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A review of methionine dependency and the role of methionine restriction in cancer growth control and life-span extension

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ABSTRACT

Methionine is an essential amino acid with many key roles in mammalian metabolism such as protein synthesis, methylation of DNA and polyamine synthesis. Restriction of methionine may be an important strategy in cancer growth control particularly in cancers that exhibit dependence on methionine for survival and proliferation. Methionine dependence in cancer may be due to one or a combination of deletions, polymorphisms or alterations in expression of genes in the methionine *de novo* and *salvage* pathways. Cancer cells with these defects are unable to regenerate methionine via these pathways. Defects in the metabolism of folate may also contribute to the methionine dependence phenotype in cancer. Selective killing of methionine dependent cancer cells in co-culture with normal cells has been demonstrated using culture media deficient in methionine. Several animal studies utilizing a methionine restricted diet have reported inhibition of cancer growth and extension of a healthy life-span. In humans, vegan diets, which can be low in methionine, may prove to be a useful nutritional strategy in cancer growth control. The development of methioninase which depletes circulating levels of methionine may be another useful strategy in limiting cancer growth. The application of nutritional methionine restriction and methioninase in combination with chemotherapeutic regimens is the current focus of clinical studies.

Introduction

Cancer is characterized by uncontrolled cellular growth as a result of changes in the expression of tumor promoting and tumor suppressing genes.[1] While a small percentage of cancers are a direct result of inherited mutations associated with cancer, the majority of cancers result from alterations in DNA integrity which accumulate over time and are caused by endogenous and environmental genotoxic factors.[1] Specifically, there is increasing evidence that dietary macronutrients and micronutrients are important environmental factors in the development and growth of cancers (see report by the World Cancer Research Fund/American Institute for Cancer Research).[2] A common feature of some cancers is the absolute requirement for methionine, a phenomenon known as 'methionine dependence'.[3] Therefore, restriction of methionine may be a useful strategy in limiting cancer growth. Methionine restriction may also prolong a healthy life-span.[4] This review summarizes the current understanding of the role of methionine restriction in cancer growth control and life-span extension and identifies important knowledge gaps for future experimental investigation.

Methionine and its metabolism

Methionine is an essential amino acid necessary for normal growth and development in mammals.[5] In every cell, methionine is partitioned between protein synthesis and the *de novo* pathway (also referred to as the methylation cycle or recycling pathway; Fig. 1) where it is converted to S-adenosylmethionine (SAM), the principal methyl donor.[6] SAM is converted to S-adenosylhomocysteine (SAH) during methylation of DNA and a large range of proteins and other molecules.[7] SAH is then hydrolyzed to homocysteine (Hcy) in a reversible reaction. Hcy is metabolized through two major pathways: methylation and trans-sulfuration.[6] Under normal conditions, approximately 50% of Hcy is re-methylated to form methionine which, in most tissues, occurs via methionine synthase (5-methyltetrahydrofolate-homocysteine methyltransferase, MTR). Hcy may also be converted to methionine via betaine–homocysteine S-methyltransferase which is predominantly present in the liver.[8] In the *trans-sulfuration* pathway, Hcy is metabolized to form cystathionine which is the immediate precursor to cysteine (Fig. 1). Cysteine is utilized in the synthesis of glutathionine, a

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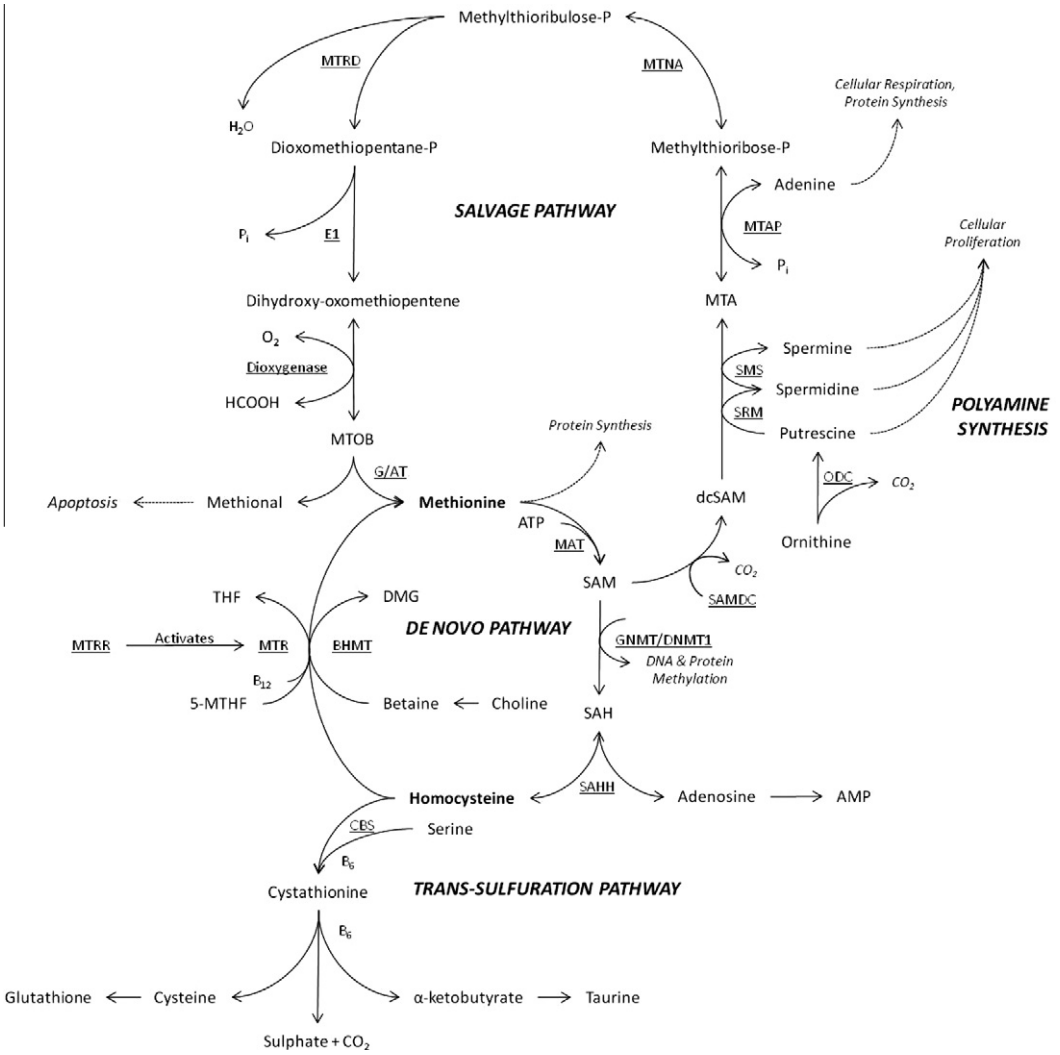

