Role of TCA Cycle-Related Enzymes in Human Diseases

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The tricarboxylic acid (TCA) cycle, also known as the citrate acid cycle, is a series of chemical reactions to form energy required for cellular function through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins. There are eight enzymes in the TCA cycle that oxidize acetyl-coenzyme A (acetyl-CoA), and genetic or non-genetic alterations in these enzymes are closely associated with human diseases, especially cancer and neurodegeneration, but the role of these eight enzymes remains unclear.

tricarboxylic acid (TCA) cycle | TCA cycle-related enzymes | human diseases | Extra-mitochondrial citrate synthase

1. Background

The tricarboxylic acid (TCA) cycle is the main source of cellular energy and participates in many metabolic pathways in cells. Recent reports indicate that dysfunction of TCA cycle-related enzymes causes human diseases, such as neurometabolic disorders and tumors, have attracted increasing interest in their unexplained roles. The diseases which develop as a consequence of loss or dysfunction of TCA cycle-related enzymes are distinct, suggesting that each enzyme has a unique function.

2. TCA-Related Enzymes and Diseases Arising from Their Dysfunction

CS is the first rate-limiting enzyme in the TCA cycle. Many studies have shown that CS is essential for maintaining energy production in all cell types [1]. Although diseases caused by CS dysfunction have not yet been reported, CS knockdown in a human embryonic kidney cell line (293T) exhibited decreased ATP production and increased oxidative damage, leading to cell death in vitro [2].

In the cytosol, ATP citrate lyase (ACLY) is involved in citrate cleavage, resulting in the formation of oxaloacetate and acetyl-CoA (for fatty acid biosynthesis) [1][3]. ACLY is associated with cardiovascular abnormalities in humans; for example, ACLY deficiency in macrophages stabilizes atherosclerotic plaques in mice [4]. The atherosclerotic plaques are due to an increase in collagen deposition and fibrous cap thickness, along with a small necrotic core.

Aconitase catalyzes the reversible isomerization of citrate to isocitrate [5]. Aconitase 1 (Aco1) and aconitase 2 (Aco2), two aconitase isoforms, present in the cytoplasm and mitochondria, respectively [3]. Deficiency of Aco1 and Aco2 are associated with increased risk of optic atrophy and encephalopathy, respectively [6][7]. These diseases are caused by autosomal recessive mutations in Aco 1 and 2. Infantile cerebellar-retinal degeneration is also associated with mutations in the mitochondrial Aco2. The patients show clinical phenotypes with ophthalmological abnormalities, such as optic atrophy.

Previous studies have demonstrated that dominant defects of three TCA cycle-related enzymes, isocitrate dehydrogenase (IDH), succinate dehydrogenase (SDH), and fumarase, lead to tumor formation [8][3].

IDH associated with $NADP^+$ is present as a monomer (IDH1) in the cytoplasm and as a dimer (IDH2) in the mitochondria. IDH1/2 are essential enzymes in the mitochondrial antioxidant system against oxidative stress as they synthesize nicotinamide adenine dinucleotide phosphate [9][3]. Mutation of IDH1/2 results in a decrease in α-ketoglutarate, leading to a disturbance of TCA cycle function. Dominant IDH1/2 mutations have been found in patients with gliomas and acute myeloid leukemia [8][3][10].

The SDH complex, also known as mitochondrial complex II, catalyzes the oxidation of succinate to fumarate in the TCA cycle [11]. This complex is composed of a heterotetramer of SDHA and SDHB subunits anchored to the inner mitochondrial membrane by SDHC and SDHD subunits [11]. Recently, it was found that dominant mutations in SDHB, SDHC, and SDHD cause susceptibility to paragangliomas [3][12]. SDHA mutations in germ cells have also been found in patients with paragangliomas [3][13]. In addition, SDHB mutations are related to renal cell carcinoma and T-cell acute leukemia [14][15], and SDHB, SDHC, and SDHD mutations result in gastrointestinal stromal tumors [16]. However, the reason for tumor development because of SDH mutations remains unclear.

Fumarase, in its homotetrameric active form, converts fumarate to malate [17]. The same gene encodes two isoforms, and recessive mutations of fumarase, in both the mitochondrial and cytosolic enzymes, causing severe encephalopathies [3]. In addition, dominant mutations in fumarase cause susceptibility to tumors, such as uterine leiomyomas, leiomyomatosis, renal cell cancer, ovarian cystadenomas, and breast cancer [18]. A study reported that in a patient with cancer caused by fumarase mutation, although there was a significant decrease in fumarase activity in the cells, mitochondrial fumarase was maintained at normal levels. However, no fumarase was detected in the cytosol, suggesting that cytosolic fumarase may function as a tumor suppressor [19].

