Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117

Perspective

Molecular Cancer Research

Mitochondrial Methylenetetrahydrofolate Dehydrogenase (MTHFD2) Overexpression Is Associated with Tumor Cell Proliferation and Is a Novel Target for Drug Development

Philip M. Tedeschi[1], Alexei Vazquez[2], John E. Kerrigan[3], and Joseph R. Bertino[4]

Abstract

Rapidly proliferating tumors attempt to meet the demands for nucleotide biosynthesis by upregulating folate pathways that provide the building blocks for pyrimidine and purine biosynthesis. In particular, the key role of mitochondrial folate enzymes in providing formate for *de novo* purine synthesis and for providing the one-carbon moiety for thymidylate synthesis has been recognized in recent studies. We have shown a significant correlation between the upregulation of the mitochondrial folate enzymes, high proliferation rates, and sensitivity to the folate antagonist methotrexate (MTX). Burkitt lymphoma and diffuse large-cell lymphoma tumor specimens have the highest levels of mitochondrial folate enzyme expression and are known to be sensitive to treatment with MTX. A key enzyme upregulated in rapidly proliferating tumors but not in normal adult cells is the mitochondrial enzyme methylenetetrahydrofolate dehydrogenase (MTHFD2). This perspective outlines the rationale for specific targeting of MTHFD2 and compares known and generated crystal structures of MTHFD2 and closely related enzymes as a molecular basis for developing therapeutic agents against MTHFD2. Importantly, the development of selective inhibitors of mitochondrial methylenetetrahydrofolate dehydrogenase is expected to have substantial activity, and this perspective supports the investigation and development of MTHFD2 inhibitors for anticancer therapy. *Mol Cancer Res; 13(10); 1361–6. ©2015 AACR.*

Introduction

In 1960, Scrimgeour and Huennekens (1) reported that, in contrast with cytoplasmic methylenetetrahydrofolate dehydrogenase (MTHFD1), which uses NADP as the coenzyme, ascites tumors contained a NAD-dependent methylenetetrahydrofolate dehydrogenase, MTHFD2. This observation went unnoticed until 1985, when Mejia and McKenzie (2) found that MTHFD2 was also expressed in embryonic liver, and not in adult tissues. This enzyme had both methylenetetrahydrofolate dehydrogenase and cyclohydrolase activity and was different from the cytoplasmic enzyme (MTHFDl), which requires NADP as a cofactor and is trifunctional, also containing cyclohydrolase and formyltetrahydrofolate synthetase activity (Fig. 1A; ref. 3). Patel and colleagues (4) then showed that this MTHFD2 was located in the mitochondria and, unlike MTHFD1, was highly expressed in embryos and decreased in activity as the embryos matured. The importance of this activity in embryos was shown by knocking out this gene (*nmdmc*) in mice. The homozygous gene knockout mice died *in utero* after day 12. Heterozygous mice were healthy, but were smaller and had pale livers, and the only abnormality detected was a reduced number of nucleated cells in the liver. However, there were no differences in the frequencies of hematopoietic precursors.

These authors postulated that "the NAD (rather than NADP) dependent dehydrogenase activity" is required to promote "a more thermodynamically favorable pathway to balance the pools of 10-formyl-THF during development" (5). The generation of one-carbon units by mitochondria in embryonic mammalian fibroblasts lacking MTHFD2 was completely blocked, and importantly, the cytoplasmic folate enzymes could not synthesize sufficient purines to allow cell growth. These MTHFD2-deficient fibroblasts were found to be glycine auxotrophs, suggested to be a consequence of the accumulation of the MTHFD2 substrate 5, 10-methylenetetrahydrofolate. The accumulation of methylenetetrahydrofolate inhibits serine hydroxymethyltransferase (SHMT2) and therefore blocks glycine generation. Thus, both glycine generation and an inability to regenerate THF would be common properties of cells with MTHFD2 deficiency (4).

