Nutriomes and Personalised Nutrition for DNA Damage Prevention, Telomere Integrity Maintenance and Cancer Growth Control

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Abstract

DNA damage at the base sequence and chromosome level is a fundamental cause of developmental and degenerative diseases. Multiple micronutrients and their interactions with the inherited and/or acquired genome determine DNA damage and genomic instability rates. The challenge is to identify for each individual the combination of micronutrients and their doses (i.e. the nutriome) that optimises genome stability, including telomere integrity and functionality and DNA repair. Using nutrient array systems with high-content analysis diagnostics of DNA damage, cell death and cell growth, it is possible to define, on an individual basis, the optimal nutriome for DNA damage prevention and cancer growth control. This knowledge can also be used to improve culture systems for cells used in therapeutics such as stem cells to ensure that they are not genetically aberrant when returned to the body. Furthermore, this information could be used to design dietary patterns that deliver the micronutrient combinations and concentrations required for preventing DNA damage by micronutrient deficiency or excess. Using this approach, new knowledge could be obtained to identify the dietary restrictions and/or supplementations required to control specific cancers, which is particularly important given that reliable validated advice is not yet available for those diagnosed with cancer.

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Keywords

Nutriomes · DNA damage · Telomere · Genome stability · Personalised nutrition

Abbreviations

MRI	Magnetic resonance imaging
TRF1; TRF2	Telomeric-repeat binding factors 1 and 2
MTHFR	Methylenetetrahydrofolate reductase
ADH1	Alcohol dehydrogenase
ALDH2	Aldehyde dehydrogenase
CBMN Cyt	Cytokinesis-blocked micronucleus cytome
MTAP	Methylthioadenosine phosphorylase

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1 Background and Current Status

DNA damage at the base sequence and chromosome level is the most fundamental cause of developmental and degenerative diseases (including accelerated ageing) and is predictive prospectively of these conditions [1–3] Hundreds of genes are involved in maintenance of genome integrity, and there is great variation amongst individuals with respect to common polymorphisms that impact on the activity of these enzymes [4–8]. The proteins encoded by those genes required for DNA replication, DNA repair or detoxification of potential genotoxins depend on essential cofactors that are obtained from the diet for optimal function [9–24]. Dietary profile differs between individuals to varying extents depending on their acquired or inherited dietary preferences and food availability; furthermore, uptake of micronutrients from the digestive system and transport into cells of the body also vary depending on genetics and altered expression of transporters that occurs with age [25–27]. Nutritional factors are required for genome maintenance not only in vivo but also in vitro which varies greatly depending on the culture

medium used [28, 29]. Maintenance of genome integrity in vitro is critical particularly in long-term culture of cells (e.g. stem cells) which may be taken out of the body for expansion and then returned to the original donor or other recipients for medical therapy reasons because DNA damage accumulated in vitro may result in oncogenic events in stem cells [30, 31]. Currently, dietary reference values (e.g. recommended daily intakes, upper safety limits) and culture medium recipes and conditions do not take into consideration impact on genome integrity and yet harm to the DNA sequence and/or the epigenome is the most fundamental and critical pathology underlying cellular and organism health and disease.

2 Nutriomes, DNA Damage and Telomere Maintenance in Vivo

Using food frequency questionnaire data and the cytokinesis-blocked micronucleus assay, one of the best validated biomarkers of DNA damage [1], we were successful in identifying nine micronutrients associated with this biomarker of chromosome breakage or loss. Increased dietary intake of vitamin E, calcium, folate, retinol and nicotinic acid was associated with reduced DNA damage, whilst increased intake of riboflavin, pantothenic acid and biotin was associated with more DNA damage and beta-carotene showed a U-shaped relationship, such that moderate intake was beneficial but excess intake was not [32]. The results also showed interactive effects amongst these factors.

Using the results of these studies, it is possible to imagine classifying preferred foods for DNA damage prevention based on their content of the nutriome consisting of vitamin E, calcium, folate, retinol, beta-carotene and nicotinic acid as shown in Fig. 1. It is evident from this that beef and bananas are a poor source of this genome-protective nutriome compared to almonds, wheat bran, cheese, broccoli and tuna. The approach of studying nutriomes may prove more efficacious in identifying those foods and dietary patterns that can best protect against fundamental pathologies at the genome level. Recently, the use of principal component analysis was reported for the first time to determine the plasma nutriomes associated with improved cognitive function and MRI measures of the brain [33]. Similar approaches could be used in relation to identifying nutrient biomarker patterns associated with DNA damage and cancer prevention.