Table 1. TCA cycle-related enzymes and diseases arising from their dysfunction.

Enzymes	Abbreviation	Localization	Diseases	References
Citrate synthase	CS	Mitochondria	Cell death (in a human cell line, in vitro)	[2]
Citrate lyase	ACLY	Cytosol	Atherosclerotic plaques (in mice, in vivo)	[4]
Extra-mitochondrial citrate synthase	eCS	Cytosol	Decrease in age-dependent male fertility (in mice, in vivo)	[20]

Enzymes	Abbreviation	Localization	Diseases	References
Aconitase	ACO1	Cytosol	Encephalopathy (in humans, in vivo)	[3][6][7]
	ACO2	Mitochondria	Optic atrophy (in humans, in vivo)	
Isocitrate dehydrogenase	IDH1	Cytosol	Gliomas, acute myeloid leukemia (in humans, in vivo)	[8][3][10]
	IDH2	Mitochondria		
Succinate dehydrogenase	SDHA	Mitochondria	Paragangliomas (in humans, in vivo)	[3][13]
	SDHB		Gastrointestinal stromal tumors, paragangliomas, renal cell carcinoma, T-cell acute leukemia (in humans, in vivo)	[3][12][14][15][16]
	SDHC		Gastrointestinal stromal tumors, paragangliomas (in humans, in vivo)	[3][12][16]
	SDHD		Gastrointestinal stromal tumors, paragangliomas (in humans, in vivo)	[3][12][16]
Fumarase (fumarase hydratase)	FH	Mitochondria	Encephalopathy, leiomyomas, leiomyomatosis, renal cell cancer, ovary cystadenomas, breast cancer (in humans, in vivo)	[3][18]
		Cytosol		
α-ketoglutarate dehydrogenase	OGDH	Mitochondria	Neurological disorder (in humans, in vivo)	[21]
Malate dehydrogenase	MDH1	Cytosol	Encephalopathy (in a human cell line, in vitro)	[22][23]
	MDH2	Mitochondria		
Malic enzyme	ME1	Cytosol	Unknown	[24][25]
	ME2	Mitochondria	Idiopathic generalized epilepsy (in humans, in vivo)	[24][26]
	ME3	Mitochondria	Unknown	[24]
Glutamate-oxaloacetate transaminase	GOT1	Cytosol	Unknown	[27]
	GOT2	Mitochondria	Neurometabolic disorder (in humans, in vivo)	[28]

(OGDH) gene [21]. OGDH is located in the mitochondria and catalyzes the conversion of α-ketoglutarate to succinyl-CoA and CO_2. Neurological disorders are caused by a decrease in the activity of OGDH and the reactive oxygen species (ROS)-induced inactivation of OGDH in brain [21].

Malate dehydrogenase (MDH) participates in the oxidation of malate to oxaloacetate. MDH 1 and MDH2 are localized in the cytosol and mitochondria, respectively [3]. In a human HEK293 cell line, MDH1 deficiency causes a

metabolic disorder of the malate-aspartate shuttle, leading to severe encephalopathy with an increase in glycerol-3-phosphate [22]. Similarly, mutations in MDH2 cause early-onset severe encephalopathy in HEK293 cells because of deleterious MDH2 variants [23].

Malic enzymes (MEs) convert malate to pyruvate (the TCA carbon source) and NADPH [29]. There are three isoforms of MEs: $NADP^+$-dependent malic enzyme 1 (ME1), NAD^+-dependent malic enzyme 2 (ME2), and $NADP^+$-dependent malic enzyme 3 (ME3). ME1 is present in the cytosol, whereas ME2 and ME3 are present in the mitochondria [24]. Inhibition of ME1 leads to a decrease in NADPH (functioning as an antioxidant) and an increase in ROS, and consequently, is lethal in ME2-unexpressed human gastric cancer cells [25]. Accordingly, ME1 knockdown in human gastric cancer cells suppressed tumor growth in vivo [25]. In humans, recessive ME2 mutations predispose to idiopathic generalized epilepsy because genetic variation of the ME2 gene confers susceptibility to idiopathic generalized epilepsy [26]. However, no evidence was found for disease arising from ME3 mutations.

Two isoforms of glutamate-oxaloacetate transaminase (GOT), GOT1 and GOT2, are known to be important regulators of glutamate levels [30]. GOT1 and GOT2 are localized in the cytosol and mitochondria, respectively. Although GOT1 inhibition promotes pancreatic cancer cell death, no evidence has been found for the disease [27]. As GOT2 metabolizes 5′-phosphate esters pyridoxal 5′-phosphate, a metabolically active form of vitamin B6, GOT2 deficiency causes its faulty metabolism, leading to an autosomal recessive neurometabolic disorder [28].

