Molecular Aspects of Medicine xxx (2016) 1–9

Contents lists available at ScienceDirect

Molecular Aspects of Medicine

journal homepage: www.elsevier.com/locate/mam

Targeting nuclear thymidylate biosynthesis

James Chon [a], Patrick J. Stover [a, b], Martha S. Field [b, *]

[a] Graduate Field of Biochemistry, Molecular and Cellular Biology, Cornell University, Ithaca, NY 14853, USA
[b] Division of Nutritional Sciences, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 26 August 2016
Accepted 18 November 2016
Available online xxx

Keywords:
Nuclear thymidylate synthesis
Antifolate
Folate-mediated one-carbon metabolism
Sumoylation
Thymidylate synthase

ABSTRACT

Thymidylate (dTMP) biosynthesis plays an essential and exclusive function in DNA synthesis and proper cell division, and therefore has been an attractive therapeutic target. Folate analogs, known as antifolates, and nucleotide analogs that inhibit the enzymatic action of the *de novo* thymidylate biosynthesis pathway and are commonly used in cancer treatment. In this review, we examine the mechanisms by which the antifolate 5-fluorouracil, as well as other dTMP synthesis inhibitors, function in cancer treatment in light of emerging evidence that dTMP synthesis occurs in the nucleus. Nuclear localization of the *de novo* dTMP synthesis pathway requires modification of the pathway enzymes by the small ubiquitin-like modifier (SUMO) protein. SUMOylation is required for nuclear localization of the *de novo* dTMP biosynthesis pathway, and disruption in the SUMO pathway inhibits cell proliferation in several cancer models. We summarize evidence that the nuclear localization of the dTMP biosynthesis pathway is a critical factor in the efficacy of antifolate-based therapies that target dTMP synthesis.

1. Overview of folate-mediated one-carbon metabolism (FOCM)

Folate functions as a family of enzyme cofactors that chemically activate and transfer one-carbon units for fundamental cellular processes including DNA replication and repair, mitochondrial protein synthesis and amino acid interconversions and catabolism (Fox and Stover, 2008). The single carbons carried by tetrahydrofolate (THF) are generated from the catabolism of the one-carbon donors that include serine, glycine, sarcosine, dimethylglycine and histidine. Folate-activated single carbons are required for *de novo* purine synthesis, *de novo* thymidylate (dTMP) biosynthesis, for the remethylation of homocysteine to methionine and for the synthesis of ^{fmet}tRNAMet (Fox and Stover, 2008). Collectively, these pathways are known as folate-mediated one-carbon metabolism (FOCM), which is a network of interconnected folate-dependent metabolic pathways that are compartmentalized in the mitochondria, cytosol and the nucleus.

In mitochondria, the inner membrane folate transporter, SLC25A32, is essential for mitochondrial folate accretion (Titus and Moran, 2000), and the mitochondrial and cytoplasmic folate pools don't freely exchange (Lawrence et al., 2014). In mitochondria, one-carbon donors are catabolized to generate formaldehyde in the form of 5,10-methylene-tetrahydrofolate, which is subsequently oxidized to 10-formyl-tetrahydrofolate. Formate is then liberated from 10-formyl-tetrahydrofolate in an ATP-generating reaction, and translocates to the cytosol and nucleus to support FOCM in those compartments (Tibbetts and Appling, 2010). Mitochondria also contain an anabolic pathway to generate dTMP for mitochondrial DNA synthesis (Anderson et al., 2011), and mitochondrial FOCM is also required for ^{fmet}tRNAMet synthesis, which is involved in the initiation of mitochondrial protein synthesis.

In the cytosol, serine and mitochondrial-derived formate are the two primary sources of folate-activated one-carbons for folate-dependent biosynthetic reactions. Formate accounts for 70–90% of total one-carbon units for cytoplasmic FOCM in MCF-7 cells (Herbig et al., 2002; Field et al., 2014), whereas serine catabolism in the cytoplasm accounts for 10–30% of total one-carbon units. Serine is a direct source of single carbons in the cytoplasm through the activity of serine hydroxymethyltransferase 1 (SHMT1) which catalyzes the transfer of the one-carbon unit from serine to tetrahydrofolate to form glycine and 5,10-methylene-tetrahydrofolate (Fig. 1).

