# Diet-Dependent Metabolic Regulation of DNA Double-Strand Break Repair in Cancer: More Choices on the Menu

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## ABSTRACT

Despite several epidemiologic and preclinical studies supporting the role of diet in cancer progression, the complexity of the diet–cancer link makes it challenging to deconvolute the underlying mechanisms, which remain scantly elucidated. This review focuses on genomic instability as one of the cancer hallmarks affected by diet-dependent metabolic alterations. We discuss how altered dietary intake of metabolites of the one-carbon metabolism, including methionine, folate, and vitamins B and C, can impact the methylation processes and thereby tumorigenesis. We present the concept that the protumorigenic effect of certain diets, such as the Western diet, is in part due to a diet-induced erosion of the DNA repair capacity caused by altered epigenetic and epitranscriptomic landscapes, while the protective effect of other dietary patterns, such as the Mediterranean diet, can be partly explained by their ability to sustain a proficient DNA repair. In particular, considering that diet-dependent alterations of the one-carbon metabolism can impact the rate of methylation processes, changes in dietary patterns can affect the activity of writers and erasers of histone and RNA methyl marks and consequently impair their role in ensuring a proficient DNA damage repair.

## Introduction

The potential importance of diet and nutrition in cancer development is widely recognized, owing to an impressive amount of evidence from epidemiologic, clinical, and laboratory research. Nutritional factors can influence cancer initiation and progression by affecting fundamental cellular processes, including those that regulate cell proliferation, cell differentiation, the expression of oncogenes and tumor-suppressor genes, cell signaling, redox homeostasis, inflammation, and other factors in the cellular microenvironment, such as immune cell infiltration and extracellular matrix (1).

Epidemiologic and preclinical studies indicate that some key aspects of diets, such as the Mediterranean diet (MD), including increased consumption of fruits, vegetables, and omega-3 fatty acids, are associated with reduced cancer risk (2, 3), while many features of other dietary patterns, such as the Western diet (WD), including high intake of saturated fats, hyperglycemic carbohydrates, and animal-derived proteins, are associated with increased risk for many common cancers (4–6). However, our mechanistic understanding of the link between diet and cancer development is still limited. To help fill this gap and deconvolute the connection between nutritional behaviors and tumorigenesis, we will analyze the implications in tumorigenesis of key components of dietary patterns, referring to the diet pyramids of MD and WD as two template diets (**Fig. 1**). Although diet can influence multiple events implicated in the process of neoplastic transformation (1), this review will focus especially on the effect of dietary patterns on genomic stability. It will provide a uniquely mechanistic perspective on the effect of diet-dependent metabolic alterations on DNA repair systems, because their role in guarding genome fidelity is of paramount importance in tumorigenesis.

Genomic instability is an integral feature of cancer, as the acquisition of cancer hallmarks largely depends on a succession of alterations in the genome of preneoplastic cells (7). The rate of genomic alterations is usually low in normal cells, thanks to the ability of the genome maintenance machinery to detect and resolve DNA lesions. However, in the course of acquiring the series of mutations necessary to promote and sustain tumorigenesis, the rate of genetic alterations is often exacerbated in cancer cells (8). This is achieved by two concomitant processes: breach in the surveillance systems that normally guard genomic integrity (i.e., loss of p53), and loss of efficiency in the DNA damage repair machinery, resulting in incomplete or defective repair of DNA lesions. Although gambling on their genome integrity could at first glance appear as a risky strategy for neoplastic cells, as DNA lesions can be genotoxic and lead to senescence or apoptosis, it is in fact a winning approach on the long term, because clonal evolution of certain mutant genotypes can confer selective advantage and enable cancer cells to

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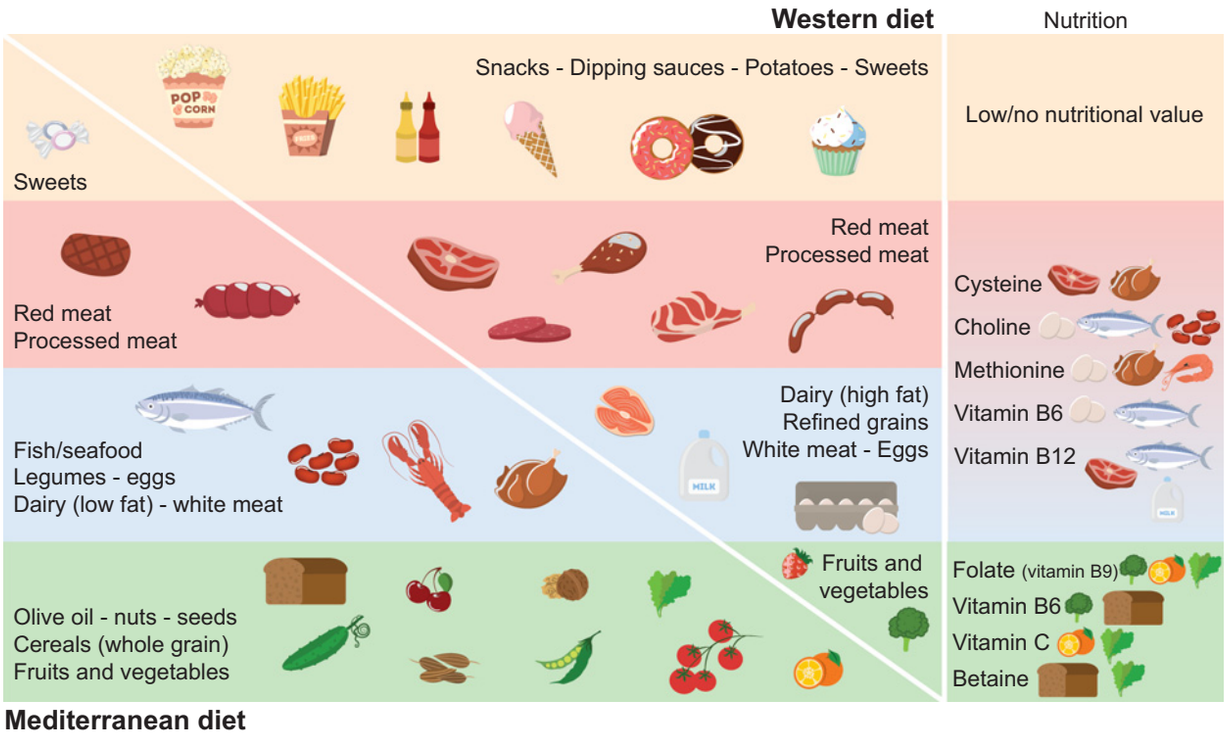


**Figure 1.**

Depiction of nutritional pyramids associated with the MD and WD. The Mediterranean dietary pattern is rich in fruits, vegetables, and wholegrain cereals. In contrast, the WD is dominated by highly processed foods, red meat, refined grains, high-fat dairy, and sweets, with a low intake of fruits and vegetables. On the right side of the figure are reported the key essential nutrients involved in the one-carbon metabolism, including their main food sources.

acquire new functional capabilities, allowing them to survive and proliferate (7, 9).

Partial inactivation of the genome maintenance machinery can be achieved through mutation of DNA repair genes (the so-called mutator hypothesis; ref. 10): for example, germline mutations in breast cancer susceptibility (BRCA1/2), ataxia telangiectasia mutated (ATM) or RAD51, all of which are genes involved in double-strand break (DSB) repair, predispose to development of various cancers (11–13). However, while germline mutations in caretaker genes can explain genomic instability in hereditary cancers, the mutator hypothesis fails to explain how the genome maintenance machinery is "silenced" in sporadic (nonhereditary) cancers. To address this question, the oncogene-induced DNA replication stress model proposes that replication stress induced by oncogene activation leads to DSBs accumulation as a result of collapsed DNA replication forks (14). This hypothesis, however, requires that the DNA repair systems are somehow eroded in their ability to effectively repair DSBs to lead to increased mutagenicity. In this review, we propose the argument that *dietary patterns, such as the WD, that have been associated with increased risk of developing certain types of cancer do so at least partly by fueling genomic instability, whereas diets that have a protective effect on cancer onset and progression, such as the MD, provide the metabolic substrates necessary to support a robust genome maintenance machinery.*

This review focuses on the repair systems of DSBs, because they are among the most genotoxic forms of DNA damage. Given the vital role of DSBs repair in guarding the integrity of the genome, cells possess complex control mechanisms to ensure that the repair system that is engaged is suited not only to the nature of the genotoxic damage, but also and importantly to the cellular context in which the damage occurs. We will present literature evidence in support of our hypothesis that metabolic changes impact the decision process of selecting the most suitable DSB repair pathway. In particular, we will discuss how dietary patterns alter the primary metabolism of cancer cells and how this can in turn impact the genome maintenance machinery by affecting both the epigenetic and epitranscriptomic landscape of cancer cells.

