DIET AND SYSTEMIC METABOLISM

Methionine metabolism in health and cancer: a nexus of diet and precision medicine

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Abstract | Methionine uptake and metabolism is involved in a host of cellular functions including methylation reactions, redox maintenance, polyamine synthesis and coupling to folate metabolism, thus coordinating nucleotide and redox status. Each of these functions has been shown in many contexts to be relevant for cancer pathogenesis. Intriguingly, the levels of methionine obtained from the diet can have a large effect on cellular methionine metabolism. This establishes a link between nutrition and tumour cell metabolism that may allow for tumour-specific metabolic vulnerabilities that can be influenced by diet. Recently, a number of studies have begun to investigate the molecular and cellular mechanisms that underlie the interaction between nutrition, methionine metabolism and effects on health and cancer.

Dietary methionine restriction
(MR). A diet characterized by reduced methionine levels compared to a standard reference diet; the degree of restriction can vary between studies.

Progeroid
Genetic predisposition that causes subjects to exhibit advanced physiological ageing.

S-adenosyl-methionine
(SAM). Methionine-derived universal methyl donor required for all cellular methylation reactions.

One-carbon metabolism
Set of biochemical reactions that allow for the transfer of single carbon units from dietary nutrients, particularly amino acids, in order to fuel critical cellular processes.

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Cancer metabolism is characterized by metabolic demands, nutritional supplies and the regulation of metabolic enzymes that differ from those in corresponding healthy tissue[1]. Although many of these alterations have been attributed to specific genetics, accumulating evidence indicates that environmental factors (particularly dietary composition) impact biological processes that promote cancer incidence and progression as much as, if not more than, genetic status does[2,3]. Therefore, novel nutritional strategies to systematically target cancer-specific vulnerabilities are a major focus in the development of cancer therapies[4–13].

An emerging aspect of cancer metabolism is the essential amino acid methionine. Its biological impact has been explored in the context of ageing and metabolic diseases, with dietary methionine restriction (MR) having been shown to extend lifespan in yeast[14], *Drosophila*[15], *Caenorhabditis elegans*[16], the mouse[17,18] and the rat[19,20], as well as to prevent accelerated ageing in progeroid mice[21]. Furthermore, methionine has also been associated with a number of metabolic benefits, including limiting accretion of fat depots, preventing high-fat diet-induced obesity, improving hepatic function, elevating resistance to oxidative stress, enhancing insulin sensitivity and preventing the development of diabetes[22–36]. In line with the widespread physiological effects modulated by methionine availability, numerous methionine-dependent processes have been implicated in cancer. In this Review, we will cover cancer-associated alterations that are observed in methionine metabolism, our present understanding of how dietary methionine contributes to cancer, and current strategies for therapeutically targeting these processes.

The one-carbon metabolic network

Methionine is an essential sulfur-containing amino acid that is catabolized and recycled in a series of metabolic reactions termed the methionine cycle (FIG. 1). Briefly, methionine is converted to the universal methyl donor S-adenosyl-methionine (SAM), which upon donation of its methyl group is converted to S-adenosyl-homocysteine (SAH). SAH is hydrolysed in order to generate homocysteine, which is then converted to cysteine via the transsulfuration pathway or, with a methyl donation from the folate cycle, back into methionine. This cycle is closely linked to the folate cycle (fuelled in large part by serine and glycine), collectively forming the two major components of what is referred to as one-carbon metabolism[37]; this metabolic network allows for the integration of nutritional carbon units in a diverse set of critical cellular processes (expanded in BOX 1). Additionally, methionine can also be recycled from the SAM-dependent polyamine biosynthesis by-product methylthioadenosine (MTA), which is further processed by the enzyme methylthioadenosine phosphorylase (MTAP) via the methionine salvage pathway. As with most metabolic processes, each of these biochemical reactions is tightly regulated and coupled to the other reactions in one-carbon metabolism, and aberrant activity within one node of this network can lead to drastic dysregulation of cellular function, as is commonly observed in disease contexts such as cancer.

Methionine metabolism in cancer

The clinical applicability of cancer-specific alterations in methionine consumption and utilization is perhaps most

