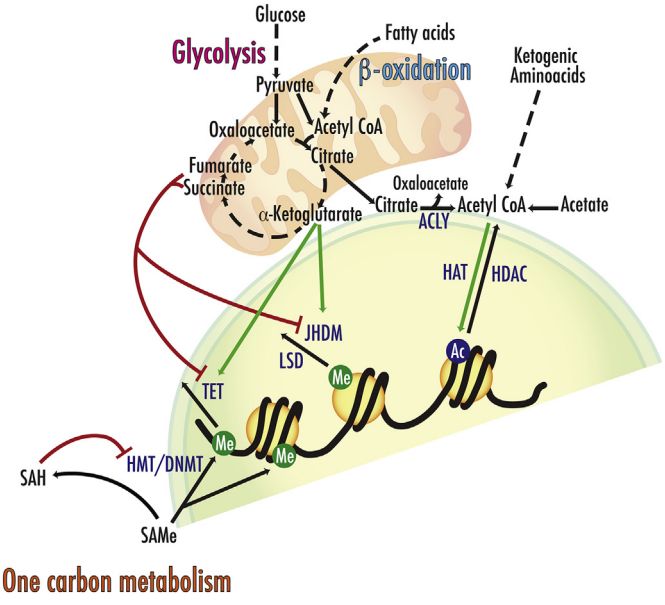

Fig. 1. Connections between metabolism and epigenetics. Metabolic intermediates are substrates of chromatin modifying enzymes. Moreover, they may also act as cofactors (green arrows) or inhibitors (red arrows) for epigenetic enzymes. Thus, the levels of metabolic intermediates affect epigenomic-mediated regulation of genome function.

Methylation of DNA or histone tails is exerted by DNA methyltransferases (DNMT) and histone methyltransferases (HMT), respectively. These enzymes catalyze the transfer of methyl groups from S-adenosyl methionine (SAMe) to DNA cytosine residues or histone lysine or arginine residues, producing S-adenosyl homocysteine (SAH) as a by-product (Fig. 2A). SAMe and SAH are intermediate metabolites of the so-called "one carbon metabolism". Mentch et al. (2015) demonstrated that SAMe/SAH levels affect histone methylation. Under their experimental conditions, histone methylation was altered within 2–4 h after methionine depletion, the amino acid precursor for SAMe production. They also found decreased H3K4me3 peaks, a marker of active transcription, and consequently reduced expression of enzymes belonging to one carbon metabolism, thus suggesting the existence of a feedback regulation to tune down SAMe consumption during methionine restriction. Moreover, in human subjects 30% of methionine variation was determined by diets, suggesting that nutrients may modulate the levels of histone methylation by altering methionine metabolism (Mentch et al., 2015). Beside methionine, also folate, vitamin B12 and B6 and choline can be mentioned as dietary compounds that influence one-carbon metabolism and SAMe levels. Changes in DNA methylation were found to be correlated with the intake of these compounds from diet (Feil & Fraga, 2012). On the contrary, histone demethylation reactions are catalyzed by different subsets of enzymes: lysine-specific histone demethylases (LSDs) and Jumonji-C domain containing histone demethylases (JHDM). FAD converted to $FADH_2$ is cofactor of LSDs, whereas oxygen, Fe(II) and α-ketoglutarate are used by JHDM enzymes with succinate as a by-product (Fig. 2B). On the other hand, DNA demethylation is presumably accomplished in two steps: first, Ten-eleven Translocation (TET) enzymes convert methyl-cytosine residues to hydroxy-methyl-cytosine, which is subsequently demethylated. TET enzymes share the same catalytic mechanism observed in JHDM (Fig. 2C). Also, succinate and fumarate compete with α-ketoglutarate, inhibiting the activity of JHDM and TET. Thus, demethylases utilize molecules with a central role in metabolism as cofactors and regulators. Studies in cancer cells demonstrated that dysregulation of TCA cycle enzymes causing altered levels of α-ketoglutarate, succinate and fumarate could impact chromatin marks (Nieborak & Schneider, 2018).

Another typical modification of histones, is the acetylation of histone tails. Histone acetylation is the result of the equilibrium between the activity of histone acetyltransferases (HAT) and histone deacetylases (HDAC). HATs transfer acetyl groups from acetyl-CoA on lysine residues, thus leading to increased chromatin accessibility, whereas HDACs, by removing them, act as corepressors of transcription. Wellen et al. (2009) demonstrated that nuclear ATP-citrate lyase (ACLY) converts citrate to acetyl-CoA and oxaloacetate, providing nuclear acetyl moieties for histone acetylation. These authors also showed that glucose availability regulates histone acetylation in ACLY-dependent manner. However, other sources can contribute to acetyl-CoA pool. In this regard, we (Ferrari et al., 2017) and other groups (McDonnell et al., 2016) demonstrated that lipids may also be used as carbon sources for histone acetylation. Interestingly, it has been demonstrated that acetate is used as acetyl donor for histone acetylation in cancer cells under hypoxia (Gao et al., 2016). On the other hand, HDACs activity can be

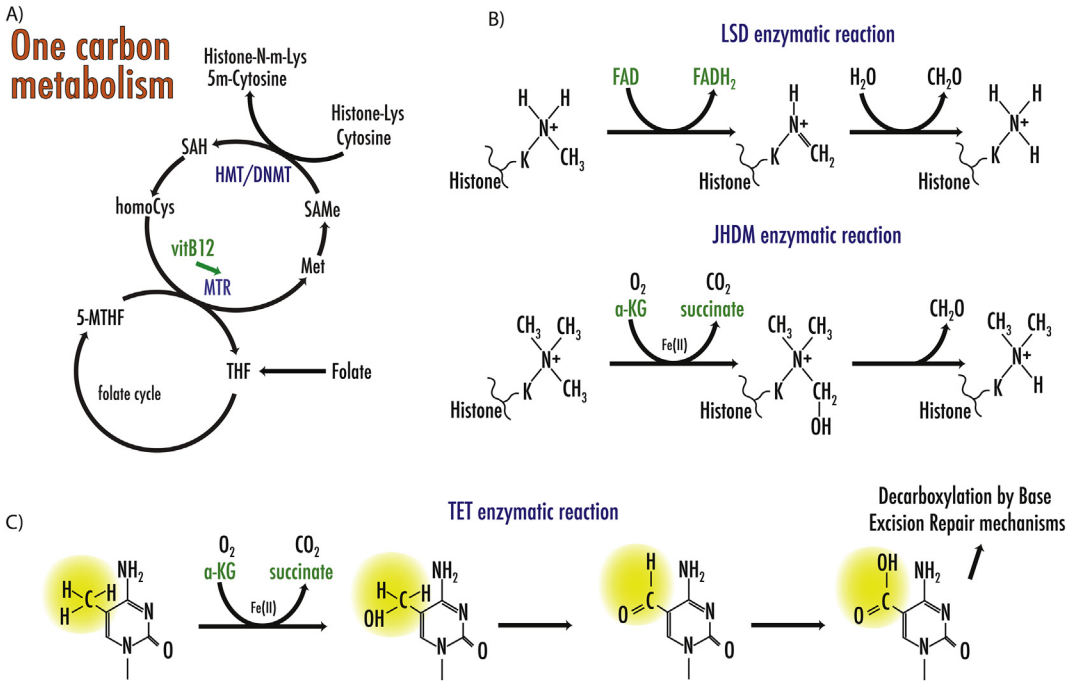

Fig. 2. Methylation and demethylation enzyme reactions. A) Methylation of DNA or histones requires recycling of Met via Methionine Synthase (*MTR*). Folate and vitamin B12 important role is shown. Green arrows indicate cofactors. B) LSD- and JHDM-mediated enzyme reactions. C) Demethylation of cytosine by TET.

modulated by metabolites. In this regard, ketone body β-hydroxybutyrate inhibits class I HDACs (Shimazu et al., 2013). Interestingly, class III HDACs, also known as sirtuins, are NAD^+-dependent: considering that their activity is linked to NAD^+/NADH equilibrium, sirtuins are considered as sensors of the energy status of the cell. Moreover, subcellular concentrations of certain metabolites or the competition between two different epigenome modifiers in using the same cofactor would definitely play a role to determine the final epigenetic outcome (Bosch-Presegué & Vaquero, 2011).

In this review, we will pinpoint how metabolic reprogramming in diseases alters epigenomic regulation of gene expression. In particular, we will focus on cancer and metabolic diseases, as these pathologies severely impact human health and the linked multiple complications represent the first cause of death worldwide. We will also explore the connections between metabolism and epigenomic regulation in stem cells. Furthermore, we will discuss possible or current therapeutic options targeting epigenome modifiers.

2. Epigenetics of metabolic disorders

Metabolic diseases, including diabetes and obesity, affect millions of people in both developed and developing countries. Besides the genetic background, they are also linked to nutritional stimuli and inherited epigenetic effects that may modify chromatin landscape, ultimately affecting gene expression. A great number of studies investigated the effect of nutrients and bioactive foods on the interaction between genome and epigenome. Diet can regulate epigenome through enzymatic inhibition, such as DNA methyltransferases and histone deacetylases (McKay & Mathers, 2011) or by providing donor groups for DNA methylations and histone post-translational modifications (Choi et al., 2004). Several evidences show that the availability of nutrients during pregnancy may carve epigenetic marks in the offspring (Sinclair et al., 2007; Waterland et al., 2006), supporting the hypothesis "we are what we eat but also what our parents ate" (Dominguez-Salas, Cox, Prentice, Hennig, & Moore, 2012).

The strong link between epigenetics and metabolism may offer attractive clinical applications to counteract the escalating prevalence of metabolic diseases, such as obesity, type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD). Addressing basic questions such as how a simple metabolite can induce changes in the epigenome, the consequent relationship with gene expression and the pathogenesis of metabolic diseases, may lead to novel therapeutic opportunities. Currently, the main challenges of epigenetic research regarding metabolic diseases are the search for epigenetic biomarkers to predict individual disease susceptibility, recognize environmental factors that may modulate gene expression through epigenetic mechanisms and develop new therapeutic strategies relying on pharmacological or nutritional agents that could alter epigenetic marks (Martínez, Milagro, Claycombe, & Schalinske, 2014). In the first part of the review, we will focus our attention on the role of some important metabolites and bioactive compounds in the modulation of epigenetic signatures related to metabolic disorders.