Fig. 1. Methionine cycle and *trans*-sulfuration pathway. Enzymes are underlined. 5-MTHF, 5-methyltetrahydrofolate; B_{12}, vitamin B_{12}; B_6, vitamin B_6; BHMT, betaine–homocysteine S-methyltransferase; CBS, cystathionine β-synthase; dcSAM, decarboxylated SAM; DMG, dimethylglycine; E1, enolase-phosphatase 1; G/AT, glutamine or asparagine transaminase; GNMT/DNMT1, glycine N-methyltransferase or DNA methyltransferase 1; MTA, methylthioadenosine; MAT, methionine adenosine transferase; MTAP, methylthioadenosine phosphorylase; MTOB, methylthiooxobutyrate; MTR, methionine synthase; MTRR, methionine synthase reductase; MTRD, methylthioribulose dehydratase; MTNA, methylthioribose isomerase; ODC, ornithine decarboxylase; SAH, S-adenosylhomocysteine; SAHH, SAH hydroxylase; SAM, S-adenosylmethionine; SAMDC, SAM decarboxylase; SMS, spermine synthase; SRM, spermidine synthase and THF, tetrahydrofolate.

tripeptide that reduces reactive oxygen species (ROS), thereby protecting cells from oxidative stress.[9]

In addition to the methylation cycle, SAM is also necessary for the production of polyamines which are synthesized as part of the methionine *salvage* pathway (Fig. 1). In this pathway, a carbon dioxide molecule is removed from SAM to form decarboxylated SAM, which, along with putrescine, is utilized for synthesis of other polyamines such as spermine and spermidine.[10] A by-product of polyamine synthesis is methylthioadenosine (MTA). MTA is catabolized by methylthioadenosine phosphorylase (MTAP) as the first in a series of steps in the salvage of methionine.[11] The immediate precursor to methionine in the *salvage* pathway is methylthiooxobutyrate (MTOB) which can be converted to methional, a potent inducer of apoptosis.[12] Genes coding the key enzymes in the methionine *de novo* and *salvage* pathways and their genetic identities are listed in Table 1.

Methionine dependence phenotype and cancer

The first evidence of methionine dependence in cancer cells was reported in 1959 from studies investigating the growth of subcutaneously transplated Walker-256 carcinosarcoma tumors in Sprague–Dawley rodents in response to a diet lacking methionine.[13] A subsequent study on methylation of transfer RNA observed a metabolic defect in Walker-256 cells suggesting a dependence of these cells on methionine.[14] To investigate if cancer cells were dependent on methionine, cultures of Walker-256, mouse lymphatic leukemia and human monocytic leukemia cells were grown in medium supplemented with Hcy in place of methionine (Met^- Hcy^+).[15] The observation that malignant cells were unable to survive and grow in Met^- Hcy^+ medium suggested an absolute dependence on methionine. In contrast, non-cancerous rodent liver epithelial and fibroblast cells, human breast and prostate fibroblasts and mouse skin fibroblasts were unaffected by the Met^- Hcy^+ medium.[15] Several later studies have reported that many malignant cell lines from different cancers (breast, bladder, colon, glioma, kidney, melanoma, prostate and others) are methionine dependent.[16–19] Furthermore, methionine dependence has been reported in fresh patient tumors derived from multiple tumor sites and grown in primary cultures.[20]

While it was initially hypothesized that methionine dependent malignant cells may be unable to methylate Hcy to form

Table 1
Known human genes encoding key enzymes of methionine metabolism.

Approved gene symbol	Approved gene name	Location	Sequence accession IDs	Previous symbols	Aliases	Pathway
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase	1q43	U73338, NM_000254		cblG	Methionine *de novo* synthesis
MTRR	5-Methyltetrahydrofolate-homocysteine methyltransferase reductase	5p15.31	AF025794		cblE	
BHMT	Betaine–homocysteine S-methyltransferase	5q13.1-q15	BC012616, NM_001713		BHMT1	
BHMT2	Betaine–homocysteine S-methyltransferase 2	5q13	NM_017614			
MAT1A	Methionine adenosyltransferase I, alpha	10q22	NM_000429		MAT, SAMS, MATA1, SAMS1	SAM synthesis and DNA methylation
MAT2A	Methionine adenosyltransferase II, alpha	2p11.2	NM_005911		SAMS2, MATA2, MATII	
MAT2B	Methionine adenosyltransferase II, beta	5q34-q35	AF182814, NM_013283		MATIIbeta, SDR23E1	
DNMT1	DNA (cytosine-5-)-methyltransferase 1	19p13.2	X63692, NM_001379	DNMT	MCMT, CXXC9	
DNMT3A	DNA (cytosine-5-)-methyltransferase 3 alpha	2p23	NM_022552			
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	20q11.2	NM_006892			
AHCY	Adenosylhomocysteinase	20q11.22	M61832, NM_000687		SAHH	
GNMT	Glycine N-methyltransferase	6p12	AF101475, NM_018960			
AMD1	Adenosylmethionine decarboxylase 1	6q21	M88006		SAMDC	Methionine salvage pathway and polyamine synthesis
MRI1	Methylthioribose-1-phosphate isomerase homolog (*S. cerevisiae*)	19p13.13	NM_032285		MGC3207, Ypr118w, MTNA	
MTAP	Methylthioadenosine phosphorylase	9p21	AB062485, NM_002451		MSAP	
ENOPH1	Enolase-phosphatase 1	4q21.3	NM_021204		MASA, E1	
SRM	Spermidine synthase	1p36-p22	BC033106, NM_003132	SRML1	SPS1	
SMS	Spermine synthase	Xp22.1	AD001528, NM_004595		SPMSY, SpS, SRS, MRSR	
SAT1	Spermidine/spermine N1-acetyltransferase 1	Xp22.1	M55580, NM_002970	SAT	SSAT	
SAT2	Spermidine/spermine N1-acetyltransferase family member 2	17p13.2	AF348524, NM_133491		SSAT2	
MSRA	Methionine sulfoxide reductase A	8p23.1	BC054033, NM_012331			Sulfoxide detoxication
MSRB2	Methionine sulfoxide reductase B2	10p12	AF122004, NM_012228	MSRB	PILB, CGI-131, CBS1, CBS-1	
MSRB3	Methionine sulfoxide reductase B3	12q14.3	BX640871, NM_198080		FLJ36866, DKFZp686C1178	