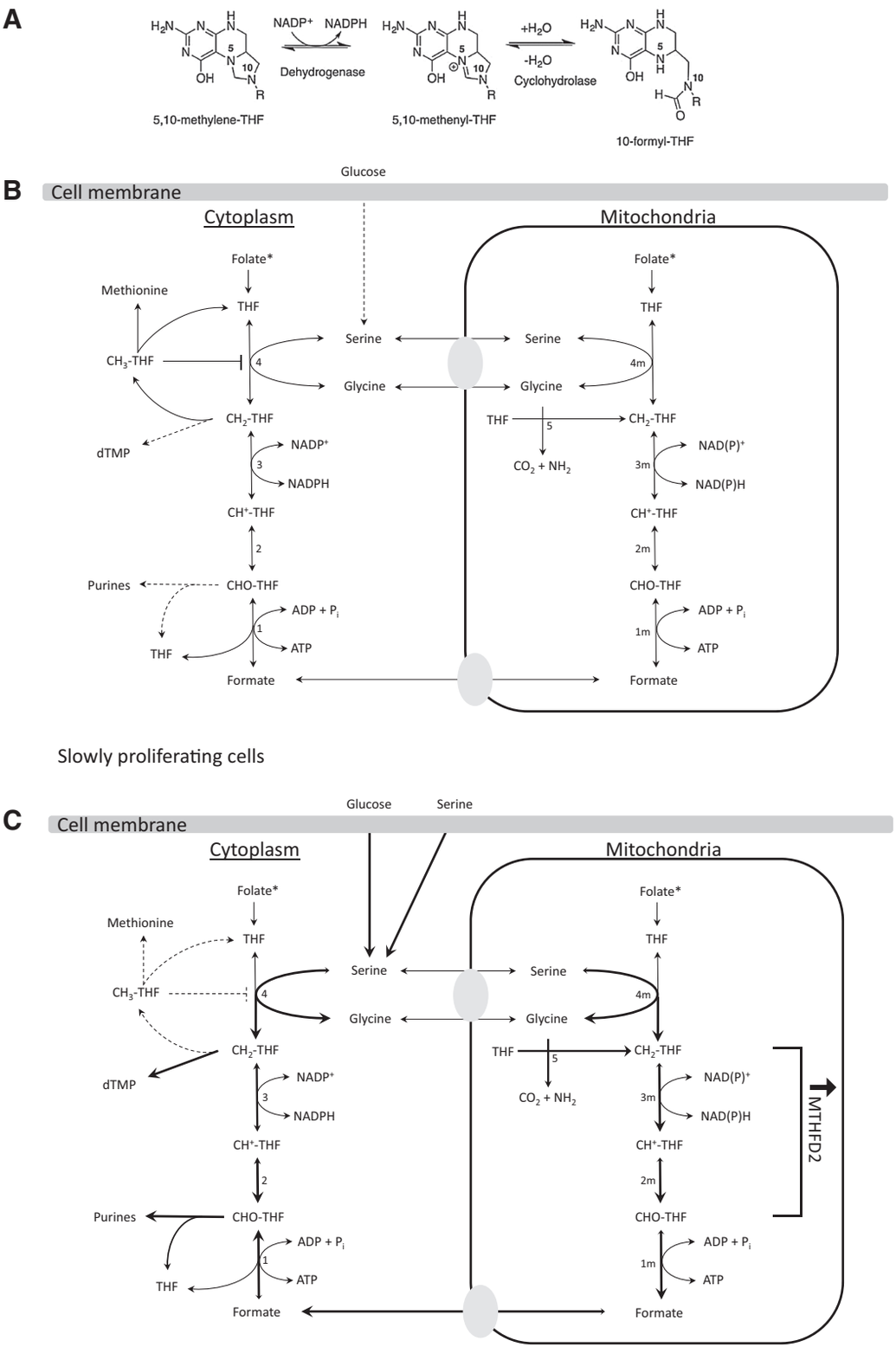
MTHFD2, shown to be overexpressed in rapidly proliferating malignant tumors, was postulated to be the "main switch" that enables mitochondria to produce additional one-carbon units for purine synthesis to enable rapid growth (6), and, as recently recognized, generates NADH necessary for protection from reactive oxygen species and macromolecular synthesis (7). In contrast, to MTHFD2, the NADP-dependent cytoplasmic trifunctional enzyme, MTHFD1, generates NADPH as well as formate, for purine biosynthesis (Fig. 1B and C). Recent studies have shown