2.1. Obesity and type 2 diabetes mellitus

Obesity is a multifactorial disorder that links genetics/epigenetics and metabolism with behavior, food habits, physical activity and social-cultural factors (Nammi, Koka, Chinnala, & Boini, 2004). In general, obesity is caused by energy imbalance, where energy intake exceeds energy expenditure leading to excessive accumulation of fat. It is a global health issue often associated with insulin resistance, glucose intolerance, hypertension, and dyslipidemia, referred as the metabolic syndrome or syndrome X (Barish, Narkar, & Evans, 2006). Obesity is also a risk factor for the development of another metabolic disorder, T2DM. This pathology is influenced by both genetic and environmental factors and is characterized by insulin resistance at the onset and impaired insulin secretion at later stages, which are responsible for the compromised glucose homeostasis (DeFronzo et al., 2015).

Even though lifestyle and environmental factors play a crucial role in the development of obesity and T2DM, an increasing number of studies identified the presence of a clear genetic and epigenetic predisposition. In recent years, the combination of genome-wide association studies (GWAS) with metabolomics (mGWAS) enabled the investigation of genetic influences on metabolic traits (Adamski & Suhre, 2013). This strategy offered an opportunity to establish a connection between genetic variants and metabolism. Kim et al. (2016) demonstrated that a specific

single nucleotide polymorphism (SNP) of the *FTO* (fat mass and obesity associated) gene influences the circulating levels of seven metabolites relevant to phospholipid and amino acid metabolism that are associated with obesity and T2DM. *FTO* encodes for a 2-oxoglutarate-dependent nucleic acid demethylase, which is involved in energy balance and appetite, being mainly expressed in the hypothalamus (Gerken et al., 2007). *Fto* overexpression in mice was reported to increase body and fat mass, due to increased food intake (Church et al., 2010), whereas *Fto* knock-in mice expressing a loss of function mutant or *Fto* knockout mice resulted in lean phenotypes (Church et al., 2009; Fischer et al., 2009). These data highlight the importance of certain metabolites in the biochemical networks underlying the progress of metabolic disorders.

Lately, significant advances were made to further understand the molecular mechanism regulating pathways mediated by FTO. Jia et al. (2012) reported that the N6-methyl-adenosine (m^6A) on the nuclear RNA molecule is the major substrate of FTO. Furthermore, they unraveled the m^6A RNA demethylase activity of FTO *in vivo* and *in vitro* for the first time. The discovery of this novel biological function of FTO in epigenetic regulation raised some questions about whether the reversible chemical m^6A alteration on RNA could be an important epitranscriptomic marker (Saletore et al., 2012; Zheng et al., 2013). In fact, it has been reported that such alteration is a mark in RNA transcripts and is regulated in response to different stimulus such as energy and nutrient availabilities (Mizuno, Pei, Yanming, & Leckstrom, 2017; Nowacka-Woszuk, Pruszynska-Oszmalek, Szydlowski, & Szczerbal, 2017). Besides modifications on posttranscriptional levels, FTO regulates gene expression at transcriptional level by acting as transcriptional co-activator, enhancing the transcription of other genes by interacting with CCAAT/enhancer binding proteins (C/EBPs) (Wu, Saunders, Szkudlarek-Mikho, Serna Ide, & Chin, 2010). Recently, it was demonstrated that FTO functions as a transcriptional repressor of its own gene promoter in hypothalamus (Liu et al., 2018). In this study, it was demonstrated that FTO binds to its own promoter, decreasing its activity. Moreover, free Fe^{2+} impedes FTO binding to the promoter, leading to *Fto* increased expression. These observations revealed an unknown function of FTO as a Fe^{2+}-sensitive transcriptional repressor of its own gene that may be potentially associated with homeostasis of body weight.

Recent studies exploiting cutting-edge technologies focused on epigenetics of metabolic alterations underlying obesity and overweight. The development of high-throughput techniques provided important insights about the way dietary habits and lifestyle contribute to epigenetic changes and how these alterations are linked to metabolic dysfunctions using epigenome-wide association studies (EWAS). A very large EWAS aiming to explore the correlation between adiposity and DNA methylation at numerous CpG sites in blood cells, found several biological pathways enriched for methylation in association with obesity, such as those involved in lipid and energy metabolism (Demerath et al., 2015). Dick and co-workers found an inverse correlation between the expression of HIF3A in adipose tissue and its DNA methylation in blood leukocytes (Dick et al., 2014). A recent characterization of the genome-wide DNA methylation profile associated with obesity, identified CpG sites in circulating leukocytes that mirrors adipose tissue methylation patterns (Crujeiras et al., 2017). The finding that these methylation signatures can be detected in circulating blood cells, most likely mirroring the epigenetic patterns of metabolic tissues as aforementioned in adipose tissue, may result in an easier and quicker identification of these epigenetic marks that may become interesting biomarkers of obesity and related disorders. In 2017, another study validated 94 and 49 CpGs associated with BMI and waist circumference, respectively (Sayols-Baixeras et al., 2017). Moreover, the authors found 70 new CpGs related with BMI and 33 CpGs linked to waist circumference. Among others, hypermethylation in a CpG on cut-like homeobox 1 (*CUX1*) was observed and associated with higher BMI. This gene has been proposed as a regulator of *FTO* and retinitis pigmentosa GTPase regulator interacting protein 1 like (*RPGRIP1L*) expression (Stratigopoulos, LeDuc, Cremona, Chung, & Leibel, 2011), ultimately reducing leptin sensitivity and causing an increase in appetite and food intake that leads to obesity.

However, in spite of the studies attempting to identify genetic and epigenetic predisposing variants, they only explain a small portion of the susceptibility to develop obesity and T2DM. Now we will review how different metabolites control transcriptional responses involved in the onset of obesity and related metabolic diseases via epigenetic mechanism.

2.1.1. TCA cycle intermediates

The TCA cycle intermediates α-ketoglutarate (α-KG), succinate and fumarate play a key role in the regulation of epigenetic modifications. As mentioned above, α-KG is a cofactor for DNA demethylation, a reaction catalyzed by TET, while, succinate and fumarate accumulation, caused by inhibition of succinate dehydrogenase and fumarate hydratase, lead to inhibition of TET (Wenyi Xu, Wang, Yu, & Xin, 2016). Besides TET, another demethylase also requires α-KG as a co-factor, JHDM. Aberrant histone modifications have been related to metabolic impairment, including the development of T2DM and obesity. For example, loss-of-function of histone H3 lysine 9 (H3K9)-specific demethylase *Jhdm2a* gene is associated with obesity and hyperlipidemia in mice (Tateishi, Okada, Kallin, & Zhang, 2009). Deficiency in this demethylase led to an increased methylation of H3K9 at enhancer of the gene encoding the peroxisome proliferator-activated receptor α (*Ppara*) and uncoupling protein 1 (*Ucp1*), two targets of JHDM2A. The important role of JHDM2A as regulator of genes involved in energy expenditure and fat storage was also observed by (Takeshi Inagaki et al., 2009) using a *Jhdm2a*-deficient mice. Notably, Tateishi et al. (2009) also reported a decreased expression of *Ucp2* and *Ucp3* in skeletal muscle and BAT, respectively, in *Jhdm2a*-deficient mice. Cold-induced *Ucp1* upregulation was almost completely blocked in the *Jhdma2a* KO BAT. To assess the hypothesis that JHDM2A could directly regulate *Ucp1* expression in response to cold exposure, the authors only performed ChIP analysis for this UCP isoform. Therefore, it cannot be ruled out a possible involvement of the other UCP isoforms in the epigenetic regulation of energy metabolism mediated by JHDM2A.

TCA cycle also contributes to define NAD^+/NADH ratio and thus influences the chromatin landscape by modulating the activity of sirtuins, NAD^+-dependent histone deacetylases also known as class III HDACs. These enzymes play an important role in energy metabolism (Fiorino et al., 2014). Picard et al. (2004) reported that calorie restriction (CR), a strategy to counteract obesity, increases the NAD^+/NADH ratio inducing SIRT1 activity. As a consequence of SIRT1 activation, downregulation of PPARγ (peroxisome proliferator-activated receptor γ) and its target genes associated with fat storage was observed.

2.1.2. Acetyl-CoA

2.1.2.1. Acetyl-CoA availability influences chromatin remodeling and metabolism of fat. Histone deacetylases (HDACs) are important epigenome modifiers and form repressor complexes involved in the regulation of several signaling pathways. Their action is counterbalanced by histone acetyltransferases (HATs). As mentioned before, citrate and acetyl-CoA play as a central role in regulating these enzymatic reactions as they are, respectively, the substrate and the product of the reaction catalyzed by ACLY, which provides acetyl-CoA directly in the nucleus as a source of acetyl groups for histone tail modification. Moreover, HATs, HDACs and coregulators display tissue-specific role, whose relevance in the context of obesity has been highlighted by means of specific knock out model. A member of class I HDACs, HDAC3, orchestrates the circadian clock that controls hepatic lipid metabolism. The disruption of this circadian rhythm resulted in obesity and insulin resistance in mice (Feng et al., 2011). In our laboratory we have demonstrated a role of HDAC3 in the regulation of lipid metabolism as well. Genetic ablation of *Hdac3*

in the adipose tissue resulted in metabolic rewiring characterized by the concurrent activation of fatty acid β-oxidation and *de novo* lipogenesis. This apparently futile cycle actually sustained increased thermogenesis and browning of white adipose tissue. In this context, citrate and acetyl-CoA played a crucial role both for *de novo* lipogenesis and for hyperacetylation of histone H3 lysine 27 (H3K27ac), which marks activation of poised enhancers. Interestingly, by proteomics experiments with [^{13}C]-palmitate we have also demonstrated that acetyl carbons for histone acetylation originated from fatty acid β-oxidation. In line with these results, we showed that selective inhibition of class I HDAC by a chemical inhibitor, MS-275, counteracted weight gain in genetically and diet induced obesity, through increased oxidative capacity of white adipose tissue (Ferrari et al., 2017; Galmozzi et al., 2013).