The gene list was obtained via the HUGO gene list web site (www.genenames.org). The genes encoding the following enzymes were not found on the HUGO list: methylthioribulose dehydratase, Dioxygenase, glutamine transaminase and asparagine transaminase.

methionine, Hoffman and Erbe[21] demonstrated that Walker-256 and the SV40-transformed SV80 and W18VA2 human cell lines were able to synthesize methionine from Hcy at rates that were at least as high as normal human skin fibroblast strains MGF316 and MGF323. These data suggested that methionine dependence may be caused by an altered utilization of methionine as opposed to an inability to synthesize methionine from Hcy.[21] In a subsequent study, Walker-256, SV80 and W18VA2 cells were cultured in Met^{-} Hcy^{+} medium for an extended period allowing for the isolation of rare methionine independent cells.[22] Characterization of the reverted cultures suggested that methionine synthesis was not a principle factor in reversion to methionine independence.[22] An in-depth analysis of reversion of methionine dependent cancer cells revealed that it was possible to select for heterogeneously transformed revertants by selecting for methionine independent cells, suggesting a possible relationship between altered methionine utilization and oncogenic transformation.[23]

The mechanisms responsible for methionine dependence in malignant cell lines are not fully understood. Early studies reported that methionine dependent cancer cells may synthesize a normal amount of endogenous methionine but have a reduced ability to utilize endogenous methionine for SAM synthesis.[24] In addition, the level of free methionine in cultures of methionine dependent SV40-transformed human fibroblasts grown in Met^{-} Hcy^{+} medium is low compared to normal human diploid fibroblasts grown in Met^{-} Hcy^{+} medium.[25] Taken together, these data suggested that free methionine was preferentially shunted to protein synthesis resulting in lower synthesis of SAM.[24,25] One possible mechanism for reduced free methionine may be deletions of key genes in the methionine *salvage* pathway.

There is emerging evidence suggesting that loss of MTAP expression from the methionine *salvage* pathway is a major factor of methionine dependence in cancer cells. Loss of MTAP expression has been observed in many cancer cell lines including those

Table 2
Co-deletion of MTAP and p15 and/or p16 tumor suppressor genes in cancer.

Citation	Ref. No.	Type of cancer	Cells or human primary tissue	Type of deletion	No. of samples with deletions	%
Chondrosarcoma						
Jagasia et al. (1996)	92	Cells derived from tumors varying in stage, grade and site	Cells	MTAP, p16	3 of 7	43
Jagasia et al. (1996)	92	Cells derived from tumors varying in stage, grade and site	Cells	MTAP, p15, p16	1 of 7	14
Jagasia et al. (1996)	93	Myxoid chondrosarcoma cell lines	Cells	MTAP, p16	4 of 4	100
Esophageal cancer						
Powell et al. (2005)	94	Invasive adenocarcinoma and metastases	Human	MTAP, p16	25 of 114	22
Kim et al. (2006)	95	Xenographs of thoracic duct lymph of esophageal squamous cell carcinoma patients	Human	MTAP, p16	6 of 8	75
Gastric cancer						
Kim et al. (2011)	96	Gastric cancer cell lines	Cells	MTAP, p16	2 of 10	20
Huang et al. (2009)	97	Gastrointestinal stromal tumor samples	Human	MTAP, p15, p16	2 of 22	9
Glioma						
Suzuki et al. (2004)	98	Primary glioblastoma samples	Human	MTAP, p16	15 of 30	50
Zhang et al. (1996)	99	Glioma cell lines	Cells	MTAP, p16	4 of 6	67
Perry et al. (1997)	100	Diffuse gliomas	Human	MTAP, p16	7 of 30	23
Olopade et al. (1992)	101	Glioma cell lines	Cells	MTAP, p15, p16	5 of 15	33
Leukemia						
Efferth et al. (2002)	102	T-cell acute lymphoblastic leukemia cell lines	Cells	MTAP, p16	5 of 13	38
Bertin et al. (2003)	103	Acute lymphoblastic leukemia	Human	MTAP, p16	80 of 284	28
Kamath et al. (2008)	104	Acute myeloid leukemia	Human	MTAP, p15, p16	1 of 1	100
Usvasalo et al. (2010)	105	Acute lymphoblastic leukemia	Human	MTAP, p15, p16	25 of 140	18
Zhang et al. (1996)	99	Leukemia cell lines	Cells	MTAP, p16	4 of 6	67
Hori et al. (1998)	106	Adult T-cell leukemia	Human	MTAP, p16	5 of 27	19
Hori et al. (1998)	106	Adult T-cell leukemia cell lines	Cells	MTAP, p16	3 of 3	100
M'Soka et al. (2000)	29	Adult T-cell leukemia	Human	MTAP, p16	6 of 29	21
M'Soka et al. (2000)	29	Childhood T-cell acute lymphoblastic leukemia	Human	MTAP, p16	15 of 39	39
Mirebeau et al. (2006)	107	B-lineage childhood acute lymphoblastic leukemia	Human	MTAP, p16	24 of 227	11
Lung cancer						
Zhang et al. (1996)	99	Lung carcinoma cell lines	Cells	MTAP, p16	2 of 6	33
Schmid et al. (1998)	31	Non-small cell lung cancer samples	Human	MTAP, p16	9 of 50	18
Lymphoma						
Marce et al. (2006)	108	Mantle cell lymphoma	Human	MTAP, p16	6 of 52	12
Dreyling et al. (1998)	109	Diffuse large cell lymphoma samples	Human	MTAP, p15, p16	6 of 16	38
Mesothelioma						
Krasinskas et al. (2010)	110	Peritoneal mesothelioma	Human	MTAP, p16	9 of 26	35
Illei et al. (2003)	111	Pleural mesothelioma	Human	MTAP, p16	64 of 95	67
Neuroblastoma						
Mora et al. (2004)	112	Neuroblastoma samples	Human	MTAP, p16	1 of 10	10
Brownhill et al. (2007)	113	Neuroblastoma cell lines	Cells	MTAP, p15, p16	0 of 3	0
Pancreatic cancer						
Hustinx et al. (2005)	114	Pancreatic cancer tissue samples	Human	MTAP, p16	91 of 300	30
Hustinx et al. (2005)	115	Pancreatic intraepithelial neoplasia lesions varying in grades	Human	MTAP, p16	6 of 73	8
Chen et al. (1996)	116	Pancreatic cell carcinoma cell lines	Cells	MTAP, p16	5 of 8	63
Other cancers						
Wang et al. (2010)	117	Malignant embryonic muscle cell lines	Cells	MTAP, p15, p16	6 of 6	100
Worsham et al. (2006)	118	Head and neck squamous carcinoma	Cells	MTAP, p15, p16	1 of 6	17
Brownhill et al. (2007)	113	Ewing's sarcoma	Human	MTAP, p15, p16	3 of 42	7
Brownhill et al. (2007)	113	Ewing's sarcoma	Cells	MTAP, p15, p16	6 of 9	67
Zhang et al. (1996)	99	Bladder carcinoma cell lines	Cells	MTAP, p16	2 of 9	22
Conway et al. (2010)	119	Primary melanoma	Human	MTAP, p15, p16	31 of 75	41

