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Review Article

Metabolic Pathways of Endogenous Formaldehyde

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Abstract

It is generally accepted that formaldehyde (FA) is a toxic substance. It is formed in various demethylation and transmethylation reactions. And there is an intracellular system for its detoxification. Meanwhile, its effect on the cell causes a different response: apoptosis, proliferation, differentiation and depends on the concentration of FA. Evolutionarily, FA is a component of the one-carbon transport system. Oxidation of the methyl group in the folate cycle is a proton donor for the reduction of NADP+. In the cell, FA spontaneously non-enzymatically binds to pterin, arginine, glutathione, which determines its participation in the metabolism of purines, nitric oxide and polyamines, and in the redox system. This review describes a number of metabolic pathways that are linked into a single system thanks to FA. FA is the main source of formate in the cell and closely interacts with arginine metabolic pathways, influencing the synthesis of nitric oxide and polyamines. The possible role of formaldehyde dehydrogenase (ADH5) in the regulation of intracellular pH is also considered.

INTRODUCTION

The biogenic carbon redox cycle is carried out by two types of biosystems – methanogens and methanotrophs. Methanogenic archaea are strictly anaerobic Euryarchaeota that reduce carbon dioxide to methane. They use molecular hydrogen, formate, methanol, methylamines and acetate as proton donors [1]. The reduction of CO2 occurs sequentially along the line "carbon dioxide-formate-formaldehyde-methanol-methane". The formyl group is transferred from methanofuran to a pterin-containing compound, tetrahydrosarcinapterin (H4MPT), an analogue of pterin-containing tetrahydrofolic acid (THF). One-carbon compounds (C1) bind to pterin. The second half of the carbon redox cycle is the oxidation of methane by methylotrophs. Methylotrophs are a heterogeneous group of microorganisms, represented by obligate and facultative bacteria. In these bacteria, methane is oxidized enzymatically to formaldehyde (FA), which combines with two C1-compound carriers: H4MPT to form methylene-H4MPT and THF to form methylene-THF. In these microorganisms, FA also enters metabolic pathways through the ribulose monophosphate pathway, in which glyceraldehyde-3-phosphate is formed, and through the serine pathway, in which methylene-THF interacts with glycine to form serine [2]. In mammals, the pathway for transporting C1 bound to THF is preserved, and methylene-THF is possibly involved in the synthesis of serine from glycine. Spontaneous binding of FA to THF has been shown in the pH range of 5.4-8.5. In pig liver extracts, FA was incorporated into serine in the pH range of 7.7-8.0 [3]. Further evolution followed the line of methylotrophs, where FA is oxidized to formate.

SOURCES OF ENDOGENOUS FORMALDEHYDE

FA is a by-product of various transmethylation [4] and demethylation reactions, including histones that contain methyl lysines [5]. Histone demethylation is irreversible. As a result of the action of the enzyme LSD 1 (lysine-specific demethylase 1), one molecule of FA is released from monomethyl lysine, two from dimethyl lysine, and three from trimethyl lysine [6].

FA is released during the demethylation of dimethylglycine (sarcosine is formed) and during the synthesis of glycine from sarcosine. Serine and glycine are the main suppliers of methyl groups for the folate cycle [7]. As a result of the demethylation of serine with the participation of THF, 5,10-methylene-THF (CH2-THF) and glycine are formed, which breaks down in the glycine-cleavage system (GCS) also with the formation of CH2-THF [8].

Kikuchi et al.[9] found that FA can be released into GCS in the absence of THF. But then spontaneously, non-enzymatically, reacts with THF to form CH2-THF [10]. However, CH2-THF is highly unstable at pH 7.5 and also spontaneously decomposes into FA and THF, while THF returns to GCS and the glycine degradation cycle does not stop [11]. Jägerstad M. et al. [12], reported that CH2-THF can spontaneously dissociate into THF and FA at both physiological and acidic pH values, and is stabilized only at pH > 8.0 or in the presence of a very large excess of FA.

In an acidic environment, THF spontaneously undergoes oxidative degradation. THF degrades to form N-(4-aminobenzoyl)-L-glutamic acid, FA, and pterin, and as the environment becomes more acidic, FA becomes more abundant [13]. When THF interacts with FA to form CH2-THF, its degradation is slowed down. It is assumed that under physiological conditions, CH2-THF still remains stable and does not degrade, and it is more stable than THF [10]. When the intracellular FA level is high, THF is stabilized and forms CH2-THF, which simultaneously reduces FA production and its content in the cell. CH2-THF is consumed in the synthesis of thymidine and methionine. When the FA level is low, some unreacted THF is degraded and FA is produced. In this case, THF itself is a source of methyl group for the folate cycle. This means that the folate cycle can exist as a self-sustaining system, independent of the associated metabolic pathways and enzyme action, which is regulated by the concentration of PA and the pH of the environment.