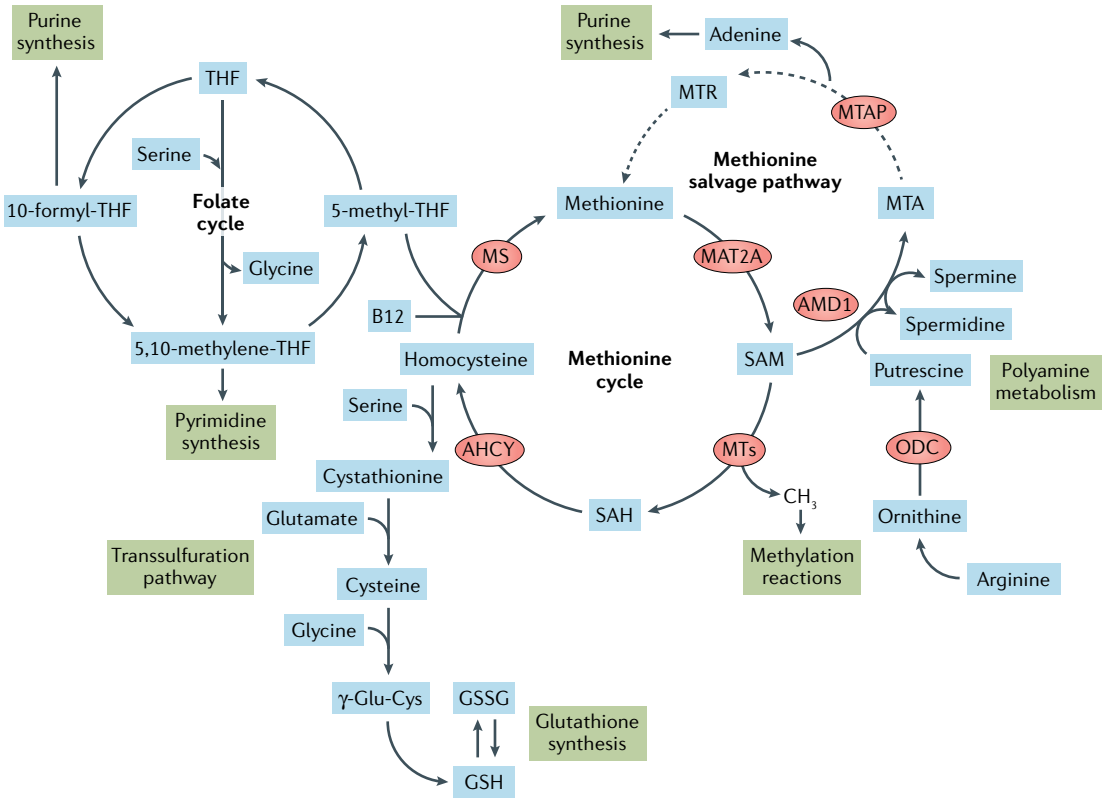


Fig. 1 | **Methionine metabolism and related metabolic processes.** The essential amino acid methionine is a critical component of the one-carbon metabolic network, which contributes to a myriad of metabolic processes, including polyamine and nucleotide (purine and pyrimidine) synthesis, as well as glutathione production. Methionine is catabolized by methionine adenosyltransferase 2A (MAT2A), producing the universal methyl donor S-adenosyl-methionine (SAM). Methyltransferases (MTs) use SAM as a methyl source, thereby producing S-adenosyl-homocysteine (SAH), which can further act as a negative regulator of SAM-dependent processes. With a donation from the tightly linked folate cycle (extensively reviewed elsewhere[3]), SAH is then converted by adenosylhomocysteinase (AHCY) to homocysteine, which can then either contribute to the transsulfuration pathway for glutathione synthesis or be converted back to methionine by methionine synthase (MS), thus completing the methionine cycle. Methionine can additionally be recycled from the polyamine biosynthesis by-product methylthioadenosine (MTA) via the methionine salvage pathway. Methionine also contributes to polyamine biosynthesis by serving as a source of SAM; the polyamine putrescine is generated from the arginine-derived molecule ornithine via the enzyme ornithine decarboxylase (ODC), upon which it can be converted to the polyamine spermidine by adenosylmethionine decarboxylase 1 (AMD1) in a SAM-dependent reaction. Spermidine can then be converted to the final polyamine, spermine, in an additional SAM-dependent process. B12, vitamin B12; γ-Glu-Cys, γ-glutamyl-L-cysteine; GSH, reduced glutathione; GSSG, oxidized glutathione; MTAP, methylthioadenosine phosphorylase; MTR, methylthioribose; THF, tetra-hydrofolate.

Polyamine
Methionine-derived polycations that interact with negatively charged moieties of DNA and other proteins and lipids.

Methylthioadenosine phosphorylase
(MTAP). Enzyme involved in the salvage of methionine and adenine from by-products of polyamine biosynthesis.

Methylation
Biochemical addition of a methyl group (composed of one carbon and three hydrogen atoms, or CH_3) to another substrate.

Methionine adenosyltransferase 2A
(MAT2A). Enzyme that catalyses the ATP-dependent conversion of methionine to SAM.

readily apparent by the observation that intratumoural methionine uptake, as evidenced by positron emission tomography (PET) imaging of ^{11}C-methionine, is at least in certain contexts more indicative of therapeutic response and overall survival than glucose uptake[38,39]. Numerous aspects of metabolism related to methionine provide links to cancer; given its placement in one-carbon metabolism, methionine metabolism may participate in the number of functions that serine, glycine and folate have been extensively shown to be linked to in cancer (reviewed elsewhere[40]). Furthermore, redox biology, chromatin and nucleic acid methylation, polyamine synthesis, and other metabolic processes connected to methionine (BOX 1) are also linked to tumour biology. In line with this finding, a study recently published has demonstrated evidence that tumour-initiating cells exhibit elevated activity of enzymes within methionine metabolism, most notably an upregulation of methionine adenosyltransferase 2A (MAT2A) expression and activity[41]. Additionally, activity of the methyltransferase nicotinamide N-methyltransferase (NNMT) was recently shown, in addition to its cell-autonomous cancer-promoting function[42], to be a driver of the oncogenic behaviour of cancer-associated fibroblasts[43]. In this context, NNMT, which uses SAM to convert nicotinamide into NAD^+ and the metabolically inert by-product 1-methylnicotinamide (1-MNA)[44], was shown to consume the available SAM pool in these cancer-associated fibroblasts, thereby diverting SAM from DNA and histone methylation processes (a phenomenon referred to as a "methyl sink"), ultimately leading to metastasis and overall cancer progression[43]. Nevertheless, we are still in the preliminary stages of understanding the role of methionine metabolism in tumorigenic mechanisms, which we discuss later in this Review. Here we survey recently appreciated genetic and environmental contexts

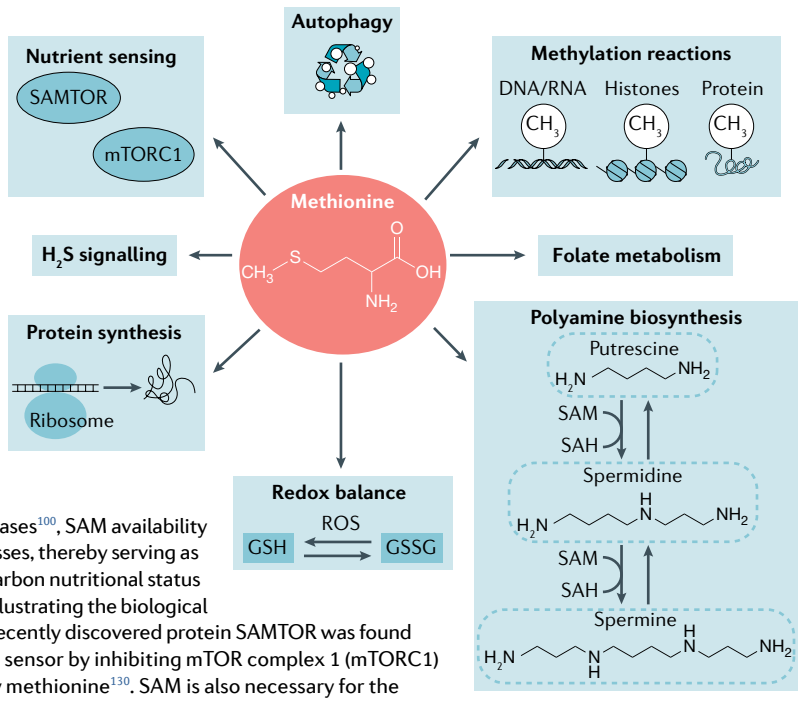
Protein arginine N-methyltransferase 5 (PRMT5). Methyltransferase that catalyses the monomethylation and symmetrical dimethylation of arginine residues of proteins.

in which the relevance of methionine metabolism in cancer has been established.

MTAP deletion. Gene deletions of *MTAP* are commonly found in tumours, due to its proximity to the *CDKN2A* locus on chromosome 9p21, which encodes one of the most frequently altered tumour suppressors, p16 (REF.[45]); ~15% of all cancers (most notably glioblastoma[46], melanoma[47], mesothelioma[48] and pancreatic cancer[49]) exhibit deletions of this chromosomal locus, with *MTAP* co-deletions occurring in ~80–90% of this subset[50]. Alterations in MTAP expression have also been found independent of *CDKN2A* deletion, due to hypermethylation of the *MTAP* promoter[51,52] or (in some rare cases) selective homozygous deletion of *MTAP*[53], suggesting that MTAP could potentially function as a tumour suppressor in addition to its established role as a passenger event[54], although more studies will be needed to definitively establish this. As mentioned previously, MTAP is an enzyme in the methionine salvage pathway that converts the polyamine biosynthesis by-product MTA ultimately into methionine and adenine (FIG. 1); loss of MTAP expression has thus been found to consistently induce intracellular accumulation of its substrate MTA[55–57].