3. Predicted Existence of Extra-Mitochondrial TCA (eTCA) Cycle

As depicted in **Figure 1**, the TCA cycle comprises eight enzymes (CS, aconitase, IDH, OGDH, succinyl-CoA synthetase, SDH, fumarase, and MDH). These enzymes are mainly distributed in the mitochondria, although most of these enzymes are also detected in the cytosolic region (**Table 1** and **Figure 2**). Pyruvate, located at the interface between glycolysis and the TCA cycle, is an important intermediate. As mentioned above, the presence of two forms of CS, catalyzing the formation of acetyl-CoA, reinforces the importance of this interface. The mitochondrial TCA cycle has been extensively studied [31], but even after these extensive studies, the roles of cytosolic TCA cycle enzymes, including CS, are not well understood.

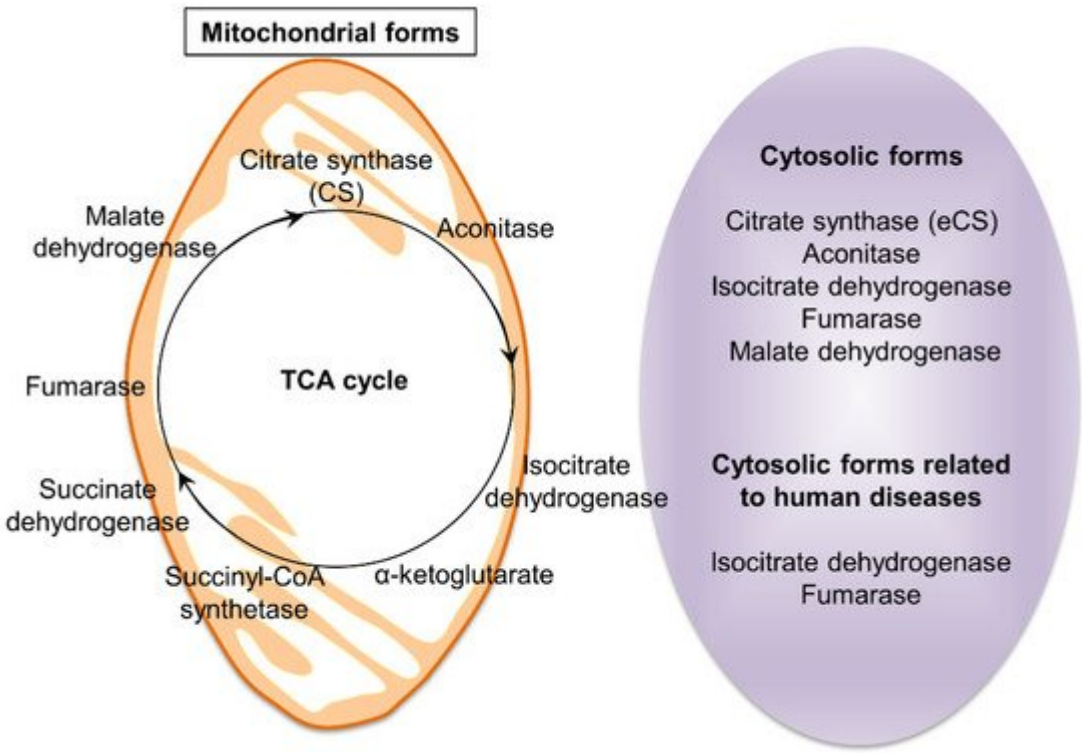

Figure 2. TCA cycle-related enzymes. The TCA cycle is the second stage of cellular respiration. This cycle occurs in the matrix of mitochondria and is catalyzed by eight enzymes. Interestingly, enzymes function in cytosolic forms, such as eCS, aconitase, isocitrate dehydrogenase, fumarase, malate dehydrogenase. Particularly, isocitrate dehydrogenase and fumarase were reported as human disease-related enzymes.

Aconitase catalyzes the isomerization of citrate and isocitrate. In mammals, the ACO1 (cytosolic aconitase), also known as iron regulatory protein 1 (IRP1), plays a role in sensing cellular iron homeostasis [32]. Cytosolic aconitase, upon losing an iron-sulfur cluster, becomes IRP1 [32]. Cytosolic aconitase belongs to a family of RNA-binding proteins that modulate iron metabolism in vertebrates, contributing to optimal cell growth [33].