CONNECTION OF METABOLIC PATHWAYS

Dihydrofolate reductase reduces dietary folate to dihydrofolic acid, which is then reduced by the same enzyme to THF. The enzyme cofactor is NADP+. Methyl groups are added to THF at N5 and/or N10 positions and have different oxidative status, which corresponds to methanol (5-methyl THF, CH3-THF), formaldehyde (5,10-methylene THF, CH2-THF) and formate (5,10-methenyl THF, CH-THF; 10-formyl THF, 10-CHO-THF and 5-formyl THF, 5-CHO-THF). n fact, the sequence of oxidation of the methyl group is built in accordance with the stages of methane oxidation: “methane-methanol-formaldehyde-formic acid-carbon dioxide”. Oxidation of the methyl group is the main proton donor (source of NADPH) in the conjugated redox system [14]. Fan et al. [15], performed a quantitative flux analysis, which revealed that NADPH is produced in the cell mainly by oxidation of cytoplasmic CH2-THF to CHO-THF.

In the cytoplasm, the enzyme serine hydroxymethyltransferase 1 (SHMT1) is active, which transfers the methyl group to glycine and, in fact, cannot participate in the formation of CH2-THF. In this case, FA can be a donor of the methyl group for binding to THF in the cytoplasm. FA may thus link the folate cycle with the cycle of glycine formation from serine via the serine-ethanolamine-choline-betaine aldehyde-betaine-dimethylglycine-sarcosine-glycine pathway. It is possible that FA released during demethylation of dimethylglycine and sarcosine is also involved in serine formation. Ethanolamine is a component of phosphatidylcholine, and choline is a component of phosphatidylcholine.

THF (Figure 1), is used as a carrier of intermediate products of the methyl group oxidation chain, thereby providing 1C-compounds for various metabolic pathways, including the biosynthesis of polyamines and phospholipids, as well as in genome methylation reactions. The metabolism of 1C-compounds is key for proliferating cells, as it provides components necessary for the synthesis of nucleic acids [16].

The folate cycle is coupled with the methionine cycle and is localized in the cytoplasm, mitochondria and nucleus [16]. CH2-THF is a substrate for methylenetetrahydrofolate reductase (MTHFR), which irreversibly converts CH2-THF to 5-methyltetrahydrofolate (CH3-THF), which plays a key role in methionine metabolism.

Thymidylate synthase (TYMS) uses CH2-THP as a methyl donor and methylates deoxyuridine monophosphate to form thymidine monophosphate [17]. Glycine, formed in the mitochondria in the reaction with SHMT2, is needed for the synthesis of purines, as well as glutathione (together with cysteine and glutamate). In addition to glycine, 10-CHO-THF is also needed for the synthesis of purines. The formyl group of 10-CHO-THF is used by phosphoribosylaminoimidazolecarboxamide formyltransferase and phosphoribosylglycinamide formyltransferase, which insert it into positions 2 and 8 of the purine ring carbons. With the participation of ATP, FA can non-enzymatically interact with THF to form 10-CHO-THF.

5-CHO-THF is not used as a cofactor in folate-dependent synthesis reactions and is considered a storage form of THF in dormant cells [18]. The enzyme methenyltetrahydrofolate synthetase (MTHFS) irreversibly converts 5-CHO-THF to CH-THF using ATP. CH-THF can non-enzymatically produce two forms of formyl THF: 5-CHO-THF and 10-CHO-THF [19].

1C-METABOLISM AND FOLATE CYCLE

In the mitochondria, CH2-THF is formed by demethylation of serine, as well as in GSC. In the cytoplasm,

