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Review

The TCA cycle as a bridge between oncometabolism and DNA transactions in cancer

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ABSTRACT

Cancer cells exploit metabolic rearrangements for sustaining their high proliferation rate and energy demand. The TCA cycle is a central metabolic hub necessary for ATP production and for providing precursors used in many biosynthetic pathways. Thus, dysregulation of the TCA cycle flux is frequently observed in cancer. The identification of mutations in several enzymes of the TCA cycle in human tumours demonstrated a direct connection between this metabolic pathway and cancer occurrence. Moreover, changes in the expression/activity of these enzymes were also shown to promote metabolic adaptation of cancer cells. In this review, the main genetic and non-genetic alterations of TCA cycle in cancer will be described. Particular attention will be given to extrametabolic roles of TCA cycle enzymes and metabolites underlying the regulation of nuclear and mitochondrial DNA transactions.

1. The TCA cycle

Mitochondria are the power house of cells providing adenosine triphosphate (ATP), the high energetic compound necessary for most of the endergonic metabolic reactions. Indeed, the inner mitochondrial membrane harbours protein complexes deputed to the transport of electrons (electron transport chain, ETC), indispensable for the mitochondrial membrane potential, the driving force for ATP production. The electrons carried along the ETC mainly derive from the reducing cofactors (*i.e.* NADH and $FADH_2$) generated by the tricarboxylic acid (TCA) cycle (also known as citric acid cycle or Krebs cycle) in the matrix of mitochondria [1].

The TCA cycle occupies a central position in metabolism and meets most of cell energy requirement by the complete oxidation of acetyl-CoA, a key product in the catabolism of carbohydrates, fatty acids and amino acids, to CO_2. For this to be achieved, the activity of the citrate synthase (CS) is fundamental to combine acetyl-CoA, obtained from both glycolysis-derived pyruvate and fatty acid β-oxidation, with oxaloacetate (OA) to form citrate. This latter is in turn rearranged to isocitrate by aconitase 2 (ACO2), also designated as mitochondrial aconitase to distinguish it from the cytosolic isoform ACO1. Then, isocitrate is decarboxylated to α-ketoglutarate (α-KG) by the isocitrate dehydrogenase (IDH) enzymes. IDH enzymes consist of two classes: IDH1 and IDH2, $NADP^+$-dependent, and IDH3, NAD^+-dependent. IDH1 localizes in the cytosol while IDH2 and IDH3 actually participate to the TCA cycle being located in the mitochondrion. A further decarboxylation performed by the α-KG dehydrogenase (α-KGDH) complex drives the conversion of α-KG to succinyl-CoA. This ensemble of reactions is responsible for producing two molecules of NADH per molecule of acetyl-CoA. The following reactions of the cycle are deputed to the oxidation of succinyl-CoA to OA, thus regenerating the starting molecule which allows the cycle to repeat. In particular, succinyl-CoA synthetase (SCS) releases guanosine triphosphate (GTP) and succinate. The latter is oxidized to fumarate by the succinate dehydrogenase complex (SDH) with a concomitant reduction of FAD to $FADH_2$. After fumarate hydration to malate by the fumarate hydratase (FH), malate dehydrogenase 2 (MDH2) finally catalyses the oxidation of malate to OA producing NADH [1] (Fig. 1).

Notably, most of the reactions of the TCA cycle are reversible apart from those performed by CS and the α-KGDH complex [1,2]. This contributes to a more dynamic and versatile vision of the cycle that is further supported by the involvement of TCA cycle substrates in accessory reactions. Indeed, many intermediates of the TCA cycle can be replenished by anaplerotic reactions. Instead, when intermediates are drawn off as precursors in biosynthetic pathways (cataplerotic reactions), the complete cycle does not operate. For instance, citrate is exported across mitochondrial membrane and converted to acetyl-CoA in the cytosol by the ATP citrate lyase (ACLY), starting lipid biosynthesis. Notably, α-KG and OA represent key intermediates in the TCA cycle plasticity. In fact, α-KG can enter the TCA cycle through glutamate