## Diet and Genomic Stability

Diet-induced loss of genomic fidelity emerges from a combination of DNA damage accumulation and deficient DNA damage repair. The increase in genotoxic load as a result of metabolic alterations can be due to (i) a decrease in redox capacity/increased oxidative stress, or (ii) the activation of

mitotic pathways. In the first case, dietary patterns characterized by excessive or inappropriate nutrient intake can alter cellular redox homeostasis by deregulating mitochondrial metabolism (15), leading to accumulation of reactive oxygen species (ROS), which cause accumulation of single-strand breaks, stalled replication forks and as a result DSBs, thereby increasing the burden on the DNA repair machinery (16, 17). In the latter case, dietary patterns characterized by a high intake of hyperglycemic carbohydrates and insulinotropic dairy overstimulate insulin/IGF-1 pathways, which promote tumor cell proliferation and therefore can induce replication stress. However, replication stress *per se* is not sufficient to increase mutagenicity if not coupled with at least a partial loss of efficiency in the repair of DNA lesions. Similarly, oxidative stress has the potential to produce genomic instability only if the genotoxic load posed by ROS-induced accumulation of DNA lesions exceeds the repair capacity of cancer cells. Hence, a compromised DNA repair machinery is a *sine qua non* condition for increasing the rate of genetic alterations through loss of genomic fidelity. *We therefore propose that, on the one hand, those dietary patterns that have been associated with increased tumorigenicity, fuel genomic instability not only by increasing the DNA damage load of cancer cells, but also, crucially, by eroding their DNA repair efficiency and, in particular, by channeling cancer cells toward more error-prone repair pathways. On the other hand, the cellular metabolic landscape fostered by "healthier diets" promotes more robust DNA repair by favoring DNA repair pathways that are less likely to lead to genotoxic alterations.*

## Overview of DSB Repair Pathways

Misrepaired DSBs have a high tendency to result in insertions, deletions, translocations, and copy-number variations in the genome, while failure to repair DSB leads to cell cycle arrest or cell death. Hence, their effective repair is critical for preventing carcinogenesis. The two major systems to repair DSBs are homologous recombination (HR) and classical nonhomologous end joining (cNHEJ). HR utilizes the sister chromatid as a template for repair, is virtually error free and can occur only in S- and $G_2$-phases, while cNHEJ joins two DNA ends with minimal reference to the DNA sequence, hence is more error prone and can occur throughout the cell cycle (18).

In both pathways, a key event that initiates the cellular responses to DNA damage is the rapid phosphorylation of H2A.X on serine 139 (γH2A.X) by the kinase ATM (19). The MDC1 complex is then recruited to γH2A.X and promotes the nucleation of various E3 ubiquitin ligases, which mediate the polyubiquitination of histones H1 and H2A (20, 21). Histone H2A monoubiquitination at Lys15, as well as histone H4 monomethylation at Lys20 (H4K20me1) are two important posttranslational modifications in the cascade of repair events, as they are required for the recruitment of p53-binding protein 1 (53BP1; refs. 22 and 23). At this stage, the retention of 53BP1 [as part of a complex named shieldin (24, 25)] at the break site favors cNHEJ repair, while 53BP1 displacement by the binding of the BRCA1–BARD1 complex promotes HR repair: thus, 53BP1 displacement or retention represents a key node in the repair-pathway choice. cNHEJ occurs through binding of the Ku70-Ku80 heterodimer, which sequesters the broken DNA ends and recruits other factors, including the DNA-dependent protein kinase catalytic subunit, the scaffolding factor XRCC4, the DNA Ligase IV (LIG4) and the nuclease Artemis to process and ligate the DNA ends (26, 27).

As for HR, although it comes in different flavors, or subpathways, all HR pathways share the DNA resection step, which consists in the extensive processing, by different nucleases (notably, the MRE11 nuclease, part of the MRN complex), of the DNA ends, resulting in the formation of a long single-stranded DNA (ssDNA; refs. 28 and 29). After the long-range resection of the broken DNA into ssDNA, the replication protein A (RPA) complex avidly binds to the ssDNA to prevent its spurious binding to other ssDNAs. The RPA complex must then be displaced by recombination mediators to allow the coating of the DNA filament with RAD51 protein, which in turn allows the invasion of the ssDNA overhang into the double-stranded sister chromatid (18). The sister chromatid is then used as a template to repolymerize the resected strand for subsequent ligation, thereby preventing any loss of information.

Balancing between the fast cNHEJ and the high-fidelity HR responses is a context-dependent decision process that involves the quick evaluation of several factors, including the type of break (one-ended as opposed to two-ended break; long vs. short ssDNA), the phase of the cell cycle during which the damage occurs and the chromatin status (i.e., histone modifications) surrounding the break. In addition, damage-related RNA transcripts (known as DDRNA), formed either before or after the DSB, are emerging as novel players in the DNA damage repair game and can contribute to the repair-pathway selection. Because RNA is subjected to posttranscriptional modifications that affect its function, ranging from splicing to export and protein interaction ability, and given that these posttranscriptional modifications are modulated by the availability of diet-dependent metabolites and cofactors (30), just like for histones and DNA, DDRNA represents another important link between cellular metabolism and the genome maintenance machinery.

## Role of Chromatin Status in DSB Repair

Chromatin is subjected to numerous posttranslational modifications that regulate its structure and thereby DNA accessibility to DNA-binding factors, including DNA repair components. Upon DSB, both the initial chromatin context and the chromatin landscape induced by the damage play a central role in the repair process (31). The histone modifications involved in the so-called "repair histone code" include acetylation of histone H3, H4, and H2A, methylation of histone H4 at Lys20

(H4K20), and others. After a first phase of chromatin compaction upon DSB, an important requirement for DSB repair initiation is chromatin relaxation, which is achieved in part through the acetylation of histones H3, H4, and H2A around the DSB (32). Besides favoring chromatin openness, acetylated histones also participate in the selective recruitment of DSB repair proteins. For instance, TIP60-dependant acetylation of histone H4 diminishes 53BP1 binding to H4K20me1/2 and commensurately increases BRCA1 recruitment to DSBs (33). Moreover, nuclear ACLY, the enzyme responsible for converting citrate into acetyl-CoA, the substrate of histone acetyltransferases, is phosphorylated at Ser455 downstream of ATM and AKT upon DSB. Phosphorylated ACLY then facilitates histone acetylation around DSB sites, impairing 53BP1 localization and enabling BRCA1 recruitment, thereby favoring HR repair (34). In addition, Yasuda and colleagues found that RAD52 acetylation is required for RAD51 sustained colocalization at DSBs and is therefore essential for HR (35). Overall, acetylation of histones surrounding DSBs represents an example of how chromatin status can influence DSB repair-pathway choice.