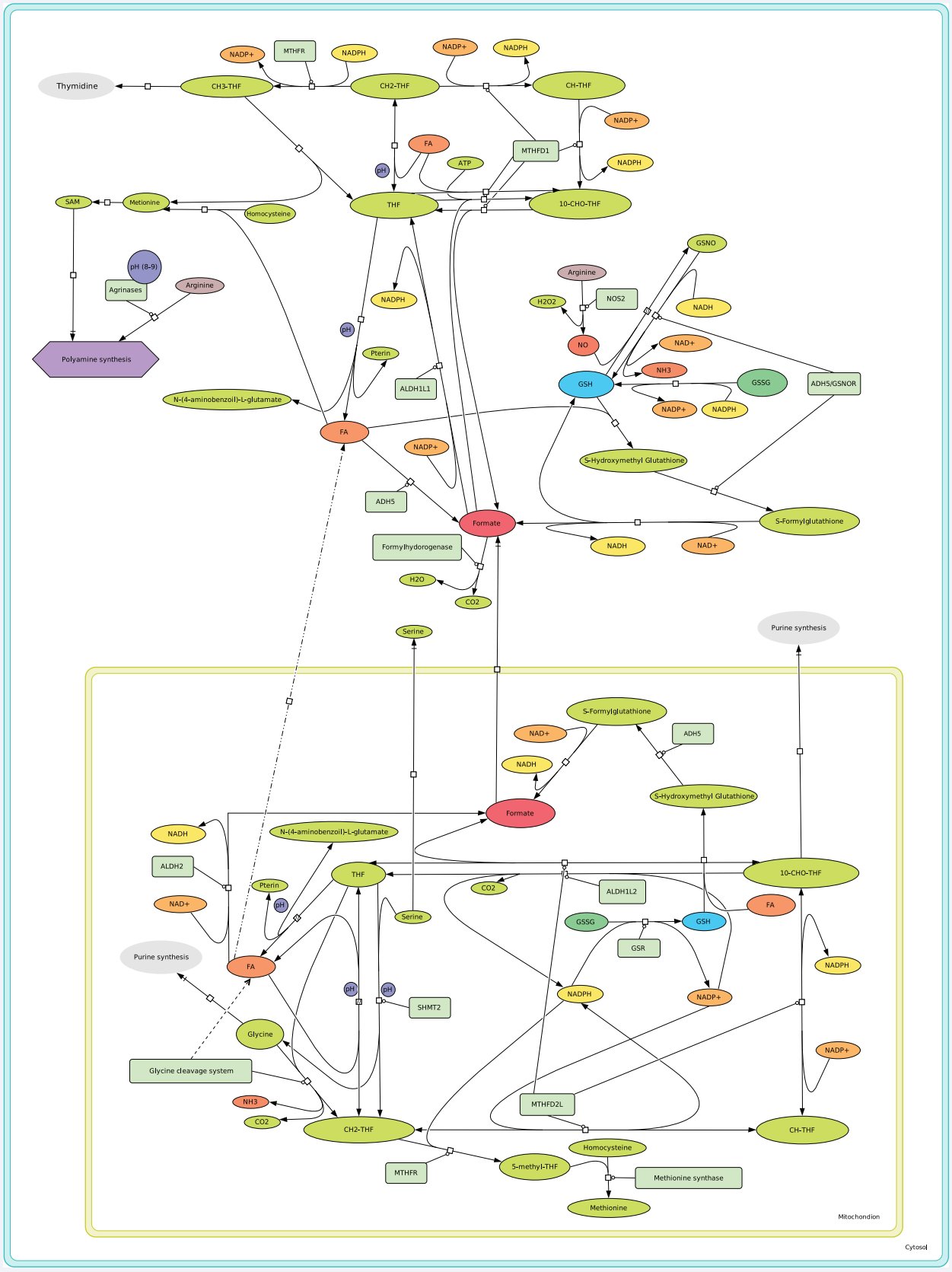

Figure 1 Coupling of metabolic pathways of formate formation in the cytoplasm and mitochondria.

the source of CH2-THF may be the non-enzymatic interaction of THF and FA. CH2-THF is enzymatically and sequentially converted into CH-THF and 10-CHO-THF. 10-CHO-THF is used in the synthesis of purines or is a source of formate and THF, which is returned to the folate cycle. Formate is oxidized with the release of carbon dioxide. The reversible oxidation of CH2-THF to CHO-THF is catalyzed by the enzyme methylenetetrahydrofolate dehydrogenase (MTHFD). There are two isoforms of this enzyme: cytoplasmic MTHFD1 and mitochondrial MTHFD2. MTHFD1 is a trifunctional enzyme, it exhibits in addition to CH2-THF dehydrogenase activity also CH2-THF cyclohydrolase and 10-CHO-THF synthetase activity, which uses $NADP^+$ as a cofactor, i.e. NADPH is formed in this reaction. MTHFD1 catalyzes not only the reversible two-step conversion of CH2-THF to CHO-THF [20], but also the ATP-dependent synthesis of CHO-THF from formate and THF.

MTHFD2 is a bifunctional enzyme, it exhibits only dehydrogenase and cyclohydrolase activities and uses NAD+ as a cofactor. However, knockdown of MTHFD2 showed that this enzyme is also involved in the production of NADPH, although it preferentially uses NAD+ [21]. A mitochondrial analogue of MTHFD2, MTHFD2L, has also been identified, which uses NADP+ as a cofactor. It is expressed at a low level in the early stages of mouse embryo development, and its expression increases by day 10. It is then found in all organs of the adult animal, most prominently in the lungs and brain [22,23]. Mammalian fibroblasts deficient in MTHFD2 were glycine auxotrophs due to accumulation of CH2-THF, a substrate of MTHFD2, which inhibits SHMT2 and blocks glycine formation [24]. Expression of GCS genes is accompanied by increased levels of folate pathway enzymes, including MTHFD2.