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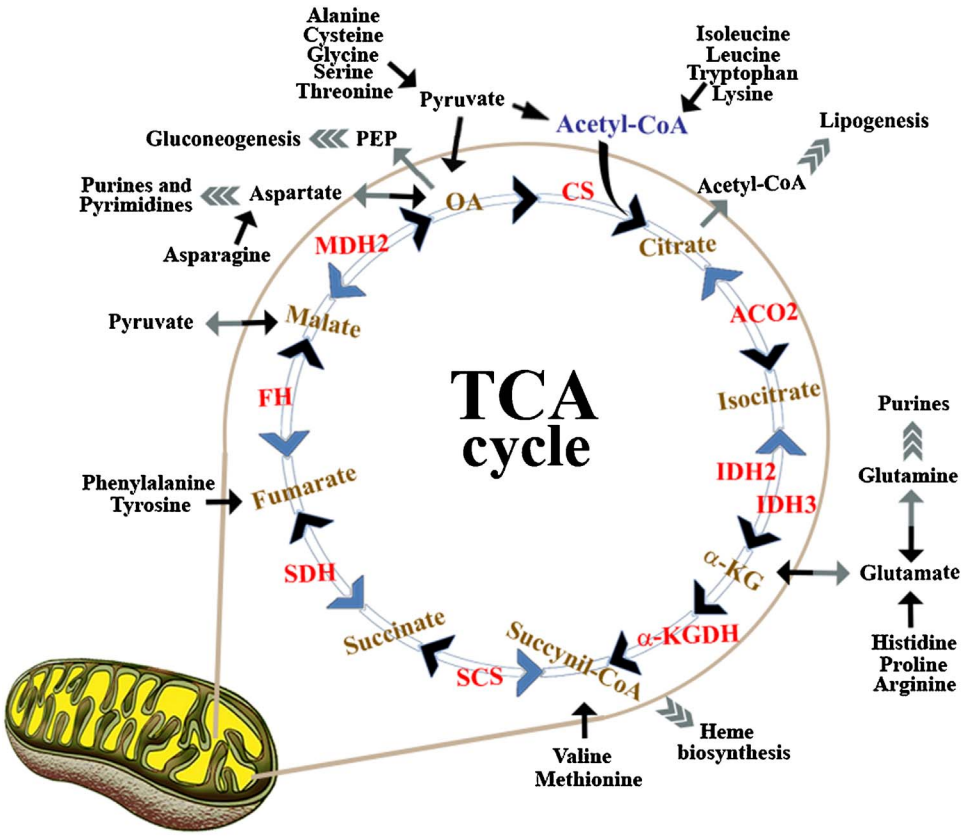

Fig. 1. Schematic overview of the TCA cycle. Black arrowheads indicate the "canonical" direction of metabolites through the cycle starting from pyruvate-derived acetyl-CoA. Blue arrowheads indicate the reverse steps of TCA cycle reactions. The enzymes and the metabolites of the cycles are depicted in red and brown, respectively. Black arrows indicate the anaplerotic reactions, including the entry of amino acids into the TCA cycle and the conversion of pyruvate into oxaloacetate or malate. Finally, grey arrows and arrowheads indicate cataplerotic reactions for the biosynthesis of lipids, glucose, nucleotides and heme group. α-KG: α-ketoglutarate; α-KGDH: a-ketoglutarate dehydrogenase; ACO2: aconitase 2; CS: citrate synthase; FH: fumarate hydratase; IDH2/3: isocitrate dehydrogenase 2/3; MDH2: malate dehydrogenase 2; OA: oxaloacetate; PEP: phosphoenolpyruvate; SCS: succinyl-CoA synthetase; SDH: succinate dehydrogenase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

deamination by the reversible reaction of glutamate dehydrogenase. On the other side, α-KG, in combination with aspartate, may provide glutamate and OA by the activity of aspartate transaminase. OA may also derive from carboxylation of pyruvate by pyruvate carboxylase or be converted to phosphoenolpyruvate (PEP) by the PEP carboxykinase contributing to gluconeogenesis [1,3] (Fig. 1).

2. Alterations of TCA cycle in cancer

Given the paramount importance of the TCA cycle in the maintenance of cell homeostasis, its contribution to the onset of several diseases is quite predictable. For instance, impairment of the TCA cycle has been linked to pathological conditions ranging from neurodegeneration to diabetes [4–6]. However, its involvement in carcinogenesis has remained elusive for long time until recent years, when a causal connection between dominant mutations of some enzymes of the TCA cycle and cancer occurrence was described. This review will provide a snapshot of the most characterized cancer-linked alterations of TCA cycle enzymes, both in terms of genetic mutations and changes in expression levels (Table 1), and of the molecular mechanisms by which they contribute to tumour formation and progression. Moreover, the impact of TCA cycle deregulation on the control of epigenetic events and mitochondrial DNA (mtDNA) maintenance will be discussed.