2.1.2.2. Modulation of histone acetylation by bioactive compounds. Natural substances, such as polyphenols and other plant compounds, are considered potential therapeutic agents to combat metabolic diseases including obesity, T2DM and atherosclerosis (Fraga, Galleano, Verstraeten, & Oteiza, 2010). Polyphenols are commonly found in vegetables, fruits, green tea and red wine, displaying mainly antioxidant properties. Notably, some of their beneficial effects have been also associated to modulation of histone deacetylase activity (Remely et al., 2014). For instance, resveratrol displays an anti-obesogenic potential by inhibiting adipocyte differentiation and *de novo* lipogenesis and by stimulating lipolysis in a sirtuin 1-dependent manner (Fischer-Posovszky et al., 2010). Another polyphenol called curcumin induces chromatin remodeling by altering histone acetylation/histone deacetylation balance under diabetic conditions (Yun, Jialal, & Devaraj, 2011). These are just two examples among several bioactive compounds, showing that their consumption may contribute to the development of nutritional programs to prevent and treat metabolic diseases by altering the epigenome. Although promising, our knowledge on epigenetic regulation of obesity and related diseases is still insufficient to propose environmental exposures or medications that could modify chromatin signature in a helpful manner.

2.1.3. One carbon metabolism

Regulation of methylation by nutritional and dietary factors occurs mainly through two mechanisms: (i) changes in the availability of methyl donors and (ii) alterations in the enzymatic activity of enzymes involved in the DNA methylation (McKay & Mathers, 2011). Several studies suggested that changes in the cellular availability of methyl groups may disrupt histone and DNA methylation, inducing adverse phenotypic alterations. Besides histone and DNA methylation, methyl groups are also required for post-translational modifications of proteins, synthesis of hormones and other small molecules such as creatine, carnitine and phosphatidylcholine (PC) (da Silva, Kelly, Al Rajabi, & Jacobs, 2014). One-carbon metabolism is a network of biochemical pathways that donate and regenerate carbon units, mediating the methylation of all biological molecules. A growing body of evidence suggests that histone and DNA methylation can be affected by unbalanced availability of nutrients interfering with the proper functioning of one carbon metabolism. Among others, folate, choline, vitamin B12 and methionine have been implicated in this mechanism (McKay & Mathers, 2011).

2.1.3.1. Folate and vitamin B12. The folate cycle is tightly connected to the methionine cycle and regulates the supply of methyl groups (CH_3) by the conversion of S-adenosylmethionine (SAMe) to S-adenosylhomocysteine (SAH) and homocysteine. SAMe is produced from methionine, which in turn can be regenerated in the presence of a methyl donor such as 5-methyltetrahydrofolate (5-MTHF) and methionine synthase (Finer, Saravanan, Hitman, & Yajnik, 2013).

Methionine synthase, encoded by *MTR* gene (5-methyltetrahydrofolate-homocysteine methyltransferase) is a key enzyme for the regeneration of methionine, an important player in the central methylation cycle. Vitamin B12 has an important role in this process, working as a co-factor of methionine synthase. Moreover, vitamin B12 is also essential to produce succinyl-CoA, an intermediate of TCA cycle, via the mitochondrial enzyme methylmalonyl-CoA mutase (Luciano-Mateo et al., 2017). Increased levels of malonyl-CoA and concomitantly, inhibition of carnitine palmitolyltransferase (CPT1) activity and ultimately, inhibition of β-oxidation are associated with deficiency of vitamin B12 (Rush, Katre, & Yajnik, 2013).

2.1.3.2. Fetal programing: effects of folate and vitamin B12. There is evidence that nutritional status during early life induce epigenomic modifications that increase the risk of developing metabolic diseases later in life. These indications support the idea that epigenetic marks in adult life are often established during the early stages of development.

Both paternal under- and overnutrition have been associated with increased risk for adult onset of metabolic disorders in the offspring. Children of obese fathers have higher risk of developing obesity later in life when compared with children of lean fathers. Donkin et al. (2016) reported similar spermatozoal histone positioning in lean and obese men, however they observed significant differences in small non-coding RNA expression and DNA methylation patterns. Primates fed high-fat diet during pregnancy showed higher levels of histone H3 acetylation in the offspring compared to the offspring from mothers on low-fat diet, accompanied by elevated triglyceride levels in fetal liver and histologic evidence of non-alcoholic fatty liver disease (Aagaard-Tillery et al., 2008).

Prenatal exposure to famine during the Dutch Hunger Winter resulted in different methylation patterns of genes linked to growth and metabolic diseases in individuals later in life when compared with their unexposed siblings (Heijmans et al., 2008). Among those genes, decreased DNA methylation of the imprinted insulin-like growth factor 2 (*IGF2*) gene was observed. IGF2 has been associated with an increased risk of obesity, dyslipidemia and insulin resistance (Martínez et al., 2014). Steegers-Theunissen et al. (2009) investigated the correlation between maternal supplementation with folic acid and the methylation at the *IGF2* gene of the child. In this study, these authors observed that periconception folic acid supplementation increased methylation status at the *IGF2* locus in the offspring.

Bariatric surgery is a procedure to counteract obesity. However, vitamins (i.e. vitamin B12 and folate) and iron deficiency are inherent problems in the post-operative period (Majumder, Soriano, Louie Cruz, & Dasanu, 2013). Vitamin B12 is one of the most common nutritional deficiencies affecting patients undergoing this surgery. In fact, vitamin B12 deficiency has been associated with increased risk of diabetes and obesity (Finer et al., 2013). Accordingly, DNA methylation undergoes remodeling after bariatric surgery, supporting the hypothesis of the reversibility of DNA methylation after weight loss. It was also demonstrated that children born after maternal bariatric surgery are less obese when compared with siblings born before maternal surgery (Guenard et al., 2013).

As opposed to maternal overnutrition, maternal low carbohydrate intake during pregnancy alters the methylation status of CpGs in the promoter region of retinoid X receptor α gene (*RXRA*), increasing child's later adiposity in two independent cohorts (Godfrey et al., 2011). Moreover, restricting the supply of vitamin B12, folate and methionine from the diet of pregnant sheep triggers DNA hypomethylation in the offspring, which was associated with insulin resistance in adult life (Sinclair et al., 2007). Furthermore, it has been showed that a promethylation dietary supplement (choline, betaine, folic acid and vitamin B12) prevented the transgenerational amplification of obesity (Waterland, Travisano, Tahiliani, Rached, & Mirza, 2008). These evidences contribute to support the hypothesis that early-life environmental conditions can cause epigenetic changes in humans that persist throughout lifetime and may be critical determinants of metabolic disorders in adulthood.

Apart from bariatric surgery, deficiency of such vitamins is observed in human population mainly due to increased requirements or decreased availability. Insufficient nutritional intake is considered the

main cause of low vitamin B12 serum level, especially in population following vegan or vegetarian diet in developing countries (Allen, Rosenberg, Oakley, & Omenn, 2010). Also, chronic alcoholism is an example of folate deficiency caused by poor dietary intake, intestinal malabsorption or impaired hepatic uptake with reduced storage of endogenous folates (McNulty, Pentieva, Hoey, Strain, & Ward, 2012). The elderly is a subpopulation in general at risk of macro- and micronutrients deficiency due to undernutrition, mainly owing to reduced intake associated to illness but also due to psychological factors such as depression. However, atrophic gastritis is thought to be the most common factor of vitamin B12 deficiency in the elderly. This is an autoimmune disease in which the mistakenly destruction of parietal cells leads to impaired vitamin B12, folate and iron absorption. Consequently, there is the development of megaloblastic anaemia and neurological and systemic signs and symptoms collectively known as pernicious anaemia (Neumann, Coss, Rugge, & Genta, 2013). In addition, vitamin B12 and folate deficiency is associated with degeneration of the spinal cord, optic nerves, cerebral tissue and peripheral nerves.

According to clinical reports and population studies, vitamin B12 metabolism has been connected with cardiomyopathies (Guéant et al., 2013). Both folate and vitamin B12 deficiency induced heart hypertrophy alongside with impaired mitochondrial fatty acid oxidation (Garcia et al., 2011). These findings were associated with an imbalance acetylation/methylation of PGC1α by SIRT1 and decreased expression of PPARα which led to decreased expression of enzymes involved in mitochondrial fatty acid oxidation and respiration.

Most of the body's reserves of vitamin B12 are stored in the liver. Studies have demonstrated the deleterious effects of vitamin B12 deficiency in the liver and patients with genetic disorders of vitamin B12 metabolism developed hepatic steatosis (Russo, Doyon, Sonsino, Ogier, & Saudubray, 1992). The link between methyl donor deficiency and hepatic problems will be discussed in greater detail in the following section.

2.2. Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a hepatic disorder tightly connected to obesity and T2DM. NAFLD is characterized by the pathological accumulation of fat within hepatocytes, displaying different levels of severity, ranging from simple steatosis to fibrosis and cirrhosis (Younossi et al., 2016). A subgroup of NAFLD, called non-alcoholic steatohepatitis (NASH), may progress to more severe stages with a significantly higher risk of developing hepatocellular carcinoma (HCC). Liver cancer is considered the sixth most common cancer and efficient treatment strategies are currently lacking, making it the third leading cause of cancer-related death (European Association for the Study of the, European Organisation for, & Treatment of, 2012). The diagnosis is mainly dependent on invasive methods (i.e. liver biopsy) with limited accuracy, leading to the urgent need for the identification of more specific and sensitive noninvasive molecular markers that may facilitate the early diagnosis and prognosis of NAFLD and HCC. It has been suggested that the DNA methylation status at specific CpGs could be a good parameter to evaluate the progression of NAFLD to NASH. 69,247 differentially methylated CpG sites (76% hypomethylated, 24% hypermethylated) were reported in patients with advanced versus mild NAFLD (Murphy et al., 2013). Mwinyi and colleagues reported that NAFLD is associated with methylation shifts near the transcriptional start site impacting the expression of genes involved in lipid and energy homeostasis (Mwinyi et al., 2017). Among those genes, the authors reported changes in methylation of apolipoprotein (*APO*) family members (lipid transport) and steroidogenic acute regulatory protein related lipid transfer domain (*STARD*), associated with lipid and cholesterol transporter, respectively. Moreover, consistent with other studies, alteration in the methylation pattern of the gene encoding the metabolic hormone fibroblast growth factor 21 (*FGF21*) was also observed. FGF21 is a hormone of the fibroblast growth factor family, mainly expressed in the liver and white and brown adipose tissue, which regulates systemic energy levels playing a role in macronutrient metabolism. *FGF21* expression is known to be induced by both fasting and PPARα agonists in the liver (T. Inagaki et al., 2007) and plays a role in the induction of weight loss in obese animals and in the amelioration of glucose imbalances (Takeshi Inagaki, 2015).

