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Anaplerotic reactions in tumour proliferation and apoptosis

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Abstract

Our aim in this commentary is to provide evidence that certain oxoacids formed in anaplerotic reactions control cell proliferation/apoptosis. In tumour cells with impaired Krebs cycle enzymes, some anaplerotic reactions do compensate for the deficit in oxoacids. One of these, oxaloacetate, derived from the transamination of asparagine but not of aspartate, is decarboxylated 4-fold more efficiently in polyoma-virus transformed cells than in their non-transformed counterparts. The deamidation of asparagine, in the cell culture medium, to aspartate by asparaginase decreases asparagine transamination and inhibits concomitantly the growth of asparaginase-sensitive lymphoma cells, suggesting a causal relationship between asparagine transamination and growth. Another oxoacid that can provide ATP when metabolised in mitochondria, but by the branched-chain oxoacid dehydrogenase complex (BCOADC), is 2-oxobutanoate. It has two origins: (a) deamination of threonine, and (b) cleavage of cystathionine, a metabolite derived from methionine. 2-Oxobutanoate in the presence of insulin promotes growth in G1/S arrested cells. But methionine also gives rise to another substrate of BCOADC, 4-methylthio-2-oxobutanoate (MTOB), which is synthesised exclusively from methylthioadenosine (MTA) by the action of MTA phosphorylase. In Met-dependent tumour cells with defective MTA phosphorylase, 2-oxobutanoate production would exceed that of MTOB. Further, BCOADC also has 3-fold greater affinity for 2-oxobutanoate than for MTOB; hence, the deficiency in 3-methylthio propionyl CoA, the final product of MTOB decarboxylation, would be exacerbated. Methional, the transient metabolic precursor in 3-methylthio propionyl CoA biosynthesis, is apoptogenic for both normal and bcl_2-negative transformed cells in culture. Investigations of other causal relationships between the genes/enzymes mediating the homeostasis of anaplerotic oxoacids and cell growth/death may be worthwhile.
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Keywords: Anaplerotic reactions; Oxoacids; Proliferation; Apoptosis

1. Introduction

The aim of this commentary is to summarise several different lines of evidence from the literature that support a role for oxoacids formed in anaplerotic reactions, in controlling proliferation and apoptosis of mammalian cells.

Mutations in three nuclear genes encoding the subunits of SDH have been found in hereditary and sporadic cases of head and neck tumours, such as paraganglioma, and in tumours of the adrenal medulla, such as pheochromocytoma [1,2]. More recently, mutations in a gene encoding another Krebs cycle enzyme (FH) have been described in 60% of the cases of benign smooth muscle tumours such as skin leiomyomata and uterine fibroids and of malignant tumours such as renal cell cancer [3]. In view of the cause-and-effect relationship that has been reported to exist between these mutated genes and the development of tumours, be they of benign or malignant origin, these housekeeping genes, although sometimes classified as ''tumour suppressor genes'' are more accurately defined as genes whose defective enzymes increase susceptibility to the tumours mentioned [2]. Indeed, to compensate for the deficiencies in the products (fumarate and malate) of SDH and FH respectively, other pathways that contribute to the synthesis of these products or of their downstream metabolites can be activated, and it is the enzymes of these activated pathways that may play a role in tumorigenesis.

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Abbreviations: Adohcy, *S*-adenosyl homocysteine; Adomet, *S*-adenosyl methionine; BCAT, branched-chain aminoacid aminotransferase; BCOADC, branched-chain oxoacid dehydrogenase complex; BHK, baby hamster kidney cells; BHKPy, baby hamster kidney cells transformed by polyoma virus; CEF, chick embryo fibroblasts; FH, fumarate hydratase; Hcy, homocysteine; MTA, methylthioadenosine; MTOB, 4-methylthio-2-oxobutanoate; SDH, succinic dehydrogenase; Spd, spermidine; Spm, spermine.