Similarly, histone methyltransferases and demethylases can regulate the recruitment and stability of repair factors. A key histone modification in the repair pathway choice is H4K20 methylation, which oscillates during the cell cycle, affecting chromatin condensation and accessibility to DNA repair factors (36). In the $G_1$-phase, nucleosomes are methylated at H4K20, with more than 80% being dimethylated (H4K20me2; ref. 37). During S-phase, unmethylated histones H4 (H4K20me0) are mixed in a 1:1 ratio with old H4K20me2. H4K20me0 is therefore a mark of postreplicative state and indicates the presence of a sister chromatid until $G_2$–M-phase, when a new surge in methyltransferase activity will catalyze the monomethylation of H4K20 (H4K20me1) first, followed by H4K20me2 (37). This cell cycle–regulated oscillatory pattern of H4K20 methylation is exploited by the cells to modulate the recruitment of DDR factors. In particular, H4K20me1/2, present throughout the cell cycle in variable proportion, is recognized by the cNHEJ-promoting 53BP1 (38). Conversely, H4K20me0, restricted to S- and $G_2$–M-phases, is recognized by BRCA1, which, as part of the BRCA1–BARD1 complex, antagonizes 53BP1 loading and promotes HR repair (39). H4K20me0 is also recognized by the HR complex TONSL–MMS22L, which then remains on the replicated locus until late $G_2$–M (37). Altogether, these data highlight how H4K20 methylation allows the DNA repair machinery to distinguish between the prereplicative and postreplicative status of a genomic locus and engages the repair pathway most suited to the replicative state.

Thanks to the elegant work of the Legube group, a broader picture of chromatin role in DSB repair has emerged, whereby actively transcribed loci display a unique chromatin signature when they undergo a break, which preferentially channels their repair toward HR, while nontranscribed regions display a different chromatin landscape and are mostly repaired through cNHEJ. By complementing chromatin immunoprecipitation followed by sequencing characterization of histone modifications at multiple DSBs with mapping of repair proteins (i.e., RAD51 as proxy of HR and XRCC4 of cNHEJ), they were able to assign a specific chromatin signature to either HR or cNHEJ repair pathway. These data revealed two distinct chromatin signatures, defined as "HR-prone" and "NHEJ-prone." Among the histones affected by DSB induction, some modifications, such as the histone H2B Lys120 (H2BK120) switch from ubiquitination to acetylation, appears to participate in DSB repair irrespective of the pathway of choice, whereas other marks were uniquely associated to either HR or cNHEJ, hence highlighting the role of chromatin status in DSB repair-pathway choice (40).

From these studies, it emerges that the timely modulation of both acetylation and methylation during DSB induction and repair is critical to ensure an effective repair by either pathway, depending on the phase of cell cycle and the transcriptional status of the genetic locus. However, depletion of essential metabolites, such as acetyl-CoA or methyl donors, might impair these dynamics and aberrantly direct DSB repair to the less suited pathway. Given that the deposition and removal of histone marks is mediated by writers and erasers whose activity requires metabolites and cofactors derived from the diet and/or from the primary metabolism, it is apparent that metabolic alterations resulting from different dietary patterns can impact the repair-pathway choice by modulating the availability of essential metabolites required for methylation or acetylation processes (41). Critically, the metabolic pathways that modulate the activity of writers and erasers of histone modifications can also regulate the activity of the enzymes responsible for the deposition and removal of the RNA posttranscriptional modifications.

## Role of RNA in DSB Repair

Recent developments have highlighted that RNA cooperates with canonical DNA repair factors to facilitate DSBs repair and therefore ensure genomic stability. Meta-analysis of proteomics studies suggests that RNA-processing enzymes are not only contributing to the DNA damage response (DDR) by being recruited to damaged chromatin and nucleating the recruitment of canonical DDR components, but are in fact an integral part of an orchestrated DDR response, as they are the direct target of DDR signals (42). Besides RNA-related proteins, RNA itself is strongly implicated in DDR: thus far, there are two models regarding the origin and function of DDRNA, dissenting mostly on the timing of DDRNA synthesis relative to the DSB occurrence.

### Postdamage DDRNA

According to some studies, the DDRNA is formed from transcription events that occur after the break and produces *de novo* an RNA transcript around the broken DNA locus (43–45).

This line of research is supported, among others, by the work of the d'Adda di Fagagna's lab. They demonstrated that, after DSB induction, RNA polymerase II is recruited to the damaged site by binding to the MRN complex and synthesizes damage-induced long noncoding RNA (dilncRNA), which then forms DNA:RNA hybrids that are processed by the RNAses Drosha and Dicer to form mature DDRNA (46, 47). The DDRNA then acts as a sequence-specific signal and allows propagation of canonical DDR pathways (44, 48). Antisense oligonucleotides matching dilncRNAs and DDRNAs, inhibition of RNA polymerase II or depletion of Dicer and Drosha activity have been shown to suppress dilncRNA production and impair site-specific DDR foci formation, as evidenced by decreased 53BP1 recruitment (44). In addition, suppression of DNA:RNA hybrids formation in cells that are in S/$G_2$-phase prevents the recruitment of BRCA1, BRCA2, and RAD51 and impairs HR-mediated repair (49). Hence, these and other (47) reports indicate that dilncRNA and DDRNA are essential factors in DSB repair initiation and, at least when cells are in S- or $G_2$-phase, are required for the proper execution of HR repair. Although more research is needed to deconvolute the role of *de novo* DDRNA in repair-pathway choice, *these studies overall suggest that postdamage transcribed DDRNA and dilncRNA favor HR and, specifically, the most conservative HR pathway.*

### Predamage DDRNA

Another line of research suggests that DDRNA derives from an RNA transcript produced at transcriptionally active loci before the break (50–52). DDRNA could then hybridize to the broken DNA and be used as a template for a high-fidelity repair (RNA-templated mechanism; refs. 52–55). Löbrich and Jeggo proposed a mechanism whereby the RNA transcript homologous to the damaged DNA site is used as a template to synthesize the new ssDNA during $G_1$-phase (i.e., when a sister chromatid is not available) in a process mediated by cNHEJ repair factors and, notably, by Artemis (53). Along the same line, Chakraborty and colleagues presented a model of cNHEJ-mediated error-free DSB repair that utilizes locally transcribed RNA as template for error-free repair (50). Collectively, these studies propose a "hybrid" repair pathway that bypasses the lack of a sister chromatin by using a homologous RNA transcript instead, hence still ensuring a high-fidelity repair even during $G_1$-phase. Other studies demonstrate a role of predamage formed DDRNA in facilitating DNA end resection and HR. For example, in postmitotic neurons, DNA:RNA hybrids have been shown to bind to RAD52, which is required for the assembly of RAD51 filaments around resected ssDNA during HR (56). Hence, although some discrepancies exist between studies, probably due to the different cellular and experimental conditions, *even in the case of predamage synthesized DDRNA it appears that its involvement in DSB repair ensures a higher level of genome fidelity, whether it is by providing a template when no sister chromatid is available or by favoring the recruitment of HR factors.*

From these studies, it emerges that damage-related ncRNA plays an integral role in DSB repair. The involvement of RNA in the DNA repair process adds a layer of complexity in the repair-pathway choice, which is starting to appear less as a dichotomic decision process, determined by fixed decision nodes, and more as a dynamic and multitiered system of choice in which more options are available for the cells, especially in suboptimal conditions (e.g., in transcriptionally active regions of nonmitotic cells). Given that its engagement in DSB repair influences the efficiency of DNA repair by preferentially contributing to the virtually error-free HR-mediated repair, it follows that RNA modifications that alter RNA function can also impair its role in DNA damage repair and consequently affect genome stability. Indeed, recent developments in epitranscriptomic studies (described in the following section) have revealed the engagement of methyl-marked RNA in DDR responses.

## $m^6A$ RNA Methylation and its Role in Genomic Stability

Cellular RNAs contain more than 160 structurally distinct posttranscriptional modifications (57), including N6-Methyladenosine ($m^6A$), N1-Methyladenosine ($m^1A$), 2′-O-methylation (2′O Me/Nm), and 5-Methylcytosine ($m^5C$). Although epitranscriptome homeostasis is often disrupted in cancer (58), little is known about the metabolic control of epitranscriptomic writers and erasers in normal as well as cancer cells. The most abundant, dynamic and well-studied modification of mRNA and ncRNA is $m^6A$, an epitranscriptomic modification that affects many aspects of RNA function, including its structure, stability, and protein interaction (59, 60). The $m^6A$ mark is generated by a methyltransferase complex, comprising the methyltransferase-like 3 (METTL3)/METTL14 (methyltransferase-like 14) heterodimer core subunit and other cofactors, including WTAP, KIAA1429, ZC3H13, and RBM15/RBM15 (61, 62). Among them, METTL3 is the only subunit of the complex that has catalytic activity and requires as a methyl-group donor the metabolite S-adenosylmethionine (SAM), an intermediate of the one-carbon metabolism (63). The RNA $m^6A$ modification can be reversibly removed by the RNA demethylases fat mass and obesity associated (FTO; ref. 64) and AlkB family member 5 (ALKB5; ref. 65), both alpha-ketoglutarate (α-KG)-dependent enzymes tied to the tricarboxylic acid (TCA) cycle.