Although no specific mutation on *CS* gene has been found up to date, its expression is frequently altered in some tumour types (Table 1). Indeed, increased CS levels were found in ovarian malignant tumours and cancer cell lines and they were associated with drug resistance. In particular, *CS* silencing by RNA interference resulted in dampened cell proliferation, migration and invasion indicating that the enzyme may play a major role in cancer progression [7]. This result is also corroborated by previous studies revealing augmented CS activity during ovarian cancer progression [8] and in pancreatic tumour specimens compared to non-tumour adjacent tissues [9]. On the other side, low protein levels of CS were described in some cervical cancer cell lines [10]. The explanation of this discrepancy can be found in CS enzymatic role as it irreversibly produces citrate, which can also prime lipid biosynthesis once extruded from mitochondria. Hence, lipid metabolism-depending tumours, such as pancreatic ones, may need to rely on augmented CS activity. Conversely, decreased expression of *CS* can be employed by those tumours that need to enhance glycolytic rates in aerobic conditions at the expense of mitochondrial oxidative metabolism [10]. This phenomenon, known as aerobic glycolysis or "Warburg effect", is a metabolic hallmark of many tumours [11].

The importance of citrate in tumour metabolism is also confirmed in prostate cancer cells, which display a peculiar use of this metabolite. Indeed, while normal epithelial prostate cells inhibit ACO2 activity to

Table 1
List of typical alterations of TCA cycle enzymes in human cancer.

Gene	Alteration	Tumour	References
CS	Overexpression/Increased Activity	RO, Pancreatic and Ovarian Cancer	[7,9,90]
ACO2	Overexpression/Increased Activity	Prostate Cancer	[12,91]
	Down-regulation	Gastric Cancer, GCC	[14,15]
IDH2	Somatic Mutations	AML, AITL, Glioma, Osteosarcoma	[18,19,92–94]
	Down-regulation	Gastric Cancer	[27]
*SDHs**	Germline Mutations	PCC/PGL, RCC, GIST	[20,21,95,96]
FH	Germline Mutations	MCUL, HLRCC, PCC/PGL, LCT, ccRCC	[24,25,97–99]
	Down-regulation	GCC, GM	[14,100]
MDH2	Overexpression	Prostate Cancer	[43]

(*) *SDHA-D* subunits. Abbreviations: AITL: Angioimmunoblastic T-cell lymphomas; AML: Acute myeloid leukemia; ccRCC: conventional clear renal cell carcinoma; GCC: Gastric cardia cancer; GIST: Gastrointestinal stromal tumour; GM: Glioblastoma multiforme; HLRCC: Hereditary leiomyomatosis and renal cell cancer; LCT: Leydig cell tumors; MCUL: Multiple cutaneous and uterine leiomyomas; PCC/PGL: Pheochromocytoma and paragangliomas; RCC: renal cell carcinoma; RO: Renal Oncocytoma.

produce and secrete large amounts of citrate, malignant transformation is accompanied by reactivation of ACO2 [12]. Strikingly, lower citrate levels are indicative of high ACO2 activity in prostate and can be considered a reliable tumour marker in this tissue [13]. In contrast to this cancer-supporting function, *ACO2* was significantly decreased both at the mRNA and protein levels in the clinical records of a large number of gastric cancer and gastric cardia cancer specimens [12,14] (Table 1). The observed ACO2 level alterations were associated with increased aggressiveness and poor prognosis [15]. It is noteworthy that human pluripotent stem cells (hPSCs), like diverse tumour types, use aerobic glycolysis to produce ATP and that down-regulation of *ACO2* contributes to this metabolic feature in hPSCs [16].

The first body of evidence of a direct connection between TCA cycle defects and cancer came from the observation that many tumours arise from genetic mutations of *IDH2*, *SDH* subunits and *FH* [17]. Somatic *IDH2* mutations are frequently observed in acute myeloid leukaemia (AML) [18,19]. *SDH* deficiency characterizes more than 7% of total gastrointestinal stromal tumours (GIST) [20–22], while germline *SDH* mutations are responsible for hereditary paragangliomas and adrenal gland pheochromocytomas [23]. Loss of *FH* has been identified in conventional clear renal cell carcinoma (ccRCC) [24] and germline *FH* mutations are typical of hereditary leiomyomatosis and renal cell cancer (HLRCC), which is a syndrome characterized by growth of fibrotic benign tumours in the skin and increased incidence of kidney carcinoma [25]. Besides frequent mutations, also expression levels of all these enzymes are altered in several malignancies [14,26,27] (Table 1).