2.2.1. One carbon metabolism

Given its function mediating energy production and detoxification, liver is the first organ facing dietary exposures. The pathogenesis of NAFLD is multifactorial and it has been associated with aberrant epigenetic alterations. Perturbations of one-carbon metabolism are usually associated with alterations in the normal hepatic metabolism. Methyl donor deficient diets result in the accumulation of triglycerides in the liver, leading to NAFLD and the progression to NASH (Kajikawa et al., 2011) whereas supplementation with dietary methyl donors improves liver steatosis by reversing the methylation status at CpG sites in specific promoters of genes involved in fatty acid metabolism (e.g., *Fasn*) (Cordero, Gomez-Uriz, Campion, Milagro, & Martinez, 2013). Accordingly, Pogribny and coworkers observed the development of hepatic steatosis, accompanied by epigenetic abnormalities such as histone modifications and DNA methylation in mice fed methyl-deficient diet (Pogribny et al., 2009).

Dietary folate depletion led to increased expression of genes involved in hepatic lipid synthesis contributing to hepatic steatosis (Champier, Claustrat, Nazaret, Fevre Montange, & Claustrat, 2012). Moreover, high fat diet-induced NAFLD in rats was prevented by a dietary methyl donor supplementation and such effects were associated with changes in the methylation pattern of hepatic genes, including fatty acid synthase *(Fasn)* and sterol regulatory element-binding transcription factor 2 (*Srebf2*) (Cordero et al., 2013). Epigenetic modulation in fetal programming seems also to play a role since folic acid supplementation during *in utero* life increased PPARγ expression in rat liver due to decreased methylation of the *Pparg* promoter, contributing to hepatic steatosis (Sie et al., 2013).

2.2.2. SAMe/SAH

SAMe and SAH are key enzymes in the central methylation cycle and folate is a key player to maintain a correct balance of SAMe/SAH. Several studies have shown a clear link between folate, choline and lipid metabolism (Henkel, Dewey, Anderson, Olivares, & Green, 2012; Rinella & Green, 2004). These findings suggest that dietary folate deficiency diminishes choline and PC levels resulting in accumulation of hepatic triglycerides (da Silva et al., 2014). Phosphatidylethanolamine *N*-methyltransferase (PEMT) is the enzyme responsible for the synthesis of PC, a process that consumes a significant portion of SAMe in the liver (Stead, Brosnan, Brosnan, Vance, & Jacobs, 2006). *Pemt* knock out mice showed steatosis and liver failure when fed a high fat and choline-deficient diet, respectively (Vance, Li, & Jacobs, 2007). Glycine N-methyltransferase (GNMT) deficiency has also been connected to steatosis. *Gnmt* knock out mice demonstrated a 40-fold increase in hepatic SAMe that is linked to steatosis (Varela-Rey et al., 2010). In the same study, the normalization of SAMe levels using a methylated compound, nicotinamide, reduced hepatic steatosis.

2.2.3. Acetyl-CoA as mediator of histone acetylation

DNA methylation analysis from morbidly obese patients demonstrated differences in expression and methylation of genes involved in metabolic regulation (Ahrens et al., 2013). Among these, the authors identified also ATP citrate lyase (*ACLY)*: decreased DNA methylation of this gene, along with higher *ACLY* mRNA expression, points to the activation of a lipogenic pathway in NAFLD patients. These results demonstrate how different epigenetic pathways are interconnected and contribute to fine tune the regulation of liver pathophysiology. The progression of hepatic steatosis is in fact influenced also by histone acetylation. p300, a member of the HAT family, was originally reported to

activate the carbohydrate-responsive element-binding protein (ChREBP) and to induce the onset of NAFLD by increasing the expression of lipogenic genes by both histone and non-histone protein acetylation (Tian, Wong, Chan, & Cheng, 2013). HDACs also play an important role in the development of NAFLD. Methyl CpG-binding protein 2 (MeCP2) binds DNA at CpG islands and interacts with HDAC3, leading to chromatin condensation and gene repression. Genetic ablation of *Mecp2* in the liver was reported to induce dyslipidemia and NAFLD due to the disruption of HDAC3 recruitment on specific loci and increased H3K27ac marks at the squalene epoxidase (*Sple*), fatty acid synthase (*Fasn*) and cluster of differentiation 36 (*Cd36*) (Kyle, Saha, Brown, Chan, & Justice, 2016). Given the crucial relevance of histone acetylation in the control of NAFLD progression, is tempting to speculate that a modulation of acetyl-CoA may be a helpful and novel approach in the treatment of this metabolic disorder.

2.2.4. RNA-based epigenetic mechanisms

Besides modifications in DNA and histones, microRNAs (miRNAs) are also epigenetic regulators, and their role in NAFLD progression has been highlighted (Afonso, Rodrigues, Simão, & Castro, 2016). Circulating miRNAs are important regulators of liver homeostasis, which have been proposed as possible biomarkers and attractive therapeutic targets for liver injury and hepatocarcinogenesis. miR-122 is highly expressed in the liver where it regulates the expression of multiple targets involved in hepatic metabolism (Esau et al., 2006). Abnormal expression of miR-122 has been associated with several liver diseases. In NASH patients, miR-122 levels are strongly reduced in comparison to steatosis patients and healthy individuals, leading to an abnormal expression of genes related to lipid metabolism (Onpan et al., 2008). Another miRNA used as a NAFLD biomarker is miR-21. Increased expression of liver miR-21 was demonstrated in NAFLD patients compared to healthy controls (Yamada et al., 2013). Interestingly, folate supplementation may influence miRNA expression linked to fatty liver disease, most probably by inducing changes in methylation levels of promoter regions in the genome. It has also been shown that choline- and folate-deficient diet in mice led to NAFLD-induced liver injury and circulating levels of miR-34a, miR-122, miR-181a, miR-192, and miR-200b correlates with the severity of the pathologic condition (Tryndyak et al., 2012). Taking all these observations together, epigenetic alterations could be considered as possible therapeutic targets and non-invasive molecular markers of NAFLD and good predictors of initial risk assessment and disease progression.

2.3. Future perspectives in the epigenetics of metabolic disorders

In spite of all the general awareness, the rising incidence of metabolic disorders affects the length and quality of life and thus urges the design of safe and effective strategies to face this problem. Lifestyle intervention, medications or even surgery are some of the strategies used to counteract obesity and related disorders. However, the final outcome is way too far from meeting the expected goal and seems to vary from patient to patient.

Over the last few years, the increasing knowledge on the genetics and epigenetics of these metabolic disorders stimulated a great number of studies investigating the connection between treatments and outcomes to establish more personalized therapies. Several epigenetic modifiers including acetyltransferases, kinases and methyltransferases use cellular metabolites as sources of acetyl, phosphate or methyl groups, respectively. It is conceivable that fluctuations in the availability of these metabolites, mainly through changes in nutrition, may have an important impact on gene expression patterns, leading in some cases to the progression of metabolic diseases. Many of these epigenetic marks are theoretically reversible, suggesting possible interventions with bioactive molecules as promising chemopreventive agents to rescue favorable epigenomic profiles. A growing body of evidence suggests that nutriepigenetics/nutriepigenomics, which investigates the impact of such bioactive food compounds in epigenetic modifications, might be an important player in this field. As described early in this section, epigenetic alterations involving histone and DNA modifications play a crucial role in the development of metabolic diseases. Nevertheless, we are still far from understanding how to normalize the epigenome modifications responsible for the onset of such disorders. In this respect, the development of epigenetic drugs targeting epigenome modifiers holds great promise. For instance, it could be possible to target some epigenome modifiers to reestablish a healthy chromatin landscape. This switch could reverse the disease phenotype, especially during the early stages of progression.

However, there are still a lot of questions to be addressed. We need to better understand how plastic is the chromatin landscape to possible agents and how this plasticity is associated with the development of metabolic diseases.

3. Metabolites as epigenetic modifiers in cancer

The metabolic rewiring occurring in tumor cells is very deep and it is considered a relevant hallmark of cancer (Hanahan & Weinberg, 2011). Traditionally the altered metabolism of cancer cells has been seen as a secondary consequence of the high metabolic and energy demands of tumors. However in most recent years there were accumulating evidences in favor of the hypothesis whereby mutations in proto-oncogenes and tumor suppressor genes primarily cause metabolic rewiring of cells. Moreover, during tumorigenesis selection of specific metabolic enzyme isoforms or mutation in metabolic enzymes further causes alteration of metabolism. The metabolic rewiring in tumor cells sustains biomass production and high proliferation through different utilization of nutrients (Ward & Thompson, 2012). Tumor microenvironment contributes to define oxygen and nutrient availability, deeply impacting metabolic rearrangements of cancer cells. Furthermore, with respect to stromal cells, on the one hand tumor cells may compete for the same nutrient with surrounding stromal cells, on the other hand these cells may constitute a symbiotic relation favoring tumor proliferation (DeNicola & Cantley, 2015). The metabolic re-organization of tumor cells involves several pathways. First, these cells preferentially metabolize glucose via glycolysis to generate lactate (Vander Heiden, Cantley, & Thompson, 2009; Warburg, 1956). Compared with oxidative phosphorylation, glycolysis is characterized by a faster turnover and makes it available a significant amount of intermediates that can be used for biosynthesis of macromolecules and redox homeostasis (Wong, Qian, & Yu, 2017). Another metabolic signature that distinguishes cancer cells is glutaminolysis: these cells are addicted to glutamine since they use it to refill the TCA cycle (anaplerosis), and as a precursor for biosynthesis of nucleotides, proteins and glutathione (C. V. Dang, 2010; Jin, Alesi, & Kang, 2016). Moreover, mitochondrial activity in cancer cells has a central anabolic role in producing precursors for macromolecule biosynthesis rather than the classical oxidative and catabolic role of energy production. Intriguingly the remodeling of cancer cell metabolism deeply impacts epigenetics. As elegantly described by Wong et al., epigenetics and metabolism are strictly interconnected in cancer because the metabolic remodeling occurring in tumor cells modulates the availability of metabolites with epigenetic functions (Wong et al., 2017). On the other hand, epigenetic dysfunction impacts metabolism by directly affecting the expression of genes encoding metabolic enzymes. Starting from the analysis of the epigenetic role of different metabolites, now we will depict how cancer affects the link between epigenetics and metabolism and perturbs this fragile balance that dictates cellular physiology and function.

