Tricarboxylic acid cycle dysfunction as a cause of human diseases and tumor formation

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The tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, has long been considered so crucial for the basal metabolism of cell that no significant and primary defect in any of its enzyme components could be compatible with life (59, 63). On the basis of this assumption, anomalies in intermediate metabolites were regarded as secondary phenomena, e.g., high fumaric aciduria in patients. However, genetic studies finally demonstrated that high fumarate excretion in the urine of patients with progressive encephalopathy indeed resulted from primary mutations in the fumarase hydratase (FH)-encoding gene (7, 47, 65). It was further shown that, far from being restricted to rare cases of encephalopathy, the total loss of FH unexpectedly results in uterine fibroids, skin leiomyomatosis, and papillary renal cell cancer (58). This raised a number of new questions on the intricate and intriguing role of TCA cycle enzymes, organic acid metabolism, and oxygen handling in controlling cell proliferation (49). Integrating these new perspectives on the TCA cycle function motivates this review.

Tumorigenesis and intermediary metabolism have a long common history. As early as 1926, Otto Warburg pioneered a large field of researches devoted to the metabolism of tumor cells (62). He thus reported a spectacular shift from a normal aerobic metabolism to a highly glycolytic metabolism (despite aerobic conditions) associated with the low respiration rate that characterizes tumor cells. A few years later, in 1937, Hans Krebs, one of his former students (and yet a mediocre one; Otto Warburg judged that Hans Krebs’s knowledge in chemistry was simply inadequate for a biochemist and advised him to return to medicine...; Ref. 53), elucidated the cycle of reactions today known as the TCA cycle (33), which turned to be the main source of the reducing molecules NADH and FADH₂ sustaining mitochondrial respiratory chain activity and therefore the major source of energy in living organisms (Fig. 1). Gathering these two major discoveries led to the belief that tumorigenesis was linked to a change in the balance between the TCA cycle and the glycolysis activity. But this oversimplified view was afterwards challenged by a number of observations, including the high requirement of tumor cells for an active lipogenesis [see recent review by Costello and Franklin (19)].

THE TCA CYCLE: STRUCTURE AND FUNCTION

Since its formalization by Hans Krebs, the TCA cycle has proved to be a major turntable of the cell metabolism (33). The conversion of reducing power provided by the carboxylic acids into the respiratory chain-usable reduced coenzymes NADH and FADH₂ constitutes a main function of the TCA. However, a number of cells, including mammalian cells, can survive the nonutilization of these cofactors by the respirator chain [rho zero cells devoid of respiratory chain due to the lack of mitochondrial DNA (mtDNA)] (31). The TCA also ensures a central role in an endless series of metabolic paths in particular, thanks to transamination reactions (24). Several major anaplerotic pathways require the TCA cycle-catalyzed breakdown of acetyl coenzyme A (acetyl-CoA) and the multistep interconversion of carbon skeletons delineated by Krebs (60). Finally, TCA cycle should also be considered as a water-splitting process generating oxygen for acetyl-CoA oxidation (64). Two reactions of the TCA cycle consume one H₂O molecule, each furnishing oxygen for CO₂ generation and oxidative reactions, namely, the synthesis of citrate from acetyl-CoA and oxaloacetate (OOA) and the formation of malate from fumarate (Fig. 1B). Atmospheric oxygen is only used by the terminal oxidase of the respiratory chain, the cytochrome oxidase, to ensure the reoxidation of reduced coenzymes.
The TCA cycle comprises a series of eight reactions resulting in the progressive oxidative decarboxylation of the acetyl-CoA, mostly produced by the activity of the pyruvate dehydrogenase (Fig. 1B). In addition to pyruvate arising from the glycolysis, the TCA cycle can be fed by carbon compounds derived from fatty acid degradation or from the several amino acids (glutamate, alanine, etc.). This conventional view of the TCA cycle has nevertheless been challenged to account for the complexity and the variety of the physiological conditions encountered in different tissues. Thus TCA cycle-related enzyme equipment of mitochondria varies between species, and, in one given species, between organs. Thus mitochondria endowed with matrix malic enzyme can use malate as a major fuel molecule for TCA cycle running, with malate then used as a source of both pyruvate and OAA, through the reactions catalyzed by the NAD⁺-malic enzyme and the malate dehydrogenase, respectively (34, 41). On the other hand, a number of experimental data would be best accounted for if the TCA cycle comprised two independent segments, allowing different fluxes, extending from α-ketoglutarate (α-KG) to OAA and from OAA to α-KG, respectively (Fig. 1B). Working in close connection with the aspartate amino acid transferase (reversibly producing aspartate and α-KG from OAA and glutamate or the glutamate-pyruvate transaminase), it would consume or produce glutamate and aspartate, requiring only catalytic amounts of OAA or α-KG.