This more detailed knowledge may help to refine dietary recommendations for optimal health. For example, the most recent WHO/FAO report recommends a minimum of 400 g of fruit and vegetables per day for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries [34]. This recommendation could be misleading as shown in Table 1, using folate as an example of a key micronutrient required for genome integrity and normal foetal development [35, 36]. There are essentially four different types of vegetables some of which are actually fruits. Which vegetables one

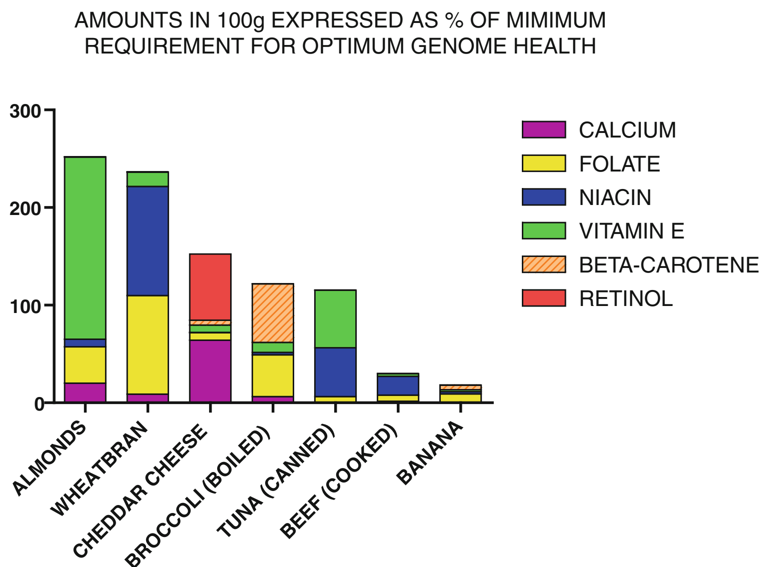

Fig. 1 Content of micronutrients associated with reduced DNA damage in selected common foods. The height of each bar for each micronutrient within the separate foods corresponds to the amount of the micronutrient expressed as the percentage of the minimum daily intake associated with a reduced micronucleus frequency index in lymphocytes as determined in the study of Fenech et al. [32]. The relative contribution of each of the micronutrients (if present) is indicated by the height of each specifically coloured bar. The nutrient content of the foods was determined using published food content tables such as the USDA Nutrient Database (http://www.nal.usda.gov/fnic/foodcomp/search/) and McCance and Widdowson's Composition of Foods Sixth Summary Edition

chooses or prefers can make a great difference to their folate intake or the amount consumed to achieve the daily requirement of folate. For high folate vegetables (i.e. pulses and/or leafy or cruciferous vegetables), it is sufficient to consume 400 g per day to meet the recommended dietary allowance of 400 μg folate per day, but if one prefers root/tuber vegetables or "fruit vegetables", then it is necessary to consume 2.5 kg per day which is impractical and could be prohibitively expensive. Furthermore, the folate level in fruit vegetables is even less than half that of roots and tubers. This example alone indicates the evident inadequacy of current recommendations with respect to maximising the efficiency of obtaining the required intakes of important micronutrients such as folate. We urgently need more precise recommendations based on nutrient dense foods such as pulses and grains and particularly those foods and combinations that deliver the nutrient combinations for genome integrity maintenance.

A critical region of the genome that is now attracting more attention by nutritional genomics scientists is the telomere. Telomeres are a TTAGGG tandem repeat sequence that caps the ends of chromosomes and have the unique function, together with the shelterin proteins associated with them (e.g. TRF1 and TRF2), of

preventing fusion of chromosome ends which would cause chromosomal instability by the formation of dicentric chromosomes, anaphase bridges and cycles of chromosome breakage and further fusions due to the generation of uncapped chromosome ends. This is the so-called breakage–fusion–bridge cycle fuelled by excessive telomere shortening and/or dysfunction of the telomere/telosome complex [37, 38]. It is likely that nutrition may play a protective role because (1) oxidation of guanine in the telomere sequence can prevent TRF1 and TRF2 binding required for telomere stability and function, and oxidation of guanine could be prevented by adequate dietary antioxidant intake; (2) folate is required to prevent accumulation of uracil in the telomere sequence that could lead to breaks within the telomere or subtelomere leading to telomere deletions and loss, whilst subtelomere hypomethylation can lead to loss of telomere length control leading to telomere dysfunction and (3) niacin is required to provide NAD for tankyrase activity which is essential for accessibility of telomerase for telomere maintenance [39, 40].

In vivo studies have shown that folate, vitamin D, omega-3 fatty acids, multivitamin use, weight loss by caloric restriction and cereal fibre intake tend to be associated with longer telomeres whilst oxidative stress, high plasma homocysteine, intake of processed meat and linoleic acid, psychological stress and obesity tend to be associated with shorter telomeres [41–47]. The significance of these results remains unclear as they are largely data from single studies and the association of telomere shortening with unhealthy ageing has recently been questioned given that both excessively short and long telomeres have been associated prospectively with cancer risk [48].