3.1. SAMe/SAH

3.1.1. SAMe/SAH in the regulation of epigenetic enzymes

As described in the previous sections, the ratio of the SAMe and SAH controls the activity of methyltransferases. For this reason, the

production of these metabolites must be fine-tuned to guarantee the physiological cellular activity (Mentch et al., 2015), since any alteration of SAMe/SAH ratio could result in detrimental effects. In this regard it has been reported that a genetic deficiency of glycine N-methyltransferase (GNMT) leads to a significant increase of SAMe levels (Mudd et al., 2001). Accordingly, mice lacking GNMT showed a 40-fold increase in hepatic SAMe. As a consequence of this dramatic imbalance, the authors detected increased CpG methylation at the promoter of tumor suppressor genes such as RASSF1 (Ras association family member 1) and SOCS2 (suppressor of cytokine signaling 2), leading to their transcriptional silencing. Moreover, they also described elevated H3K27me3 at these promoters, consistent with silencing of specific genome regions. Phenotypically, GNMT knock out determined the activation of oncogenic pathways, resulting in a higher incidence of hepatocellular carcinoma (Martinez-Chantar et al., 2008). Another mechanism allowing cancer cells to raise SAMe level is via promoting one carbon metabolism, including folate and methionine cycles. This allows cells to generate one-carbon units as methyl groups, used as source for the biosynthesis of anabolic precursors and for methylation reactions (Newman & Maddocks, 2017). In human cancer cells, on one side the overexpression of amino-acid transporters LAT1 and LAT4 determines the increased uptake of methionine (Haase, Bergmann, Fuechtner, Hoepping, & Pietzsch, 2007). On the other hand, these cells often feature higher expression of 3-phosphoglycerate dehydrogenase (PGDH) that preferentially redirect glycolysis intermediates to the serine-glycine biosynthesis pathway (Haase et al., 2007). Serine can donate its side chain to tetrahydrofolate, fostering the folate cycle that in turn recycles methionine from homocysteine (Lukey, Katt, & Cerione, 2017). Alterations in SAMe/SAH ratio not only affects DNA methylation, but it can also dictate changes in histone methylation in cancers. SAMe is in fact methyl donor in the reaction catalyzed by nicotinamide N-methyl-transferase (NNMT), which converts nicotinamide to 1-methylnicotinamide (1-MNA) (Luo et al., 2017). Several types of cancer, including lung, liver, kidney, bladder and colon cancers (Tang et al., 2011; Thomas et al., 2013; Wu, Siadaty, Berens, Hampton, & Theodorescu, 2008; Zhang, Wang, Li, Yu, & Xie, 2014), are marked by overexpression of NNMT that exerts an oncogenic effect. NNMT depletes cellular SAMe pool, profoundly altering SAMe/SAH ratio and reducing the availability of SAMe for HMTs. As a result, NNMT-expressing cancer cells show an altered epigenetic profile, marked by reduced H3K4, H3K9, H3K27 and H4K20 methylation (Ulanovskaya, Zuhl, & Cravatt, 2013). These results suggest NNMT as a novel potential drug target to modulate cancer epigenome for therapeutics.

3.1.2. Modulators of SAMe/SAH ratio in cancer therapy cycle

Given the relevance of SAMe availability for the activity of DNMTs and HMTs, modulation of SAMe cycle in tumor cells, by affecting DNA and histone methylation, could modulate epigenetic hallmarks and cancer progression. In this regard, an interesting target is represented by the enzyme s-adenosylhomocysteine hydrolase (AHCY) which catalyzes the hydrolysis of SAH into adenosine and homocysteine and participates in the maintenance of methylation homeostasis (Tehlivets, Malanovic, Visram, Pavkov-Keller, & Keller, 2013). The first identified SAH hydrolase inhibitor was 3-deazaneplanocin A (DZNep) (Glazer et al., 1986) that, when administered to cancer cell lines, is able to inhibit DNA and histone methylation (Miranda et al., 2009). Moreover, DZNep in cancer cells targets the oncogenic HMT histone-lysine N-methyltransferase EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) via SAH hydrolase inhibition (Girard et al., 2014). It has also been shown that the combination of DZNep with the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (5-Aza) is able to re-activate genes that are aberrantly silenced in leukemia and colorectal cancer, exerting a relevant anticancer activity (Momparler, Cote, Momparler, & Idaghdour, 2014; Momparler, Idaghdour, Marquez, & Momparler, 2012).

3.2. TCA cycle metabolites

3.2.1. TCA cycle metabolites in the regulation of DNA and histone demethylation

As already mentioned above, DNA and histone methylation are regulated by both DNA and histone methyltransferase and by demethylases as well. TETs and JHDMs require α-KG as a cofactor and are competitively inhibited by the oncometabolite 2-hydroxyglutarate (Xu et al., 2011) and by the TCA cycle intermediates, such as succinate and fumarate (Xiao et al., 2012). Interestingly, cancer cells with mutations in metabolic genes can accumulate these metabolites, determining a deep epigenetic remodeling further sustaining tumorigenesis.

3.2.1.1. 2-hydroxyglutarate. In certain types of tumors, including gliomas (Balss et al., 2008) and angioimmunoblastic T-cell lymphoma (Paschka et al., 2010), genetic mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) isoforms IDH1 and IDH2 have been described. These metabolic enzymes are respectively cytosolic and mitochondrial and catalyze the $NADP^+$-dependent oxidative decarboxylation of isocitrate to α-KG (Hurley, Dean, Koshland Jr., & Stroud, 1991). Moreover, these enzymes are also implicated in reductive carboxylation of glutamine-derived α-KG to citrate. (Hurley et al., 1991). Mutated form of IDH1 or IDH2 cannot synthesize isocitrate from α-KG, but they catalyze the NADPH-dependent reduction of α-ketoglutarate to R(−)-2-hydroxyglutarate (2HG) (L. Dang et al., 2009; Ward & Thompson, 2012), as shown in Fig. 3A. Structure of 2-HG is similar to α-KG, but it acts as a competitive inhibitor of TETs and JHDMs (Lu et al., 2012). Thus, IDH mutations deeply affect epigenome (Sasaki et al., 2012). Interestingly, it has been observed that some forms of cancer without genetic mutations of IDH also show high levels of 2-HG: in breast cancer this is the result of MYC (myelocytomatosis oncogene) pathway activation (Terunuma et al., 2014). In renal cancer the elevation of 2-HG L-enantiomer is mediated in part by reduced expression of L-2HG dehydrogenase (*L2HGDH*), preventing conversion of 2-HG back to α-KG (Shim et al., 2014). The conversion of 5-methylcytosine (5mC) to 5-hydroxymethylcitosine (5hmC) by TET1/2 is potently inhibited by 2-HG (Chowdhury et al., 2011; Xu et al., 2011). Importantly, it has been observed that IDH1/2 mutations in human patients with glioma or acute myeloid leukemia are able to determine CpG island methylated phenotype, marked by hypermethylation in a large number of genes (Turcan et al., 2012). Since DNA hypermethylation mediated by increased 2-HG is reversible, this pathway represents a therapeutic target in IDH1/2-mutant cancers (Wong et al., 2017). 2-HG levels in IDH1/2 mutant tumors not only regulate DNA methylation, but also modulate histone demethylase activity: 2-HG in fact strongly inhibits several histone demethylases including JMJD2A/KDM4A, JMJD2C/KDM4C, JHDM1A/KDM2A (Chowdhury et al., 2011) and KDM7A (Xu et al., 2011). Xu and co-workers in fact demonstrated that 2-HG binds to catalytic core of histone demethylases and competes with α-KG (Xu et al., 2011). Accordingly, it has been reported that overexpression of mutant IDH1 or administration of 2-HG in human glioma cells increased H3K9, H3K27 and H3K79 dimethylation and H3K4 trimethylation. Also, mutations in IDH1 in oligodendroglioma patients correlate with higher H3K9me3 compared with those with wild-type IDH1/2 (Venneti et al., 2013).

3.2.1.2. Succinate and fumarate. These two TCA cycle metabolites antagonize the effect of α-KG, since they are competitive inhibitors of TETs catalyzed hydroxylation of 5mC and of the histone demethylases KDM2A and KDM4A (Xiao et al., 2012). For this reason, inactivating mutations in TCA cycle enzymes fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) mediate epigenetic reprogramming and are a hallmark of distinct human cancer (Hao et al., 2009; Hensen & Bayley, 2011; Janeway et al., 2011; Pollard, Wortham, & Tomlinson, 2003; Ricketts et al., 2012). SDH is made of four subunits and catalyzes oxidation of succinate to fumarate (Rustin, Munnich, & Rötig, 2002).

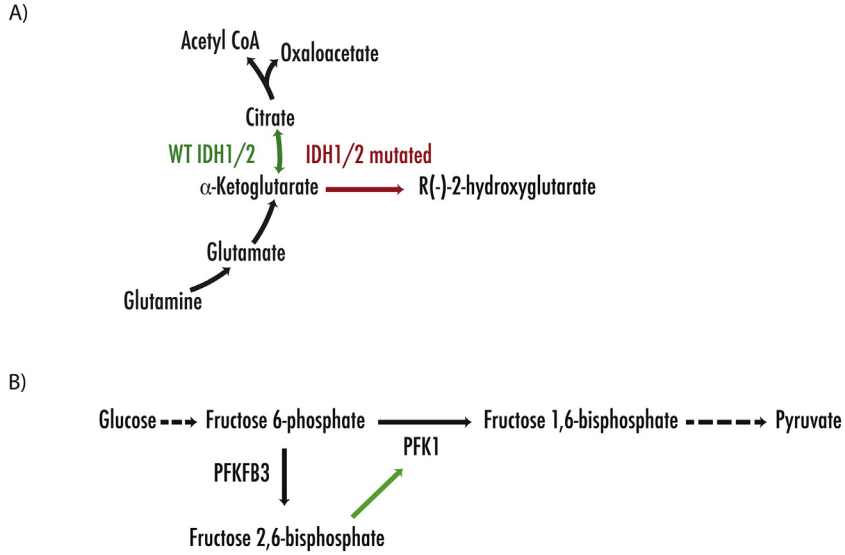

Fig. 3. PFKFB3 and IDH enzyme reactions. A) WT IDH (green) produces citrate from α-KG; whereas mutations of IDH (red) in cancer lead to production of 2-HG from α-KG. B) PFKFB3 positively regulates glycolysis by producing fructose 2,6 bisphosphate, which allosterically activates PFK1.