derived from primary ductal carcinoma, gliomas, osteosarcoma, melanoma, non-small cell lung cancer and T-cell acute lymphocytic leukemia.[26–31] MTAP is encoded by the *MTAP* gene which is located on human chromosome 9p21, approximately 100 kb telomeric to the *p16* tumor suppressor gene that is deleted in many cancers.[32,33] Several studies have reported that the *MTAP* gene is frequently co-deleted with *p16* and in some cases with the *p15* tumor suppressor genes in a variety of cancer cell lines and tumor samples (Table 2). These data suggest that deletion of the *MTAP* gene may simply be due to its proximity to the p16 and p15 loci.

However, there is now some evidence suggesting that MTAP itself may act as a tumor suppressor. Loss of *MTAP* has been observed in the absence of loss of *p16* in both non-small-cell lung cancer and gliomas.[31,34] In addition, reintroduction of MTAP into MTAP

deficient MCF-7 breast adenocarcinoma cells inhibits their growth and MTAP expressing MCF-7 cells are suppressed for tumor formation when implanted into SCID mice.[35] MTAP deletion may not be a direct cause of methionine dependence, despite the two frequently occurring together. A study by Tang et al.[36] demonstrated that MCF-7 cells, which are methionine dependent and MTAP deficient, did not grow in Met^{-} Hcy^{+} medium when *MTAP* cDNA was transfected into the cells. These data suggest that mutations in genes other than *MTAP* in the methionine *salvage* and/or *de novo* pathways may be involved in the methionine-dependence phenotype of cancers.

Control of methionine dependent cancers by MTA and adenine analogs

Cells which lack MTAP are unable to catabolize MTA to generate adenine, a purine derivative with many roles including cellular respiration and protein synthesis (Fig. 1), presenting a possible therapeutic target for cancer treatment. A recent study proposed a novel strategy for selectively killing MTAP deficient tumor cells via a combination of a toxic adenine analog (such as 2,6-diaminopurine, 6-methylpurine or 2-fluoroadenine) and MTA.[37] Normal cells will generate adenine from MTA and block conversion of the toxic analog to its active nucleotide form, whereas MTAP deficient tumor cells are unable to block conversion of the analog and are killed. However, a recent phase II clinical trial demonstrated that L-alanosine, a potent inhibitor of adenine biosynthesis, was ineffective in patients with advanced tumors deficient in MTAP.[38] A possible explanation for a lack of effect of L-alanosine is that synthesis of adenine from MTA in cancers may not be limited by the absence of MTAP within the cell. Surrounding normal tissues may act as a source of adenine, thereby severely limiting the effectiveness of L-alanosine. An alternate approach to target adenine biosynthesis in cancers is to globally inhibit MTAP. A recent study by Basu et al.[39] demonstrated that systemic inhibition of MTAP with methylthio-DADMe-Immucillin-A (MTDIA), a transition state analog inhibitor of MTAP, is effective in inhibiting growth and metastases of human lung cancers in mouse xenographs.

There is evidence suggesting that deletion of MTAP may lead to increased activity of ornithine decarboxylase (ODC).[40] ODC is the rate-limiting enzyme in the production of polyamines, which stimulate cellular proliferation and therefore cancer growth (Fig. 1). *Saccharomyces cervisiae* (yeast) cells lacking MTAP have elevated ODC activity and the introduction of MTOB or MTAP represses ODC levels.[40] Similarly, introduction of MTAP into MTAP deficient MCF-7 cells significantly reduces ODC activity.[40] These data suggest that other enzymes in the methionine *salvage* pathway may regulate tumor growth in addition to MTAP and require further investigation.

Defects in the folate metabolism pathway that may contribute to the methionine dependence phenotype

Methionine dependence has been linked to reduced MTR activity as observed in methionine dependent HTC liver cancer cells.[41] However, other studies have previously reported similar levels of MTR in methionine dependent malignant cells relative to normal cells.[42] To perform its enzymatic activity, MTR requires 5-methyltetrahyrofolate (5-MTHF) as a methyl donor and cobalamin (vitamin B_{12}) as a cofactor.[43] 5-MTHF synthesis is catalyzed by methylenetetrahydrofolate reductase (MTHFR) from 5,10-MTHF as part of the folate cycle (Fig. 2). A recent study by Beetstra et al.[44] reported that the methionine dependence of human lymphocytes tended to be higher in *BRCA1* and *BRCA2* gene mutation carriers with breast cancer compared to those without cancer and was significantly increased in *MTHFR* C677T T allele carriers relative to C allele carriers. However, in studies utilizing methionine dependent Walker-256 carcinosarcoma cells, 5-MTHF had no effect on methionine dependence suggesting that MTHFR does not contribute to this phenotype in these cells,[45] although it should be noted that there is no data on *MTHFR* polymorphisms in Walker-256 cells. The effect of the C677T polymorphism on *MTHFR*