Germline *SDH* and *FH* mutations can be found throughout the gene and generally cause the expression of inactive enzymes or their total loss, with consequent accumulation of succinate and fumarate, respectively [28]. On the contrary, *IDH2* gene mainly harbours somatic and monoallelic mutations causing changes in the amino acid residues Arg172 or Arg149 [18,19]. Notably, also the cytosolic IDH1 isoform is frequently mutated at the Arg132 residue in glioma [17,29]. Mutated IDHs acquire a neomorphic catalytic activity allowing them to convert α-KG into the R-enantiomer of 2-hydroxyglutarate (R-2HG), which can raise up to millimolar concentration thus becoming an oncometabolite [30]. Surprisingly, other mechanisms than *IDH* mutations have been discovered for 2HG accumulation, such as MYC-driven metabolic reprogramming in breast cancer [31] and hypoxic conditions in paediatric glioblastoma cells [32].

The most well characterized oncogenic effect of 2HG accumulation is the inhibition of α-KG-dependent dioxygenase enzymes, among which the prolyl hydroxylases domain proteins (PHDs) play a role in tumour metabolic adaptations. A part of these enzymes includes negative regulators of the transcription factor hypoxia-inducible factor-1α (HIF-1α), which drives the hypoxic response known to promote metabolic rearrangements, angiogenesis and metastasis [17]. Upon adequate oxygen levels, HIF-1α is repressed through the hydroxylation of two proline residues catalysed by O_2-dependent PHDs, with a concomitant oxidation of α-KG to succinate [33]. Following hydroxylation, HIF-1α becomes a substrate for the E3-ubiquitin ligase von Hippel-Lindau tumour suppressor protein (pVHL) and committed to proteasomal degradation [34]. Under hypoxic conditions HIF-1α is stabilized and localizes into the nucleus where it promotes the transcription of target genes orchestrating the hypoxic response [34]. Constitutive HIF-1α activation under normal oxygen levels can occur in cancer, as observed in *IDH1/2* mutated tumours, and this condition is known as pseudohypoxia [35].

α-KG-dependent dioxygenases are not only inhibited by aberrant products of the TCA cycle, as in the case of 2HG, but even by canonical intermediates when present in altered concentrations. In fact, since succinate and fumarate are structurally similar to α-KG, their accumulation elicits a pseudohypoxic response in *SDH* and *FH* mutated tumours, respectively [28,36,37]. Moreover, a recent report has demonstrated that the unexpected decrease of α-KG levels due to the overexpression of the α subunit of the heterotetrameric IDH3 complex was also able to promote HIF-1 activation. Notably, increased IDH3α levels were associated with poor prognosis in various cancer types [38].

Therefore, the setting of a HIF-driven pseudohypoxic environment seems to represent a common feature of several defective TCA cycle enzymes in cancer. However, this hypothesis has been threatened by two recent studies arguing for other mechanisms than HIF-1α activation. In fact, it has been also shown that the PHD/HIF pathway was not responsible for the development of renal cysts, a hallmark of the *FH*-deficiency associated tumours [39]. Moreover, another report suggested that R-2HG can activate PHDs rather than inhibiting them [40], thus paradoxically favouring HIF-1α degradation. This raises the possibility that some effects of mutated IDHs may be independent of HIF in certain contexts and may possibly involve other α-KG-dependent dioxygenases. For instance, it was shown that brain-specific IDH1 Arg132 mutation knock in mice caused R-2HG-mediated inhibition of collagen prolyl 4-hydroxylases, thus impairing the correct maturation of collagen. This resulted in an altered membrane basement and consequent glioma progression [41]. An alternative mechanism is also provided by the deregulation of α-KG-dependent dioxygenases involved in epigenetic control (see below).

Finally, also a role for MDH2 in cancer has recently emerged (Table 1). Besides a report highlighting a decrease of *MDH2* expression in pheochromocytoma/paraganglioma due to a germline mutation [42], others mainly claim that overexpression of MDH2 in cancer is implicated in resistance to chemotherapy. In particular, high levels of MDH2 in prostate tumours are associated with a reduction of the relapse-free survival period of patients after chemotherapy. Consistently, *in vitro* experiments in prostate cancer cells showed that *MDH2* silencing leads to docetaxel-mediated apoptosis [43]. Similarly, *MDH2* abrogation impaired cell viability in doxorubicin-resistant uterine cancer cells but in this case the action was independent of the apoptotic pathway [44].