Mutations in any of the four subunits inactivate the SDH complex, leading to marked accumulation of succinate (Wang, Gu, & Ji, 2015). Mitochondrial FH mediates the reversible conversion between fumarate and malate, and the loss-of-function mutation of *FH* results in high levels of fumarate (Gaude & Frezza, 2014). Hence, overexpression of *FH* and *SDH* mutants recapitulate the effects of succinate and fumarate. SDH-related paragangliomas show increased 5-mC/5-hmC ratio and enhanced histone methylation at H3K9 and H3K27 with a consequent downregulation of key genes involved in neuroendocrine differentiation (Letouzé et al., 2013). Moreover, knock out of *Sdhb* determines profound epigenetic changes in cells leading to a transcriptional downregulation of genes involved in the suppression of metastasis, and consequently to increase tumor invasiveness. *In vivo* results from SDH mutant gastrointestinal stromal tumors (GISTs) revealed comparable measures of global hypo- and hypermethylation with *IDH*-mutant glioma (Killian et al., 2013). This evidence is in line with the observation by Letouzé et al. (2013), describing that in paraganglioma patients with *SDH* or *FH*-deficiency the deep epigenetic dysregulation leads to a worse prognosis compared with other molecular subtypes, favoring tumor development and progression.

3.2.2. Modulation of TCA cycle metabolites as a cancer therapy

To counteract the production of the oncometabolite 2-HG, a possible strategy is the inhibition of IDH1/2. A selective inhibitor of mutant IDH1, AGI-5198, with no activity on wild type IDH1 and on IDH2, in IDH1-mutant glioma cells, was able to reduce 2-HG production and cell growth *in vitro* and *in vivo*. This compound had no effect on DNA methylation but induced demethylation of H3K9me3 and restored the expression of genes associated with gliogenic differentiation (Rohle et al., 2013). Subsequently, novel molecules able to inhibit mutant IDH1 exhibiting promising selectivity also over wild type IDH1 have been reported (Wong et al., 2017). It has also been described a molecule, AG-221, able to suppress 2-HG levels in IDH2 mutant hematopoietic cells (Shih et al., 2014) and in murine models of *Idh2*-mutant leukemia. AG-221 ameliorates survival rate in primary human *IDH2* mutant acute myeloid leukemia xenografts, reversing the block in cellular differentiation conferred by high levels of 2-HG (Wang et al., 2013). AG-221 is also highly effective in decreasing plasma and bone marrow 2-HG levels in patients with *IDH2* mutant advanced hematologic malignancies, contributing to durable remission of the tumors. Finally, Kernytsky et al. (2015) reported that AGI-6780 (Wang et al., 2013), another selective inhibitor of mutant IDH2, determines histone and DNA demethylation *in vitro*, restoring the normal transcriptional profile that was altered by metabo-epigenetic alterations.

3.3. Acetyl-CoA

3.3.1. The relevance of acetyl-CoA in histone acetylation

Acetyl-CoA is a key metabolite that feeds the TCA cycle. Acetyl-CoA derives from glucose derived pyruvate in the reaction catalyzed by the pyruvate dehydrogenase complex or from fatty acid β-oxidation. Other sources of acetyl-CoA are ketogenic amino acids (leucine, isoleucine, tryptophan, tyrosine, threonine) and fatty acid β-oxidation. Cytosolic and nuclear levels of this metabolite are regulated by two enzymes: acetyl-CoA synthetase short-chain family 1 (AceCS1), converting acetate into acetyl-CoA, and ACLY, which converts cytosolic citrate into acetyl-CoA (Wellen et al., 2009). Acetyl-CoA is particularly relevant in correlating epigenetics and metabolism because is a substrate for HATs, whose activity is in fact dictated by the availability of acetyl-CoA. As mentioned above, this is modulated both by the expression of ACLY and by the level of available citrate as a substrate for this enzyme. Wellen et al. (2009) demonstrated that ACLY protein is also localized in the nucleus, where it plays a crucial role in providing a nuclear pool of acetyl-CoA. The same authors demonstrated that ACLY silencing in HCT116 cells suppresses histone acetylation, whilst no effect was observed after AceCS1 knockdown. Importantly, this study showed that acetylation status of non-histone proteins was unaltered in response to ACLY knockdown, highlighting the specificity of ACLY activity in regulating the production of acetyl-CoA required for histone acetylation. Lee et al. (2014) reported that acetyl-CoA is regulated by glucose availability in cancer cells and that global histone acetylation levels are regulated by the ratio of acetyl-CoA:coenzyme A. The authors also reported that the overexpression of oncogenic *Kras* (Kirsten rat sarcoma viral oncogene homolog) or *Akt* in mouse pancreas stimulated histone acetylation changes in the early stages of tumor development. They demonstrated that ACLY mediates the effects of Akt on histone acetylation and that phosphorylation of AKT on serine 473 correlates significantly with histone acetylation marks in human gliomas (AcH4) and prostate tumors (H3K18ac, H3K9ac, and H4K12ac). Another important factor involved in the regulation of histone acetylation is MYC, which has been shown to upregulate the expression of *HAT-GCN5* (Nishimura et al., 2015). MYC also mediates gene expression of metabolic enzymes linked to acetyl-CoA synthesis, including glycolysis and glutaminolysis. It has also been shown that *Myc* increased the

mitochondrial export of acetyl-groups through citrate in rat fibroblasts, favoring the incorporation of acetyl equivalents in histone H4K16 (Nishimura et al., 2015). These results thus highlight acetyl-CoA metabolism as a key determinant of epigenome remodeling in cancer cells, demonstrating that several oncogenic signals regulate specific gene expression trough the modulation of histone acetylation.

3.3.2. Therapeutic opportunities based on modulation of acetyl-CoA levels

Histone acetylation is regulated by the glycolytic flux in a dose dependent manner (Cluntun et al., 2015). It has been shown that accelerated glycolysis in cancer determines histone hyperacetylation via citrate and acetyl-CoA (Liu, Little, & Yuan, 2015). Thus, inhibition of glycolysis represents a potential pharmacological approach to modulate histone acetylation. Deoxyglucose (2-DG), a glucose analog that can be metabolized by the rate-limiting enzyme for glycolysis, hexokinase (HK), to form 2-deoxy-D-glucose-6-phosphate (2-DG-P), has been proposed as interesting molecule. 2-DG-P in fact cannot be further metabolized by phosphohexose isomerase (Chen & Gueron, 1992) leading to a feedback inhibition of HK. Interestingly, acetyl-CoA and acetylation levels of histone H3, H4, H2A and H2B are significantly reduced upon treatment with 2-DG in multiple cancer cell lines, determining compromised DNA repair capacity of cancer cells, which are then more susceptible to DNA-damaging therapeutics (Liu et al., 2015). Beyond epigenetics effects, we should mention that other therapeutic approaches targeting glycolysis were proposed. In this regard, Cantelmo et al. (2016) showed that inhibition of 6-phosphofructo kinase/2,6-fructose bisphosphatase 3 (PFKBF3), a mediator of glycolysis (Fig. 3B), in tumor endothelial cells induced tumor vessel normalization. Inhibition of glycolysis in these cells, in fact, reduced VE-cadherin (vascular endothelial- cadherin) endocytosis and inflammation and increased pericytes coverage, thus leading to healthier vessel architecture and integrity, that reduced tumor cell hypoxia and metastasis.

Another pathway frequently elevated in cancer is glutaminolysis, which generates α-KG and acetyl-CoA. As mentioned above, cancer cells rely on glutaminolysis to feed TCA cycle, to produce ATP and carbon precursors and as nitrogen donor for biosynthesis of macromolecules. This pathway also contributes to maintain redox homeostasis and reduces ROS production. Moreover, glutaminolysis has a role in epigenetic and metabolic rewiring of cancer cells by generating α-KG and acetyl-CoA. For these reasons, glutaminase (GLS), catalyzing the deamination of glutamine to glutamate, has been studied as drug target to modulate epigenetic status in cancer (Jin et al., 2016). Several inhibitors of GLS have been described, including bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide (BPTES) (Robinson et al., 2007) which decreases ATP production and increased ROS levels (6), CB-839 (currently undergoing Phase I dose escalation trials for solid and hematological malignancies), and compound 968 (Robinson et al., 2007). This compound alters histone H4K16 acetylation and histone H3K4 methylation leading to downregulation of numerous cancer-related genes in breast cancer cells (Simpson, Tryndyak, Beland, & Pogribny, 2012). Another inhibitor of GLS emerged from an unbiased screen by Elhammali et al. (2014) these authors identified a phosphodiesterase 5 inhibitor, Zaprinast, as a potent inhibitor of mutant IDH1-mediated 2-HG biosynthesis in HT1080 cells. However, they surprisingly reported that Zaprinast did not target mutant IDH1 as expected, but it efficiently suppressed GLS. The molecular mechanism of this drug is indeed not related to the modulation of histone acetylation, but to the ability to remodel histone methylation: since GLS-mediated glutaminolysis plays a crucial role in providing α-KG, Zaprinast treatment resulted in a marked reduction in histone H3K9me2 and H3K9me3 methylation. However, it is important to underlie that recent studies have downsized the enthusiasm about GLS inhibitors, as it has been proven that nutrient preferences are different in tumors *in vivo* compared with cultured cancer cells (Muir & Vander Heiden, 2018). In cultured lung and brain cancer cells, glutamine is in fact a primary source to feed metabolic pathways necessary for growth, but tumors formed from the same cells in mice are less relying on glutamine catabolism (Wolpaw & Dang, 2018). Also, genetic screens of human cells in culture and in xenograft tumors have shown that cancer cells require different metabolic processes depending on their microenvironment and that, consequently, microenvironment affects drug response (Yau et al., 2017). In this regard, inhibitors of GLS are able to slow the proliferation of most cancer cells in culture, but this is poorly predictive of tumor response to glutaminase inhibitors in human or mouse cancer models.