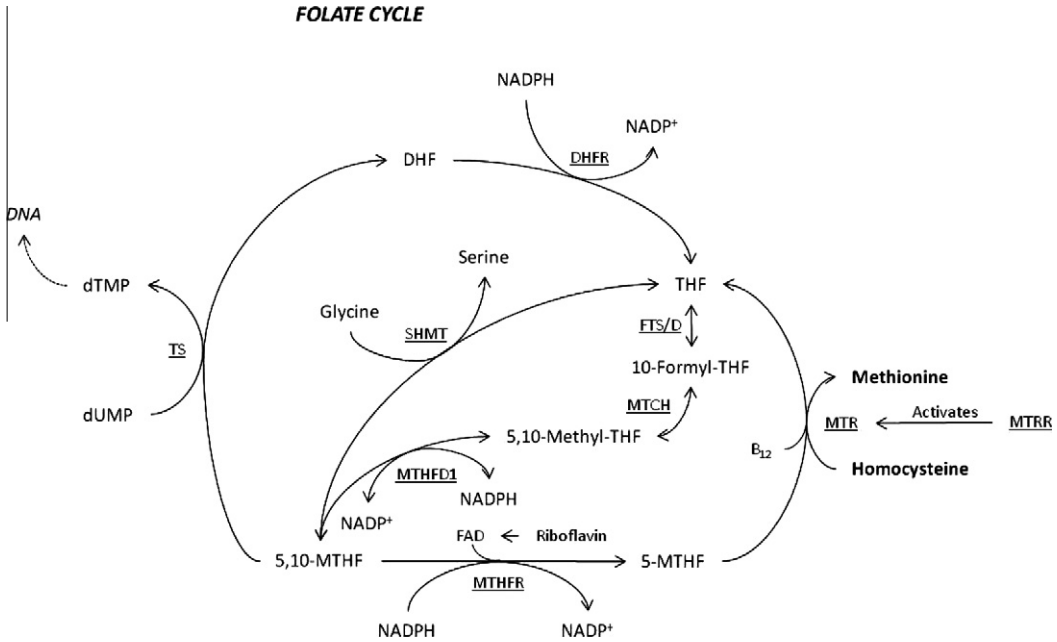

Fig. 2. Folate cycle. Enzymes are underlined. 5-MTHF, 5-methyl-tetrahydrofolate; 5,10-MTHF, 5,10-methenyl-tetrahydrofolate; B_{12}, vitamin B_{12}; DHF, dihydrofolate; DHFR, dihydrofolate reductase, dTMP, deoxy-thymidine-monophosphate; dUMP, deoxy-uracil-monophosphate; FAD, flavin adenine dinucleotide; FTS/D, 10-formyl-tetrahydrofolate synthase or 10-formyl-tetrahyrofolate dehydrogenase; MTCH, 5,10-methenyl-tetrahydrofolate cyclohydrolase; MTHFD1, 5,10-methenylenetetrahydrofolate dehydrogenase; MTHFR, 5,10-methylene-tetrahydrofolate reductase; MTR, methionine synthase; MTRR, 5-methyl-tetrahydrofolate-homocysteine methyl-transferase reductase; SHMT, serine-hydroxy-methyl transferase; THF, tetrahydrofolate and TS, thymidine synthase.

activity is modified by the concentration or intake of riboflavin (vitamin B_2) which is a cofactor of MTHFR. Culture of human lymphocytes in medium rich in riboflavin negates the impact of the *MTHFR* C677T polymorphism and high riboflavin intake *in vivo* prevents high plasma Hcy in homozygous carriers of the T allele of *MTHFR*.[46,47]

A possible mechanism for methionine dependence via diminished MTR activity is the impairment of cobalamin metabolism which lowers MTR activity as observed in melanoma and glioma cells.[48,49] MeWo-LC1 cells, which possess impaired cobalamin metabolism due to methylation of a CpG island at the 5′-end of the methylmalonic aciduria (cobalamin deficiency) cblC type with homocystinuria (*MMACHC*) gene are rescued from methionine dependence by addition of wild-type *MMACHC* (the specific function of *MMACHC* is currently unknown).[50] In contrast, a study by Watkins[51] which utilized a panel of 14 tumor and leukemia cell lines reported that impairment in cobalamin metabolism is unlikely to be a common cause of methionine dependence.

The *MTR* A2756G polymorphism has been implicated in breast cancer risk in Brazilian women and may be associated with breast cancer risk in carriers of the *BRCA1* and *BRCA2* germline inactivating mutations which are evident in 5–10% of all breast cancer cases.[44,52] Despite these findings, a recent meta-analysis suggested that the association between *MTR* A2756G polymorphism and breast cancer risk was specific to European women with no associations being identified in other ethnicities.[53] Most studies suggest that individuals with the rarer *G* allele have lower plasma Hcy levels than those with the more common *A* allele (see review by Sharp and Little).[54] Carriers of the *MTR* A2756G allele are also reported to have lower levels of chromosome damage measured as micronuclei (biomarkers of whole chromosome loss and/or damage) and are more likely to live to 100 years.[55,56] It is not clear if this polymorphism results in increased *de novo* synthesis of methionine (therefore lowering Hcy levels) or if it is a contributing factor in the methionine dependence phenotype in breast cancer.[44]

Methionine dependence in cancer cells may be due to an increased requirement for methionine as opposed to a specific metabolic block in the *salvage* or *de novo* pathways. For example, human gliomas are often characterized by a high accumulation rate of methionine in comparison to normal tissues, allowing for positron emission tomography using biologically active ([^{11}C]methyl)-L-methionine to image tumor size in patients.[57] The increased requirement for methionine in tumor cells is likely to be due to elevated rates of transmethylation and possibly lead to the silencing of key genes regulating growth inhibition and apoptosis by hypermethylation.[7,58] The observation that tumor cells are unable to maintain a high level of transmethylation in Met^- Hcy^+ medium suggests that these cells are dependent on exogenous methionine.[59] Therefore, some tumor cells may lack the ability to increase *MTR* expression or activity in Met^- Hcy^+ medium to maintain an elevated level of transmethylation, although the mechanism for this remains unclear.

Dietary methionine restriction therapy for cancer growth control *in vivo*

The observation that some human tumors are methionine dependent *in vivo* presents a therapeutic target in cancer growth control.[60] As methionine is sourced mainly from food, a strategy to lower methionine levels *in vivo* is to restrict or remove methionine from the diet. When dietary methionine is restricted, methionine already in the system is conserved, presumably by a reduction of cystathionine synthesis in the *trans-sulfuration* pathway, leading to a temporary increase in levels of total Hcy for *de novo* methionine synthesis (Fig. 1).[61] Therefore, there is often a lag between dietary methionine restriction and the reduction in serum methionine levels. A phase I clinical trial of enteral methionine restriction for 18 weeks in adults with a variety of metastatic cancers reported a 58% decline in plasma methionine within 2 weeks and an overall weight loss of approximately 0.5 kg per week.[62] These data suggest that methionine restriction in humans is relatively safe and tolerable over a period of 18 weeks, but the consequences of further weight loss in such cancer patients have not been explored.