Due to the difficulty of therapeutically targeting tumour suppressor pathways, *MTAP* deletion has gained considerable interest as a modifier of cancer-specific metabolic vulnerabilities. Some preliminary work has shown that *MTAP*-deleted cells exhibit enhanced sensitivity to inhibitors of de novo purine metabolism[58–60]. Interestingly, recent studies have identified that protein arginine N-methyltransferase 5 (PRMT5) exhibits a high degree of sensitivity to intracellular MTA levels, due to the high affinity of MTA for the SAM binding pocket of PRMT5 (REF.[57]), and is selectively required for cell growth across diverse MTAP-deficient cell lines[55,57]. Importantly, in these studies the supplementation of MTA in MTAP-expressing cells was found to induce sensitivity to PRMT5 inhibition, demonstrating that

Box 1 | **Role of methionine in biological processes**



One of the most prominent functions of methionine (see the figure) is its contribution to intracellular methylation by serving as the sole source of the universal methyl donor S-adenosyl-methionine (SAM); SAM is a necessary substrate for all methylation reactions, including those that modulate gene expression (via methylation of DNA, RNA and histones), phospholipid integrity, the activity of signalling pathways and polyamine biosynthesis. Due to the intracellular concentration of SAM relative to the Michaelis constant (K_m) values of cellular methyltransferases[100], SAM availability can directly impact these processes, thereby serving as a metabolic link between one-carbon nutritional status and cellular behaviour. Further illustrating the biological importance of methionine, the recently discovered protein SAMTOR was found to specifically function as a SAM sensor by inhibiting mTOR complex 1 (mTORC1) activity under conditions of low methionine[130]. SAM is also necessary for the biosynthesis of polyamines (including putrescine, spermidine and spermine), which function to maintain protein, DNA and RNA stability; protect against oxidative stress; and regulate the activity of ion channels[170]. Importantly, major disruptions in a subset of SAM-consuming reactions can significantly drive aberrant methylation patterns in other methylation-dependent processes[171].

Beyond mediating these SAM-dependent reactions, methionine also contributes to essential metabolic pathways that regulate nucleotide biosynthesis and intracellular redox balance. Via the contribution of homocysteine, methionine directly contributes to the folate cycle, which provides multiple inputs to both purine and pyrimidine biosynthesis (FIG. 1); furthermore, the methionine salvage pathway produces adenine from the polyamine metabolic by-product methylthioadenosine (MTA), creating an additional substrate for purine metabolism. Methionine also contributes to the maintenance of cellular redox status by providing homocysteine as a substrate for the transsulfuration pathway, which ultimately produces the antioxidant glutathione (GSH). The subsequent reversible oxidation of GSH to oxidized glutathione (GSSG) effectively combats cellular damage caused by reactive oxygen species (ROS)[172]. Further contributing to its role as a regulator of cellular oxidative stress, methionine also functions as a source of sulfur for the production of the critical signalling molecule hydrogen sulfide (H_2S)[131]. Finally, methionine plays a particularly important role in autophagic processes[173] and in protein synthesis via multiple mechanisms[174].

SAH, S-adenosyl-homocysteine.

the metabolic vulnerability induced by *MTAP* deletion is specifically due to MTA accumulation. Another study has also shown evidence that the reduction of PRMT5 activity found in multiple MTAP-deficient cell lines creates additional vulnerabilities in methionine metabolism that can be targeted therapeutically[56]. Although an understanding of how PRMT5-mediated arginine methylation regulates cellular processes (and by extension, why *MTAP*-deleted cells exhibit enhanced dependence on its activity) is currently lacking, aberrations in PRMT5 activity and expression have been implicated in numerous cancer types[61–63], thereby providing support for the therapeutic potential of targeting its activity. The current status of these therapeutic approaches is detailed in our later discussions of ongoing clinical investigations.

While these collateral vulnerabilities observed in *MTAP*-deleted cells may provide promising therapeutic opportunities, it is important to note that metabolism is largely determined by external factors[64], and these have been shown in certain contexts to override genetically driven phenotypes[65]. A recent investigation of how nutrient availability impacts the metabolic status of diverse pan-tissue cancer cell lines showed that *MTAP* deletion was non-predictive of metabolic responsiveness to methionine, serine or cysteine restriction; furthermore, methionine restriction was sufficient to abrogate the accumulation of MTA to levels found in MTAP-expressing cells[66]. Additionally, re-expression of MTAP protein had heterogeneous consequences on global metabolism in different cell lines. Given that dietary methionine levels are highly variable and correlate with circulating plasma concentrations of both methionine and methylated metabolites[67], these results, while not ruling out methionine metabolism as a reasonable drug target, shed light on the importance of considering potential gene–environment interactions that may impact the efficacy of therapies targeting these potential vulnerabilities. As an extension of this concept, a discussion of dietary methionine availability and the regulation of health and disease is expanded on later in this Review.

Polyamine metabolism. Elevated activity of polyamine metabolism, which directly branches from the methionine cycle (FIG. 1), has long been associated with rapid cell proliferation[68]. Since this discovery, genetic alterations that lead to changes in the expression or activity of enzymes that regulate polyamine metabolism have been found to be highly prevalent in cancer cells. Overexpression of the polyamine biosynthesis enzyme ornithine decarboxylase (ODC) is frequently observed across numerous cancer types[69–71] and has been shown to promote cancer cell growth in preclinical studies[72–74]. Furthermore, ODC expression has been shown to be predictive of the degree of tumorigenicity as well as of therapy resistance[75–77]. Of particular interest is the finding that ODC activity is regulated by 2-keto-4-methylthiobutyrate (MTOB), an intermediate in the MTAP-mediated conversion of MTA to methionine[49]; it was later found that ODC overexpression is frequently associated with concurrent deletion of *MTAP* in pancreatic cancers[78].

Another polyamine enzyme found to be dysregulated in cancer, adenosylmethionine decarboxylase 1 (AMD1), has gained considerable interest. AMD1 serves as an enzymatic link between the methionine cycle and polyamine biosynthesis via decarboxylation of the universal methyl donor SAM, ultimately controlling the interconversion of the polyamine putrescine to spermidine (FIG. 1). One investigation identified AMD1 as a putative tumour suppressor in a murine model of lymphoma, and subsequently discovered that heterozygous deletions of *AMDI* are prevalent in human lymphomas[79]. It was more recently shown that the expression of AMD1 is regulated by mTOR complex I (mTORC1), a growth factor and nutrient-sensing protein complex (BOX 1) that has been implicated in numerous tumour-associated processes; in line with this, AMD1 was found to be frequently upregulated in mTORC1-driven prostate cancers, and that tumours excised from patients treated with an mTORC1 inhibitor exhibit decreased AMD1 expression and reduced proliferation[80].

Methionine and epigenetic mechanisms

One role of methionine in the regulation of cancer-associated phenotypes is likely through epigenetic mechanisms. Although the conceptual definition of epigenetics is controversial, it is generally considered that the intersection of fixed genotypes (that is, DNA sequences) with external factors (such as chromatin status) mediates gene expression, ultimately driving the emergence of diverse and plastic phenotypes[81]. These dynamic relationships are typically characterized by modifications on nucleic acids and histones (FIG. 2). The bulk levels, positional locations (especially relative to promoter, enhancer and repressor binding) and geometrical properties of methylation modifications have been shown to correlate with and contribute to a number of developmental processes[82,83] and have also been implicated in a myriad of cancer phenotypes[84,85]. Given that the methyl donor SAM is generated from methionine, a major area of investigation in the epigenetics field is how methionine metabolism and the related environmental nutrient availability influence these processes[86].

DNA and RNA methylation. Methylation of cytosine residues in cytosine–phosphate–guanine (CpG) islands by DNA methyltransferases (DNMTs) is widely observed across various stages of development (FIG. 2) and has historically been associated with the repression of gene expression[87], although recent genome-wide methylation studies suggest that DNA hypermethylation does not necessarily correspond to gene repression[88,89]. These methylation events are highly dependent on methionine metabolism[90–92], with alterations in dietary methionine found to have both temporal[93,94] and tissue-specific effects on DNA methylation[83]. These contextual factors have made it difficult to determine how methionine metabolism globally impacts DNA methylation patterns, particularly those that may be associated with cancer. It has also been shown that adaptations in one-carbon metabolism resulted in a phenotype characterized by global DNA hypomethylation with concomitant increases in repressive histone methylation

Collateral vulnerabilities
Co-deletion of a gene proximal to a tumour suppressor gene, resulting in a targetable vulnerability independent of the tumour suppressor deletion.