The TCA cycle takes place in the semifluid matrix space of the mitochondria, cluttered with folded membranes, proteins,
and RNA and DNA molecules, spatially and kinetically compartmented. Increasing efficient operation of the cycle, metabolically related enzymes are associated into metabolons ensuring channeling of substrates through selected sets of enzymes (Fig. 1A (55)). Accordingly, the TCA cycle enzymes are consistently found in balanced proportions depending on different tissues (44), suggesting a concerted expression of the genes coding for TCA cycle enzymes to fit tissue-specific metabolic demand. The semifluid state of the matrix also results in a kinetic compartmentation of soluble oxidation cofactors, such as NAD⁺ (50), together with the several dehydrogenases.

Citrate synthase, isocitrate, and α-KG dehydrogenase (α-KGDH) activities are the major regulatory steps controlling the flux through the entire cycle (59). Regulation also involves the substrate supply, the pyridine nucleotide redox poise and concentration (NADH/NAD⁺), the coenzyme A availability, the matrix phosphorylation potential (Pᵢ + ADP/ATP ratio), and the Ca²⁺ concentration that regulate several steps of the TCA cycle.

Finally, a wealth of evidence has accumulated establishing that thyroid hormones and glucocorticoids influence the activity of the TCA cycle. Thyroid hormone-induced increase of mitochondrial Ca²⁺ and substrate supply would stimulate the overall TCA cycle activity. In addition, the control of mitochondrial biogenesis by thyroid hormones and glucocorticoids, exerted at both transcriptional and translational levels, results in the broader term effect of hormones on TCA cycle activity (54). The TCA cycle enzymes are all nuclearly encoded. The 15 nuclear genes coding for the protein moieties constitutive of the 9 TCA cycle mitochondrial enzymes have now been localized in humans, and most of the corresponding cDNAs have been cloned and sequenced (46). Four of the enzymes possess both a mitochondrial and a cytosolic form. As far as the fumarase is concerned, two isoenzymes have been identified and shown to be encoded by a single gene. Their distribution varies among tissues, the cytosolic form being absent in the brain (1). The mechanism of their distribution raises intriguing questions that have been a matter of debate (29, 57).

**TCA CYCLE ENZYMOPATHIES CAUSING NEURODEGENERATIVE DISEASES**

The autosomal recessive disease due to mutation in the fumarase gene, previously known as fumaric aciduria, is a severe and early encephalopathy with seizures and muscular hypotonia leading to growth and developmental delay, with excessive excretion of urinary fumarate (7, 47). Because of the similarity of the clinical features associated with succinate dehydrogenase (SDH) and FH gene mutations, it was later suggested that decreased SDH activity due to the accumulation of fumarate consecutive to a loss of fumarase activity was the link between the two genetic diseases (48).