3. Influence of TCA cycle on DNA transactions in cancer

Multiple lines of evidence shed light on TCA cycle enzymes and metabolites in the regulation of both nuclear and mitochondrial DNA transactions, including replication, repair, and transcription. Primarily, it is commonly known that alterations of mitochondrial metabolism can lead to excessive oxidative DNA damage due to higher production of ROS [45]. In this context, mutations of *SDH* subunits in cancer have been shown to increase steady-state level of superoxide, which was associated with increased mutation frequency and thus genome instability [46,47]. More recently, also DNA repair mechanisms have been described to be affected by defective TCA cycle. In fact, accumulation of R-2HG due to tumour-derived *IDH1/IDH2* mutations inhibits the activity of α-KG-dependent alkB homolog (ALKBH) DNA repair enzymes, which remove methylated lesions of DNA caused by alkylating agents [48].

The impact of TCA cycle on other DNA transactions has been largely investigated especially in the context of transcriptional regulation, where it mainly concerns epigenetic regulatory mechanisms. In addition, numerous reports have also shown that TCA cycle enzymes could contribute to mtDNA homeostasis. Hence, hereafter, the influence of TCA cycle on epigenetics and mtDNA copy number in cancer will be discussed.

3.1. Epigenetics

Epigenetic mechanisms, particularly covalent modification of DNA and histones, are involved in the organization of chromatin structure thus regulating gene transcription and genome stability. DNA epigenetic modifications mainly occur on cytosines present at the CpG dinucleotides. The non-random addition of a methyl group on cytosines (5-methylcytosine-5mC) by the DNA methyltransferase enzymes shapes DNA methylation patterns, the distribution of which is the results of co-

regulated epigenetic events. In particular, the Ten-eleven translocation (TET) family of DNA hydroxylases catalyses the stepwise oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), which function as proper epigenetic marks and intermediates of DNA demethylation as well. As far as concerns histones, several residues of N-terminal histone tails undergo different types of epigenetic modifications (*e.g.* acetylation, methylation), the turnover of which is controlled by the activity of modifying and de-modifying enzymes [49,50].

The sophisticated cross-talk between DNA and histone epigenetic modifications arranges transcriptional programs assuring cell type identity. Deregulation of epigenetic events, which can depend on altered expression/activity of epigenetic machinery or of their cofactors [51–54], has been implicated in physiological and pathological conditions such as aging and cancer [55]. Notably, malignant cells undergo extensive epigenetic reprogramming that sustains transformation mining transcriptional networks and genome stability. In particular, a typical feature of cancer cells is the silencing of tumour suppressor genes mediated by DNA hypermethylation and repressive histone marks. Beyond the aberrant expression of epigenetic enzymes and/or readers of epigenetic marks, the metabolic dysfunctions occurring in cancer may trigger alteration of epigenetic landscapes. In fact, diet, environment and lifestyle are well-known drivers of epigenetic events because substrates and cofactors of enzymes involved in epigenetics originate from metabolic pathways [50,56].

Looking at the TCA cycle-related enzymes, the impact of *IDH2* mutations on epigenetic events is now well established. In fact, the aberrant production of the oncometabolite R-2HG impinges on the activity of epigenetic enzymes that utilize α-KG as cofactor. Among those, TET enzymes are α-KG/Fe(II) dioxygenases whose activity is inhibited by R-2HG in a competitive manner (Fig. 2). In particular, R-2HG has been demonstrated to inhibit *in vitro* both TET1 and TET2, with a more pronounced effect against TET2 catalytic activity [57]. Consistently, overexpression of IDH2 mutants determined 5hmC loss [57,58], which is now recognized as an epigenetic hallmark of cancer [50]. A decrease of wild-type IDH2 protein can also account for 5hmC decline as demonstrated in gastric cancer cells [27]. It is worth mentioning that tumours bringing *IDH2* mutations can exhibit the molecular epigenetic feature named “hypermethylator phenotype”. This phenomenon consists of a simultaneous and directional methylation of multiple gene promoters [59,60], that can distinguish a subtype of tumour with specific clinical features [61]. Notably, the hypermethylator phenotype evidenced in AML with mutated *IDH2* mirrors the epigenetic profile observed when *TET2* is mutated in the same tumour type [58], thus confirming the functional connection of TET enzymes with α-KG-producing pathways. Moreover, the evidence that *IDH1/2* and *TET2* mutations are mutually exclusive in AML strongly suggests that TET2 enzyme is a key pathogenic target of R-2HG [58].