In animals, methionine restriction may impair cancer growth and carcinogenesis. An early study by Breillout et al.[63] reported that female Wistar AG rats bearing rhabdomyosarcoma pulmonary metastases had a lower number of median metastases when fed a low methionine diet. In another study, diets deficient in methionine, Hcy and choline extended the survival of Yoshida sarcoma bearing nude mice to approximately 30–38 days, whereas mice on a diet containing methionine were all deceased by day 12.[64] Extended survival of methionine restricted mice in the latter study may be due to a significant decrease in plasma methionine. Furthermore, a recent study reported that azoxymethane treated male F344 rats fed a methionine restricted diet (0.17% [wt/wt] methionine) had up to 80% fewer colon preneoplastic aberrant crypt foci than rats fed the control diet (0.86% [wt/wt] methionine).[65] Despite these promising data, it should be noted that prolonged use of diets extremely deficient in methionine and its precursors could be lethal.[66] Therefore, more attention needs to be given to determine the safe, tolerable limit of methionine restriction.

In humans, methionine restriction may be achieved using a predominately vegan diet.[67] A vegan diet is based almost exclusively on plant derived products and therefore contains no meat, fish, dairy or eggs. Vegan diets tend to be high in fiber, vitamin B_1, folate, vitamin C, vitamin E, magnesium and iron and low in retinol, vitamin B_{12}, vitamin D, calcium and zinc.[68] While vegan diets are typically low in methionine, some nuts and legumes (such as Brazil nuts and kidney beans) are rich in methionine (Table 3). Therefore, careful choice of food based on methionine content is required to achieve a reliable methionine restriction regimen that is not deficient in other essential nutrients.

A recent study by Ornish et al.[69] utilized a vegan diet as part of an intensive lifestyle program for prostate cancer patients who had not elected any conventional cancer treatment. The lifestyle program included activities such as meditation, yoga and moderate aerobic exercise. Serum from the patients, collected at baseline and following 1 year of the lifestyle program was used *in vitro* to investigate growth inhibition of LNCaP prostate cancer cells. The serum from patients on the vegan diet and undergoing lifestyle changes inhibited growth of LNCaP cells by up to 70% compared to serum from patients not on a vegan diet and not undergoing an intensive lifestyle program.[69] These data suggested that intensive lifestyle changes in combination with a methionine restricted diet may lower the risk of progression of some prostate cancers. Furthermore, a recent study has demonstrated that 40% dietary methionine restriction in male Wistar rats decreases production of ROS in the brain and kidney mitochondria without inhibition of body weight gain which may occur at 80% dietary methionine restriction.[70] Therefore, a 40% methionine restricted diet may be safer than an 80% methionine restricted diet as a long term regimen for humans.

Dietary restriction of methionine in combination with other nutrients that are known to aid cancer growth may have an additive effect in limiting growth and metastases of cancers. For example, glucose restriction has recently been reported to extend the life-span of normal WI-38 fetal lung fibroblasts while impairing immortalized WI-38/S precancerous cells through epigenetic mechanisms.[71] Therefore, a combination of dietary methionine restriction and caloric restriction, by limiting glucose, may prove beneficial in cancer growth control. Other combinations of

Table 3
Methionine, vitamin B_{12} and protein content of important food groups ranked according to methionine content within each food group.