Mitochondrial folate enzymes produce formate to a greater extent than cytoplasmic ones. This is due to the mitochondrial enzyme MTHFD1L, which exhibits only 10-CHO-THF synthetase activity, supplying the substrate for nucleotide synthesis [25]. However, it catalyzes the reverse reaction with greater efficiency, releasing formate from 10-CHO-THF [26].

The formyl group in 10-CHO-THF can be oxidized with the release of CO2 and the simultaneous reduction of NADP+ to NADPH in both the cytoplasm and mitochondria [27]. The oxidative degradation of 10-CHO-THF to THF and CO2 is catalyzed by the enzyme 10-formyltetrahydrofolate dehydrogenase (10-FDH). Since 10-CHO-THF is a substrate for purine biosynthesis reactions, it was suggested that by reducing the intracellular purine content, 10-FDH inhibits cell proliferation [28,29]. At the same time, it appears to regulate the 1C content of THF metabolites, as this is the last reaction of the folate pathway. The reaction product, CO2, leaves the cell. This enzyme could be considered in terms of regulating formate concentration in the cell [30], if there were no pathways for direct oxidation of FA to formate.

10-FDH contains pentaglutamyl-THF as a tightly bound non-catalytic cofactor. The 10-FDH gene is very similar in nucleotide sequence to the genes of enzymes of the aldehyde dehydrogenase family, so its other name is aldehyde dehydrogenase (ALDH).

There are two isoforms of the enzyme: cytosolic ALDH1L1 and mitochondrial ALDH1L2 [27,30]. ALDH1L1 is the most abundant cytosolic enzyme of 1C metabolism, its amount reaches 1.2% of the total protein in rat liver [27,30,31]. ALDH1L1 is expressed in many tissues at levels several times higher than ALDH1L2 expression [30]. The contribution of this enzyme to the cytosolic pool of intracellular NADPH has not yet been established. In mitochondria, NADPH production in 1C metabolism is coupled with the enzymatic activity of ALDH1L2 [15,32].

It has been suggested that this reaction is the main supplier of NADPH to mitochondria [33]. ALDH1L2 is the product of the ALDH1L2 gene. It has approximately 74% similarity with the cytosolic 10-FDH gene, and the identity of the dehydrogenase domain reaches 79% [34]. Unlike its homologue, ALDH1L2 is expressed in malignant tumors and is involved in the regulation of metastasis [35].

Since the sequential oxidation reactions of THF metabolites, including 10-CHO-THF, are reversible, it is believed that 10-FDH may be a major regulator of the folate cycle and related metabolic pathways.

FA is one of the sources of formate in the cell [36]. When mammalian cells were incubated in a medium containing physiological concentrations of FA (20-40 mM), it was the source of formation of 10% to 50% of all cellular formate [37]. In the cytosol, the enzyme formate-THF ligase, the activity of the trifunctional enzyme MTHFD1, binds it to THF in an ATP-dependent reaction to form CHO-THF [38]. It is unknown under what conditions the enzymatic and non-enzymatic binding of FA to THF using ATP occurs.

FORMALDEHYDE DETOXIFICATION

FA spontaneously reacts with sulfhydryl groups of cysteine, particularly in reduced glutathione (GSH). The cytosolic zinc-containing enzyme alcohol dehydrogenase class 5 (ADH5), also known as alcohol dehydrogenase class

III (ADH3) and formaldehyde dehydrogenase (FALDH), oxidizes the resulting hydroxymethyl-GSH (HMGSH) using NAD+ to formyl-GSH [39]. Formyl-GSH hydrolase (esterase D hydrolase activity) then hydrolyzes formyl-GSH to formate and GSH [40]. Formate binds to THF (CH-THF) or is oxidized to CO2. FA, which in an acidic environment (pH < 7.4) is formed as a result of the breakdown of THF and CH2-THF, at pH> 7.5 spontaneously interacts with both THF and GSH. GSH and THF compete for binding to FA [41,42]. However, THF has been shown to be significantly more efficient at capturing FA than GSH, indicating that CH2-THF can persist in a glutathione-rich environment [43]. The folate cycle and the glutathione system are coupled not only at the level of NADPH formation, which is used to reduce oxidized GSSG to GSH, but also at the level of maintaining certain FA concentrations in the cell, including through the formation of HMGSH. The interaction of FA with GSH, in this case, should not be considered as its detoxification.

Metabolomic analysis shows that formate, as a product of FA oxidation associated with glutathione, is actively involved in DNA and ATP synthesis, since the radioactive label in nucleotides did not appear in ADH5-deficient cells [44] (Km = 0.12–6.5 μM). However, the culture of chicken DT40 cells lacking the ADH5 gene can grow normally, and their sensitivity to exogenous FA does not differ from that of wild-type cells [44]. The lifespan (LS) of Adh5−/− mice did not differ from that of wild-type animals [45]. Moreover, Adh5−/− mice of both sexes were born without defects and developed normally [46]. It was suggested that there is another metabolic pathway of FA not associated with ADH5. FA can be oxidized to formate by mitochondrial aldehyde dehydrogenase 2 (ALDH2, Km = 170–400 μM) [47]. ALDH2 with a relatively low Km can be considered a compensatory enzyme in relation to ADH5.