Beyond TET enzymes, also some histone lysine demethylases (KDMs) are α-KG-dependent enzymes and thus negatively influenced by R-2HG accumulation (Figure 2). Consistently, IDH mutants overexpression impaired the activity of the H3Lys9 demethylase KDM4C and this events was associated with a failure of cell differentiation [62]. A crystallographic study of CeKDM7A, a *Caenorhabditis elegans* histone demethylase, in association with α-KG or R-2HG demonstrated that these molecules adopt a comparable orientation in the catalytic core of the enzyme in coordination with the Fe(II), and this justifies their competitive nature [57]. In the same way, also the activities of KDM2A, a human histone H3Lys36 demethylase, and of KDM5B, a H3Lys4 specific demethylase, were affected by R-2HG [57]. Experiments performed using oxalomalate, a competitive inhibitor of IDHs, demonstrated an increase of several histone methylation marks further supporting that α-KG production is necessary for maintaining epigenetic enzyme activity [57].

As observed for PHD enzymes, also α-KG-dependent dioxygenases involved in epigenetics are inhibited by elevated levels of fumarate and succinate in *FH* and *SDH* mutated tumours [63,64] (Fig. 2). Moreover, *SDH* mutations in paragangliomas were also frequently associated with nuclear exclusion of TET1 protein [65] and were able to establish a hypermethylator phenotype. Surprisingly, the hypermethylator phenotype was stronger in tumours with *SDHB* mutations, which show poor prognosis probably as consequence of DNA methylation-mediated repression of anti-metastatic genes [66]. Abrogation of TET enzymatic activity by fumarate accumulation instead mediated the activation of epithelial-to-mesenchymal transition (EMT) pathways via silencing of *miR-200* [67]. EMT was also demonstrated to occur in ovarian cancer cells after *SDHB* silencing, which led to histone hypermethylation due to the up-regulation of genes involved in the metabolism of S-adenosyl methionine (SAM), the methyl donor of histone and DNA methyltransferase enzymes [26].

A different kind of epigenetic mechanism, partially dependent on TCA cycle activity, is histone acetylation, a modification associated with active gene expression. Acetylation is introduced by the histone acetyltransferase enzymes, which catalyse the transfer of acetyl group from acetyl-CoA to lysine residues. One of the sources of acetyl-CoA is citrate. Indeed, exogenous administration of citrate is able to increase global levels of H3 and H4 acetylation [68]. Glucose-derived citrate is produced by CS in mitochondria and, once exported to the cytosol, it is converted into acetyl-CoA by ACLY. Notably, ACLY is frequently overexpressed in many kinds of tumours [69,70] and histone acetylation dependent on ACLY-derived acetyl-CoA was shown to contribute to the transcription of glucose metabolism genes, including *glucose transporter 4*, *hexokinase-2*, *phosphofructokinase-1* and *lactate dehydrogenase A* [71]. These observations suggest that epigenetic modulation of histone acetylation by ACLY may sustain aerobic glycolysis in cancer cells. On the other hand, metabolic alterations based on KRAS-AKT oncogenic pathways have been shown to induce histone hyperacetylation through ACLY, thus favouring cancer epigenetic reprogramming [72] (Fig. 2).

3.2. mtDNA

Mitochondrial genome consists of double-stranded circular DNA compacted in a nucleoprotein complex known as mitochondrial nucleoid. The mtDNA encodes for 13 essential components of oxidative phosphorylation complexes and for several rRNAs and tRNAs. Genetic mutations or decrease in mtDNA content are responsible for several neuropathies and contribute to the aging process [73]. Moreover, a number of papers have also highlighted an association between risk of cancer development and alterations of mtDNA sequence and content. Detrimental mtDNA mutations occurring in cancer have been identified in genes for subunits of each respiratory chain complex as well as in regulatory regions [74]. On the other hand, variations in mtDNA copy number are dependent on tumour-associated mutations of nuclear-encoded genes involved in mtDNA replication and repair, including mitochondrial transcription factor A (TFAM) and p53 [75–77]. A recent study performed on 22 tumour types has disclosed that most of them undergo loss of mtDNA in comparison to normal surrounding tissue and this event was frequently associated with down-regulation of respiratory genes [78].