In 2017, Xiang and colleagues published a study reporting $m^6A$ RNA recruitment to DNA damage sites (66). They observed that, upon ultraviolet-induced DNA damage, $m^6A$ RNA and its writers METLL3 and METLL14 nucleated at γH2A.X-marked foci quickly after damage. Importantly, depletion of METLL3 delayed the resolution of the break and reduced cell survival after DNA damage. If we consider these results in light of the work by Liu and colleagues, who showed how $m^6A$ methylation induces a structural switch in the RNA transcript that in turn affects its protein interactions (67), we can appreciate how these data collectively point toward a

potential mechanistic link between epitranscriptomic alterations and DNA repair. Moreover, in 2020, Abakir and colleagues proposed a new mechanism linking $m^6A$ RNA and R-loops, which are tripartite structures formed by a DNA:RNA hybrid and an unpaired ssDNA (68). By combining DNA:RNA hybrid immunoprecipitation and sequencing and $m^6A$ DNA immunoprecipitation and sequencing, they observed that $m^6A$ and DNA:RNA hybrids displayed preferential accumulation to the same genomic features (i.e., transcribed regions and repeated sequences). They went on to dissect a model whereby $m^6A$ deposition by METLL3 on the RNA moieties of DNA:RNA hybrids contribute to R-loops removal. Remarkably, depletion of METLL3, as well as YTHDF2, an $m^6A$ reader, increased the levels of γH2A.X, thus suggesting that pathological R-loop accumulation concomitant to $m^6A$ depletion can challenge genomic stability (69). In line with these data, Yang and colleagues observed a positive correlation between METLL3-dependent $m^6A$ accumulation and R-loop formation, although in the context of transcription termination (70). These observations were further substantiated by the recent observation that METTL3 is phosphorylated by ATM after DSB induction and recruited to DNA damage sites in an RNA polymerase II–dependent manner. METTL3 then catalyzes the $m^6A$ methylation of DDRNAs, favoring the formation of RNA:DNA hybrids and the subsequent execution of HR-mediated repair (71). As Marnef and Legube also pointed out (72), these studies reveal an unexpected interplay between the epitranscriptome and genomic stability. *If we place this newly described model in the context of DNA:RNA hybrids and their role in HR-mediated repair, we can envision a link between metabolism and cancer in which dietary patterns that impair $m^6A$ deposition, either by cofactor depletion or competitive inhibition, reduce the efficiency of the DNA repair machinery, especially considering that replication stress (fueled by oncogene activation, metabolic changes, or both) can promote pathological R-loops formation* (73).

Altogether, these data suggest that $m^6A$ RNA methylation must be buffered to maintain RNA homeostasis and its functional outcome is context dependent. Specifically, the putative oncogenic role of its metabolic modulation must be investigated considering the impact that global $m^6A$ levels have on gene expression, beyond DNA repair. While the role of nutritional metabolism in regulating $m^6A$, through the modulation of SAM levels and METLL3, FTO, and ALKB5 activity, has been reviewed elsewhere (74), the effect of manipulating dietary intake of one-carbon metabolites on $m^6A$ methylation of DDRNA in cancer cells remains a yet unexplored area of investigation. A mechanistic understanding of this link would allow establishing dietary guidelines specific to the maintenance of RNA homeostasis to maximize genomic stability.

## Metabolic Regulation of Methylation

Many enzymes involved in methylation and acetylation processes use diet-dependent metabolites and cofactors (41). While this phenomenon was first studied mostly through the prism of histone and DNA posttranslational modifications (the epigenome), recent work indicates that metabolism can also modulate the balance of RNA posttranscriptional modifications (the epitranscriptome). Given that both the epigenome and the epitranscriptome play a crucial role in DNA repair, their modulation through dietary intake of key metabolites and cofactors is of relevance for cancer prevention. Because acetylation of RNA transcripts have been scantly studied so far (75), we chose to focus only on methylation processes and their corresponding metabolic pathways.

### The one-carbon metabolism

All methylation reactions, whether they target histones, DNA or RNA molecules, are performed by methyltransferases and require SAM as the ultimate methyl donor. This metabolite is derived from methionine, an essential amino acid that can be either obtained from the diet or generated from homocysteine in a process that utilizes carbon derived from dietary folate, choline, or betaine. This cellular process is called one-carbon metabolism and is a bicyclic metabolic pathway composed of the folate and methionine cycles (76). The transfer of a methyl group from SAM to the substrate results into another metabolite, S-adenosylhomocysteine (SAH), which can act as a competitive inhibitor of methyltransferases or be restored as SAM thanks to the coordinate action of the methionine and folate cycles. Thus, the intracellular SAM:SAH ratio, which is regulated by the one-carbon metabolism, dynamically modulates methyltransferases activity (77). The active removal of methylated marks can be chemically mediated by demethylases that use as a cofactor α-KG, an intermediate metabolite of the TCA that requires vitamin C as a cofactor (ref. 78; **Fig. 2**). Importantly, the intracellular levels of metabolites that act as cofactors for cellular methyltransferases are often in their Michaelis constant ($K_m$) range, suggesting that their availability can indeed be a limiting factor for enzymatic activity (78). *It is therefore apparent that one-carbon metabolites and vitamins are central to methylation processes and intimately integrated with diet-dependent regulation of cancer metabolism.* In particular, dietary intake of methionine, folate, and vitamin C are among the most studied dietary alterations in the context of cancer prevention.

### Dietary methionine restriction

The contribution of dietary patterns to systemic methionine levels is highly variable. At the two extremes of the spectrum, we find the vegan diet (low methionine) and the WD (high methionine), while the MD falls in between, with a methionine intake on average 40% lower than the WD (79, 80). This is explained by the low levels of methionine found in vegetables and fruits (of which the MD is rich), as opposed to the high methionine content in meat, eggs, and cheese (staples of the WD; ref. 81).

The metabolic dependency of cancer cells to circulating methionine is exemplified by the observation that intratumoral