The very first case of inherited SDH deficiency was found in two sisters with a neurodegenerative disorder presenting as a leukodystrophy (8). CT scan and MRI demonstrated small symmetrical foci of necrosis in the substantia nigra and in the basal ganglia typical of Leigh syndrome as well as diffuse cerebral white matter abnormalities. These patients were homozygous for an Arg554Trp mutation within the Fp subunit (SDHA subunit) causing a significant decrease in SDH activity. Thereafter, mutations of SDHA have been reported in only four families (6, 8, 27, 43). Three cases presented with autosomal recessive Leigh syndrome. In one case, a child died at 5 mo of age from a severe deterioration of neuromuscular, cardiac, and hepatic symptoms after an intermittent infection. Patients with an inherited deficiency of the α-KGDH present a progressive, severe encephalopathy with axial hypotonia, psychotic behavior, and pyramidal symptoms (25). Children exhibit permanent lactic acidosis, with acute episodes during emotional stress, and various infections associated with elevated lactate/pyruvate ratio and slightly decreased ketone body ratio in the plasma.

An abnormal urinary excretion of organic acids was frequently noticed in those patients with TCA cycle enzyme deficiency, with occasional peaks of α-KG observed whatever the enzyme deficiency (α-KGDH, SDH, or FH). This excretion of α-KG suggested that in all these cases, the activity of the α-KHHDH is decreased, potentially due to sequestration of CoA between various CoA esters in case of SDH and fumarase deficiency.

Succinyl-CoA synthetase (ADP forming) mutations result in autosomal recessive encephalopathy and Leigh syndrome (21). Secondary depletion of mtDNA was also noticed in these patients. The physical interactions between the succinyl-CoA synthetase and the nucleoside diphosphate kinase, which might be involved in the phosphorylation of the mitochondrial deoxyribonucleotide diphosphates, suggest a role for the former enzyme in the synthesis of the deoxynucleotide triphosphates of the mitochondria. This may account for mtDNA depletion and the consecutive loss of the activity of respiratory chain complexes containing subunits encoded by the mitochondrial genome. Because of the occurrence of a second succinyl-CoA synthetase (GDP-forming succinyl-Coa synthetase), the activity of the TCA cycle should be less affected than in the case of SDH, FH, or α-KGDH mutations. As a result, a defect in the respiratory chain rather than TCA cycle dysfunction may be at the origin of the disease.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene</th>
<th>Disease</th>
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<tbody>
<tr>
<td>α-Ketoglutarate dehydrogenase</td>
<td>OGDH</td>
<td>Severe encephalopathy, hypotonia, psychotic behavior, pyramidal symptoms</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>SDHA</td>
<td>Leigh syndrome</td>
</tr>
<tr>
<td></td>
<td>SDHB</td>
<td>Paraganglioma and pheochromocytoma</td>
</tr>
<tr>
<td></td>
<td>SDHC</td>
<td>Paraganglioma</td>
</tr>
<tr>
<td></td>
<td>SDHD</td>
<td>Paraganglioma and pheochromocytoma</td>
</tr>
<tr>
<td>Fumarase</td>
<td>FH</td>
<td>Early encephalopathy, seizures, and muscular hypotonia</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase (ADP forming)</td>
<td>SUCLA2</td>
<td>Leiomymatosis and papillary renal cell cancer</td>
</tr>
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<td></td>
<td></td>
<td>Encephalomyopathy and mtDNA depletion</td>
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To this list of TCA cycle-specific enzymopathies (Table 1 and Fig. 2), one should add Friedreich ataxia, which associates a severe aconitase and SDH defect to a respiratory chain disorder (complex I and III deficiencies) (48). All the defective enzymes in Friedreich ataxia share iron-sulfur clusters as essential components in their catalytic activity. Accordingly, the frataxin protein lacking its function in Friedreich ataxia has been shown to be involved in the biosynthesis of iron-sulfur cluster (56) due to improper handling of iron, which renders cells hypersensitive to oxidative insults (14). As a matter of fact, there is compelling evidence that iron-sulfur clusters are exquisitely sensitive to superoxides and thus are often found to be more or less severely affected in cases of oxidative insults (40, 42). Accordingly, SDH activity is often found to be decreased in case of mitochondrial ATPase defects known to result in the release of large amounts of superoxides (12). Finally, a consistent observation that can be made in patients suffering any of these TCA cycle defects deals with the tissue specificity of these diseases, which has not yet received a rational explanation (10).