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Such compensatory pathways or anaplerotic reactions, first described in 1966 by Kornberg [4] studying bacterial energetics, have been defined as follows: ‘‘reactions that enable the provision of energy to be maintained under conditions in which the removal (or loss) of biosynthetic precursors would otherwise interrupt the pathways of energy supply’’ [5]. The term ‘‘loss’’ in parentheses is that of the authors.

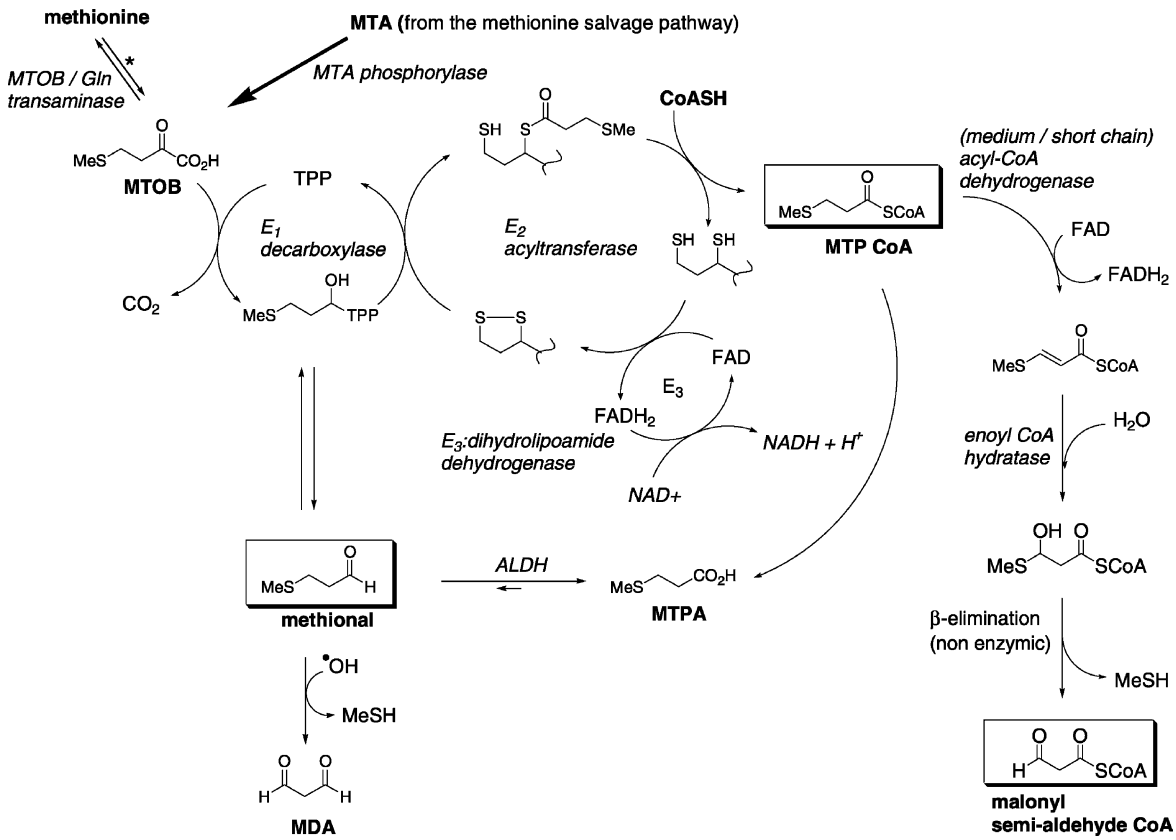
In this commentary, which is by no means exhaustive, we will attempt to provide evidence from the literature which shows that the oxoacids produced in some anaplerotic reactions such as Asn → oxaloacetate or Met → 2-oxobutanoate do play a causative role in cell proliferation, while others such as Met → 4-methylthio-2-oxobutanoate give rise to metabolites that are potent apoptogenic compounds.