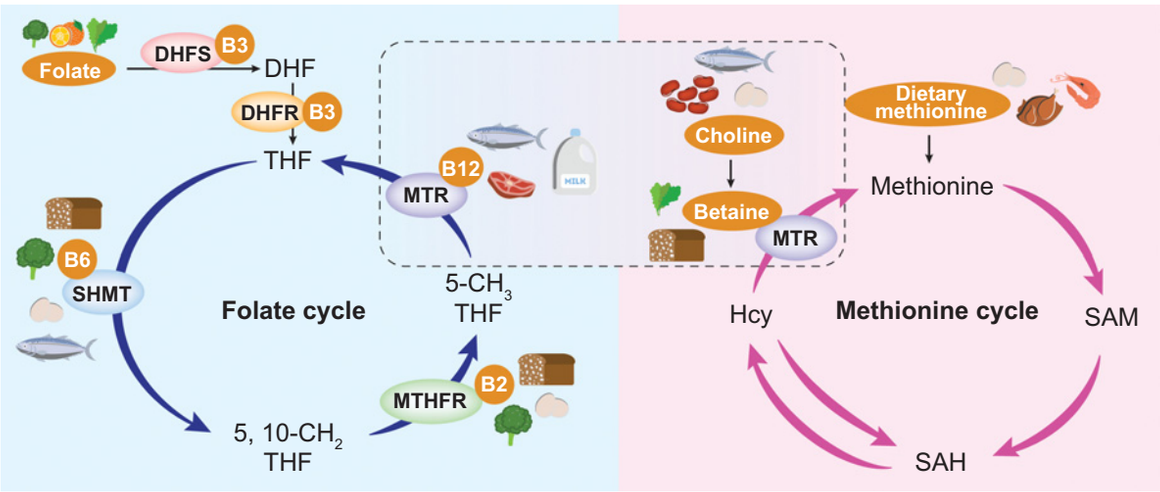


**Figure 2.**

The folate and methionine cycles highlighting the contribution of vitamins B to enzymatic activity. Micronutrients (and respective nutrient sources) that are involved in the one-carbon metabolism. In the folate cycle, dietary folate is converted to dihydrofolate (DHF) via the dihydrofolate synthase (DHFS), and then to tetrahydrofolate (THF) by the dihydrofolate reductase (DHFR). In both steps, vitamin B3 acts as a cofactor. The one-carbon transfer from the amino acid serine to THF is catalyzed by the enzyme serine hydroxymethyltransferase (SHMT), which uses vitamin B6 as a cofactor. The product of this reaction is 5,10-$CH_2$ THF, which in turn is transformed into 5-$CH_3$ THF by the methylene tetrahydrofolate reductase (MTHFR), which uses vitamin B2 as a cofactor. 5-methyl THF is the primary methyl donor for the reaction that remethylates homocysteine (Hcy) into methionine, catalyzed by the methionine synthetase (MTR) and requiring vitamin B12 as a cofactor. The metabolite S-adenosylmethionine (SAM) is derived from methionine, an essential amino acid that can be either obtained from the diet or generated from Hcy in a process that utilizes carbon derived from dietary folate, choline or betaine. The transfer of a methyl group from SAM to the substrate results into S-adenosylhomocysteine (SAH), which can be then restored as SAM. It is overall apparent that vitamins of the B family are key nutrients participating in the one-carbon metabolism.

methionine uptake, as evidenced by positron emission tomography, can be used to monitor tumor burden (e.g., in patients with multiple myeloma) and is more indicative of overall survival than glucose uptake (82). In addition, tumor-initiating cells exhibit elevated activity of enzymes associated with methionine metabolism (83). Hence, restricting dietary methionine is a viable strategy for reducing cancer risk and progression. Indeed, dietary methionine restriction has been shown to extend health and life span, prevent obesity and diabetes and inhibit tumor growth in rodents (80).

The mechanism underlying these phenotypes is partly due to its SAM-depleting effect: besides impairing methylation, SAM depletion can negatively impact phospholipid integrity (84), reduce protein, DNA and RNA stability (by impairing polyamines synthesis; ref. 85) and suppress, through the SAM-sensor protein SAMTOR, the mTOR anabolic pathway (86), a master regulator of cell metabolism whose aberrant activation reduces lifespan and promotes tumor growth (87). Moreover, beyond mediating these SAM-dependent reactions, methionine restriction can impact the regulation of nucleotide biosynthesis, via the folate cycle, and the maintenance of cellular redox homeostasis by reducing the levels of homocysteine, a substrate of the transsulfuration pathway, which ultimately produces the antioxidant glutathione (88). Hence, although dietary intake of methionine is essential for the maintenance of cellular homeostasis, its restriction can hold some beneficial effects in the context of cancer prevention, especially in light of the importance of 'buffering' the histone and RNA methylation status to properly execute DNA damage repair.

## Folate and dietary folate deficiency

Folate, or vitamin B9, is contained in dark green leafy vegetables, legumes, and oranges. It is essential for DNA synthesis, where it is required for the *de novo* synthesis of purines and thymidylate. In addition, it contributes to methylation through the folate cycle, where it participates in the remethylation of homocysteine to methionine, in conjunction with vitamin B6 and vitamin B12 (**Fig. 2**). The role of folate on cancer risk is still controversial, as various epidemiologic studies suggest opposite effects. This could be due to differences between cancer stages, because suppression of folate synthesis with methotrexate is a viable anticancer strategy in established tumors (89), while its role in cancer prevention seems tumor type dependent, as more actively proliferating cancer cells are considered to be more sensitive to folate deficiency/supplementation than slow-growing cancers. Most studies suggest that increased dietary availability of folate can modulate DNA and histone methylation and have anticarcinogenic effects (90). Specifically, epidemiologic studies have found that higher intakes of folic acid, as well as vitamin B6 and B2, are associated with a reduced risk of developing colon, rectal, and breast cancer (91–93). Conversely, low folate intake was associated with increased risk of cancer development through DNA damage, hypomethylation and inhibition of DNA methyltransferases (94). Given that folate-dependent enzymes and folate-binding proteins exhibit binding constants ($K_m$ values) in the nanomolar range, folate-dependent pathways must compete for a limiting pool of folate cofactors. Therefore, all folate pathways are anticipated to be sensitive to folate deficiency (95).

Before cereal grains became fortified with folate in 1998, in the United States it was estimated that about 10% of the population had low intakes of folic acid, and its insufficient dietary intake due to low intake of vegetables seems to correlate with WD (95, 96), whereas adherence to a MD ensures a sufficient intake of folate, as well as other methyl donors (97).

### Vitamin C as a cofactor for α-KG–dependent dioxygenases

Vitamin C is an essential dietary requirement, contained at high concentrations in oranges, tomatoes, crucifers, and leafy vegetables (98). In addition to its well-known role as an antioxidant, vitamin C is a cofactor for α-KG–dependent dioxygenases, including epigenetic regulators such as the Jumonji-C domain-containing histone demethylases (99), the ten-eleven translocation (TET) family of DNA hydroxylases and the RNA demethylases of the AlkB homolog family (100, 101). Among those, the FTO enzyme is a nutrient-sensitive RNA demethylase that is positively associated with obesity and increased risk of breast and gastric cancers (102–104). Epidemiologic studies have also reported a correlation between FTO polymorphisms and prostate and endometrial cancers, but the correlation is no longer significant after adjusting for body mass index (105, 106).

Suboptimal intake of vitamin C has been reported in patients with cancer and has recently been shown to accelerate cancer progression in disease models (107). The ability of vitamin C to potentiate the activity of histone and DNA demethylating enzymes has clinical applications in the treatment of cancer (98). In particular, treatments involving administration of supraphysiologic doses of vitamin C are currently being explored as a therapeutic intervention in TET2-deficient tumors to activate TET3 enzyme and compensate for the lack of TET2 activity, hence targeting aberrant histone and DNA methylation patterns associated with leukemia progression (108). α-KG–dependent enzymes have relatively high $K_m$ values for vitamin C (140–300 μmol/L) and may require above 1 mmol/L intracellular levels for optimal activity. Therefore, vitamin C is often essential for maximal α-KG activity (109). To maintain optimal physiologic levels of vitamin C, the recommended daily intake is 200 mg/day, which can be readily sustained from the consumption of a variety of fruits and vegetables (110). Whether the main anticancer function of vitamin C is due to its role as a cofactor of epigenetic regulators or to its antioxidant function, it is evident that maintaining a diet proficient in vitamin C could help restrain cancer progression. In this respect, a vegetable-rich diet, such as the MD, could sustain adequate levels of vitamin C, while the WD would require dietary supplements [i.e., in the United States, it is estimated that 8.4% of the population is deficient in vitamin C (111)]. Similarly, maintaining physiologic levels of vitamins B through consumption of fruits and vegetables is a necessary requirement to sustain cellular metabolism and support methylation/demethylation processes that are associated with reduced cancer risk.

### Future perspectives on dietary manipulation of one-carbon metabolites

While preserving a balanced intake of nutrients essential for the one-carbon metabolism is recommended to reduce cancer risk, modulation of some specific nutrients (i.e., vitamin C supraphysiologic dose and methionine restriction) might also be, in certain contexts, a viable therapeutic strategy to reduce cancer progression. What emerges overall is the relevance of nutritional metabolism in contributing to the maintenance of an effective DNA repair machinery. If this can, on the one hand, be applied to shape dietary guidelines for cancer prevention, it can also be used to develop a dietary manipulation as a precision nutrition tool to direct cancer cells toward the desired DSB repair system once cancer has emerged.