SDH B-, C-, AND D-SUBUNIT-ENCODING GENES ACT AS TUMOR SUPPRESSOR IN NEUROENDOCRINE TISSUES

Three of the four subunits of complex II, namely, SDHB, SDHC, and SDHD, have been involved in the tumorigenesis resulting in paragangliomas and pheochromocytomas (4). Most probably, the presence of two SDHA forms in neuroendocrine tissues accounts for the absence of paraganglioma associated with SDHA mutations (11). All SDH-hereditary paragangliomas are transmitted according to an autosomic dominant mode of inheritance. However, in the case of SDHD-related syndrome, a maternal genomic imprinting is associated with the dominant transmission, and the disease is only transmitted by the paternal branch (4). Theses tumors are mostly benign, highly vascularized, and slow growing. They result from a germline heterozygous mutation associated with a loss of the wild type allele in the tumoral tissue, inducing a specific loss of SDH enzymatic activity (23). Paragangliomas caused by SDH deficiency generally occur from parasympathetic ganglia in the head and the neck (mostly in the carotid body) and from sympathetic ganglia in the thorax, the abdomen, and the pelvis. Pheochromocytomas are derived from the chromaffin cells of the adrenal medulla. The only curative therapy is the early surgical resection of the tumors (22). Noticeably, paragangliomas of the carotid body can be caused by chronic hypoxia as well, as observed in patients living in high altitude or suffering from chronic obstructive pulmonary disease (30). Mutations in SDHB and SDHD genes have been found in patients harboring both paragangliomas and pheochromocytomas (3, 5), whereas SDHC mutations have only been found in patients with head and neck paragangliomas to date (51).

FUMARASE MUTATION PREDISPOSES TO CUTANEOUS AND UTERINE LEIOMYOMAS AND PAPILLARY RENAL CELL CANCER

More than 40 mutations in the fumarase gene have been reported to cause uterine and/or cutaneous leiomyomas (58). In a subset of these cases, leiomyomas are associated with pap-
illary renal cell cancer, resulting in the so-called hereditary leiomyomatosis and renal cell cancer (HLRCC) syndrome. Recent studies found that a vast majority of patients presenting skin leiomyomas (up to 85%) had deleterious mutations in the fumarase gene (2).

**SDH AND FH TUMORS, A COMMON PATHWAY?**

Hypoxia-inducible factors (HIFs) are major elements of the cellular response to hypoxia. These transcription factors acti-

vate glycolysis, angiogenesis, and numerous targets when the cell is short of oxygen (36). The involvement of HIFs has been observed in numerous types of tumors, playing an active role in the progression of the neoplasia (38). HIFs are composed of two subunits, an α-subunit, which is regulated by oxygen, and a β-subunit (HIF-1β, also called the aryl hydrocarbon receptor nuclear translocator, or ARNT), which is constitutively and ubiquitously expressed. Three α-subunits have been identified so far (HIF-1α, HIF-2α and HIF-3α), each encoded by a different gene (61). Under normoxic conditions, HIF-α are continuously ubiquitinated and subsequently degraded by the proteasome (9). The process of ubiquitination is started by their recognition by the von Hippel-Lindau (VHL) protein, which requires the hydroxylation of two proline residues on HIF-α (39). The very first step of HIF-α degradation under normoxic conditions is thus dependent of this hydroxylation, which is catalyzed by HIF prolyl hydroxylases (PHDs). PHDs belong to the superfamily of Fe(II)-dependant oxygenases and require reduced iron as a cofactor, α-KG and oxygen as cosubstrates, with carbon dioxide and succinate being the products of the reaction (13). Under hypoxic conditions, the absence of oxygen prohibits PHD activity, and HIF-α are thus stabilized, allowing for their nuclear translocation and the subsequent activation of their target genes.