2. Evidence for the existence of anaplerotic reactions in mammalian cells

Defective SDH and/or FH in tumour cells should bring about the complete interruption of the Krebs cycle with a concomitant decrease in ATP production from Krebs cycle intermediates via NAD and FAD. This does not occur, as shown below. Indeed, in MCF-7 breast cancer cells, the contribution to total ATP turnover over 5 days was 80% oxidative and 20% glycolytic. Contributions to the oxidative component were: 10% glucose, 14% glutamine, 7% palmitate, 4% oleate, and 65% from unidentified sources, and, more importantly, were not different from those of other non-transformed cells [6]. The contribution to total turnover (glycolysis and oxidation) was: 28.8% from glucose and 10.7% from glutamine. Therefore, as glucose and glutamine taken together contribute only 40% of total ATP turnover, 60% of ATP is unaccounted for [6]. Hence, two questions arise: (a) Does the missing 60% of ATP turnover arise from anaplerotic reactions? (b) If so, to which Krebs cycle intermediates do they give rise?

3. 2-Oxoacids from amino acids as anaplerotic reactions

It is already well established, on the one hand, that the 2-oxoacids (2-oxoglutarate, pyruvate, and oxaloacetate, which are substrates for Krebs cycle enzymes) can also be formed from the transamination of non-essential amino acids; on the other hand, 2-oxobutanoate, 3-methyl-2-oxobutanoate, 3-methyl-2-oxopentanoate, 4-methyl-2-oxopentanoate, and 4-methylthio-2-oxobutanoate, which are products of the transamination/deamination of essential amino acids, are substrates of the mitochondrial BCOADC



Scheme 1. Intracellular metabolism of 4-methylthio-2-oxobutanoate (MTOB). Key: ALDH, aldehyde dehydrogenase; MDA, malondialdehyde; MTA, methylthio adenosine; MTOB, 4-methylthio-2-oxobutanoate; MTPA, 3-methylthiopropanoate; MTP CoA, 3-methylthiopropionyl CoA; TPP, thiamine pyrophosphate; →*, excess methionine. Scheme was based on material from Refs. [14,18,19,28,40,41].

whose activity can also generate ATP via NAD and H^+ for the electron transport chain. BCOADC has three distinct activities: E1, decarboxylase; E2, acyl transferase; E3, dihydrolipoamide dehydrogenase, which work in the sequence E1 → E2 → E3 (see Scheme 1).

4. Evidence for oxoacids as growth factors

Normal human fibroblasts in culture in a serum-deprived medium require the presence of one of the oxoacids (glyoxylate, pyruvate, 2-oxoglutarate, or oxaloacetate) for their proliferation, and, of these, glyoxylate is the most effective [7]. A similar observation has been made for the Walker carcinoma [8].

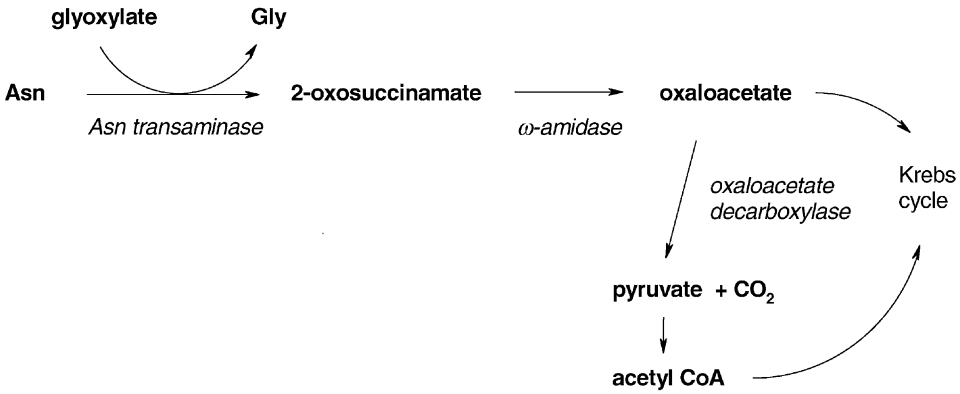
CEF arrested in the G1/S phase by a protein synthesis inhibitor (cycloheximide) overcome this block in DNA synthesis by the addition to the culture medium of insulin plus one of the oxoacids in decreasing order of efficacy: pyruvate → 2-oxobutanoate → oxaloacetate. The addition of insulin alone or of pyruvate alone is much less effective [9]. Based on the results cited in Section 3 and this section, SDH and FH may not be tumour suppressors, but rather the enzymes of the anaplerotic pathways may be tumour promoters.