[1]Department of Pharmacology, Rutgers Cancer Institute of New Jersey, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey. [2]Cancer Research UK Beatson Institute, Glasgow, United Kingdom. [3]Department of Bioinformatics, Rutgers Cancer Institute of New Jersey, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey. [4]Departments of Medicine and Pharmacology, Rutgers Cancer Institute of New Jersey, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, New Jersey.

Corresponding Author: Joseph R. Bertino, The Cancer Institute of New Jersey, 195 Little Albany Street, Room 3033, New Brunswick, NJ 08903. Phone: 732-235-5810; Fax: 732-235-8181; E-mail: bertinoj@cinj.rutgers.edu

doi: 10.1158/1541-7786.MCR-15-0117

Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117



Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117

that mitochondrial rather than cytoplasmic folate enzymes generate most of the formate used for purine synthesis via the mitochondrial enzyme MTHFD1L, which only has formyl THF synthetase activity (8). The mitochondrial counterpart of MTHFD2 has now been identified as MTHFD2L. This enzyme uses NADP as a cofactor and, unlike MTHFD2, is expressed at low levels in early mouse embryos, increases by day 10.5, and is found in all adult tissues, with highest levels in lung and brain (9) and is likely a housekeeping enzyme.

MTHFD2 as a Target for Drug Development

In a study of mRNA profiles spanning 19 cancer types, Nisson and colleagues (10) showed that the mitochondrial folate pathway and in particular MTHFD2 mRNA and protein expression is elevated in highly proliferating cancers. What makes MTHFD2 a novel target for drug development is its high expression in rapidly proliferating tumors and its restricted expression in adult differentiated and proliferating tissues (2). As it has a redundant function to the cytoplasmic NADP-dependent trifunctional cytoplasmic enzyme (MTHFD1), as well as the bifunctional mitochondrial enzyme MTHFD2L, the question arises as to the role MTHFD2 plays in cell metabolism. MTHFD2 may supply the increased demand in rapidly proliferating cells for formate and ATP generation (11). Knockdown of this enzyme led to inhibition of most tumor cell lines, providing the rationale for inhibitor development (10).

In a recent study of KRAS mutant non–small cell lung cancer cells, there was a correlation between expression of MTHFD2 and response to pemetrexed, a potent thymidylate synthase inhibitor (12). Kras mutant–related metabolic genes were identified as transcriptional targets of c-Myc, the transcription factor that we have suggested may regulate mitochondrial folate enzyme transcription (11).

Fan and colleagues (7) used quantitative flux analysis to show that a major contribution to generation of NADPH is via oxidation of cytoplasmic methylenetetrahydrofolate to 10 formyl THF, and conversion of NADP to NADPH. Surprisingly, knockdown of MTHFD2 demonstrated that it also contributed to NADPH production, as the enzyme primarily uses NAD rather than NADP as the coenzyme partner. Glycine is a source of one-carbon units via the glycine cleavage system, which is reported to be increased in lung cancer tumor-initiating cells (13). The addition of glycine decreased the cellular NADP:NADPH ratio, indicating that at least in this cell line serine, not glycine, is essential for growth and NADPH production (13). In fact, higher concentrations of glycine have been reported to inhibit proliferation, proposed to be due to the utilization of one-carbon units needed to synthesize serine from glycine, creating a one-carbon deficiency (14).

In recent studies (11, 15), increased activity of the serine biosynthesis pathway, one-carbon metabolism, and the glycine cleavage system (SOG pathway) was examined in a subset of tumors, and was found to be upregulated in tumors in breast and prostate tumors with poor prognosis and was correlated with increased sensitivity to antifolate inhibitors methotrexate and pemetrexed (11). In tumors with high rates of proliferation, the SOG pathway contributed to increased ATP synthesis, which is necessary for *de novo* purine synthesis. As shown in lymphoma and other tumor subtypes, expression of genes in the SOG pathway showed increased levels of mitochondrial folate enzymes, including MTHFD2.