## Concluding Remarks

Although the ability of certain diets to prevent (2, 3) or fuel (4–6) cancer progression is supported by several epidemiologic and preclinical studies, the complexity of the diet-cancer link makes it challenging to deconvolute the underlying mechanisms, which remain scantly elucidated. In this review, we highlighted the key role of genomic instability in mediating the effect of diets on cancer progression. We presented here evidence from the literature supporting the idea that the oncogenicity of "WD-like diets" is in part due to a diet-induced loss of DNA repair capacity caused by altered epigenetic and epitranscriptomic landscapes, while the protective effect of "MD-like diets" can be partly explained by their ability to maintain a proficient DNA repair system and therefore reduce genomic instability. In particular, considering that diet-dependent alteration of metabolites of the one-carbon metabolism can impact the rate of methylation processes, we argued that changes in dietary patterns can affect the activity of writers and erasers of histone and RNA methyl marks and consequently impair their role in orchestrating DNA damage repair (**Fig. 3**). However, more research is still needed to investigate the link between dietary patterns, cancer cell epigenetic and epitranscriptomic status, and DSB repair system in one single comprehensive perspective. The reticence of the scientific community to address this knowledge gap could be due to the fact that, while dietary manipulation is mostly studied *in vivo*, the mechanisms of DSB repair, including the role of epigenetic and epitranscriptomic status on the DNA repair machinery, are traditionally investigated *in vitro*, with few exceptions (e.g., ref. 112). This technical challenge could be overcome by exploiting the power of omics tools, such as metabolomics and lipidomics, to translate the complexity of the *in vivo* settings into a more defined *in vitro* context. In parallel, the impact of dietary patterns on DNA repair processes should be interrogated at a population level, by combining the use of food questionnaires with analyses of DNA damage markers (i.e., γH2A.X, RAD51, 53BP1) and histone or methyl marks (i.e., $m^6A$ or H4K20me2). With a

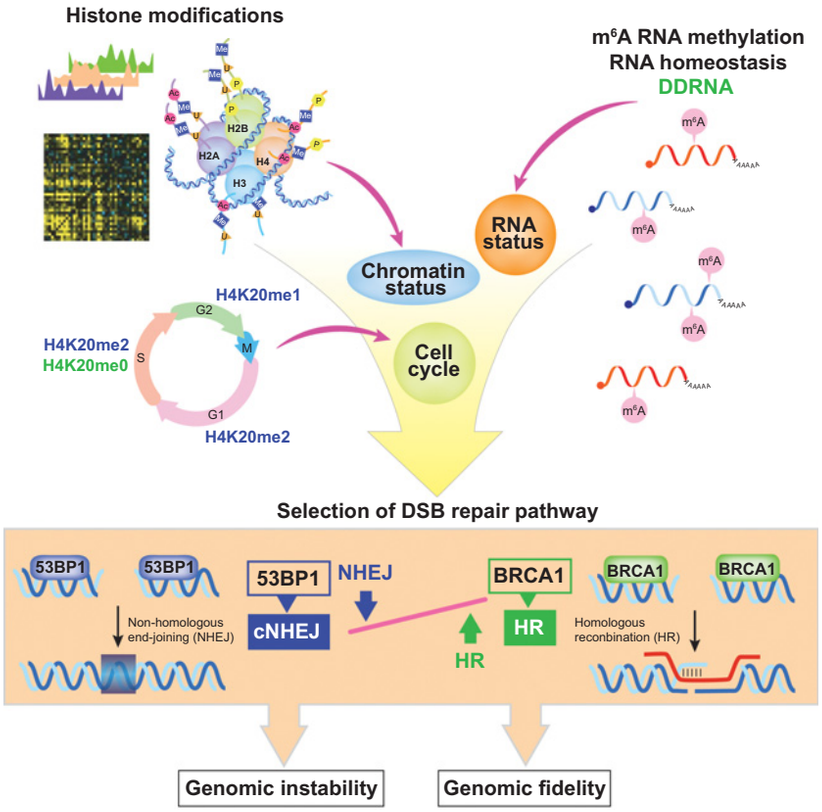


**Figure 3.**
Key metabolic-dependent posttranslational modifications involved in ensuring a proficient DNA damage repair. Histone modifications, $m^6A$ methylation of DDRNA and cell cycle are major contributors in the selection of DSB repair pathway, hence affecting the genomic stability of cancer cells.

better understanding of the dietary basis to DNA repair processes, this raises the possibility that precision nutrition could be used to reduce cancer risk.

## Authors' Disclosures

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## References

1. David CD and Milner JA. Diet and cancer prevention. In: Temple NJ. Wilson T, Jacobs DR Jr, editors. Nutritional health: strategies for disease prevention. 2nd ed. Totowa (NJ): Humana Press; 2006. p 465.
2. Toledo E, Salas-Salvado J, Donat-Vargas C, Buil-Cosiales P, Estruch R, Ros E, et al. Mediterranean diet and invasive breast cancer risk among women at high cardiovascular risk in the PREDIMED trial: a randomized clinical trial. JAMA Intern Med. 2015;175:1752–60.
3. Galbete C, Schwingshackl L, Schwedhelm C, Boeing H, Schulze MB. Evaluating Mediterranean diet and risk of chronic disease in cohort studies: an umbrella review of meta-analyses. Eur J Epidemiol 2018; 33:909–31.
4. Grosso G, Bella F, Godos J, Sciacca S, Del Rio D, Ray S, et al. Possible role of diet in cancer: systematic review and multiple meta-analyses of dietary patterns, lifestyle factors, and cancer risk. Nutr Rev 2017;75: 405–19.
5. Dandamudi A, Tommie J, Nommsen-Rivers L, Couch S. Dietary patterns and breast cancer risk: a systematic review. Anticancer Res 2018;38:3209–22.
6. Castello A, Amiano P, Fernandez de Larrea N, Martin V, Alonso MH, Castano-Vinyals G, et al. Low adherence to the western and high adherence to the mediterranean dietary patterns could prevent colorectal cancer. Eur J Nutr 2019;58:1495–505.
7. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646–74.
8. Salk JJ, Fox EJ, Loeb LA. Mutational heterogeneity in human cancers: origin and consequences. Annu Rev Pathol 2010;5:51–75.