The oxoacids pyruvate and 2-oxoglutarate arise primarily from glucose and Gln/Glu metabolism, respectively, but, as stated previously, account for only 40% of ATP production. Potential candidates for the remaining 60% of the anaplerotic reactions are oxaloacetate and the substrates of BCOADC (see Section 3).

5. Evidence for an oxoacid anaplerotic pathway specific for transformed cells in culture

In BHK cells in culture transformed by polyoma virus (BHKPy), $[^{14}C]CO_2$ production from [U-^{14}C]L-Asn is 4-fold greater than that in non-transformed BHK controls [10], and [^{14}C]2-oxosuccinamic acid is one of the intermediates in this reaction [10]. In non-transformed BHK, the $[^{14}C]CO_2$ is isotopically diluted by 88% by the addition of unlabelled Asp to the medium [10], whereas in BHKPy the $[^{14}C]CO_2$ is not isotopically diluted by the addition of unlabelled Asp to the medium but rather increases by 20% [10]. It is therefore clear that the CO_2 arising from Asn does not come from the prior deamidation of Asn to Asp but from the transamination of Asn via 2-oxosuccinamate, which is then converted to oxaloacetate by the action of ω-amidase [11].

However, for Asn transaminase to be active, an oxoacid is necessary. The oxoacid for which Asn transaminase shows the highest affinity is glyoxylate [12], which has been shown to be an amine receptor in the transamination reaction outlined below:



In mouse lymphoma asparaginase-sensitive cells in culture, the addition of asparaginase to the culture medium inhibits growth and decreases intracellular glycine levels [13]. On the contrary, in their asparaginase-resistant counterparts, cellular glycine levels were unaffected by treatment with asparaginase [13].

6. Anaplerotic reactions that give rise to oxoacid substrates of BCOADC

These reactions fall into three main categories:

6.1. Transamination of aminoacid (2-oxoglutarate as amine acceptor)

Val $\xrightarrow{BCAT}$ 3-methyl-2-oxobutanoate

Ile $\xrightarrow{BCAT}$ 3-methyl-2-oxopentanoate

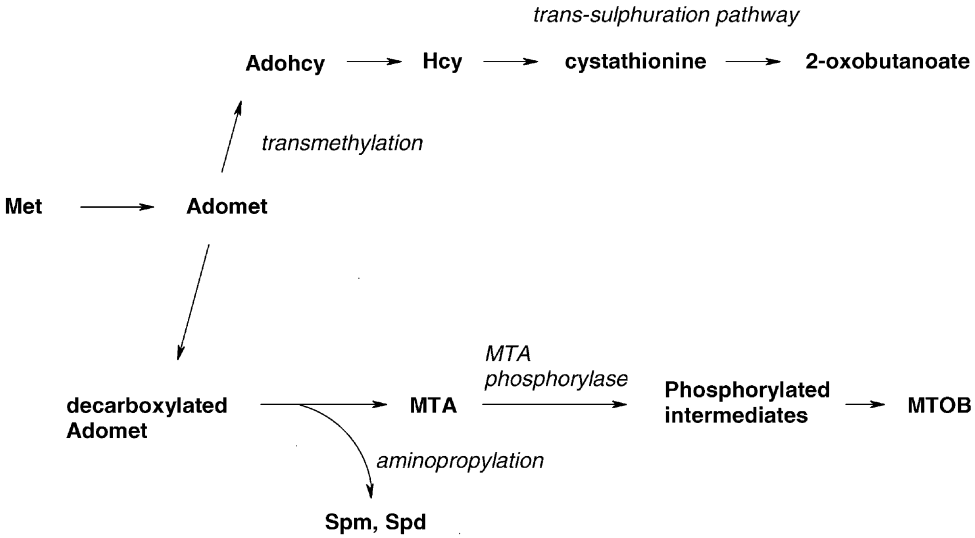
Leu $\xrightarrow{BCAT}$ 4-methyl-2-oxopentanoate