Among the proteins that contribute to the maintenance and integrity of mtDNA, also some TCA cycle enzymes have to be included. Studies performed on *Saccharomyces cerevisiae* have identified aconitase, IDH, subunits of α-KGDH and SCS complexes as components of the mitochondrial nucleoid structure [79,80]. Absence of most of these genes in yeast produced severe growth defects that were associated with loss of mtDNA [81]. In particular, aconitase regulates mtDNA stability similarly to the abundant mtDNA packaging protein ARS-binding factor 2 (Abf2p), the yeast ortholog of TFAM. Consistently, the degree of association of aconitase with mitochondrial nucleoids mirrors the one of Abf2p and the constitutive expression of aconitase is able to rescue the mtDNA defects arising from genetic ablation of Abf2p [82,83]. Moreover, aconitase is able to preserve the integrity of mtDNA

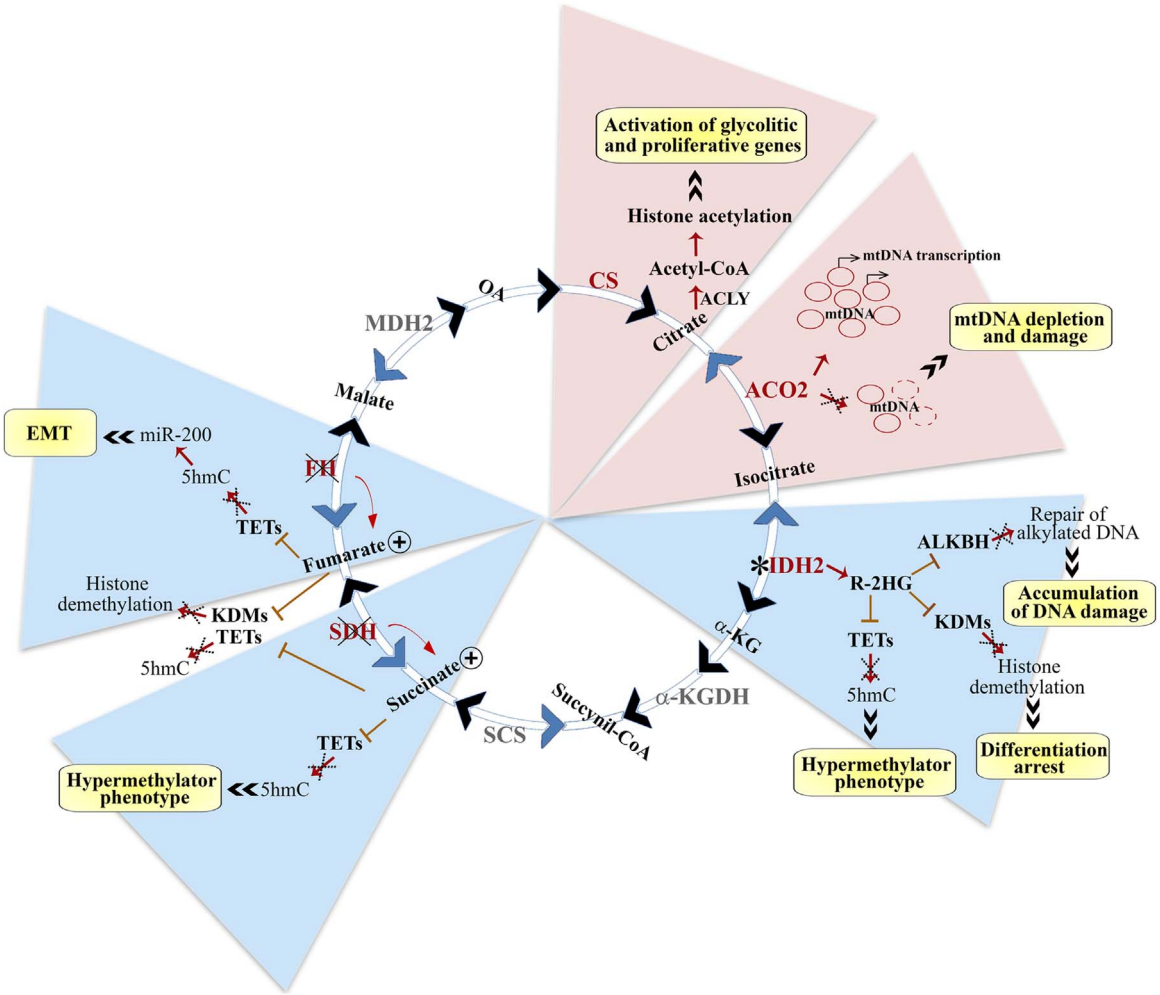

Fig. 2. Impact of altered TCA cycle on DNA transactions in cancer. Schematic representation of the enzymes deregulated in cancer (red text) with proven/potential effects on biological processes including DNA transactions (yellow boxes). In particular, the inhibition of α-KG-dependent enzymes by R-2HG, succinate and fumarate in cancer and the impact of citrate on histone acetylation are shown. The role of ACO2 in the control of mtDNA is also reported. Violet background: genes with altered expression in cancer; light blue background: genes mutated in cancer. *: neomorphic mutation. 5hmC: 5-hydroxymethylcytosine; α-KG: α-ketoglutarate; α-KGDH: α-ketoglutarate dehydrogenase; ACLY: ATP citrate lyase; ACO2: aconitase 2; ALKBH: alkB homolog; CS: citrate synthase; EMT: epithelial-mesenchymal transition; FH: fumarate hydratase; IDH2: isocitrate dehydrogenase 2; KDMs: histone lysine demethylase enzymes; MDH2: malate dehydrogenase 2; mtDNA: mitochondrial DNA; OA: oxaloacetate; R-2HG: R-enantiomer of 2-hydroxyglutarate; SCS: succinyl-CoA synthetase; SDH: succinate dehydrogenase; TETs: ten-eleven translocation enzymes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from the occurrence of point mutation and DNA breaks through direct binding of mtDNA [84]. Recent reports have highlighted the actual contribution of aconitase in the regulation of mtDNA transactions also in human cells. *ACO2* ectopic expression was able to rescue mtDNA depletion observed in cells deriving from a patient with missense *ACO2* variants [85]. ACO2 was also shown to be implicated in the preservation of mtDNA from oxidative damage in concert with 8-oxoguanine DNA glycosylase (OGG1), a key enzyme of the base excision repair pathway. In this context, OGG1 also avoided the decrease of ACO2 expression and activity mediated by pro-oxidant stimuli in order to prevent apoptosis and mitochondrial dysfunction caused by oxidative stress [86,87].

For other TCA cycle enzymes taking part to mitochondrial nucleoid composition, no clear evidence for a role in mtDNA regulation has been provided. On the other side, a deleterious effect of 2HG on mtDNA stability has been demonstrated. In fact, the introduction of cancer-associated mutations of human *IDH* genes into *Saccharomyces cerevisiae* orthologs was accompanied by 2HG accumulation, which induced extensive loss of mtDNA and thus reduced respiratory capacity [88].