Ornithine decarboxylase
(ODC). Enzyme that catalyses the conversion of ornithine to putrescine, the initial and committing step of polyamine biosynthesis.

Adenosylmethionine decarboxylase 1
(AMD1). Enzyme responsible for the decarboxylation of SAM for polyamine biosynthesis.

Histones
DNA-interacting proteins responsible for organizing DNA into structural units called nucleosomes.

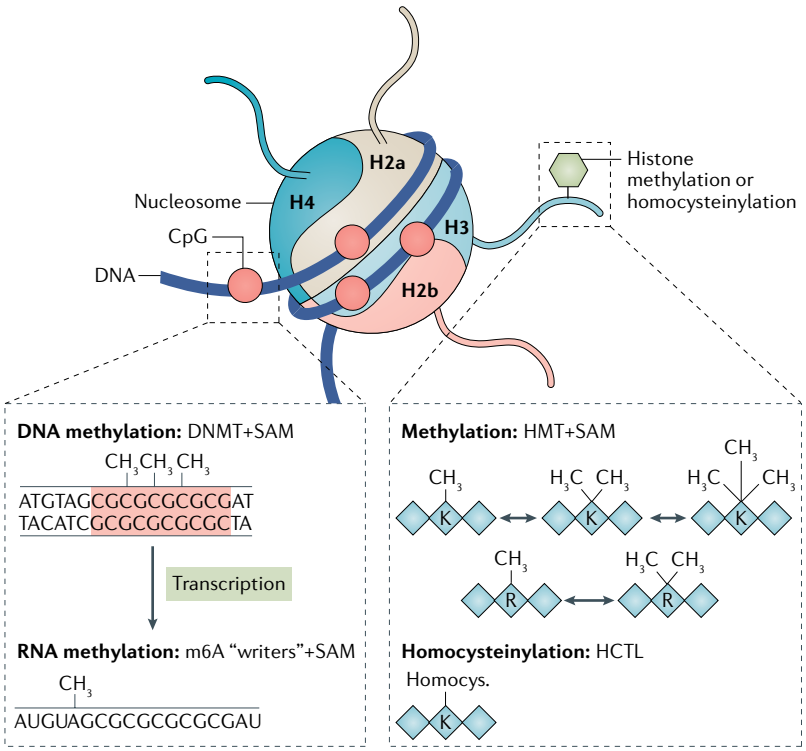


Fig. 2 | **Epigenetic modifications that are methionine-dependent.** By yielding the universal methyl donor S-adenosyl-methionine (SAM), methionine is critical for the regulation of chromatin dynamics. Chromatin is characterized by structural units called nucleosomes, which are composed of an octameric complex of histone proteins as well as the DNA associated with this complex. Both histones and DNA can be methylated to varying degrees by diverse methyltransferases, and the resulting arrangement and composition of these methylation marks are widely believed to contribute to gene expression patterns. Furthermore, RNA molecules can also be methylated at particular residues (most notably at the N6 position of adenosine, referred to as m6A), which can affect the relative propensity of an encoded protein to be translated. Additionally, homocysteinylation of specific histone residues has recently been discovered, which may also contribute to the epigenetic regulation of gene expression. CpG, cytosine–phosphate–guanine; DNMT, DNA methyltransferase; HCTL, homocysteine thiolactone; HMT, histone methyltransferase; Homocys., homocysteine.

marks (unpublished data, REF.[95]). Although these effects appear somewhat contradictory, they also underscore the lack of current molecular understanding of the role of histone and DNA modifications in mediating gene expression and chromatin biology. Nevertheless, given the widespread, recurrent observations of mutations in histone- and DNA-modifying enzymes, these modifications have important (although poorly understood) functions in oncogenesis.

Further confounding the complexity of methionine-associated epigenetic programs, expression of the SAM-producing enzyme MAT2A has been shown to be regulated by the post-transcriptional methylation of its RNA, thereby promoting its subsequent translation[96,97]; these findings illuminate an additional layer of feedback regulation in methionine metabolism. Furthermore, methionine availability has been shown to directly impact overall cellular translational capacity via regulation of tRNA modifications[98], while restriction of other nutrients within the one-carbon network was also found to globally reduce both DNA and RNA methylation due to an imbalance of substrates within the methionine cycle[99].

N-homocysteinylation
Addition of a thiol-containing homocysteine molecule to proteins via acylation of a lysine residue.

Although the relationship of these increasingly complex interrelated processes to cancer incidence and progression is poorly understood, these studies collectively provide additional support for the concept that methionine metabolism can play a major role in the regulation of cancer-associated epigenomic programs.

Histone modifications. Histone methylation is considered to be another factor that mediates chromatin state and subsequent gene expression. Lysine residues of histones can be mono-, di- or tri-methylated, while arginine residues can be mono- or dimethylated, by a family of over 30 enzymes referred to as histone methyltransferases[100] (FIG. 2). An overwhelming body of evidence suggests that the coordinated deposition and removal of these methyl groups contribute to regulating gene expression[101], in many cases independent of DNA methylation[102,103]. Intriguingly, these methylation events are also increasingly becoming characterized and implicated in tumorigenic settings[104,105]. As mentioned, while alterations in methionine availability and metabolism have been shown to exert substantial effects on histone methylation[42,67,106], resulting in striking biological phenotypes (such as altered immune reactivity[107] and embryonic development[82,108]), the impact of dietary methionine availability on histone methylation dynamics in the context of tumour initiation and progression is poorly understood but currently an intriguing area of investigation. Additionally, the lack of availability of other nutrients, such as glutamine, within the microenvironment has also been shown to influence intratumoural histone methylation levels[104], further supporting the notion that alterations in dietary nutrient composition can have epigenetic consequences in the context of cancer.

N-homocysteinylation of protein lysine residues has been previously observed[109] and was recently identified as an additional histone modification. The accumulation of homocysteine, an intermediate in the methionine cycle (FIG. 1), is associated with a multitude of pathologies[110,111] and has been shown to be effectively induced by high dietary methionine concentrations[112]. A recent study showed that homocysteinylation of the K79 lysine residue on histone 3 (H3K79) plays a critical role in neural tube development, and abnormally high levels of this mark were associated with substantial neurodevelopmental defects[113]. While the function of this post-translational modification beyond developmental biology remains to be determined, it may eventually be implicated in tumorigenesis, because of pervasive overlap in the processes involved in cancer and development.

Dietary methionine in cancer

Pathological phenotypes associated with dietary methionine availability. A number of studies have demonstrated a connection between dietary MR, which reduces but does not completely eliminate methionine, and improvement of health as well as reversal of pathology, by means including lifespan extension, attenuation of high fat diet-induced obesity and prevention of diabetes (FIG. 3). Following a key finding that MR can extend lifespan in yeast[14], numerous studies have further

Age-related disorders
Physiological states or diseases (including metabolic, neurological or other types) whose incidence is more prevalent in ageing populations.