MTHFD2 Structure and Characteristics for Rational Drug Design

Given the importance of MTHFD2 for cell survival and proliferation, this enzyme could represent a possible target for antineoplastic agents. Accordingly, we have looked into existing structures for strategies to rationally target MTHFD2 selectively. As both the cofactors NAD/NADP and the substrate MTHF are required for enzyme activity, competitive inhibitors based on either the cofactor or the substrate molecules can be considered for drug design/development.

Several structural studies (mainly X-ray crystallography) of the three methylenetetrahydrofolate dehydrogenase enzymes have been reported in past years. Cygler and colleagues (16, 18) have published several structures based on DC301, the human cytoplasmic trifunctional enzyme with the isolated bifunctional methylenetetrahydrofolate dehydrogenase/cyclohydrolase domain crystalized. The crystal structure of DC301 (MTHFD1) in complex with an inhibitor (LY345899) based on the 5,10-methenyl-THF intermediate provides a nice illustration of the placement of substrate analogue relative to the cofactor (Fig. 2A).

The bifunctional mitochondrial MTHFD enzymes are known to be aggregate dimers (17, 18). Note the additional stabilizing interaction of residue asparagine N189(B) of chain B with arginine R173 of chain A in the protein dimer (Fig. 2B). Other key stabilizing interactions are noted between the two arginine residues (R173 and R198) with the phosphate of NADP. Schmidt and colleagues propose that lysine (K56) plays a role in the proton transfer reaction in the conversion of the hydroxymethyl intermediate to 10-formyl tetrahydrofolate (see Fig. 1). The authors additionally noted that mutations of K56 to residues that provide a free electron pair enhanced dehydrogenase activity and the K56R mutation abolished cyclohydrolase activity (18).

In Fig. 2C is depicted the theoretical model of human MTHFD2 (1ZN4.pdb) dimer published by Mackenzie and colleagues (3). The arginine R166 in chain A is stabilized by aspartate D190 (B) of chain B of the dimer. The phosphate ion (P_i) is stabilized by

Figure 1.

A, reactions catalyzed by the dehydrogenase and cyclohydrolase activities of MTHFD enzymes. B, the cytoplasmic and mitochondrial folate pathway in slowly proliferating (normal) cells. C, the cytoplasmic and mitochondrial folate pathway modifications found in rapidly proliferating (transformed) cells. In the cytoplasm, reactions 1, 2, and 3 are carried out by the trifunctional enzyme MTHFD1 (methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, and formyltetrahydrofolate synthetase activities). In the mitochondria, reaction 1m is catalyzed by monofunctional MTHFD1L (formyltetrahydrofolate synthetase activity). Reactions 2m and 3m are catalyzed by bifunctional NAD-dependent MTHFD2 or bifunctional NADP-dependent MTHFD2L (methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase activities). The interconversion of serine and glycine and the entry of one-carbon units into the pathway (reactions 4 and 4m) are catalyzed by serine hydroxymethyltranferase 1 and 2, respectively. Reaction 5 depicts the glycine cleavage system. THF, tetrahydrofolate; CH_2-THF, 5,10-methylenetetrahydrofolate; CH-THF, 5,10 methenyltetrahydrofolate; CHO-THF, 10-formyl tetrahydrofolate; CH_3-THF, 5-methyl tetrahydrofolate. *Folate is used here as a generic term; in plasma, the major form of folate is 5-methyl THF.

Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117

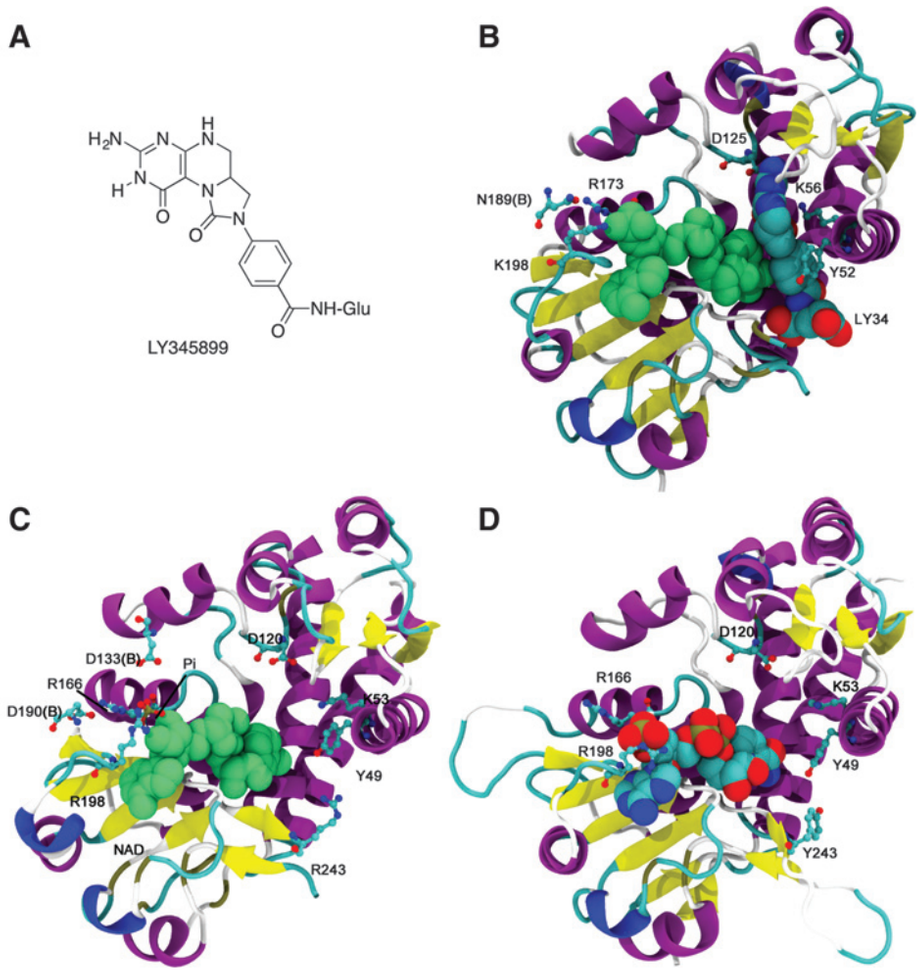


Figure 2.
A, structure drawing of LY345899. B, ribbon diagram representation of DC301 (MTHFD1; 1DIB.pdb) chain A of the dimer colored by secondary structure showing relative location of cofactor NADP (green colored spheres) to inhibitor LY34 (LY345899; CPK colored spheres). Key interacting protein residues are depicted in ball-stick and labeled. C, human MTHFD2 (1ZN4.pdb) with NAD cofactor (green spheres) and phosphate (Pi). The key residues are depicted in ball-stick. D, homology model of the human MTHFD2L enzyme with NADP cofactor (spheres with CPK coloring) bound and key residues depicted as ball-stick. Illustrations were prepared using the VMD molecular graphics software package (27).

arginine R166 and R198. The authors speculate that the role of Mg^{2+} ion might be similar to that of arginine R198 in stabilization of the P_i. Alternately, the authors pose the possibility that the Mg^{2+} ion might coordinate with aspartate D133 (B) of the opposite chain of the dimer and P_i. In the structure above, note the key tyrosine (Y49), lysine (K53) and aspartate (D120) in the MTHF binding site. In addition, MTHFD2 has an arginine (R243) in the MTHF binding site, which is a tyrosine residue in the MTHFD1 (not fully resolved in this crystal structure) and MTHFD2L enzymes.