9. Greaves M, Maley CC. Clonal evolution in cancer. Nature 2012;481:306–13.
10. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability–an evolving hallmark of cancer. Nat Rev Mol Cell Biol 2010;11:220–8.
11. Page EC, Bancroft EK, Brook MN, Assel M, Al Battat MH, Thomas S, et al. Interim results from the IMPACT study: evidence for prostate-specific antigen screening in BRCA2 mutation carriers. Eur Urol 2019;76:831–42.
12. Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 1997;386:761–3.
13. Roth DB, Gellert M. New guardians of the genome. Nature 2000;404:823–5.
14. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. Science 2008;319:1352–5.
15. Saha SK, Lee SB, Won J, Choi HY, Kim K, Yang GM, et al. Correlation between oxidative stress, nutrition, and cancer initiation. Int J Mol Sci 2017;18:1544.
16. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol 2014;24:R453–62.
17. Panieri E, Santoro MM. ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis 2016;7:e2253.
18. Scully R, Panday A, Elango R, Willis NA. DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat Rev Mol Cell Biol 2019;20:698–714.
19. Caron P, Choudjaye J, Clouaire T, Bugler B, Daburon V, Aguirrebengoa M, et al. Non-redundant functions of ATM and DNA-PKcs in response to DNA double-strand breaks. Cell Rep 2015;13:1598–609.
20. Schwertman P, Bekker-Jensen S, Mailand N. Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. Nat Rev Mol Cell Biol 2016;17:379–94.
21. Mattiroli F, Vissers JH, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, et al. RNF168 ubiquitinates K13–15 on H2A/H2AX to drive DNA damage signaling. Cell 2012;150:1182–95.
22. Meas R, Mao P. Histone ubiquitylation and its roles in transcription and DNA damage response. DNA Repair 2015;36:36–42.
23. Shibata A. Regulation of repair pathway choice at two-ended DNA double-strand breaks. Mutat Res 2017;803–805:51–5.
24. Noordermeer SM, Adam S, Setiaputra D, Barazas M, Pettitt SJ, Ling AK, et al. The shieldin complex mediates 53BP1-dependent DNA repair. Nature 2018;560:117–21.
25. Mirman Z, Lottersberger F, Takai H, Kibe T, Gong Y, Takai K, et al. 53BP1-RIF1-shieldin counteracts DSB resection through CST- and Polalpha-dependent fill-in. Nature 2018;560:112–6.
26. Nick McElhinny SA, Snowden CM, McCarville J, Ramsden DA. Ku recruits the XRCC4-ligase IV complex to DNA ends. Mol Cell Biol 2000;20:2996–3003.
27. Ahnesorg P, Smith P, Jackson SP. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 2006;124:301–13.
28. Jasin M, Rothstein R. Repair of strand breaks by homologous recombination. Cold Spring Harb Perspect Biol 2013;5:a012740.
29. Greene EC. DNA sequence alignment during homologous recombination. J Biol Chem 2016;291:11572–80.
30. Thomas JM, Batista PJ, Meier JL. Metabolic regulation of the epitranscriptome. ACS Chem Biol 2019;14:316–24.
31. Miller KM, Jackson SP. Histone marks: repairing DNA breaks within the context of chromatin. Biochem Soc Trans 2012;40:370–6.
32. Dhar S, Gursoy-Yuzugullu O, Parasuram R, Price BD. The tale of a tail: histone H4 acetylation and the repair of DNA breaks. Philos Trans R Soc Lond B Biol Sci 2017;372:20160284.
33. Tang J, Cho NW, Cui G, Manion EM, Shanbhag NM, Botuyan MV, et al. Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. Nat Struct Mol Biol 2013;20:317–25.
34. Sivanand S, Rhoades S, Jiang Q, Lee JV, Benci J, Zhang J, et al. Nuclear acetyl-CoA production by ACLY promotes homologous recombination. Mol Cell 2017;67:252–65 e6.
35. Yasuda T, Kagawa W, Ogi T, Kato TA, Suzuki T, Dohmae N, et al. Novel function of HATs and HDACs in homologous recombination through acetylation of human RAD52 at double-strand break sites. PLoS Genet 2018;14:e1007277.
36. Beck DB, Oda H, Shen SS, Reinberg D. PR-Set7 and H4K20me1: at the crossroads of genome integrity, cell cycle, chromosome condensation, and transcription. Genes Dev 2012;26:325–37.
37. Saredi G, Huang H, Hammond CM, Alabert C, Bekker-Jensen S, Forne I, et al. H4K20me0 marks post-replicative chromatin and recruits the TONSL-MMS22L DNA repair complex. Nature 2016;534:714–8.
38. Botuyan MV, Lee J, Ward IM, Kim JE, Thompson JR, Chen J, et al. Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. Cell 2006;127:1361–73.
39. Nakamura K, Saredi G, Becker JR, Foster BM, Nguyen NV, Beyer TE, et al. H4K20me0 recognition by BRCA1-BARD1 directs homologous recombination to sister chromatids. Nat Cell Biol 2019;21:311–8.
40. Clouaire T, Rocher V, Lashgari A, Arnould C, Aguirrebengoa M, Biernacka A, et al. Comprehensive mapping of histone modifications at DNA double-strand breaks deciphers repair pathway chromatin signatures. Mol Cell 2018;72:250–62 e6.
41. Wang Z, Long H, Chang C, Zhao M, Lu Q. Crosstalk between metabolism and epigenetic modifications in autoimmune diseases: a comprehensive overview. Cell Mol Life Sci 2018;75:3353–69.
42. Bader AS, Hawley BR, Wilczynska A, Bushell M. The roles of RNA in DNA double-strand break repair. Br J Cancer 2020;122:613–23.
43. Bonath F, Domingo-Prim J, Tarbier M, Friedlander MR, Visa N. Next-generation sequencing reveals two populations of damage-induced small RNAs at endogenous DNA double-strand breaks. Nucleic Acids Res 2018;46:11869–82.
44. Michelini F, Pitchiaya S, Vitelli V, Sharma S, Gioia U, Pessina F, et al. Damage-induced lncRNAs control the DNA damage response through interaction with DDRNAs at individual double-strand breaks. Nat Cell Biol 2017;19:1400–11.
45. Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, et al. A role for small RNAs in DNA double-strand break repair. Cell 2012;149:101–12.
46. Francia S, Michelini F, Saxena A, Tang D, de Hoon M, Anelli V, et al. Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature 2012;488:231–5.
47. Lu WT, Hawley BR, Skalka GL, Baldock RA, Smith EM, Bader AS, et al. Drosha drives the formation of DNA:RNA hybrids around DNA break sites to facilitate DNA repair. Nat Commun 2018;9:532.
48. Gao M, Wei W, Li MM, Wu YS, Ba Z, Jin KX, et al. Ago2 facilitates Rad51 recruitment and DNA double-strand break repair by homologous recombination. Cell Res 2014;24:532–41.
49. D'Alessandro G, Whelan DR, Howard SM, Vitelli V, Renaudin X, Adamowicz M, et al. BRCA2 controls DNA:RNA hybrid level at DSBs by mediating RNase H2 recruitment. Nat Commun 2018;9:5376.
50. Chakraborty A, Tapryal N, Venkova T, Horikoshi N, Pandita RK, Sarker AH, et al. Classical non-homologous end-joining pathway utilizes nascent RNA for error-free double-strand break repair of transcribed genes. Nat Commun 2016;7:13049.
51. Keskin H, Shen Y, Huang F, Patel M, Yang T, Ashley K, et al. Transcript-RNA-templated DNA recombination and repair. Nature 2014;515:436–9.
52. Wei L, Nakajima S, Bohm S, Bernstein KA, Shen Z, Tsang M, et al. DNA damage during the G0/G1 phase triggers RNA-templated,