Dietary methionine depletion
A diet characterized by total removal of methionine.

Walker-256 carcinosarcoma
A rat-derived transplantable carcinosarcoma cell line; tends to exhibit carcinoma characteristics when transplanted in younger rats, and sarcoma characteristics in older rats.

Yoshida sarcoma
A transplantable allograft sarcoma tumour model derived from ascites; one of the first cancer cell lines successfully generated.

Metabolomics
Systematic identification and quantification of metabolic products (metabolites).

demonstrated the evolutionary conservation of lifespan extension across *Drosophila*[15], *C. elegans*[16], mouse[18,21] and rat[19]. These observations provided one of the first definitive links between specific dietary amino acid composition and longitudinal health maintenance, and they further supported the scarcely tested theory that dietary MR could potentially provide a therapeutic benefit in diseases such as cancer.

Lending further support to this hypothesis are the health benefits that have been observed in numerous studies of MR in mice, most notably in reduced adiposity[23], improved cardiac function[24] and increased insulin sensitivity[114] (FIG. 3). It is currently unclear whether these health-promoting properties of MR are driven by cell-autonomous changes in methionine metabolism (FIG. 1) or are a result of systemic alterations in metabolic regulation. Nevertheless, these MR-mediated benefits in age-related disorders further illustrate the notion that methionine metabolism is linked to cancer biology.

The antineoplastic effect of the complete removal of methionine from the diet (that is, dietary methionine depletion) was first reported in Sprague-Dawley rats carrying the Walker-256 carcinosarcoma, where animals were fed diets lacking individual amino acids and were subsequently shown to exhibit significantly reduced tumour growth under a methionine-deprived diet[115]. Following this observation, a number of additional animal studies have reported similar findings in various settings. For instance, MR was shown to effectively induce a cell cycle blockade and overall tumour regression in Yoshida sarcoma-bearing nude mice[116], as well as in a xenograft model of glioma[117]. Other reports further demonstrated that depleting dietary methionine could induce sensitivity to cytotoxic agents such as cisplatin[118] and doxorubicin[118,119] in drug-resistant xenograft tumours in mice. A more recent study additionally observed enhanced efficacy of lexatumumab when combined with dietary MR in an orthotopic triple-negative breast cancer (TNBC) mouse model[120], an observation followed by another study that showed that a reduction in dietary methionine alone was effective in suppressing lung metastasis in a TNBC xenograft mouse model[121]. Although the consistency of the antineoplastic effects found with MR is promising, many of these studies have yet to draw definitive conclusions about what aspects of molecular metabolism are driving the observed effects and whether these outcomes may extend to more advanced preclinical models.

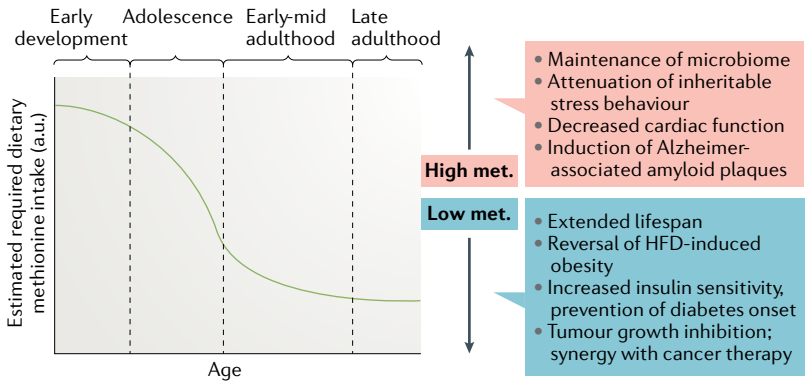


Fig. 3 | **Dietary methionine intake has age-dependent effects on health.** Dietary methionine restriction has been shown to exert a myriad of beneficial health effects in preclinical studies, including lifespan extension[18,21,175], prevention of obesity[23,36] and diabetes[18,35,114], and tumour growth inhibition[121]. Furthermore, high levels of dietary methionine have been linked to negative health outcomes, such as reduced cardiac function[161] and potentiation of Alzheimer disease phenotypes[162]. However, total restriction of methionine can be relatively toxic, and dietary methionine has been shown to maintain healthy microbiome populations[92], as well as to contribute to the attenuation of inheritable stress behaviours[163]. Therefore, it will be important to consider the relative trade-offs of dietary methionine restriction for desired health outcomes. Additionally, methionine is necessary for normal developmental processes, and restriction during embryonic, postnatal or adolescent development can thus have deleterious consequences; this further illustrates the potential contextual factors that must be taken into consideration for determining the relative health benefits of methionine restriction. The graph shown on the left-hand side is a model of the relationship between required dietary intake of methionine and age, taking into account the increased requirement of methionine for early development compared with the maintenance of physiological processes in adults. HFD, high-fat diet; met., methionine.

Possible mechanisms of antitumour effects of methionine restriction. Our group has shown that dietary MR at specific doses alters methionine metabolism in circulation and in liver after 12 weeks in healthy mice[67], a long-term interventional time frame that had been previously reported to improve metabolic health in patients with metabolic syndrome, as evidenced by decreased adiposity and elevated insulin sensitivity[122]. However, it was unclear whether these therapeutic benefits could be achieved in a more acute setting in preclinical models, which would need to be demonstrated to support the clinical applicability of MR. In a recent study from our group, a comparative metabolomics approach to profile the metabolic dynamics brought about by MR in detail in C57BL/6J mice revealed that within 24 hours, MR reduced methionine and its derivatives methionine sulfoxide and 2-keto-4-methylthiobutyrate by over 50%. Importantly, MR led to a reduction in levels of circulating methionine without consistently altering the levels of other circulating amino acids or markers of oxidative stress, providing evidence that MR enables a rapid and specific metabolic perturbation of methionine and sulfur metabolism on a systemic level in healthy mice.

In this same study, dietary MR alone resulted in tumour regression in two patient-derived xenograft (PDX) mouse models of colorectal cancer (CRC) driven by RAS mutations (*KRAS*^{G12A} or *NRAS*^{Q61K}), which was not attributable to caloric restriction[123]. Metabolomics analyses have revealed alterations in cysteine and methionine metabolism with MR in both tumour and liver tissue as well as plasma, although an integrated analysis demonstrated a significantly greater degree of methionine-related metabolic alterations within tumours than in other tissues. MR also exerted synergistic effects on tumour growth when combined with current frontline cancer therapies, including chemotherapy (5-fluorouracil, 5-FU) and radiation, that interact with nucleotide and redox metabolism, and thus one-carbon metabolism. Importantly, given that colorectal cancers frequently exhibit resistance to 5-FU, MR significantly increased the efficacy of 5-FU treatment when 5-FU was given at a chemoresistant dose. This combination therapy induced the strongest global metabolic effects

Sulfur metabolism
Biological processes involving methionine and cysteine.

Patient-derived xenograft
(PDX). Preclinical cancer model whereby patient-excised tumour cells are directly implanted into immunodeficient mice.

5-fluorouracil
(5-FU). A pyrimidine analogue that inhibits nucleotide synthesis, functioning as an antimetabolite chemotherapy.