Four years after the seminal observation in 2001 of the putative implication of HIFs in a paraganglioma due to a mutation in the SDHD gene (23), it was recently shown that the high concentration of succinate accumulated in tumors (45), or in SDH-deficient cells (11) directly linked to the loss of SDH activity, was sufficient to affect PHD activity in vitro (28, 52). Similar to hypoxia, succinate accumulation could therefore induce the inhibition of the PHD, allowing for the nuclear translocation of HIF-1α. Recently, a very similar mechanism was described for FH deficiency in a renal cell cancer (28, 45). In this latter case, fumarate, accumulated due to fumarase inactivation, was found to act as a competitive inhibitor of the PHD, thus inducing the abnormal stabilization of HIF-1α. Noticeably, other structurally related organic acids can also inhibit the PHD (20). Therefore, a TCA cycle blockade may result in the induction of angiogenesis and in the tuning up of the glycolysis during tumorigenesis, being at the origin of the Warburg effect. These observations support the view that hypoxia-inducible factors play a central role in tumorigenesis of the neuroendocrine system. Accordingly, in the VHL syndrome, characterized by the presence of hemangioblastomas, renal cell carcinomas, and pheochromocytomas, abnormal ac-

tivation of HIF-2α caused by mutations in the VHL gene has been suggested to be directly responsible for tumorigenesis (32, 37). However, this proposal has been challenged by the observation that in pheochromocytomas, HIF activity is not per-

se the tumorigenic cause of VHL mutation (35).

**A PROVISIONAL CONCLUSION**

So far only a subset of the components of the TCA cycle has been found to be defective in humans. Yet, the clinical spec-

trum encompasses a large variety of diseases, ranging from devastating encephalopathies to tumor formation and cancers. The predictable consequences of a TCA cycle blockade are multiple, but it already appears that the impairment of each of the TCA cycle steps is susceptible to result in highly specific features. Indeed, if severe defects of several of the enzymes result in overlapping clinical features already observed in cases of pyruvate dehydrogenase and respiratory chain deficiency, suggestive of a common mechanism presumably linked to energetic failure, some of the defects result in much more specific features such as tumor formation and/or cancer. In these latter cases, the mechanism that triggers cell proliferation appears to be mainly associated with imbalance in the organic acids controlling the prolyl hydroxylase enzyme. Specific or-

ganic acids ultimately cause HIF-1α nuclear translocation, allowing for angiogenesis and cell proliferation.

Initially disregarded as a primary cause of human diseases because of a predicted lethality, several inherited TCA cycle deficiencies were proved in the mid-1990s to result in enceph-

alopathies in humans. Even more unexpected was the link recently established between organic acid accumulation and abnormal cell proliferation, resulting in tumor and/or cancer formation. Our poor understanding of the highly variable

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**Table 2. TCA cycle proteins and mtDNA stability in the yeast**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Gene</th>
<th>mtDNA Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitate</td>
<td>Aco1</td>
<td>mtDNA total loss (rho 0)</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>Idh1</td>
<td>Moderate instability</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase</td>
<td>Kgd1</td>
<td>Moderate instability</td>
</tr>
<tr>
<td>Kgd2</td>
<td></td>
<td>Moderate instability</td>
</tr>
<tr>
<td>Lpd1</td>
<td></td>
<td>Moderate instability</td>
</tr>
<tr>
<td>Succinyl-Coa synthetase</td>
<td>Lsc1</td>
<td>Stable</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase</td>
<td>Pda1</td>
<td>Moderate instability</td>
</tr>
<tr>
<td>Pdh1</td>
<td></td>
<td>Moderate instability</td>
</tr>
</tbody>
</table>

TCA, tricarboxylic acid; mtDNA, mitochondrial DNA. Data are from Ref. 15.
phenotypes associated with an impairment of the TCA cycle may stand from our substantial ignorance of a number of issues related to TCA cycle, especially dealing with tissue-to-tissue differences in enzyme activity and the role of organic acids. Finally, the potential multifunctional roles of these enzymes, already partially recognized in yeast (15) (Table 2), suggests that new surprises are to be found around the corner in the years to come.

GRANTS

We thank the Association Française contre les myopathies, the GIS-Institut des Maladies Rares for the PGL.NET Network, and the Integrated Project EuMitocombat for their support.

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