Fumarase (also known as fumarate hydratase) is an enzyme found in both the mitochondria and the cytoplasm, and is extensively found in microorganisms, plants, and animals [34]. In mitochondria, fumarase catalyzes the reversible formation of l-malate from fumarate. In plants, cytosolic fumarase is also involved in fumarate formation [35]. Plants inhabiting cold environments are adapted to cold and freezing temperatures. Cytosolic fumarase-mediated accumulation of fumarate is essential for adaptation of *Arabidopsis thaliana* to cold [36].

Molecular cues of fumarases have been obtained from microorganisms [37]. In microorganisms, fumarases are divided into two classes, I and II, with distinct properties. Bacteria have three fumarase genes: *fumA*, *fumB*, and *fumC*. Their products, FUMA, FUMB, and FUMC, are biochemically divided into two distinct classes. Class I fumarases, FUMA and FUMB, are homologous to fumarases identified in *Euglena*. FUMA and FUMB are differentially regulated; FUMA functions in the TCA cycle, while FUMB supplies fumarate as an anaerobic electron acceptor. The class II fumarase, FUMC, is homologous to fumarases identified in *Bacillus subtilis*, *Saccharomyces*

cerevisiae, and mammals. Class II fumarases are structurally conserved, with highly homologous sequences across species.

Human fumarase exists in both cytosolic and mitochondrial forms with extended N-terminus, differing only in the translation initiation site [34]; however, its role in the cytoplasm is unclear. Recent evidence has demonstrated that fumarase functions as a tumor suppressor in mammals [34]. Fumarase functions in the mitochondria, but in recent studies, it has emerged as a participant in the response to DNA double-strand breaks in the nucleus [34]. In humans, fumarase deficiency causes the formation of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) [18]. HLRCC is a rare genetic disease with smooth muscle growth on the skin and uterus, and is associated with a risk of developing kidney (renal) cancer. A mutation in a gene encoding fumarase is believed to cause all known cases of HLRCC. Specifically, the cytosolic form of fumarase is involved in the onset of this disease. Cytosolic fumarase plays a role in repairing DNA double-strand breaks, both through its movement from the cytoplasm to the nucleus, and enzymatic activity [38]. In other words, when fumarase is absent from cells, the DNA repair mechanism is impaired, but the administration of a high concentration of fumarate reverses this abnormality [34]. This result raises the possibility that fumarate moves actively or passively across organelles, including the mitochondria and the nucleus, and possibly other organelles.

Cytoplasmic and mitochondrial forms of isocitrate dehydrogenases (IDH1 and IDH2) dehydrate isocitrate to form oxalosuccinate [10]. Point mutations in both *IDH1* and *IDH2* have been frequently associated in the pathogenesis of a subset of gliomas, mainly low-grade gliomas and secondary glioblastomas [10]. Somatic mutations in *IDH1* cause disturbances in cell metabolism, a common feature of gliomas [10]. In addition, a novel inactivating mutation in *IDH* has been discovered in high-grade astrocytomas [39].

MDH is an enzyme that converts l-malate to oxaloacetate. In eukaryotic cells, MDH has two isoforms, MDH1 and MDH2 [29]. MDH1 is a cytoplasmic protein that transports malate into mitochondria, whereas MDH2 is a mitochondrial protein, which is part of the TCA cycle. In honeybees, three alleles encode cytosolic MDH: F, M, and S [40]. These alleles have temperature-dependent fitness benefits.

On the other hand, the cytoplasmic forms of the three enzymes, OGDH, succinyl-CoA synthetase, and SDH, remain unidentified.

Generally, ATP production using the TCA cycle occurs under aerobic conditions. Notably, cancer cells rely on glycolysis for ATP production, even under aerobic conditions [41], referred to as the Warburg effect (aerobic glycolysis) [20][21]. Although cancer cells mainly generate ATP via this process, its functions remain unclear [42]. As citrate synthesis is essential for a switch from glycolysis to the TCA cycle, dysfunction or gain-of-function of CS could be a possible cause for the Warburg effect.

Due to the lack of MTS in eCS, it is predominantly located in the sperm acrosome and not in the mitochondria. Therefore, eCS may be involved in energy production for sperm function via the eTCA cycle independently. The findings obtained from oximetric and biochemical analyses of retinal rod outer segments support the possible

existence of the eTCA [43]. Additionally, *eCs*-KO male mice exhibited decreased fertility with aging (>6-month-old), suggesting an increase in eCS contribution for sperm function in older mice. This implies the possibility of the existence of the TCA cycle in the extra-mitochondrial space [43].

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