Gene–environment interactions
Relationships through which genetic status influences how a given cell/organism responds to environmental variation.

in tumours compared with either therapy alone, suggesting that the observed reduction in tumour growth was likely due in large part to disrupted nucleotide and redox metabolism, which was confirmed to be causally implicated in primary cell lines derived from these tumours. Furthermore, MR also showed synergistic effects when combined with radiation[123], which as a monotherapy had previously been shown to exert only a modest effect[124]. In an autochthonous mouse model of soft-tissue sarcoma, tumour progression was reduced by combining MR with a single dose of 20 Gy focal radiation that otherwise produces a minimal effect in this model. As seen in the combined treatment with the chemotherapy 5-FU, MR together with radiation also resulted in a cumulative disruption to nucleotide metabolism and cellular redox balance[123]. Altogether, this study provides a novel characterization of the metabolic consequences of dietary MR in both healthy and malignant tissue across multiple cancer models, as well as a preliminary but nonetheless promising mechanistic understanding of how MR may exert its antitumour effects. However, these results do not rule out other methionine-related mechanisms that also contribute to this phenotype.

Considering the multifaceted functions of methionine (BOX 1), the antitumour effects of MR are likely to be manifold. Following the early work of Robert Hoffman, which demonstrated the methionine dependence of transformed rat and human cells in cell culture, it was originally hypothesized that the pervasive dependence on exogenous methionine in cancer was due to a defect in methionine synthesis (termed "methionine auxotrophy")[125]. Subsequent studies using these rat- and human-derived cells demonstrated that malignant and transformed cells were still able to endogenously synthesize methionine at rates similar to those found in normal cell counterparts[126]. Another hypothesis for this dependence on exogenous methionine is an increased reliance on transmethylation reactions[41,127], although neither SAM nor SAH was significantly altered by MR in several in vivo settings[123]. However, the epigenetic consequences of dietary MR within tumours remain to be fully elucidated and may indeed contribute to cancer in some contexts. It is also possible that altered protein synthesis could play a role in the observed antineoplastic phenotype, but studies directly examining this possibility are lacking. As the MR-mediated extension of lifespan appears to be autophagy-dependent in both yeast and progeroid mouse models[14,21], these metabolic differences may be due to differential activation of autophagic processes. Additionally, it is possible that nutrient-sensing signalling pathways might also play a part in shaping the metabolic responsiveness to MR[114,128–130]. This is supported by the finding that hydrogen sulfide (H_2S) production mediates the stress resistance phenotype imparted by MR in hepatic tissue, which was abrogated by mTORC1 hyperactivation[131].

The mechanisms driving the antitumour effects of MR could also be dependent on the contextual factors (that is, gene–environment interactions) that shape individual tumours. For example, the metabolic dependencies of *KRAS*-driven tumours have previously been shown to be dependent on the originating tissue[65]. Therefore, as an extension of this concept, it is possible that MR may primarily exert antineoplastic effects via differential mechanisms that can be dependent on both intrinsic and extrinsic factors specific to individual tumours. For instance, *PI3K* mutations have been shown to enhance the methionine dependence phenotype via differential activity of the cysteine–glutamate antiporter[132], suggesting that this subset of tumours may exhibit sensitivity to MR due to the subsequent alterations in the availability of other amino acids. Interestingly, we observed that MR achieved a stronger inhibitory effect on tumour growth when given two weeks prior to tumour engraftment in a CRC PDX model than when the dietary intervention was initiated only after tumour formation[123]. Given the temporal nature of the effect of the diet in this model, there are intriguing possibilities that MR exerts its effects at discrete developmental stages of tumorigenesis, when presumably different genetic statuses are present. These considerations illuminate the degree to which our mechanistic understanding of methionine dependence in cancer is currently lacking, but the highly robust antitumour efficacy of MR across multiple subtypes creates a compelling need for continued characterization of its biological effects in diverse physiological settings.

Targeting methionine metabolism

Efforts have been made to develop various methionine analogues (that is, "antimetabolites") in hopes of selectively targeting cancer cells, as had been done with other efficacious therapies using antimetabolites such as antifolates[133]. While one of these analogues, ethionine, appeared to demonstrate preclinical efficacy[134], it was ultimately found to be toxic, and clinical investigations of its use have subsequently been abandoned. A similar approach involved the administration of methioninase (and its more stable recombinant form rMETase), which degrades methionine to α-ketobutyrate, methanethiol and ammonia rather than SAM[135]. Although a phase I clinical trial demonstrated that its administration was tolerable and effectively lowered serum methionine levels[136,137], over 20 years have passed since this initial phase I trial, and it has yet to advance to subsequent clinical development. It is worth noting, however, that one group has recently published a number of studies demonstrating the efficacy of rMETase in patient-derived xenograft mouse models of melanoma[138] and sarcoma[139], as well as in an orthotopic model of osteosarcoma[140]. Additionally, a very recent independent pilot phase I clinical trial of rMETase was conducted with no toxicities reported, suggesting a possible resurgence of interest in this therapeutic approach[141]. Nonetheless, a number of novel strategies to therapeutically target methionine metabolism are also currently under active investigation.

Therapies targeting the methionine salvage pathway. The inability of *MTAP*-deleted cells to synthesize adenine (a purine derivative) from MTA initially provided an attractive therapeutic approach of targeting purine synthesis in this subset of tumours. Studies of this approach focused on identifying ways to take advantage of this vulnerability, primarily via the administration of

adenine analogues that inhibit the formation of critical intermediates for nucleotide synthesis[142,143]. However, as was noted previously, a phase II trial investigating the adenine analogue L-alanosine (also referred to as SDX-102) failed to show efficacy in advanced-stage tumours exhibiting *MTAP* deletion[144]. Although efforts using this approach have mostly been abandoned, preclinical investigations of MTAP-associated vulnerabilities within purine metabolism remain ongoing.

The recent discoveries of additional metabolic vulnerabilities induced by *MTAP* deletion create a multitude of potential avenues for clinical investigation. Upregulation of PRMT5 expression and/or activity has been extensively implicated in numerous cancer types[61–63], which has resulted in three ongoing phase I clinical trials, with one phase II clinical trial already approved (TABLE 1). Although these investigations are not specific to tumours with *MTAP* deletion, it is likely that the applicability of those compounds to *MTAP*-deleted tumours will be an active area of investigation in the near future. Another therapeutic approach that has gained considerable attention recently is an inhibitor of MAT2A, which was shown to exhibit substantial efficacy in a preclinical patient-derived xenograft mouse model of *MTAP*-deleted non-small-cell lung cancer[145]. A phase I clinical trial investigating the efficacy of this compound (AG-270) in *MTAP*-deleted advanced solid tumours was initiated as a result of these findings and is currently ongoing (TABLE 1). However, given the enhanced methionine dependency of tumours independent of *MTAP* status, it is likely that other tumours may also exhibit sensitivity to MAT2A inhibition; it will be interesting to see the future clinical applications this therapeutic approach may have.

Therapies targeting polyamine metabolism. Given the role of polyamines in cancer (see the "Polyamine metabolism" subsection above), targeting the metabolic processes associated with their regulation is another potentially promising therapeutic strategy. Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase (ODC), was developed shortly after the requirement of polyamines for cellular growth was discovered[146]. This compound was considered to be a particularly attractive therapeutic agent, due to its apparent selective cytotoxicity against malignant cells[147]; however, it failed to demonstrate efficacy as a single agent in early clinical trials[148,149], potentially due to upregulation of polyamine uptake from the microenvironment[150], although a phase II clinical trial recently demonstrated its efficacy as a chemopreventative agent in reducing the incidence of relapse in neuroblastoma[151].