3.4. Oxygen

During the growth of solid tumors, cancer cells are exposed to reduced levels of both nutrient supplies and oxygen (hypoxia). To face up the reduced oxygen availability cancer cells promote angiogenesis, forming new blood vessels from surrounding host tissues. Particularly relevant during this process is the role played by Hypoxia-inducible factors (HIFs), that drive the transcriptome of cells to adapt to low-oxygen conditions (Semenza, 2014). These transcriptional events are epigenetically regulated as it has been reported that the histone demethylase genes JMJD1A, JMJD2B, and JARID1B are HIF targets, indicating that HIFs indirectly influence gene expression at the level of histone methylation under hypoxia (Krieg et al., 2010). It has been shown that hypoxia inducible JMJD1A is downregulated in clinical human germ cell-derived tumors, such as seminomas, yolk sac tumors, and embryonal carcinomas. The authors demonstrated that JMJD1A is not essential for stem cell self-renewal but acts as a tumor suppressor in opposition to the hypoxia-regulated oncogenic H3K9 methyltransferase G9a. Accordingly, loss of Jmjd1a results in increased tumor growth, while loss of G9a is related to reduced growth and results in smaller tumors (Ueda et al., 2014). These findings raise the possibility that hypoxia effectuates long-term adaptation to low-oxygen conditions by altering the epigenetic landscape of cancer cells.

4. Metabolomics and epigenetics in stem cells

Every cell type is characterized by a distinct metabolic phenotype and by unique epigenome landscape. Also, during the different stages of growth and differentiation, peculiar epigenomic remodeling dictates the acquirement of specific features contributing to cell development, fate and function (Festuccia, Gonzalez, & Navarro, 2017). This means that a "pluripotent epigenome" will maintain transcription of pluripotency-related genes, while the expression of the same genes will be switched off rapidly upon differentiation. At this step, in fact, reshaping the epigenome landscape will favor the activation of lineage-specific genes. During the physiological process of cellular maturation and differentiation cells are also exposed to various extracellular signals, including nutrient availability, and consequently they adapt their metabolic state (Vernardis, Terzoudis, Panoskaltsis, & Mantalaris, 2017). This metabolic rewiring in turns modulates various epigenetic processes, further influencing the acquirement of a specific phenotype. Recently, particular attention has been focused to the understanding of metabolic and epigenomic features that underlie the pluripotency of embryonic stem (ES) cells. In these cells chromatin is typically open and highly dynamic, while progression of differentiation and imprinting toward a specific cellular identity determines relevant changes in chromatin architecture (Boland, Nazor, & Loring, 2014). It is important to underline that two different pluripotent states are observed in embryonic stem cells: the first state is the ground or naïve state, characterized by an unlimited self-renewal capacity and by the ability to differentiate into tissues of all three germ layers. These cells also show pluripotency *in vivo*, as when they are injected into early embryos contribute to all somatic lineages including the germline. On the other hand, primed pluripotent stem cells also have unlimited potential to self-renew and differentiate into three germ layers *in vitro*, but they cannot give rise to germline chimeras (Kumari, 2016). Distinction of naïve and primed

embryonic stem cells is sustained also by different metabolic and epigenetic hallmarks.

4.1. Amino acids and their contribution to SAMe/SAH ratio

Metabolism in ES cells is strictly dependent on several amino acids, which play a key role in the epigenetic regulation of pluripotency. Among these, threonine is particularly relevant because it has been demonstrated that when cells are cultured in a threonine-depleted medium they proliferate slowly and show increased differentiation (Wang et al., 2009). Threonine catabolism contributes to cellular glycine, which is required for SAMe production via the SAMe cycle. It has been reported that mouse ES cells depleted of threonine from the culture medium or knocked-down for the enzyme threonine dehydrogenase (*Tdh*) showed reduction of SAMe levels (Alexander, Wang, & McKnight, 2011). The drop of SAMe level determines reduced H3K4me3 and consequently transcriptional repression (Shyh-Chang et al., 2013). Interestingly SAMe/SAH ratio and H3K4me3 level could be rescued by supplementing medium with threonine or with glycine and pyruvate. Another important amino acid for the survival and maintenance of ES cells is methionine. Shiraki and colleagues (Shiraki et al., 2014) reported that methionine deprivation from human ES cell culture medium induces higher cell death accompanied by reduction in SAMe levels and by a decreased expression of *NANOG*. Of note, NANOG is a transcription factor required for maintenance of pluripotency in ES cells (Mitsui et al., 2003). These authors also demonstrated that knockdown of the methionine adenosyltransferases, *MAT2A* and *MAT2B*, catalyzing the conversion of methionine to SAMe, similarly decreases cell number after 48 h. These results suggest that SAMe rather than methionine, is essential for cell survival. It has been shown that methionine deprivation decreases H3K4me3 and moderately reduces global DNA methylation, but these effects could be reversed upon supplementation with SAMe (Shiraki et al., 2014), confirming that SAMe is a major methyl donor in pluripotent cells and that reduction of SAMe levels impairs histone methylation. In this regard, it is important to bear in mind that metabolic homeostasis results from complex interactions of several pathways, influencing each other. Shyh-Chang et al. (2013) observed that after 6 h of threonine depletion a major metabolic remodeling occurred in the cells, marked by a reduction of NADH/NAD^+ ratio and of glycine levels and increased ATP, glucose-6-phosphate and fructose-6-phosphate levels. Thus, cell death could be the result of a rapid depletion of several nutrients leading to cell starvation and not simply the consequence of reduced SAMe levels. In support of this concept, it has been shown that human ES cells are able to replenish SAMe through recycling of homocysteine, which thus may be crucial in the modulation of cell survival (Harvey, Rathjen, & Gardner, 2016). These results demonstrate the relevance of essential amino acids in the modulation of pluripotency of stem cells. However, further investigations will be required to elucidate the mechanism whereby deprivation of these substrates potentiates differentiation. Apart from essential amino acids, glutamine plays a relevant role in the epigenetic regulation of stem cells physiology. Glutamine has been shown to regulate pluripotency and histone methylation (Ryu, Lee, Seong, & Han, 2015). Interestingly, naïve, but not primed, ES cells are able to proliferate in a glutamine deficient medium, even though cellular proliferation is slower upon glutamine withdrawal (Carey, Finley, Cross, Allis, & Thompson, 2015). In fact, Carey and coworkers demonstrated that this ability of naïve ES cells relies on the increase in glutamate production from glucose (Carey et al., 2015). These authors also reported that supplementation of medium of primed ES cells with precursors of glutamine synthesis enabled proliferation of these cells also in glutamine-depleted medium. This suggests that the transition from naïve to primed ES cells imposes a metabolic rewiring toward oxidative metabolism, which makes cells more dependent on glutamine availability and on an intense TCA cycle activity to support proliferation and to satisfy epigenomic requirements. Another amino acid important in the metabolic-epigenetic features of stem cells is L-proline. The amino acid L-proline has been shown to induce the differentiation of ES cells to pluripotent early primitive ectoderm-like (EPL) cells, characterized by low expression of ES-specific markers, and by increased expression of selective primitive ectoderm markers (Rathjen et al., 1999; Tan, Lonic, Morris, Rathjen, & Rathjen, 2011). Intriguingly, analysis of histone methylation patterns in L-proline-treated cells showed an epigenome remodeling marked by increased H3K9 and H3K36 methylation (Comes et al., 2013). These epigenetic alterations were sufficient to determine an embryonic-stem-to-mesenchymal-like transition, characterized by the switch from compacted/clustered ESCs to motile pluripotent stem cells. This transition was instead reverted by the addition of ascorbic acid through an unknown mechanism.

4.2. α-ketoglutarate: metabolite and epigenetic regulator of pluripotent stem cell identity

Since α-ketoglutarate is a key metabolite in physiology of stem cells and it is known to regulate DNA and histone methylation as a cofactor of JHDM and TET enzymes, we will briefly discuss its role in stem cell identity. *Tet1* and *Tet2* are highly expressed in mouse ES cells (Koh et al., 2011) and accordingly mouse ES cells show increased levels of 5hmC, that are instead reduced after differentiation (Walter, 2011). It has also been shown that *Jmjd1a (lysine (K)-specific demethylase 3A)* and *Jmjd2c (lysine (K)-specific demethylase 4C)* knockdown leads to mouse ES cell differentiation, by reducing the expression of ES cell-specific genes and inducing lineage marker genes (Loh, Zhang, Chen, George, & Ng, 2007). A study by Carey and coworkers elegantly demonstrated the relevance of α-KG in the maintenance of pluripotency in naïve embryonic stem cells, elucidating that these cells are characterized by higher α-KG/succinate ratio (Carey et al., 2015). Their experiments demonstrated that the pool of α-KG in naïve ES cells is sustained both by anaplerotic conversion of glutamine-derived glutamate and by glucose that can be converted into pyruvate and enters the TCA cycle. However, part of glucose derived α-KG exits TCA cycle and is converted into glutamate. Given the importance of α-KG in regulating the activity of histone demethylases, these authors tested whether glutamine deprivation affected histone lysine methylations. They demonstrated that cells cultured in glutamine-free medium exhibited reversible increase of trimethylation and reduction of monomethylation on H3K9, H3K27, H3K36 and H4K20, with changes in H3K4 methylations. On one hand, these results demonstrate the exceptional selectivity of α-KG-mediated epigenomic remodeling. On the other hand, the authors were able to show that the increase in H3K27me3 and H4K20me3 is reversed upon supplementation of culture medium with cell-permeable α-KG (Carey et al., 2015), and that this correlates with maintenance of ES cells in an undifferentiated state. These data demonstrate that the cellular α-KG/succinate ratio contributes to the ability of ES cells to suppress differentiation and that dynamic epigenome remodeling allows cells to be highly responsive to metabolite availability. More recently, TeSlaa et al. (2016) focused on the relevance of α-KG in primed pluripotent stem cells (PSCs) and demonstrated that the increased α-KG/succinate ratio accelerated primed PSC differentiation inducing global histone and DNA demethylation in primed PSCs. Conversely succinate levels delayed primed PSC differentiation and impaired global histone and DNA demethylation. These results highlight that α-KG, TET enzymes and JHDMs show a context-specific activity, and thus may play multiple roles in determining self-renewal and differentiation of stem cells. However, a full comprehension of mechanisms underlying the metabolic-epigenetic control of stem cells physiology is still lacking, thus future investigations should focus on the identification of novel actors involved in these processes. In this regard, it has been recently shown that phosphoserine aminotransferase 1 (*Psat1*), an Oct4/Sox2/Nanog (*Osn*) target protein, determines the fate of mouse ESCs (mESCs) by regulating α-KG availability (Hwang et al., 2016). These authors reported that a moderate knock down of *Psat1* was sufficient to impair levels of α-KG, thereby reducing DNA hydroxymethylcytosine

and increasing H3K9me3 and H3K36me3. This epigenetic remodeling was able to promote cellular differentiation. Based on these results the authors conclude that the maintenance of PSAT1 levels was essential for self-renewal and pluripotency. This enzyme is indeed particularly interesting because it converts glutamine-derived glutamate + glucose-derived 3-P-Hydroxy-Pyruvate to α-KG + 3-P-Serine, thus linking pathways related to two key metabolites (glucose and glutamine) and epigenetic remodeling.