The main substrate for ALDH2 is acetaldehyde, the cofactor is NAD+. ALDH2 oxidizes acetaldehyde to acetate and is important in ethanol metabolism [48]. Mice knocked out in two genes, mitochondrial ALDH2 and cytoplasmic ADH5, had a very short lifespan, developed leukemia, had profoundly impaired hematopoiesis, and had decreased levels of hematopoietic stem cells and lymphoid progenitors, which led to the loss of acquired immunity [49]. These mice had an 11-fold increase in blood FA levels, which correlated with both FA-induced DNA damage and mutagenesis similar to that observed in human cancer cells [50]. Decreased ALDH2 activity is associated with decreased oxygen consumption rate. ALDH2-deficient mice had decreased mitochondrial respiration, despite unchanged expression of electron transport chain (ETC) proteins [51]. This may be due to inhibition of ETC complex 4 by nitric oxide.

The effect of FA on the cell is dose-dependent. At a concentration of 1.0–10 μg/ml of analytically pure FA, apoptosis was observed in the culture of cancer and endothelial cells, and at a concentration of 0.1–0.01 μg/ml, cell proliferation was observed [52,53]. When rat hepatocytes were incubated in a medium with a low concentration of FA, a dose-dependent decrease in the membrane potential of mitochondria was observed, accompanied by the formation of ROS. The content of GSH also decreased dose-dependently [54].

Thus, manipulations that change the intracellular FA content can affect the functioning of the folate cycle and cell proliferation. In normal cells, the intracellular pH is <7.4, and then the folate cycle does not function. It is only triggered in proliferating cells at alkaline intracellular pH. How can formate formation during a functioning folate cycle affect intracellular pH?

METHIONINE CYCLE AND POLYAMINES

Ectopic expression of 10-FDH results in decreased levels of CH3-THF and S-adenosyl methionine (SAM) [55]. MTHFR, with the participation of NADPH, irreversibly reduces CH2-THF to CH3-THF, which couples the folate cycle with the methionine cycle. CH3-THF is a methyl group donor for methionine synthase, which catalyzes the synthesis of methionine from homocysteine. Of all the metabolites of the folate cycle, CH3-THF is the one that is formed in the greatest quantity [56]. In mammals, methionine synthesis is the only reaction involving CH3-THF [57]. MTHFR is allosterically inhibited by SAM [58,59]. The methionine cycle supplies methyl groups to methylases that methylate DNA. CH3-THF allosterically inhibits glycine N-methyltransferase (GNMT) [60]. GNMT catalyzes the conversion of SAM to S-adenosylhomocysteine (SAH) and plays an important role in maintaining the SAM/SAH ratio [61]. GNMT activity is regulated by the concentrations of both SAM, which inhibits MTHFR and the formation of CH3-THF, and CH3-THF itself [62]. Deletion of the GNMT gene results in a significant increase in SAM levels and changes in methylation status in vivo. GNMT is one of the factors regulating proliferation and carcinogenesis [63,64]. Demethylation of SAM in the methionine cycle releases FA [65]. At the same time, FA methylates homocysteine to form methionine. SAM is involved in the synthesis of polyamines. Arginase is active at alkaline pH (7.5-8.0) and functions in proliferating cells.

NITRIC OXIDE AS A COMPONENT OF THE CONJUGATED REDOX SYSTEM

Nitric oxide (NO) exhibits two opposing effects: on the one hand, it inhibits proliferation, on the other, it activates it. This paradox is explained by its concentrations that were used in different experiments. High concentrations of NO suppress, while low concentrations activate the proliferation of both normal and tumor cells [66]. In the microenvironment of tumor cells, the content of NO is recorded in nanomolar (nmol/L) quantities. It is these concentrations that support carcinogenesis.

In the search for intracellular targets of nitric oxide that induce proliferation in human breast cancer cell lines MDA-MB-231 and MCF-7, the NO donor DETA/NO, characterized by a slow release of NO and a half-life of about 20 hours, was used. NO in nanomolar concentrations significantly increased protein synthesis, primarily the translation of cyclin D1, without affecting the level of their mRNA [67]. When using DETA/NO in a culture of hematopoietic stem and progenitor cells, its biphasic effect was revealed. At low concentrations DETA/NO activated classical NO signaling and proliferation, at high concentrations DETA/NO slowed cell division and initiated their differentiation. Exposure of embryonic stem cells to low concentrations of NO (from 2 to 20 μmol/l) can slow differentiation [68]. In another range of low concentrations (10 - 50 μM/l), the NO donor protected mouse bone marrow stromal cells from spontaneous apoptosis [69]. The arrest of keratinocyte proliferation under the influence of NO occurs simultaneously with an increase in the activity of cytosolic superoxide dismutase 1 (SOD1) [70]. In mitochondria, SOD2 neutralizes superoxide anion, increasing NO production, which is observed in fibrosarcoma cells [71]. NO is involved in proliferation, including through pathways associated with polyamine metabolism [72].