A resurgence of interest in DFMO as an adjuvant chemotherapeutic agent has recently occurred, particularly in neuroblastomas that are characterized by MYC overexpression[152], as ODC expression has been found to be directly regulated by *Myc* activation in murine fibroblasts[153], with two ongoing phase I clinical trials examining DFMO co-administration with either cyclophosphamide or the polyamine transporter inhibitor AMXT-1501 (TABLE 1). Interestingly, preclinical evidence suggests that the combination of DFMO with AMXT-1501 may exert antitumour effects in an immune-dependent manner by preventing T cell immune repression[154], providing further support for future investigations of this therapeutic approach. Finally, as we mentioned above (see the "Polyamine metabolism" subsection), *MTAP*-deleted cells have also been shown

Table 1 | **Cancer therapies targeting methionine metabolism**

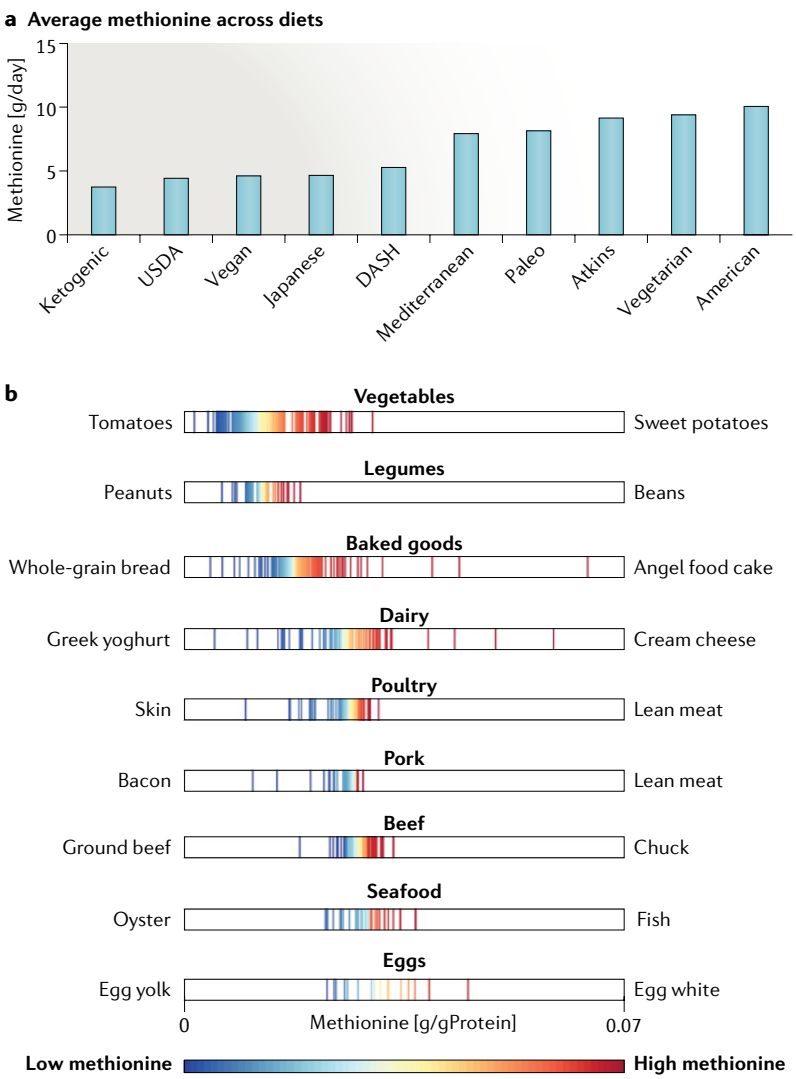
Molecular target	Compound	Current stage	Applications	Ref.
MAT2A	AG-270	Phase I	*MTAP*-deleted cancers (advanced solid tumours, lymphoma)	176
	PF-9366	Preclinical	Cancers exhibiting upregulated MAT2A expression	157
	AKBA	Preclinical	Preclinically validated in keratinocytes; potential for use in melanoma	156
PRMT5	GSK3326595	Phase I/II	Advanced solid tumours, AML	177
	PF06939999	Phase I	NSCLC, head + neck, oesophageal, endometrial, cervical, urothelial cancers	178
	JNJ64619178	Phase I	Advanced solid tumours, lymphoma	179
ODC	DFMO + CP + topotecan	Phase I	Relapsed neuroblastoma	180
Polyamine transporter	AMXT-1501 + DFMO	Phase I	Advanced solid tumours	181
MetAP2	M8891	Phase I	Advanced solid tumours	182
Nucleotide synthesis (antifolate)	Pemetrexed + avelumab	Phase II	*MTAP*-deleted metastatic urothelial cancers	183
Circulating methionine	Methioninase, recombinant methioninase	Phase I	High-stage cancers	141

Numerous clinical trials investigating compounds that directly or indirectly target methionine metabolism are currently ongoing; additionally, some compounds that have been characterized in preclinical studies could be clinically investigated in the near future. AKBA, acetyl-11-keto-β-boswellic acid; AML, acute myeloid leukaemia; CP, cyclophosphamide; DFMO, difluoromethylornithine; MAT2A, methionine adenosyltransferase 2A; MetAP2, methionyl aminopeptidase 2; MTAP, methylthioadenosine phosphorylase; NSCLC, non-small-cell lung cancer; ODC, ornithine decarboxylase; PRMT5, protein arginine N-methyltransferase 5.

Box 2 | **Variation of methionine levels in human foods and diets**

Methionine content is highly variable between (as well as within) different food groups (see the figure). According to the US Department of Agriculture (USDA) Food Composition Database, the most methionine-rich foods are eggs (containing 0.032 g of methionine per gram of protein) and seafood (containing 0.029 g of methionine per gram of protein), while vegetables and legumes (both containing 0.013 g of methionine per gram of protein) have the lowest amounts. A vegan diet is thereby more efficient in restricting dietary methionine intake than a vegetarian diet, which does not strictly limit the consumption of methionine-rich animal products. Methionine abundance also varies substantially among foods within the same category; for instance, the average abundance of methionine in egg yolk is 0.025 g of methionine per gram of protein, compared to 0.037 g in egg white.

The variation in methionine concentration in foods results in considerable flexibility of methionine intake in human diets, thus allowing people to achieve dietary methionine restriction by choosing foods lower in methionine without changing their adherence to a certain diet. As is shown in the figure, among ten different diets, all but the American diet (with a lower limit of methionine intake of 0.37 g per day) allow daily methionine intake as low as 0.12 g (for the USDA-recommended diet) or lower (for other diets), which is equivalent to 2 mg or less of methionine per kilogram of body weight per day for a 60-kg human subject. Hence, dietary methionine restriction is feasible under almost all popular human dietary schemes. Nevertheless, there are still significant differences between diets in their allowed range of daily methionine intake. Diets that tend to be more methionine-restricted include the mentioned vegan diet and those that rely on fat (ketogenic diet) or carbohydrates (Japanese, DASH and USDA-recommended diets) instead of protein as the major source of energy. Given that the amounts of methionine in human foods and diets are highly variable, the development of novel computational approaches will be important for achieving precise control of dietary methionine intake.



to exhibit enhanced ODC activity[49]. Although preclinical studies have failed to demonstrate the efficacy of DFMO in *MTAP*-deleted cells[155], ongoing efforts to identify contextual factors that could potentially enhance tumour sensitivity in *MTAP*-deleted cells could provide novel therapeutic strategies for the clinical use of DFMO as an antineoplastic agent.