Cockayne syndrome B-dependent homologous recombination. Proc Natl Acad Sci U S A 2015;112:E3495–504.
53. Lobrich M, Jeggo P. A process of resection-dependent nonhomologous end joining involving the goddess artemis. Trends Biochem Sci 2017;42:690–701.
54. Mazina OM, Keskin H, Hanamshet K, Storici F, Mazin AV. Rad52 inverse strand exchange drives RNA-templated DNA double-strand break repair. Mol Cell 2017;67:19–29.e3.
55. Storici F, Bebenek K, Kunkel TA, Gordenin DA, Resnick MA. RNA-templated DNA repair. Nature 2007;447:338–41.
56. Welty S, Teng Y, Liang Z, Zhao W, Sanders LH, Greenamyre JT, et al. RAD52 is required for RNA-templated recombination repair in post-mitotic neurons. J Biol Chem 2018;293:1353–62.
57. Cantara WA, Crain PF, Rozenski J, McCloskey JA, Harris KA, Zhang X, et al. The RNA modification database, RNAMDB: 2011 update. Nucleic Acids Res 2011;39:D195–201.
58. Chen XY, Zhang J, Zhu JS. The role of m(6)A RNA methylation in human cancer. Mol Cancer 2019;18:103.
59. Lewis CJ, Pan T, Kalsotra A. RNA modifications and structures cooperate to guide RNA-protein interactions. Nat Rev Mol Cell Biol 2017;18:202–10.
60. Meyer KD, Jaffrey SR. Rethinking m(6)A readers, writers, and erasers. Annu Rev Cell Dev Biol 2017;33:319–42.
61. Huang H, Weng H, Chen J. The biogenesis and precise control of RNA m(6)A methylation. Trends Genet 2020;36:44–52.
62. Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, et al. A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-adenosine methylation. Nat Chem Biol 2014;10:93–5.
63. Wang X, Feng J, Xue Y, Guan Z, Zhang D, Liu Z, et al. Structural basis of N (6)-adenosine methylation by the METTL3-METTL14 complex. Nature 2016;534:575–8.
64. Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011;7:885–7.
65. Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 2013;49:18–29.
66. Xiang Y, Laurent B, Hsu CH, Nachtergaele S, Lu Z, Sheng W, et al. RNA m(6)A methylation regulates the ultraviolet-induced DNA damage response. Nature 2017;543:573–6.
67. Liu N, Dai Q, Zheng G, He C, Parisien M, Pan T. N(6)-methyladenosine-dependent RNA structural switches regulate RNA-protein interactions. Nature 2015;518:560–4.
68. Antson AA. Single-stranded-RNA binding proteins. Curr Opin Struct Biol 2000;10:87–94.
69. Abakir A, Giles TC, Cristini A, Foster JM, Dai N, Starczak M, et al. N (6)-methyladenosine regulates the stability of RNA:DNA hybrids in human cells. Nat Genet 2020;52:48–55.
70. Yang X, Liu QL, Xu W, Zhang YC, Yang Y, Ju LF, et al. mA promotes R-loop formation to facilitate transcription termination. Cell Res 2019;29:1035–8.
71. Zhang C, Chen L, Peng D, Jiang A, He Y, Zeng Y, et al. METTL3 and N6-methyladenosine promote homologous recombination-mediated repair of DSBs by modulating DNA-RNA hybrid accumulation. Mol Cell 2020;79:425–42 e7.
72. Marnef A, Legube G. mA RNA modification as a new player in R-loop regulation. Nat Genet 2020;52:27–8.
73. Kotsantis P, Silva LM, Irmscher S, Jones RM, Folkes L, Gromak N, et al. Increased global transcription activity as a mechanism of replication stress in cancer. Nat Commun 2016;7:13087.
74. Wu J, Frazier K, Zhang J, Gan Z, Wang T, Zhong X. Emerging role of m (6) A RNA methylation in nutritional physiology and metabolism. Obes Rev 2020;21:e12942.
75. Sas-Chen A, Thomas JM, Matzov D, Taoka M, Nance KD, Nir R, et al. Dynamic RNA acetylation revealed by quantitative cross-evolutionary mapping. Nature 2020;583:638–43.
76. Kaelin WG Jr, McKnight SL. Influence of metabolism on epigenetics and disease. Cell 2013;153:56–69.
77. Janke R, Dodson AE, Rine J. Metabolism and epigenetics. Annu Rev Cell Dev Biol 2015;31:473–96.
78. Su X, Wellen KE, Rabinowitz JD. Metabolic control of methylation and acetylation. Curr Opin Chem Biol 2016;30:52–60.
79. Tosti V, Bertozzi B, Fontana L. Health benefits of the mediterranean diet: metabolic and molecular mechanisms. J Gerontol A Biol Sci Med Sci 2018;73:318–26.
80. Sanderson SM, Gao X, Dai Z, Locasale JW. Methionine metabolism in health and cancer: a nexus of diet and precision medicine. Nat Rev Cancer 2019;19:625–37.
81. U.S. Department of Agriculture ARS. FoodData Central; 2019. Available from:<https://fdc.nal.usda.gov>.
82. Luckerath K, Lapa C, Albert C, Herrmann K, Jorg G, Samnick S, et al. 11C-Methionine-PET: a novel and sensitive tool for monitoring of early response to treatment in multiple myeloma. Oncotarget 2015;6:8418–29.
83. Wang Z, Yip LY, Lee JHJ, Wu Z, Chew HY, Chong PKW, et al. Methionine is a metabolic dependency of tumor-initiating cells. Nat Med 2019;25:825–37.
84. Ye C, Sutter BM, Wang Y, Kuang Z, Tu BP. A metabolic function for phospholipid and histone methylation. Mol Cell 2017;66:180–93 e8.
85. Pegg AE. Functions of polyamines in mammals. J Biol Chem 2016;291:14904–12.
86. Gu X, Orozco JM, Saxton RA, Condon KJ, Liu GY, Krawczyk PA, et al. SAMTOR is an S-adenosylmethionine sensor for the mTORC1 pathway. Science 2017;358:813–8.
87. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;168:960–76.
88. Hosios AM, Vander Heiden MG. The redox requirements of proliferating mammalian cells. J Biol Chem 2018;293:7490–8.
89. Hagner N, Joerger M. Cancer chemotherapy: targeting folic acid synthesis. Cancer Manag Res 2010;2:293–301.
90. Pembrey M, Saffery R, Bygren LO, Network in Epigenetic Epidemiology; Network in Epigenetic Epidemiology. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. J Med Genet 2014;51:563–72.
91. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Cholewinski S, Wilkinson G. Folate intake and carcinogenesis of the colon and rectum. Int J Epidemiol 1991;20:368–74.
92. Benito E, Stiggelbout A, Bosch FX, Obrador A, Kaldor J, Mulet M, et al. Nutritional factors in colorectal cancer risk: a case-control study in Majorca. Int J Cancer 1991;49:161–7.
93. Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999;281:1632–7.
94. Yamaji T, Inoue M, Sasazuki S, Iwasaki M, Kurahashi N, Shimazu T, et al. Fruit and vegetable consumption and squamous cell carcinoma of the esophagus in Japan: the JPHC study. Int J Cancer 2008;123:1935–40.
95. Herbig K, Chiang EP, Lee LR, Hills J, Shane B, Stover PJ. Cytoplasmic serine hydroxymethyltransferase mediates competition between folate-dependent deoxyribonucleotide and S-adenosylmethionine biosyntheses. J Biol Chem 2002;277:38381–9.
96. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for

cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290–5.
97. Fischer M, Stronati M, Lanari M. Mediterranean diet, folic acid, and neural tube defects. Ital J Pediatr 2017;43:74.
98. Cimmino L, Neel BG, Aifantis I. Vitamin C in stem cell reprogramming and cancer. Trends Cell Biol 2018;28:698–708.
99. Wang T, Chen K, Zeng X, Yang J, Wu Y, Shi X, et al. The histone demethylases Jhdm1a/1b enhance somatic cell reprogramming in a vitamin-C-dependent manner. Cell Stem Cell 2011;9:575–87.
100. Yi C, Jia G, Hou G, Dai Q, Zhang W, Zheng G, et al. Iron-catalysed oxidation intermediates captured in a DNA repair dioxygenase. Nature 2010;468:330–3.
101. Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007;318:1469–72.
102. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889–94.
103. Niu Y, Lin Z, Wan A, Chen H, Liang H, Sun L, et al. RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3. Mol Cancer 2019;18:46.
104. Xu D, Shao W, Jiang Y, Wang X, Liu Y, Liu X. FTO expression is associated with the occurrence of gastric cancer and prognosis. Oncol Rep 2017;38:2285–92.
105. Lewis SJ, Murad A, Chen L, Davey Smith G, Donovan J, Palmer T, et al. Associations between an obesity related genetic variant (FTO rs9939609) and prostate cancer risk. PLoS One 2010;5:e13485.
106. Lurie G, Gaudet MM, Spurdle AB, Carney ME, Wilkens LR, Yang HP, et al. The obesity-associated polymorphisms FTO rs9939609 and MC4R rs17782313 and endometrial cancer risk in non-Hispanic white women. PLoS One 2011;6:e16756.
107. Ames BN, Wakimoto P. Are vitamin and mineral deficiencies a major cancer risk? Nat Rev Cancer 2002;2:694–704.
108. Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, et al. Restoration of TET2 function blocks aberrant self-renewal and leukemia progression. Cell 2017;170:1079–95.
109. Young JI, Zuchner S, Wang G. Regulation of the epigenome by vitamin C. Annu Rev Nutr 2015;35:545–64.
110. Padayatty SJ, Levine M. Vitamin C: the known and the unknown and goldilocks. Oral Dis 2016;22:463–93.
111. Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 national health and nutrition examination survey (NHANES). Am J Clin Nutr 2009;90:1252–63.
112. Ke Z, Firsanov D, Spencer B, Seluanov A, Gorbunova V. Short-term calorie restriction enhances DNA repair by non-homologous end joining in mice. NPJ Aging Mech Dis 2020;6:9.