ETC components have different sensitivities to NO inhibition. At concentrations below 0.2 μM, NO reversibly inhibits COX, controlling mitochondrial respiration; in the range of 0.3–0.5 μM, it inhibits electron transfer between cytochromes b and c1 [73]. With relatively long-term exposure at a concentration of 0.5–1 μM NO, the activity of NADH dehydrogenase complex I is selectively inhibited in intact cells [74].

Extracellular pH affects the production of NO and hydroxyl radical in hepatocytes. As a result of acidification of the medium (pH 7.0), there is a significant increase in OH radicals, cell damage and a sharp decrease in glutathione content. Incubation at physiological pH 7.4 led to an increase in NO synthesis, and at pH 7.8 - to a significant increase [75]. Meanwhile, inducible NO synthase (iNOS) is active at acidic pH (7.0-7.4) and utilizes NADPH, the oxidation product of the methyl group of CH2-THF and formate in the folate cycle. At physiological (pH 7.4) and alkaline (pH 7.8) pH values, a decrease in ONOO- formation correlated with a decrease in superoxide anion formation and an increase in GSH levels [76]. Thus, at acidic pH values, NO interacts with superoxide anion to form ONOO-, and at alkaline pH values, with GSH. In many normal and cancer cells, increased GSH levels are associated with a proliferative response [77]. Elevated GSH levels have been observed in a variety of cancers including breast, ovarian, lung, head and neck cancers [78]. There is a direct correlation between GSH levels, proliferation and metastasis [79].

NO competes with FA for binding to GSH. ADH5 (FALDH) uses not only HMGSH but also nitrosoglutathione (GSNO) as a substrate, reducing it to glutathione and ammonia. GSNO is formed non-enzymatically by the interaction of ONOO- with cysteine in glutathione. This catalytic activity has given FALDH another name – nitrosoglutathione reductase (GSNOR). It can restore nitrosylated cysteine residues not only in glutathione but also in proteins [80]. Attention has been drawn to this enzyme since it became clear that NO is a signaling molecule involved in a wide variety of physiological processes [81].

GSNOR is a cytosolic zinc-containing enzyme that functions as a dimer. Loss of GSNOR activity leads to the development of nitrosative stress [82]. The rate of GSNO catalysis is 20 times higher than the rate of HMGSH catalysis [83]. The products of reactions catalyzed by GSNOR are ammonia (in the case of GSNO) and formate (in the case of HMGSH), which can affect intracellular pH depending on the concentrations of NO and FA [84]. It can be speculated that ADH5 in the coupled redox system can serve as a basal regulator of intracellular pH by releasing formate and ammonia.

NOS AND FORMALDEHYDE

Both excess and deficiency of arginine in the medium affect cell proliferation. Deficiency of arginine causes apoptosis [85]. In cells of some types of cancer, a combined activation of iNOS and arginine synthase is observed [86,87]. Arginine is a substrate for the synthesis of NO and polyamines. It is methylated at the guanidine group, yielding hydroxymethyl and methyl derivatives. Methylarginines inhibit NOS [88]. Arginine is methylated by SAM and FA [89].

The methyl group of SAM is transferred to the guanidine nitrogen of arginine residues of proteins. This transfer is catalyzed by SAM- or AdoMet-dependent methyltransferases PRMT (protein N-arginine methyltransferases) [90,91]. In mammalian cells, the main methyl group carriers are PRMT1 and PRMT5. They methylate almost all arginines in proteins. However, in

human cells and yeast, about 80% of all methyltransferase activity is accounted for by PRMT1 [92]. PRMTs are regulated by kinases. Thus, phosphorylation of CARM1 (PRMT4) leads to a decrease in its activity [93]. In proteins, arginine can be methylated in three different ways. During proteolysis in proteasomes or lysosomes, small molecules of NG-monomethyl-l-arginine (l-NMMA, MMA), NG, NG-dimethylarginine (asymmetric dimethylarginine, ADMA), and NG, NG'-dimethylarginine (symmetric dimethylarginine, SDMA) are released from proteins. ADMA is obtained by methylation of arginine by methylase PRMT1, SDMA – by methylation by methylase PRMT2.