BCAT : branched chain aminoacid aminotransferase

6.2. Deamination of amino acid (no oxoacid acceptor required)

Thr $\xrightarrow{threonine\ dehydratase}$ 2-oxobutanoate

6.3. Deamination of aminoacid metabolites



Adomet: *S*-Adenosyl methionine

Adohcy: *S*-Adenosyl homocysteine

MTA: Methylthio adenosine

MTOB: 4-Methylthio-2-oxobutanoate

Hcy: homocysteine

Spm: spermine

Spd: spermidine

Of these three anaplerotic pathways (6.1, 6.2, 6.3), that from methionine (6.3) is unique in that it gives rise to oxoacids involved in *both* apoptosis (MTOB) and cell proliferation (2-oxobutanoate).

7. Evidence of a role for methionine in an apoptotic pathway

MTOB is synthesised obligatorily from MTA, a by-product of aminopropylation (spermine and spermidine synthesis), by the action of MTA phosphorylase [14], and a deficit in MTA phosphorylase activity is found in many different human tumour cell lines in culture [15–17]. MTOB is oxidatively decarboxylated by BCOADC to 3-methylthiopropionyl CoA [18] via a transitory intermediate, methional, which is itself a potent apoptogenic compound for both normal and transformed cells in culture [19,20]. Additional evidence for a role of BCOADC in apoptosis is the observation that dexamethasone, a potent inducer of apoptosis, increases the synthesis and activity of the E2 complex of BCOADC [21].

8. Evidence of a role for branched-chain 2-oxoacid substrates of BCOADC in apoptosis

The overexpression of c-Myc can induce apoptosis in serum-deprived cultures of rat fibroblasts [22]. One of the targets of c-Myc regulation is the BCAT responsible for the transamination of valine to 3-methyl-2-oxobutanoate, isoleucine to 3-methyl-2-oxopentanoate, and leucine to 4-methyl-2-oxopentanoate [23]. The addition of 4-methyl-2-oxopentanoate to NIH3TE cells at 13 mM (the concentration found in the serum of maple syrup urine disease patients with an inborn genetic error in BCOADC) induces these 3T3 cells into apoptosis, whereas leucine at an equivalent concentration is ineffective [23]. 4-Methyl-2-oxopentanoate, like MTOB and the other branched-chain oxoacids, activates BCOADC [24] by inhibiting (IC_{50}: 20 μM) the specific BCOADC kinase responsible for the inactivation of BCOADC [25].

9. Evidence of a role for methionine in a proliferation pathway

Around 60% of human tumour cells are dependent upon methionine in the culture medium for their proliferation [26,27]. In a Met-free medium, this Met-dependence can be alleviated by the addition of MTOB to the medium at concentrations equimolar to those of the missing methionine [28] but not by the addition of equimolar homocysteine [26,27]. The inhibition by transition-state inhibitors of the Gln transaminase responsible for the transamination of MTOB → Met leads to an accumulation of cellular MTOB and concomitantly to the inhibition of proliferation [28]. This inhibition of growth is seen preferentially with