To further search for structural similarities and differences between MTHFD enzymes, we built a homology model of human MTFHD2L (MTHFD2-Like, the mitochondrial enzyme) depicted in Fig. 2D using protein sequence accession Q9H903 and the high-resolution ($R = 1.5$ Å) crystal structure of human cytosolic methylenetetrahydrofolate dehydrogenase/cyclohydrolase (DC301) (PDB: 1A4I.pdb) as the template (16). We used the Modeller (v9.13) software package (19–21) to build the homology model via the single template approach. The MTHFD2L sequence has 41% sequence similarity with the structure template sequence. The finished Ramachandran plot analysis performed using the Procheck analysis tool (22) indicates that the homology model has 98.8% of residues in allowed regions of the plot.

Like MTHFD2, the MTHFD2L enzyme is a bifunctional mitochondrial enzyme with dehydrogenase activity that can use either NAD or NADP as cofactor (23). However, Appling and colleagues note that phosphate (P_i) and Mg^{2+} ion are needed when using NAD as cofactor (23). The enzyme is more commonly expressed in brain and lung tissue, but also occurs in all other tissues. An excellent sequence alignment comparing the MTHFD enzymes is illustrated by Bolusani and colleagues (24).

The same arginine residues present in MTHFD2 (R166 and R198) are involved in stabilization of the NADP phosphate or the phosphate (P_i) when NAD is used with Mg^{2+} ion playing a role. In addition, the two aspartate residues D190(B) and D133(B) (not shown in Fig. 2) are conserved as well. Therefore, MTHFD2L can stabilize Mg^{2+} and P_i when using NAD as cofactor via the same D133 residue in an aggregate dimer as Mackenzie had proposed for MTHFD2. The same three residues (Y49, K53, and D120) that play a role in substrate/inhibitor binding in MTHFD2 are also present in MTHFD2L (Fig. 2D).

The structural alignment depicted in Fig. 3 was built using 1DIB.pdb, 1ZN4.pdb, and our MTHFD2L homology model. We used the STAMP method in the VMD Multi-Seq tool to prepare the alignment (25, 26). Note the characteristic Rossman fold shared by these enzymes that makes up the cofactor binding site. The MTHFD2L homology model (purple in Fig. 3) reveals the two

Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117

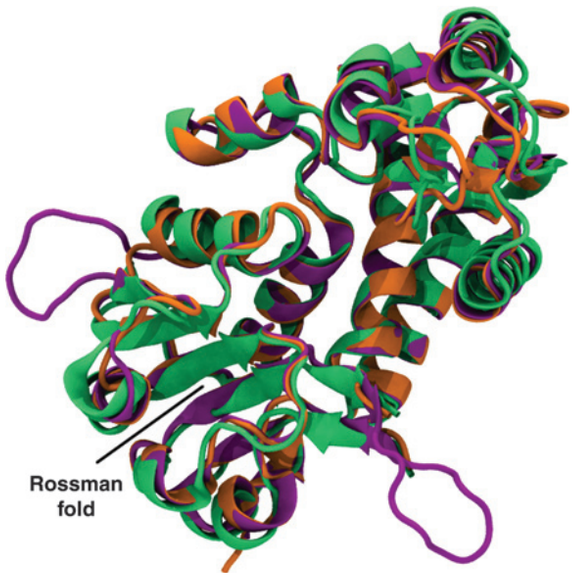


Figure 3.
Ribbon diagram depiction of STAMP structural alignment of MTHFD1 (orange), MTHFD2 (green), and MTHFD2L (purple). Illustrations were prepared using the VMD molecular graphics software package (27).

loop regions that are not resolved in the crystal structure of MTHFD1 and left out of the homology model of MTHFD2. The secondary structure appears to be well conserved in these proteins. Note the more open form of the MTHF binding site in the crystal structure of the MTHFD1 complex with LY34 (orange in Fig. 3). The key aggregate dimer-mediated interactions occur at the NAD/NADP cofactor binding region.

The conserved Rossman folds in the three enzymes make it unlikely that competitive inhibitors of NAD/NADP would have specificity. The close similarity of the MTHFD2 and the MTHFD2L enzymes may also make it difficult to find folate inhibitors that distinguish between these enzymes; however, inhibition of both the MTHFD2 and MTHFD2L mitochondrial reductases may have antitumor effects in rapidly replicating tumors without significant toxicity (3). One might also be able to take advantage of the single difference in residue position 243 of the MTHFD2/2L. In the MTHFD2 enzyme, this residue is a positively charged arginine residue, whereas in the MTHFD2L this residue is replaced with a neutral tyrosine residue.