Mitochondrially-derived formate is also a source of 5,10-methylene-tetrahydrofolate. It is condensed with tetrahydrofolate to form 10-formyl-tetrahydrofolate (Fig. 1) in an ATP-dependent reaction catalyzed by the synthase (S) activity of the trifunctional enzyme methylenetetrahydrofolate dehydrogenase (MTHFD1). 10-

* Corresponding author.
E-mail address: mas246@cornell.edu (M.S. Field).

http://dx.doi.org/10.1016/j.mam.2016.11.005
0098-2997/

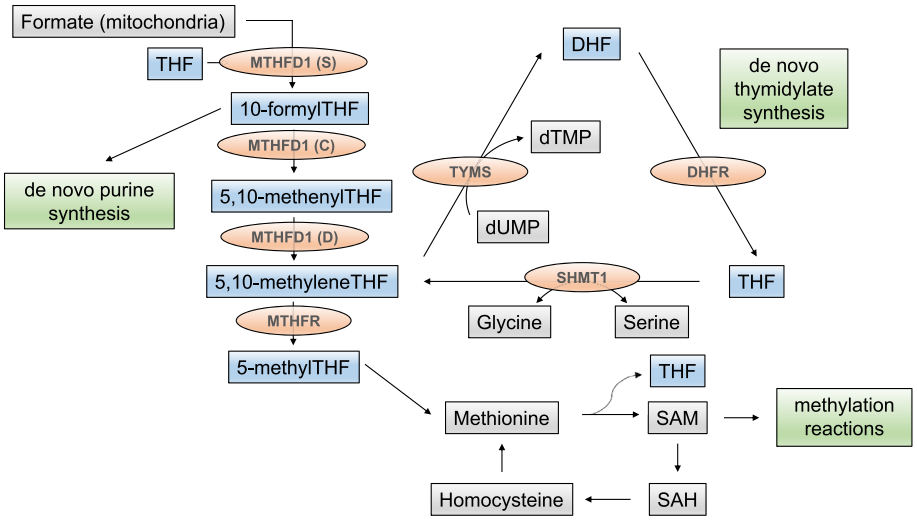

Fig. 1. Overview of folate-mediated one-carbon metabolism. Folate carries one-carbon units required for *de novo* thymidylate synthesis, *de novo* purine synthesis, and methylation reactions. THF, tetrahydrofolate; DHF, dihydrofolate; dUMP, deoxyuracil monophosphate; dTMP, deoxythymidine monophosphate; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; MTHFD1, methylenetetrahydrofolate dehydrogenase; (S), synthase; (C), cyclohydrolase; (D), dehydrogenase; SHMT1, serine hydroxymethyltransferase; TYMS, thymidylate synthase; DHFR, dihydrofolate reductase; MTHFR, methylenetetrahydrofolate reductase.

formyl-tetrahydrofolate can serve as a cofactor that supplies the 2 and 8 carbon of the purine ring for *de novo* purine synthesis. Alternatively, MTHFD1 can further process 10-formyl-tetrahydrofolate to 5,10-methenyl-tetrahydofolate through its cyclohydrolase (C) activity, and then to 5,10-methylene-tetrahydrofolate through the NADPH-requiring dehydrogenase (D) activity of MTHFD1. 5,10-methylene-tetrahydrofolate exists at a branch-point in the FOCM network. It is the substrate for 5,10-methylene-tetrahydrofolate reductase (MTHFR). The NADPH-dependent synthesis of 5-methyl-tetrahydrofolate by MTHFR is essentially irreversible *in vivo*, hence 5-methyl-tetrahydrofolate is committed to serving as a cofactor for homocysteine remethylation to methionine, catalyzed by the vitamin B12-dependent enzyme methionine synthase (MTR). Methionine is a precursor of *S*-adenosylmethionine (SAM), which is a required cofactor for over 100 cellular methylation reactions including DNA, histone, protein, and phospholipid methylation and for neurotransmitter synthesis (Fox and Stover, 2008). 5,10-methylene-tetrahydrofolate is also the source of one-carbons for *de novo* dTMP biosynthesis (Fig. 1).