NDB#	Food	Methionine (g/100 g)	Vitamin B_{12} (μg/100 g)	Protein (g/100 g)	Methionine (g)/protein (100 g)
Grains					
18035	Bread, multi-grain (includes whole-grain)	0.138	0.00	13.36	1.033
20040	Rice, brown, medium-grain, raw	0.169	0.00	7.50	2.253
20005	Barley, pearled, raw	0.190	0.00	9.91	1.917
20028	Couscous, dry	0.199	0.00	12.76	1.560
08122	Cereals, oats, instant, fortified, plain, dry	0.215	0.00	12.72	1.690
Legumes					
11052	Beans, snap, green, raw	0.022	0.00	1.83	1.202
11304	Peas, green, raw	0.082	0.00	5.42	1.513
16427	Tofu, raw, regular, prepared with calcium sulfate	0.103	0.00	8.08	1.275
16069	Lentils, raw	0.220	0.00	25.80	0.853
16056	Chickpeas (garbanzo beans, bengal gram), mature seeds, raw	0.253	0.00	19.30	1.311
16071	Lima beans, large, mature seeds, raw	0.271	0.00	21.46	1.263
16087	Peanuts, all types, raw	0.317	0.00	25.80	1.229
16027	Beans, kidney, all types, mature seeds, raw	0.355	0.00	23.58	1.506
16108	Soybeans, mature seeds, raw	0.547	0.00	36.49	1.499
Nuts and other edible seeds					
12131	Nuts, macadamia nuts, raw (1)	0.023	0.00	7.91	0.291
12098	Nuts, chestnuts, european, raw, peeled	0.038	0.00	1.63	2.331
12061	Nuts, almonds	0.151	0.00	21.22	0.712
12155	Nuts, walnuts, english	0.236	0.00	15.23	1.550
12147	Nuts, pine nuts, dried (1)	0.259	0.00	13.69	1.892
12151	Nuts, pistachio nuts, raw (1)	0.335	0.00	20.27	1.653
12087	Nuts, cashew nuts, raw	0.362	0.00	18.22	1.987
12036	Seeds, sunflower seed kernels, dried	0.494	0.00	20.78	2.377
12023	Seeds, sesame seeds, whole, dried	0.586	0.00	17.73	3.305
12078	Nuts, brazil nuts, dried, unblanched	1.008	0.00	14.32	7.039
Vegetables					
11282	Onions, raw	0.002	0.00	1.10	0.182
11143	Celery, raw	0.005	0.00	0.69	0.725
11429	Radishes, raw	0.010	0.00	0.68	1.471
11564	Turnips, raw	0.011	0.00	0.90	1.222
11485	Squash, winter, butternut, raw	0.012	0.00	1.00	1.200
11109	Cabbage, raw	0.012	0.00	1.28	0.938
11080	Beets, raw	0.018	0.00	1.61	1.118
11246	Leeks (bulb and lower leaf-portion), raw	0.018	0.00	1.50	1.200
11124	Carrots, raw	0.020	0.00	0.93	2.151
11135	Cauliflower, raw	0.020	0.00	1.92	1.042
11354	Potatoes, white, flesh and skin, raw	0.026	0.00	1.68	1.548
11507	Sweet potato, raw, unprepared	0.029	0.00	1.57	1.847
11265	Mushrooms, portabella, raw	0.029	0.05	2.11	1.374
11011	Asparagus, raw	0.031	0.00	2.20	1.409
11260	Mushrooms, white, raw	0.031	0.04	3.09	1.003
11098	Brussels sprouts, raw	0.032	0.00	3.38	0.947
11090	Broccoli, raw	0.038	0.00	2.82	1.348
11457	Spinach, raw	0.053	0.00	2.86	1.853
11215	Garlic, raw	0.076	0.00	6.36	1.195
Fruit					
09003	Apples, raw, with skin (1)	0.001	0.00	0.26	0.385
09252	Pears, raw	0.002	0.00	0.38	0.526
09316	Strawberries, raw	0.002	0.00	0.67	0.299
11529	Tomatoes, red, ripe, raw, year round average	0.006	0.00	0.88	0.682
09089	Figs, raw	0.006	0.00	0.75	0.800
09191	Nectarines, raw	0.006	0.00	1.06	0.566
11205	Cucumber, with peel, raw	0.006	0.00	0.65	0.923
09112	Grapefruit, raw, pink and red, all areas	0.007	0.00	0.77	0.909
11333	Peppers, sweet, green, raw	0.007	0.00	0.86	0.814
09040	Bananas, raw	0.008	0.00	1.09	0.734
09176	Mangos, raw (1)	0.008	0.00	0.82	0.976
09279	Plums, raw	0.008	0.00	0.70	1.143
09132	Grapes, red or green (European type), raw	0.009	0.00	0.72	1.250
09070	Cherries, sweet, raw	0.010	0.00	1.06	0.943
09236	Peaches, raw	0.010	0.00	0.91	1.099
11422	Pumpkin, raw	0.011	0.00	1.00	1.100
11209	Eggplant, raw	0.011	0.00	1.01	1.089
09181	Melons, cantaloupe, raw	0.012	0.00	0.84	1.429
09193	Olives, ripe, canned (small-extra large)	0.012	0.00	0.84	1.429
09266	Pineapple, raw, all varieties (1)	0.012	0.00	0.54	2.222
09139	Guavas, common, raw (1)	0.016	0.00	2.55	0.627
11477	Squash, summer, zucchini, includes skin, raw	0.018	0.00	1.21	1.488
09200	Oranges, raw, all commercial varieties	0.020	0.00	0.94	2.128

Table 3 (*continued*)

NDB#	Food	Methionine (g/100 g)	Vitamin B_{12} (μg/100 g)	Protein (g/100 g)	Methionine (g)/protein (100 g)
09298	Raisins, seedless	0.021	0.00	3.07	0.684
11278	Okra, raw	0.021	0.00	2.00	1.050
09087	Dates, deglet noor (1)	0.022	0.00	2.45	0.898
09148	Kiwifruit, green, raw	0.024	0.00	1.14	2.105
09037	Avocados, raw, all commercial varieties (1)	0.038	0.00	2.00	1.900
11167	Corn, sweet, yellow, raw	0.067	0.00	3.27	2.049
Dairy					
01145	Butter, without salt	0.021	0.17	0.85	2.471
01211	Milk, whole, 3.25% milkfat, without added vitamin A and vitamin D	0.073	0.45	3.15	2.317
19095	Ice creams, vanilla	0.081	0.39	3.50	2.314
01116	Yogurt, plain, whole milk, 8 g protein per 8 oz	0.102	0.37	3.47	2.939
01017	Cheese, cream	0.191	0.25	5.93	3.221
01036	Cheese, ricotta, whole milk	0.281	0.34	11.26	2.496
Meat and fish					
10123	Pork, cured, bacon, raw	0.258	0.69	11.60	2.224
07029	Ham, sliced, regular (approximately 11% fat)	0.319	0.42	16.60	1.922
15149	Crustaceans, shrimp, mixed species, raw	0.397	1.11	13.61	2.917
01124	Egg, white, raw, fresh	0.399	0.09	10.90	3.661
05111	Chicken, roasting, meat and skin, raw	0.454	0.31	17.14	2.649
17302	Lamb, Australian, imported, fresh, leg, sirloin chops, boneless, separable lean and fat, trimmed to 1/8″ fat, raw	0.469	2.72	18.33	2.559
17294	Lamb, Australian, imported, fresh, leg, shank half, separable lean and fat, trimmed to 1/8″ fat, raw	0.476	2.75	18.59	2.561
23005	Beef, short loin, t-bone steak, separable lean and fat, trimmed to 1/8″ fat, all grades, raw	0.491	2.78	19.19	2.559
15139	Crustaceans, crab, blue, raw	0.508	9.00	18.06	2.813
15007	Fish, butterfish, raw	0.512	1.90	17.28	2.963
10036	Pork, fresh, center loin (chops), bone-in, separable lean and fat, raw	0.570	0.53	20.71	2.752
05165	Turkey, all classes, meat and skin, raw	0.574	0.40	20.42	2.811
15076	Fish, salmon, Atlantic, wild, raw	0.587	3.18	19.84	2.959

Values obtained from the USDA National Nutrient Database (www.nal.usda.gov). NDB#, USDA National Nutrient Database Code Number.

nutrients may also limit cancer growth, however, the challenge is to identify which combination of nutrients and their doses are optimal. A nutrient array model has recently been proposed as a diagnostic tool for identifying, on an individual basis, the most favorable nutrient combinations (nutriomes) which may lower the risk of cancer growth and other degenerative diseases.[72]

Dietary methionine restriction may also extend life-span

Early studies by Orentreich et al.[73] and Richie et al.[74] demonstrated that Fischer 344 rats experienced an increase of greater than 40% in both mean and maximal life-span when dietary methionine content was restricted by 80% from 0.86% (wt/wt) to 0.17% (wt/wt). To confirm that these findings were not unique to Fischer 344 rats, three other strains of rats (Brown–Norway, Sprague–Dawley and Wistar–Hannover) were subjected to dietary methionine restriction with each strain experiencing prolonged life-span.[4] These effects were independent of any effect of caloric restriction as the Fischer 344 male rats fed the control diet containing 0.86% (wt/wt) methionine were pair-fed, so that their caloric intake was matched to that of rats fed the 0.17% (wt/wt) methionine diet *ad libitum*.[4] In mice, a methionine-deficient diet prolongs life-span and slows immune system and eye lens aging, improves stress resistance and alters glucose, T4, IGF-I and insulin levels.[75] It is unknown whether methionine restriction extends healthy life-span in humans.