Conclusions

The finding that the mitochondrial enzyme MTHFD2 is overexpressed in rapidly replicating tumor tissues and not in replicating normal tissues, and that knockdown studies have shown an antiproliferative effect, provides a strong rationale for finding inhibitors of this enzyme for selective cancer treatment (10). While modeling studies indicate that selective inhibition of the MTHFD2 enzyme and not the MTHFD2L enzyme expressed in adult tissues may be difficult, targeting both enzymes may be of advantage, as more complete inhibition of mitochondrial purine synthesis may result. Additional studies are necessary to show the effect of knockdown or inhibition of the MTHFD2 enzyme on *in vivo* tumor growth, and the role of metabolites, i.e., serine and folates on augmenting or relieving inhibition, as well as combination therapy with antifolates or other therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P.M. Tedeschi, J.R. Bertino
Development of methodology: P.M. Tedeschi, J.R. Bertino
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P.M. Tedeschi, J.R. Bertino
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.M. Tedeschi, J.E. Kerrigan, J.R. Bertino
Writing, review, and/or revision of the manuscript: P.M. Tedeschi, A. Vazquez, J.E. Kerrigan, J.R. Bertino
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P.M. Tedeschi, J.R. Bertino
Study supervision: J.R. Bertino

Received March 6, 2015; revised June 8, 2015; accepted June 9, 2015; published OnlineFirst June 22, 2015.

References

1. Scrimgeour KG, Huennekens FM. Occurrence of a DPN-linked, N5,N10-methylene tetrahydrofolic dehydrogenase in Ehrlich ascites tumor cells. Biochem Biophys Res Commun 1960;2:230–3.
2. Mejia NR, MacKenzie RE. NAD-dependent methylenetetrahydrofolate dehydrogenase is expressed by immortal cells. J Biol Chem 1985;260:14616–20.
3. Christensen KE, Mirza IA, Berghuis AM, Mackenzie RE. Magnesium and phosphate ions enable NAD binding to methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase. J Biol Chem 2005;280:34316–23.
4. Patel H, Pietro ED, MacKenzie RE. Mammalian fibroblasts lacking mitochondrial NAD^+-dependent methylenetetrahydrofolate dehydrogenase-cyclohydrolase are glycine auxotrophs. J Biol Chem 2003;278:19436–41.
5. Pietro ED, Sirois J, Tremblay ML, MacKenzie RE. Mitochondrial NAD-Dependent Methylenetetrahydrofolate Dehydrogenase-Methenyltetrahydrofolate Cyclohydrolase Is Essential for Embryonic Development. Mol Cell Biol 2002;22:4158–66.
6. Christensen KE, Mackenzie RE. Mitochondrial methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, and formyltetrahydrofolate synthetases. Vitam Horm 2008;79:393–410.
7. Fan J, Ye J, Kamphorst JJ, Shlomi T, Thompson CB, Rabinowitz JD. Quantitative flux analysis reveals folate-dependent NADPH production. Nature 2014;510:298–302.
8. Pike ST, Rajendra R, Artzt K, Appling DR. Mitochondrial C1-tetrahydrofolate synthase (MTHFD1L) supports the flow of mitochondrial one-carbon units into the methyl cycle in embryos. J Biol Chem 2010;285:4612–20.
9. Momb J, Lewandowski JP, Bryant JD, Fitch R, Surman DR, Vokes SA, et al. Deletion of Mthfd1l causes embryonic lethality and neural tube and craniofacial defects in mice. Proc Natl Acad Sci U S A 2013;110:549–54.
10. Nilsson R, Jain M, Madhusudhan N, Sheppard NG, Strittmatter L, Kampf C, et al. Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. Nat Commun 2014;5:3128.
11. Tedeschi PM, Markert EK, Gounder M, Lin H, Dvorzhinski D, Dolfi SC, et al. Contribution of serine, folate and glycine metabolism to the ATP,

Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117

NADPH and purine requirements of cancer cells. Cell Death Dis 2013;4: e877.
12. Moran DM, Trusk PB, Pry K, Paz K, Sidransky D, Bacus SS. KRAS mutation status is associated with enhanced dependency on folate metabolism pathways in non–small cell lung cancer cells. Mol Cancer Ther 2014; 13:1611–24.
13. Zhang WC, Shyh-Chang N, Yang H, Rai A, Umashankar S, Ma S, et al. Glycine decarboxylase activity drives non-small cell lung cancer tumor-initiating cells and tumorigenesis. Cell 2012;148:259–72.
14. Labuschagne CF, van den Broek NJF, Mackay GM, Vousden KH, Maddocks ODK. Serine, but not glycine, supports one-carbon metabolism and proliferation of cancer cells. Cell Rep 2014;7:1248–58.
15. Vazquez A, Tedeschi PM, Bertino JR. Overexpression of the mitochondrial folate and glycine-serine pathway: a new determinant of methotrexate selectivity in tumors. Cancer Res 2013;73:478–82.
16. Allaire M, Li Y, MacKenzie RE, Cygler M. The 3-D structure of a folate-dependent dehydrogenase/cyclohydrolase bifunctional enzyme at 1.5 A resolution. Structure 1998;6:173–82.
17. Mejia NR, Rios-Orlandi EM, MacKenzie RE. NAD-dependent methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase from ascites tumor cells. Purification and properties. J Biol Chem 1986; 261:9509–13.
18. Schmidt A, Wu H, MacKenzie RE, Chen VJ, Bewly JR, Ray JE, et al. Structures of three inhibitor complexes provide insight into the reaction mechanism of the human methylenetetrahydrofolate dehydrogenase/cyclohydrolase. Biochemistry 2000;39:6325–35.
19. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen M-Y, et al. Comparative protein structure modeling using Modeller. Curr Protoc Bioinforma Ed Board Andreas Baxevanis Al 2006;Chapter 5: Unit 5.6.
20. Sánchez R, Pieper U, Melo F, Eswar N, Martí-Renom MA, Madhusudhan MS, et al. Protein structure modeling for structural genomics. Nat Struct Biol 2000;7 Suppl:986–90.
21. Eswar N, John B, Mirkovic N, Fiser A, Ilyin VA, Pieper U, et al. Tools for comparative protein structure modeling and analysis. Nucleic Acids Res 2003;31:3375–80.
22. Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Crystallogr 1993;26:283–91.
23. Shin M, Bryant JD, Momb J, Appling DR. Mitochondrial MTHFD2L is a dual redox cofactor-specific methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase expressed in both adult and embryonic tissues. J Biol Chem 2014;289: 15507–17.
24. Bolusani S, Young BA, Cole NA, Tibbetts AS, Momb J, Bryant JD, et al. Mammalian MTHFD2L encodes a mitochondrial methylenetetrahydrofolate dehydrogenase isozyme expressed in adult tissues. J Biol Chem 2011;286:5166–74.
25. Eargle J, Wright D, Luthey-Schulten Z. Multiple Alignment of protein structures and sequences for VMD. Bioinformatics Oxf Engl 2006;22: 504–6.
26. Roberts E, Eargle J, Wright D, Luthey-Schulten Z. MultiSeq: unifying sequence and structure data for evolutionary analysis. BMC Bioinformatics 2006;7:382.
27. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. J Mol Graph 1996;14:33–8, 27–8.

Published OnlineFirst June 22, 2015; DOI: 10.1158/1541-7786.MCR-15-0117

Molecular Cancer Research

Mitochondrial Methylenetetrahydrofolate Dehydrogenase (MTHFD2) Overexpression Is Associated with Tumor Cell Proliferation and Is a Novel Target for Drug Development

Philip M. Tedeschi, Alexei Vazquez, John E. Kerrigan, et al.

Mol Cancer Res 2015;13:1361-1366. Published OnlineFirst June 22, 2015.