ADMA and l-NMMA are the most potent NOS inhibitors. They inhibit all three isoforms almost equally: iNOS, eNOS (endothelial), nNOS (neuronal). Due to its high concentration, ADMA inhibits NOS more actively than l-NMMA. ADMA competes with arginine not only for binding to NOS, but also for transport into the cell [94]. The Na+ transport system Y+ CAT (cation arginine transport), common to all N-methylarginines and arginine, transports ADMA and SDMA with varying efficiency [95]. The concentration of arginine in blood plasma is ~ 100 to 200 μM, the concentration of ADMA is significantly lower: 0.3–1.0 μM [96]. However, the intracellular content of ADMA is much higher than the extracellular content: 5-20 times [97]. CAT probably does not ensure equilibrium between intracellular and extracellular ADMA levels. Intracellular ADMA can inhibit CAT by limiting both ADMA efflux and arginine influx [96]. Access of extracellular arginine to NOS is facilitated by the enzyme's close association with the arginine Y+ transporter. Activation or inhibition of NOS thus depends on the L-arginine/ADMA concentration ratio [98,99]. SDMA has only one methyl group on each nitrogen of the guanidine group. This methylarginine is a structural isomer of ADMA. However, SDMA does not inhibit NO, but deactivates SAT and limits the bioavailability of arginine, thereby indirectly affecting NOS activity [100].

SDMA is excreted from the bloodstream in the urine [101], and ADMA and L-NMMA are catabolized by DDAH 1 (Dimethylarginine Dimethylaminohydrolase, two isoforms DDAH 1 and DDAH 2) to form dimethylamine and citrulline [96]. Part of the dimethylamine is demethylated to form methylamine and FA. Oxidative deamination of methylamine produces three products: ammonia, FA, and hydrogen peroxide [102]. Both isoenzymes have a higher affinity for ADMA than for L-NMMA [102,103].

DDAH is a semicarbazide-sensitive amine oxidase. It belongs to a group of copper-containing amine oxidases that are inhibited by semicarbazide. DDAH is a homodimeric transmembrane glycoprotein that has a large extracellular domain with a catalytic center containing Cu2+. The enzyme cofactor is 6-hydroxydopaquinone. The presence of Cu^{2+} in the active site indicates the possibility of direct inhibition of this enzyme by NO. S-nitrosylation of cysteines in the active site of DDAH (Cys-249) by nitric oxide also inhibits the enzyme. When ADMA accumulates, it in turn blocks NO synthesis. At the same time, accumulating methylamine and dimethylamine can enhance NO synthesis. Methylarginines can also regulate the formation of superoxide anion by NO synthases [103,104]. When BH4 is oxidized to BH2, ADMA stimulates the production of ROS, which in turn increases the concentration of intracellular ADMA. At the same time, ONOO- increases the synthesis of free FA through the modification of proteins by oxidizing their methionine residues into methionine sulfoxide. After release from proteins as a result of proteolysis, methionine sulfoxide, under conditions of hyperproduction of NO and ROS, undergoes further oxidation to form FA [105].

The guanidine group of arginine can bind one, two, or three FA molecules, resulting in the formation of mono-, di-, and tri-hydroxymethylated derivatives of arginine [106]. The hydroxymethyl groups are bound to the guanidine group reversibly, so that arginine can either accept or donate an FA molecule. Hullán et al. [107], noted that NG-hydroxymethylated arginine regulates the level of endogenous FA. Arginine interacts with FA both in free form and as part of proteins. And transfers it to the folate cycle and to the synthesis of thymidine. Hydroxymethylated derivatives of arginine are relatively stable compounds. They can serve as a reservoir and carriers of FA [4].

NG-hydroxymethylarginine dose-dependently and significantly inhibited the proliferation of HT-29 colon carcinoma cells, P-388 mouse lymphoma cells, PC-3 prostate carcinoma cells, and K-562 human erythroleukemia cells in culture [108]. In vivo experiments were performed by intraperitoneally administering 400 mg/kg hydroxymethylated arginine to mice with transplantable Ehrlich ascites carcinoma daily. Complete cessation of tumor growth was achieved after 10 days [109].

ARGINASE AND FORMALDEHYDE

Arginine is a substrate for both NOS and arginase. Increased expression of arginase has been reported in many types of human cancer [110,111]. Polyamines are small cationic molecules that are involved in cell proliferation and differentiation at millimolar concentrations [112,113], regulation of signal transduction, expression of cell cycle genes [114,115]. Increased intracellular polyamine content is associated with tumor development [113]. At the same time, intracellular accumulation of polyamines

induces apoptosis [116], through activation of caspases [117].