1.1. Overview of dTMP biosynthesis pathways

The *de novo* dTMP synthesis pathway catalyzes conversion of deoxyuridylate (dUMP) to dTMP, and involves four enzymes. SHMT1 and MTHFD1 each independently generate 5,10-methylene-tetrahydrofolate from tetrahydrofolate using serine and formate as one-carbon sources, respectively, as described above. Thymidylate synthase (TYMS) utilizes 5,10-methylene-tetrahydrofolate as a cofactor in the conversion of dUMP to dTMP (Fig. 1), oxidizing tetrahydrofolate to dihydrofolate. Dihydrofolate reductase (DHFR) catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate (Fig. 1).

In addition to folate-dependent *de novo* synthesis, thymidylate synthesis can occur through a salvage pathway. Salvage synthesis occurs through the action of thymidine kinase (TK), which catalyzes the ATP-dependent phosphorylation of the nucleoside thymidine to the nucleotide dTMP. TK1 is found in the cytoplasm and nucleus whereas TK2 is found within the mitochondria (Pontarin et al., 2003). Both TK1 expression and nuclear localization increase during S-phase of the cell cycle and in response to DNA damage (Chen et al., 2010), but TK1 activity is low in quiescent cells. TK2 is not cell cycle regulated, rather it is constitutively expressed and contributes to the mitochondrial dTMP pool to support mitochondrial DNA synthesis, which is required even in post-mitotic tissues (Dorado et al., 2011). In animal cells, salvage pathway synthesis generally does not provide sufficient levels of dTMP to meet DNA synthesis requirements.

2. Causes and consequences of impaired *de novo* thymidylate biosynthesis

Impaired *de novo* dTMP synthesis creates imbalances in dNTP pools, which are mutagenic (Niida et al., 2010), stall replication forks, and induce cell cycle arrest and apoptosis (Wataya et al., 1993). Impaired *de novo* dTMP synthesis also increases rates of uracil misincorporation into DNA (Blount et al., 1997; Goulian et al., 1980). DNA polymerases can incorporate either dTTP or dUTP during DNA replication when there is an “A” base on the template strand, and will increase dUTP incorporation into DNA when dTTP becomes limiting. Both rates of cell division and of uracil accumulation into DNA may be affected by the dUTP/dTTP ratio (Vertessy and Toth, 2009).

The main consequence of uracil in DNA is DNA single- and double-strand breaks generated by DNA repair enzymes. Mammalian cells possess redundant pathways to both limit and repair uracil misincorporation. Deoxyuridine triphosphatase (dUTPase) catabolizes dUTP making it unavailable for DNA synthesis (Vertessy and Toth, 2009). Once dUTP is incorporated into DNA, one of several uracil DNA glycosylases initiate base excision repair. Uracil DNA glycosylase (UNG2 is the nuclear isozyme and UNG1 is the mitochondrial isozyme) is considered to be the primary glycosylase that targets uracil in DNA. However, other glycosylases contribute to uracil excision including Single-Strand-Selective Monofunctional Uracil-DNA Glycosylase 1 (SMUG1). In base excision repair, DNA glycosylases cleaves uracil from the sugar phosphate backbone, leaving an abasic (AP) site. This site is then cleaved by AP endonuclease (APE1) creating a single-strand break. Single-strand breaks can either be processed by short- or long-patch repair, removing a single or several nucleotides, respectively. DNA polymerase β fills these sites (Schormann et al., 2014;

Please cite this article in press as: Chon, J., et al., Targeting nuclear thymidylate biosynthesis, Molecular Aspects of Medicine (2016), http://dx.doi.org/10.1016/j.mam.2016.11.005