4.3. Glucose-dependent regulation of epigenetic remodeling in pluripotent stem cells

Another distinctive feature of ES cells is high glycolytic flux. This metabolic pathway in fact drives the production of acetyl-CoA, another relevant metabolite with epigenetic function. Moussaieff et al. (2015) reported that the high rate of glycolysis in pluripotent cells maintains acetyl-CoA levels two-fold higher than those found in differentiating cells and that a rapid loss of this glycolytic activity occurs when cells undergo differentiation. Downstream glycolysis, pyruvate is converted to acetyl-CoA that enters the TCA cycle and, after condensation with oxaloacetate, it forms citrate. This citrate can be exported from mitochondria to cytosol, thus contributing to cytosolic pool of acetyl-CoA (Wellen et al., 2009). Accordingly, it has been reported that acetyl-CoA levels were significantly reduced upon mouse ES cell differentiation, and that the supplementation of acetate to differentiating human ES cells was able to delay cell differentiation (Moussaieff et al., 2015). Since acetyl-CoA can be used for histone acetylation, in ES cells undergoing differentiation they also reported reduced H3K9/K27 acetylation, leading to gene inactivation. Interestingly acetate was able to block early histone deacetylation in a dose-dependent manner. These results show a fast metabolic rewiring occurring during the first hours of differentiation, further demonstrating the importance of metabolic fluxes in defining the epigenetics of pluripotent stem cells.

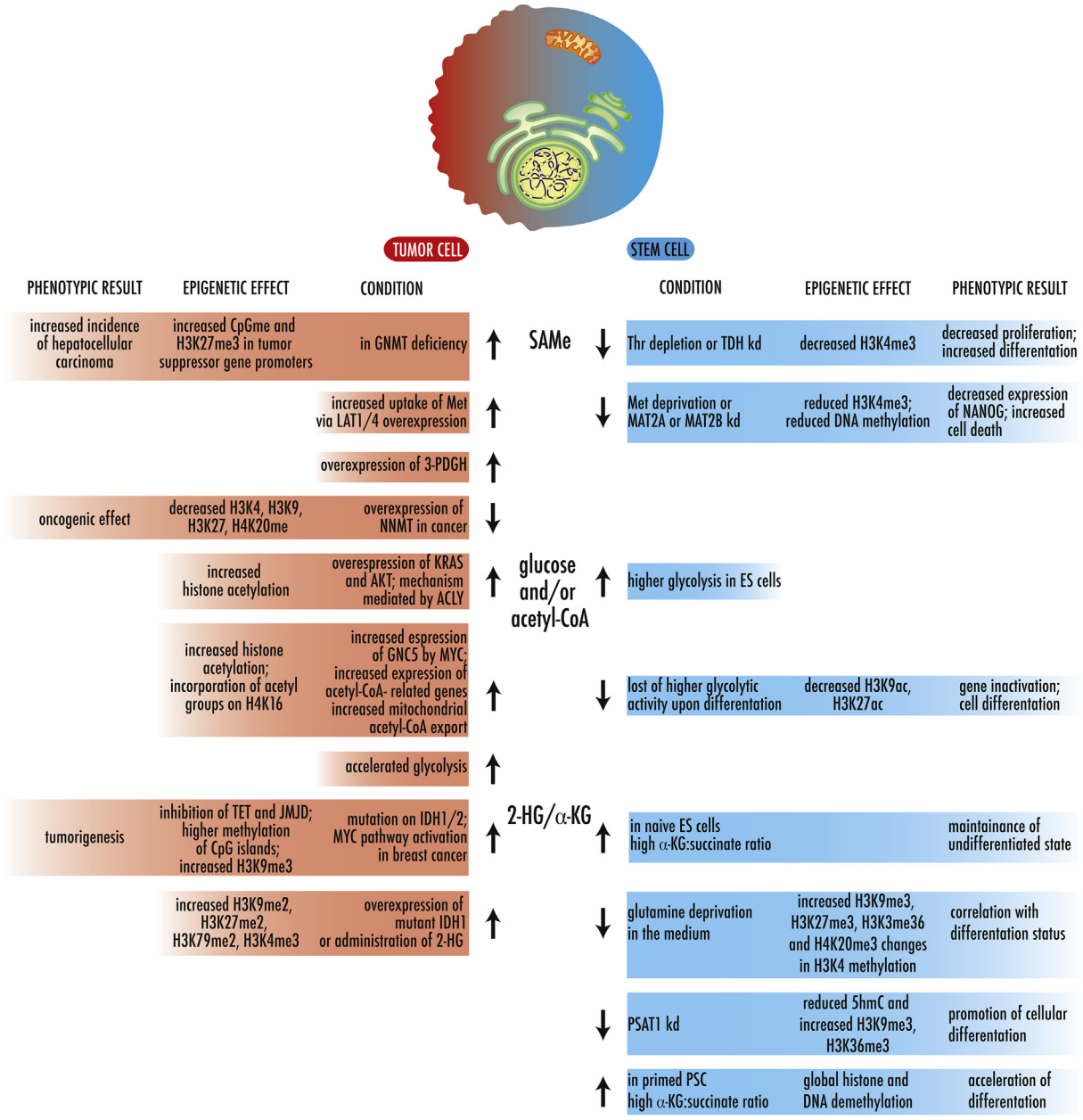

Fig. 4. Similarities and differences between tumor and stem cells. Comparison of the levels of SAMe, acetyl-CoA and 2-HG/α-KG between tumors and stem cells. These "epigenetic" metabolites drive chromatin remodeling events that confer specific phenotypic effects.

4.4. Epigenetic control of stemness pathways in cancer stem cells

Some of the key pathways of stemness crucial for embryo development, including Hedgehog (Hh) and wingless-Int1 (Wnt) pathways, are usually dormant in adult tissues, but they can be pathologically activated contributing to the progression of multiple forms of cancer (Wils & Bijlsma, 2018). It has been shown that epigenetics contribute to regulate these pathways by controlling production of ligands and by modulating extra– and intracellular pathway members. It is known that expression of the main Hh paralog, Sonic Hedgehog (SHH) is driven by Nuclear factor-κB (NF-κB) (Nakashima et al., 2006), and it has been demonstrated that DNA hypomethylation of the promoter region of SHH containing the binding site for NF-κB is correlated with upregulation of SHH in some type of cancer. Hypomethylation favors NF-κB binding thereby resulting in increased SHH production (Duan et al., 2015; Wang, Hsu, Feng, & Huang, 2012). Interestingly, hypermethylation of SHH promoter has been observed in different breast cancer cell lines and it has been correlated with an abrogation of the “classic” SHH expression (ten Haaf et al., 2011). These apparently controversial results have been explained by the study of the promoter region of the SHH gene, that showed the presence of two distinct transcription start sites (TSS). Promoter methylation of the predominant TSS leads to a shift of the transcription machinery to the alternative transcription start site, resulting in an elongated version of the SHH ligand precursor. CpG methylation seems to play an important role also in the regulation of Wnt signaling: WNT5A, WNT9A and WNT10 B promoters are frequently hypermethylated in colorectal cancer cell lines and tumors. Furthermore, also histone modification may affect the expression of these gene, but the effects of these modifications on Wnt ligand levels are still under debate and may depend on cancer type studied and by the availability of receptors (Wils & Bijlsma, 2018).

Other authors have demonstrated that the expression of core pluripotency factors OCT4 and NANOG is regulated by hypoxia-mediated epigenetic modifications in glioma cells. Particularly, it has been demonstrated that the expression of TET1 and 3 is induced upon hypoxia in these cells and that they can bind the Oct4 and Nanog regulatory regions. This contributes to the proliferation of cancer stem cells in gliomas (Pankaj, Arora, Jonita, Sujata, & Tapasya, 2017). These experimental evidences highlight the importance of epigenetics in the control of stemness pathways, and how epigenetic changes could contribute to the aberrant activation of processes that are usually silenced in physiological condition, contributing to the onset of different type of cancers. Currently, a link between metabolite availability and epigenetic-mediated activation of stemness pathways has not been provided, nonetheless given the strict connection between metabolism and epigenetics it is possible to speculate that future investigations will address this issue, potentially identifying novel targets for the treatment of forms of cancers related to the re-activation of stemness genes.

5. Conclusions

The numerous studies cited in this review highlight the tight interconnection between metabolism and epigenetics, in multiple pathophysiological contexts. Moreover, the comparison of several studies depicted common mechanisms involving metabolic-epigenetic regulation of differentiation status both in cancer and in stem cell (Fig. 4). For this reason, a full elucidation of molecular mechanisms and dynamics orchestrating cellular epigenetic responses to metabolite availability offers a unique possibility to explore novel therapeutics avenues in different medical fields, including metabolic disorders and cancer. Also, the emerging evidence of a crucial role played by metabolic-epigenetics in the control of embryonic stem cell pluripotency could pave the way for innovative approaches in multiple aspects of regenerative medicine, including regeneration of cardiac tissue and cartilage, but also of pancreatic β-cells to treat type 1 diabetes (Nishimura et al., 2015). Other potential outcome from the manipulation of stem cells metabolism/epigenetics may include treatment of glaucoma and infertility and restoration of heart rhythm trough differentiation of ES cells into pacemaker cells (Nishimura et al., 2015). Novel and in depth studies of the tight connection of metabolism and epigenetics made possible by technological innovations, including next generation sequencing, metabolomics and metabolic tracing, represent an exciting challenge for the modern medicine. The elucidations of novel mechanisms will potentially facilitate the design of innovative "metabolism-based" pharmacological tools in the treatment of severe invalidating pathological conditions with a known epigenetic basis.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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