4. Concluding remarks and perspectives

Many metabolic pathways, including glycolysis, synthesis/oxidation of fatty acids and amino acids, convey to and depart from the TCA cycle, which therefore has a central role in the maintenance of cell homeostasis. Based on this, it is not surprising that defects of TCA cycle are implicated in diverse pathologies ranging from cancer to neurological and metabolic disorders. Altered expression of *CS*, *ACO2* and *MDH2* has been shown to contribute to cancer-specific features, among which glycolysis addiction, resistance to chemotherapy and increased lipid biosynthesis. More importantly, cancer occurrence has been linked to genetic mutations of *IDH2*, *SDH* subunits and *FH*. Notably, tumours with mutations of these enzymes share common features since the accumulation of R-2HG, succinate and fumarate causes the inhibition of α-KG-dependent dioxygenases such as PHDs, which consequently trigger HIF-1-driven pseudohypoxia. Nevertheless, several lines of evidence identified other mechanisms than HIF-1 activation for the instauration of a pseudohypoxic state in tumours.

Considering that several epigenetic enzymes necessitate substrates and cofactors produced by metabolic pathways, defective TCA cycle in cancer also impinges on DNA transactions. In particular, inhibition of α-KG-dependent histone and DNA demethylases provokes transcriptional

changes that can underpin cancer formation and/or progression. This suggests that epigenetic reprogramming and metabolic rewiring, which are both typical events occurring in tumours, are strikingly intertwined.

Besides being deputed to sustain cellular energetic metabolism, an alternative function of some TCA cycle enzyme is the maintenance of mtDNA integrity and copy number. Such a control of mtDNA homeostasis could build up a bridge between metabolism and mtDNA transcription. Therefore, the deregulation of multifunctional TCA cycle enzymes is an advantageous strategy for cancer cells to target multiple pathways in mitochondria. For instance, altered levels of ACO2 in cancer, beyond impairing TCA cycle rate, can entail mitochondrial defects that are generally associated with tumorigenesis, including depletion of mtDNA and accumulation of detrimental mutations as well (Fig. 2). However, future studies aimed at investigating the actual impact of defective TCA cycle on mtDNA functionality in cancer are needed.

Over the last years, many attempts to target tumour metabolism have been made to improve anti-cancer therapy. Although limited success has been obtained by using several small molecules directed against TCA cycle enzymes, promising effects have been recently observed with inhibitors of neomorphic IDH mutants [89]. Considering that TCA cycle defects are associated with alteration of epigenetic machinery, it would be challenging to combine metabolic and epigenetic drugs as an alternative and more efficient therapeutic approach against cancer.

Conflicts of interest

The authors have no conflict of interests to declare.

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