Therapies targeting the methionine cycle. Substantial efforts have been made to identify other novel approaches to chemically disrupt methionine metabolism and the processes that are reliant on its activity. Two additional compounds targeting MAT2A have recently been described in preclinical studies. One of these compounds, acetyl-11-keto-β-boswellic acid (AKBA), is a natural MAT2A inhibitor that demonstrated activity in keratinocytes[156], while the other is a small-molecule allosteric modulator (PF-9366) that inhibits MAT2A when methionine or SAM levels are high, and activates MAT2A when levels of these metabolites are low[157]. However, chronic PF-9366 treatment can result in the compensatory upregulation of MAT2A expression[157]. This feedback mechanism, although poorly characterized and only reported for PF-9366, could potentially reduce the therapeutic potential of these compounds.

Another novel approach under investigation is inhibition of the pro-angiogenic protein methionine aminopeptidase 2 (MetAP2), a metalloenzyme responsible for the removal of N-methionine residue from nascent proteins, thereby effectively impairing protein synthesis[158]. A MetAP2 inhibitor, M8891, is currently in phase I clinical trials for advanced solid tumours (TABLE 1), although an earlier MetAP2 inhibitor (ZGN-440, or beloranib) — which was previously investigated for its ability to promote metabolic health in patients with obesity, as evidenced by weight loss and increased insulin sensitivity — was pulled from clinical trials due to vascular toxicity[159]. These approaches further illustrate the high therapeutic potential as well as the appreciable challenges in pharmacologically targeting methionine-related processes. Finally, given the prominent role of methionine in mediating epigenetic status (see the "Methionine and epigenetic mechanisms" section), it will be interesting to observe whether targeting methionine metabolism (either via pharmacological or dietary intervention) could induce or enhance sensitivity to pharmacological agents targeting epigenetic modifiers that are currently a major area of clinical interest[160].

Methionine restriction in humans

Given the significant improvement of pathological phenotypes and the cancer-specific auxotrophic methionine dependency discussed above (see the "Dietary methionine in cancer" section and FIG. 3), the potential of using MR as a cancer therapeutic has gained considerable interest. However, the relative feasibility of achieving beneficial effects in humans through MR without inducing systemic toxicities is a topic of debate. Although high dietary methionine intake has been associated with a number of adverse health outcomes in preclinical models, such as reduced cardiac function in mice[161] and elevated induction of amyloid plaques in a mouse model of

Methionine aminopeptidase 2
(MetAP2). Metallopeptidase responsible for removing N-terminal methionine residues from newly translated proteins.

Cystemustine
A chloroethylnitrosourea chemotherapy agent approved for the treatment of high-grade melanomas and gliomas.

Precision diets
Systematic development of personalized diets; can be individual-specific or more broadly orientated towards a particular nutrient or disease.

Alzheimer disease[162], dietary methionine has also been shown to be necessary for the regulation of healthy gut microbiome populations (although its supplementation was shown to increase the ratio of pathogenic to healthy bacterial populations in the gut) in mice[92] and potentially to promote psychological health, as evidenced by reversal of heightened stress responses in a rat model of inherited anxiety behaviour[163]. Additionally, recent work has demonstrated that upregulation of methionine uptake and metabolism is essential for the methylation-dependent processes required for T cell differentiation during antigen receptor stimulation in mouse T cells[164]. Therefore, identifying a clinically relevant level of dietary methionine intake that can maximize health-promoting benefits while preventing the potential toxicities that may be associated with its restriction will be crucial moving forward.

Clinically, MR (2 mg methionine/kg body weight per day) for 16 weeks in patients with metabolic syndrome has previously been shown to significantly decrease hepatic lipid content and increase fat oxidation[122]. We recently demonstrated in healthy, middle-aged individuals (both male and female) that a 3-week regimen of low dietary methionine (equivalent to ~2.92 mg/kg of daily methionine intake) is sufficient to dramatically reduce circulating methionine levels[123]. Despite the variability in global metabolite profiles arising from different dietary regimens, MR-induced alterations in circulating methionine-related metabolites are highly correlated between mouse models and humans.

Controlled clinical studies have extended the feasibility of MR treatment in humans, from observations in methionine-free diets that are toxic beyond a 24-hour regimen[165,166], to dietary methionine levels that are relatively well-tolerated over an 8- to 17-week period but that induce notable body weight loss in non-obese patients with metastatic cancer[167], and finally to levels that are not associated with significant side effects in healthy subjects over a 3-week treatment regimen[123]. It is worth noting that one phase II clinical study was able to demonstrate a well-tolerated regimen of cystemustine administration in combination with one day of a methionine-free diet (repeated every two weeks) in patients with metastatic melanoma or glioma, although haematological toxicities were reported and the combinatorial treatment did not show significant efficacy[168]. Future studies assessing how short-term MR could result in specific metabolic changes (in both patients with cancer and healthy subjects) are undoubtedly warranted. A brief elaboration on methionine composition in various foods and predefined diets is provided in this Review (BOX 2), as methionine-specific precision diets will likely increase in popularity within the near future.

Conclusion

The increased dependence on methionine and dysregulation of methionine metabolism in cancer implies that either pharmacological or environmental disruption of the methionine metabolic network could demonstrate substantial therapeutic efficacy. As we have discussed, the use of MR as an antineoplastic intervention is an attractive prospect, given the emerging preclinical evidence that it can effectively inhibit tumour growth as a single or an adjuvant agent[118–121,123], especially upon consideration of its minimal toxicity profile, as indicated by the myriad of health-promoting benefits observed with its use[22–36]. The concept of using dietary amino acid restriction, particularly in the context of one-carbon metabolism, is further supported by the finding that dietary restriction of both serine and glycine (which are both critical inputs to the folate cycle, thereby providing substantial regulation of the methionine cycle; FIG. 1) has also been shown to significantly attenuate tumour growth in a myriad of preclinical xenograft models[99,169]. However, the molecular mechanisms driving these phenotypes are just beginning to be defined, and the role of methionine and one-carbon metabolism in tumorigenesis and cancer progression is still poorly understood. Furthermore, it remains unclear whether dietary MR can effectively reduce the risk of cancer occurrence and, if so, at what biological time point it should be recommended, taking into account the effects it exerts in the course of normal development and health span. The levels of methionine intake that are required to benefit health will likely be age-dependent, with the extent of methionine intake that is required to maintain physiological processes substantially decreasing past early adulthood (left panel, FIG. 3). Nevertheless, our current understanding of how dietary methionine availability drives physiological processes is largely based on preclinical findings (right panel, FIG. 3). Additional studies aimed at characterizing the relative trade-offs in altering dietary methionine composition will be instrumental in future applications of its therapeutic use. The development of precision diets that effectively restrict methionine while encompassing a diverse array of attractive food preferences and nutritional sustenance will also significantly contribute to the potential applicability of MR. Finally, as our understanding of how the genetic status of different tumour subsets influences the impact of dietary composition on methionine metabolism continues to expand, new biomarkers (such as elevated MTA levels in plasma for *MTAP*-deleted tumours) will likely contribute to the development of novel treatment strategies.

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S.M.S. and J.W.L. outlined, wrote, revised and edited the content of the manuscript. Z.D. contributed the content related to Box 2. X.G. drafted the sections on dietary methionine.

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