transformed rather than with normal cells [28] and cannot be reversed by the addition of methionine to the medium [28]. Therefore, it must be due to the accumulation of MTOB which attains the concentration necessary for it to be oxidatively decarboxylated by BCOADC to methional [19] and finally to 3-methylthiopropionyl CoA [18]. But the conversion of MTOB to methional is dependent upon the concentration of 2-oxobutanoate because BCOADC has a 3-fold greater affinity for 2-oxobutanoate (K_m 18 μM) than for MTOB (K_m 67 μM) [25,29]. 2-Oxobutanoate has growth promoting activity (see Section 4); hence, in cancer cells with hyper DNA and RNA methylase activities [30–32], there would be increased formation of 2-oxobutanoate arising from the condensation of Hcy (the final by-product of transmethylation) with serine to give cystathionine, which is then cleaved by cystathionase to 2-oxobutanoate in the transulphuration pathway.

For each molecule of 2-oxobutanoate formed from cystathionine, there is the concomitant formation of one molecule of cysteine, which is itself an allosteric inhibitor of cystathionase, and decreases in both cystathionase content and activity have been observed in some cases of human neuroblastoma [33] and leukaemia [34].

10. Evidence of a role for cysteine in an antiapoptotic pathway

Cysteine is a component of GSH, and when cells undergo apoptosis, a decrease in cellular and, in particular, mitochondrial GSH levels is a precocious event [35,36]. Radioresistance and chemoresistance are decreased by inhibiting intracellular GSH biosynthesis [37,38].

11. Conclusions

From the results presented in Sections 2–5, it would appear that:

(a) The transamination of Asn to oxaloacetate is increased in cells transformed by polyoma virus but not in their non-transformed counterparts.
(b) Glyoxylate is the amine acceptor in spontaneously transformed mouse lymphoma cells.
(c) There is a cause-and-effect relationship between Asn transamination (as an anaplerotic reaction) and the proliferation of asparaginase-sensitive lymphoma cells.

From those presented in Sections 6–9, it would appear that:

(a) The Met-dependence of some human and murine cancer cells is alleviated by adding MTOB but not homocysteine to the culture medium.
(b) The inhibition of MTOB transamination slows down the growth of cancer cells more effectively than that of normal cells.
(c) An increase in the intracellular content of BCOADC active enzyme (responsible for converting MTOB to 3-methylthiopropionyl CoA) either by the addition of dexamethasone or by the inhibition of BCOADC kinase (responsible for inactivating BCOADC) induces apoptosis in some normal and cancer cells.
(d) Methional, a transitory metabolic precursor in 3-methylthiopropionyl CoA biosynthesis, is a potent apoptogenic compound for many normal and transformed cells in culture with one exception, cells overexpressing Bcl_2 [39].

Some remaining questions that could be addressed in future investigations by classical biochemical methods and functional proteomics studies are outlined below.

(a) Glyoxylate, an amine acceptor in Asn transamination, arises from the degradation of hydroxyproline. Are the amounts and/or activities of the enzymes involved in hydroxyproline degradation as well as those of Asn transaminase and ω-amidase increased in certain benign and malignant tumours?
(b) In Met-dependent tumours, are the amounts and/or activities of the enzymes involved in 2-oxobutanoate synthesis increased, in particular those involved in cystathionine formation? Is there a substantial decrease in 3-methylthiopropionyl CoA, the final product of MTOB decarboxylation?
(c) In Met-independent tumours, are 2-oxobutanoate levels elevated and, if so, is this due to increases in the amounts and/or activities of threonine deaminase? In Met-independent tumours are the amounts and/or activities of the BCATs or of pyruvate carboxylase, another well established anaplerotic reaction, increased?

Before undertaking such studies though, it must be stressed that the results reported here were obtained with many different cell types in culture. Therefore, the pathways involved may not all be characteristic of every type of cancer cell. Nevertheless, the concept of anaplerotic reactions, which in our opinion has not received the attention it merits, provides one rationale for selecting oxoacid pathways in each particular type of cancer cell under study in order to determine the contribution of the genes and enzymes mediating these pathways to proliferation and/or apoptosis.

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