The optimal pH for arginase is 9.0–9.5 [118]. An intermediate in nitric oxide synthesis, N-hydroxy-L-arginine (NOHA), is an arginase inhibitor [119-121]. S-nitrosylation of arginase cysteines is a post-translational modification of the enzyme, which does not reduce, but increases its activity [122]. Inhibition of arginase 1 stimulates NO production, which reduces polyamine synthesis by shutting down ornithine decarboxylase (ODC), the first enzyme in the metabolic pathway for polyamine synthesis from ornithine [123]. NO inhibits ODC by S-nitrosylation of cysteine residues in its catalytic center, thereby blocking polyamine production and cell proliferation [124]. At the same time, in cancer cells, ODC activity is constantly elevated [125,126]. In colorectal cancer cells, the content of polyamines, the activity of ODC and another enzyme of their synthesis, S-adenosylmethionine decarboxylase, is increased by 3 times compared to intact cells [127,128]. In the synthesis of methionine from homocysteine, CH3-THF and FA directly are involved. Thus, the synthesis of polyamines is associated with the synthesis of nucleotides, and with FA both through CH2-THF and directly.

Polyamines regulate MYC expression, whereas c-Myc regulates ODC expression at the transcriptional level [129,130]. ODC translation is affected by ammonia accumulation, significantly reducing it. Ammonia inhibits polyamine biosynthesis and cell proliferation.

In normal cells, the content of reduced glutathione is 98% of its total amount [131]. With an increase in the GSSG/GSH ratio, the activity of the enzyme GSH-disulfide reductase is induced, using NADPH as a cofactor [132]. NO can inhibit GSH reductase. This relationship is not well understood, since NADPH is consumed both for NO synthesis and for GSSG reduction. There are likely as yet unidentified regulations in the glutathione and NO metabolic pathways.

CONCLUSION

For a long time, the effect of FA on the biosystem was considered from the point of view of its high toxicity. The toxicity of FA is due to its interaction with DNA (genotoxicity) and with proteins, with the formation of carbonyl derivatives. On the other hand, FA not only induces apoptosis, but also affects the proliferation of normal and cancer cells and the differentiation of stem cells. This bidirectional action is due to its concentrations, both exogenous and endogenous. For normal functioning, a system must exist in the cell to maintain the concentration of FA at a certain level, i.e. the reactions in which it is released and the reactions in which it is absorbed must be balanced. During cell transformation, the FA content may be increased, but this does not cause apoptosis, but mutagenesis. Apparently, in carcinogenesis, the primary change is the balance of FA content, while mutagenesis is secondary.

FA can be oxidized to formate and reduced to methanol with equal probability, which determined its regulatory role in the functioning of the biogenic carbon redox cycle; methanogenic archaea are methylotrophic archaea (rudiments). Evolution followed the line of methylotrophy, and methyl groups (later acetate) become proton donors for reduction reactions involving NADPH. The basic pathway for both methanogens and methylotrophs is the transfer of C1 compounds, which evolves in methylotrophs into the folate cycle and is coupled with basic metabolic pathways, including nucleotide synthesis and DNA methylation.

Endogenous FA metabolism is closely related to arginine metabolism. Apparently, through the formation of methylarginines, FA controls the synthesis of NO and through the formation of methionine, the synthesis of polyamines.

Can ADH5 activity be considered as FA detoxification? FA spontaneously interacts with both THF and GSH. Both of these interactions result in formate formation. Formate is oxidized to CO2, which can be considered as a factor in the acidification of the intracellular environment. At the same time, ADH5 is 20 times more intensive in reducing GSNO to form ammonia, which can be a factor in the alkalization of the environment. Is the activity of ADH5, an evolutionarily conserved enzyme with dual redox action - oxidation of HMGSH to formate and reduction of GSNO to ammonia - fundamental to the regulation of intracellular pH? The questions remain open.

In this regard, endogenous FA metabolism can be considered from the point of view of coupling of the main metabolic pathways, regulation of the redox system functioning and intracellular pH. The scope of this review does not allow considering coupling of FA with other metabolic pathways, in particular, the participation of serine in the regulation of pyruvate kinase activity and others.

We believe that further work expanding the systemic description of these coupled regulatory pathways will shed light on the interaction of processes that determine cell fate and its functional modes.

By using data on the kinetics of biochemical reactions, including non-enzymatic ones, and the factors of their

regulation, including pH, FA, NO, and taking into account the expression of enzymes, it will be possible to construct a systemic biological model of the cell, which will allow us to obtain a description of the metabolic regimes of the cell and link them with the processes of proliferation, differentiation and apoptosis.

Conflict of Interest

Authors Laskaviy VN and Polyanina TI are founders and employees of SARBIOTEKH, which is developing a formaldehyde-based therapeutic product. This employment represents a potential conflict of interest. All other authors declare no competing interests.

Author Contributions

Tereshina EV was responsible for the conceptualization and methodology of the study, performed the formal analysis, and prepared the original draft of the manuscript. Formal analysis and critical input during manuscript revision were also provided by Alekseev AA, who was responsible for review and editing. Alekseev AA, Polyanina TI, Laskaviy VN and Sukhikh GT contributed to the discussion and interpretation of the results. All authors read and approved the final version of the manuscript.

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