Methioninase to control cancer growth

Reduction of methionine levels by dietary intervention alone has some limit as methionine may also be sourced from protein breakdown or Hcy. A pharmacological approach to lowering methionine *in vivo* is to use the enzyme L-methionine-α-amino-γ-mercaptoethane lyase (methioninase). Originally purified from *Clostridium sporogene*, methioninase degrades methionine to α-ketobutyrate, methanethiol and ammonia.[76] Methioninase was reported to be more effective at slowing growth of the Walker-256 carcinosarcoma in Wistar rats than a methionine-free diet.[77] Later, methioninase was purified from *Pseudomonas putida*, which yielded a more stable enzyme with a relatively low K_m.[78] In nude mice, intraperitoneal injection of methioninase inhibited growth of Yoshida sarcoma and slowed growth of H460 human non-small-cell-lung carcinoma.[79] In the same study, the administration of methioninase did not cause weight loss indicating that toxicity of this compound is likely to be low, however, yields of the enzyme from *P. putida* were not sufficient for clinical use.[79] To overcome the issue of low yields of methioninase, Tan et al.[80] cloned and over-expressed the methioninase gene in *Escherichia coli* producing high yields of recombinant methioninase. Methoxypolyethylene glycol succinimidyl glutarate-5000 was then conjugated to recombinant methioninase to improve the therapeutic potential of the enzyme by increasing its half life in circulation.[81] Conjugation of polyethylene glycols in protein therapeutics is also beneficial as they may reduce antigenicity.[82] Both recombinant methioninase and polyethylene glycol conjugated recombinant methioninase are reported to have a broad selective efficacy for many cancers *in vitro* as well as a high activity for killing cancer cells.[83]

Interactive effects of methionine restriction and methioninase with chemotherapy to treat cancer

Dietary methionine restriction and methioninase present two therapeutic approaches to inhibit cancer growth in methionine dependent tumors. Whether these strategies can modulate the efficacy of chemotherapeutic agents on human tumors *in vivo* has been a major focus of pre-clinical and clinical studies. Methionine

depletion in methionine dependent cancer cells can lead to cell cycle arrest in the late-S/G_2 phase both *in vitro* and *in vivo*.[64,84] Cells that arrest in late-S/G_2 phase are susceptible to spontaneous death and are hypersensitive to chemotherapeutic agents.[19] This tumor-specific metabolic defect was exploited in combination with the chemotherapeutic agents doxorubicin and vincristine *in vitro* to selectively kill tumor cells from co-cultures with normal cells.[85] Furthermore, MX-1 human breast carcinoma cells grown in nude mice were highly sensitive to the combination of a methionine depleted diet and cisplatin, but resistant to each alone.[60] A study by Machover et al.[86] demonstrated a cytotoxic synergism of recombinant methioninase in combination with 5-fluorouracil and folinic acid in CCRF-CEM human leukemia cells.

A study by Goseki and Endo[87] reported that tumor proliferation in rats was inhibited in response to methionine depleted total parenteral nutrition (TPN), an effect which appeared to enhance the anti-tumor effect of nimustine hydrochloride. In a subsequent study, 8 day methionine restriction by TPN in combination with 4 g/body weight 5-flourouracil markedly degenerated cancer tissue in humans with advanced gastric cancer.[88] Histological data from this study indicated that the methionine restricted TPN inhibited cancer growth to a greater extent than conventional TPN.[88] A phase I clinical trial investigated the association of a methionine-free diet with nitrosourea chemotherapy in metastatic and recurrent gliomas and reported that the methionine-free diet decreased plasma methionine by a maximum of 55% after 4 h without significant toxicity and impairment of nutritional status.[89] A more recent clinical trial by the same group investigated the feasibility of dietary methionine restriction in combination with the 48 h regimen of 5-flourouracil, leucovorin and oxaliplatin (FOLFOX regimen) in patients with metastatic colorectal cancer.[90] FOLFOX is a current first-line regimen for patients with metastatic colorectal cancer.[91] In response to dietary methionine restriction and the FOLFOX regimen, 3 out of 4 evaluable patients experienced a partial response and the fourth patient experienced long-lasting disease stabilization after surgery.[90] The combination of a methionine restricted diet with chemotherapeutic agents has produced promising preliminary data and should be further investigated in phase II trials.

Knowledge gaps

While current understanding of the methionine dependence phenotype in cancer is improving, there remain several questions with respect to the feasibility, safety and sustainability of methionine restriction for targeted control of cancer growth:

- What is the best way to determine whether a tumor is methionine dependent?
- What level of methionine restriction is optimal for control of cancers *in vivo*?
- Is there a genetic or epigenetic risk to normal tissue associated with severe methionine restriction or methioninase therapy?
- Which methionine restriction dietary pattern is most efficacious for cancer growth control?
- Is a combination of methionine restriction with glucose restriction on cancer growth more efficacious than either nutrient restriction alone?
- Is a transition from a methionine rich to a methionine restricted diet sustainable in the long term?

Conclusions

Despite the promising clinical data on methionine restriction either on its own or in combination with chemotherapeutic agents on cancer growth control, there is still insufficient knowledge to give reliable nutritional advice to cancer sufferers and survivors to prevent tumor growth and relapse, respectively. The dependence of some cancers on methionine and the potential susceptibility of cancers to glucose restriction present a wholly nutritional therapeutic approach to cancer growth control. A better understanding of nutrient interactions (such as glucose and methionine restriction) and the specific nutritional dependencies of an individual's cancer may allow for a targeted nutritional regimen to limit cancer growth and extend life-span. Finally, a targeted nutritional restriction regimen may be enhanced via combination with lifestyle changes and/or existing conventional cancer therapies.

Conflict of interest statement

None declared.

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