Table 1 Folate content of vegetables (DFE in μg per 100 g)[a]

High folate (HF) vegetables		Low folate (LF) vegetables	
Pulses	Leafy or cruciferous vegetables	Roots or tubers	"Fruit" vegetables
Red kidney beans (130)	Broccoli (93)	Onions (16)	Tomato (15)
Mung beans (60)	Brussel sprouts (60)	Potato (22)	Pumpkin (9)
Chickpeas (171)	Cabbage (43)	Turnip (9)	Cucumber (6)
Lentils (180)	Endive (142)	Parsnip (57)	Capsicum (11)
Peas (59)	Spinach (146)	Swede (21)	Eggplant (14)
Lima beans (50)	Lettuce (73)	Carrot (14)	Olives (0)
Mean (108)	Mean (93)	Mean (23)	Mean (10)
Mean (100)		Mean (16)	

[a] Data from USDA Nutrient Database (http://www.nal.usda.gov/fnic/foodcomp/search/)
DFE dietary folate equivalent, DFE values are shown in brackets

3 Nutritional Needs and Knowledge Gaps in Tissue Culture Systems

A critical issue in tissue culture is the evident lack of physiological conditions in terms of both composition of culture medium and oxygen tension, both of which have profound impacts on the rate of growth of cells and their level of chromosomal instability. For example, recipes of culture media can vary enormously between each other with respect to minerals and vitamins and often the concentration is supra-physiological relative to human serum or deficient depending on the micronutrient. RPMI 1640 culture medium, one of the most commonly used for culturing human cells, is supra-physiological for folate, methionine and riboflavin and deficient for iron, copper, zinc, calcium, magnesium and sulphur relative to human serum (Table 2). Whilst some of the deficiencies in culture medium may be addressed by the addition of foetal bovine serum this is only added at 5–10 % which would still render culture medium deficient if the micronutrient is absent or deficient in the recipe. It is evident that current culture media are not physiological relative to human plasma, and therefore, data obtained from in vitro experiments need to be treated with caution if attempts are made to extrapolate to in vivo predictions. The latter can only become feasible once physiological culture media are developed that are equivalent in composition to human plasma and other body fluids (e.g. cerebro-spinal fluid, interstitial fluid) and if the oxygen tension used is similar to that experienced by tissues in the body. Physiological oxygen tension is at least 2–4 times lower than that of atmospheric oxygen typically used in cell culture incubators. It was shown that cells grown under physiological oxygen conditions experience less oxidative stress and paradoxically grow more slowly compared to cells in atmospheric oxygen incubators [49, 50]. Faster growth does not necessarily result in better genome stability because the former could be due to permissiveness of cell cycle checkpoints causing a reduction in cell cycle time and/or reduced apoptosis of cells with DNA damage. We and others have shown that DNA damage, cell death and cell growth in cultured cells are strongly affected by concentration of essential micronutrients, such that both deficiency or excess within the physiological range can profoundly harm the genome and alter cell growth and survival kinetics [23, 28, 29, 51–54]. The use of excessively high concentrations of methyl donors (e.g. folate, methionine, choline, vitamin B12) in culture medium theoretically may lead to an adverse DNA methylation pattern that may inappropriately silence important house-keeping genes, although strong evidence for this hypothesis is currently lacking [55]. It is evident that, given the wide spectrum of micronutrients required for genome maintenance and repair, the development of physiological culture medium composition is an important prerequisite to enable the determination of optimal culture conditions for growth of human cells in a genomically stable state and to explore the impact of various micronutrient combinations (i.e. nutriomes) and dosages against different genetic backgrounds. These developments are also critical if we are to use in vitro data reliably to predict in vivo nutritional effects on an individual basis. In this regard, it

Table 2 Comparison of concentration of some micronutrients between a single sample of human serum and normal complete RPMI 1640 culture medium (data not previously published)

Micronutrient	Concentration	Human serum	RPMI 1640 culture medium
Folate	μmol/L	0.028	2.3
Methionine	μmol/L	30	100
Riboflavin	μmol/L	0.05	0.53
Iron	mg/L	0.84	0.19
Copper	mg/L	1.4	<0.1
Zinc	mg/L	0.94	0.17
Calcium	mg/L	98	26
Magnesium	mg/L	20	11
Sodium	mg/L	3,400	3,200
Potassium	mg/L	154	200
Phosphorous	mg/L	121	174
Sulphur	mg/L	1,110	64

is important to note that concentrations of micronutrients achievable in vitro might not be possible in vivo due to excretion and redistribution within tissues. Furthermore, with respect to body fluids, we only have good knowledge on possible micronutrient concentrations in blood plasma and our knowledge about interstitial fluids surrounding organs (e.g. cerebrospinal fluid) or within tissues is at this stage rudimentary. We need to consider optima both within the physiological and supra-physiological range but only use “physiological dose ranges” achievable in vivo for in vivo predictions using in vitro models.

With respect to optimising in vitro and in vivo cellular health, it is becoming increasingly recognised that parameters of genome and epigenome damage are exquisitely sensitive to changes in micronutrient concentration even within the “normal” physiological range [23, 28, 29, 52, 55, 56]. It is therefore practical, feasible and desirable to start re-examining dietary reference values so that recommended intakes coincide with the attainment of tissue concentrations that are consistent with minimised DNA damage. For a detailed recent review on the status of validation of DNA damage, biomarkers for measuring the genomic impact of malnutrition and a proposed roadmap for determining nutrient and nutriome requirements for optimal genome maintenance refer to Fenech [1, 18].

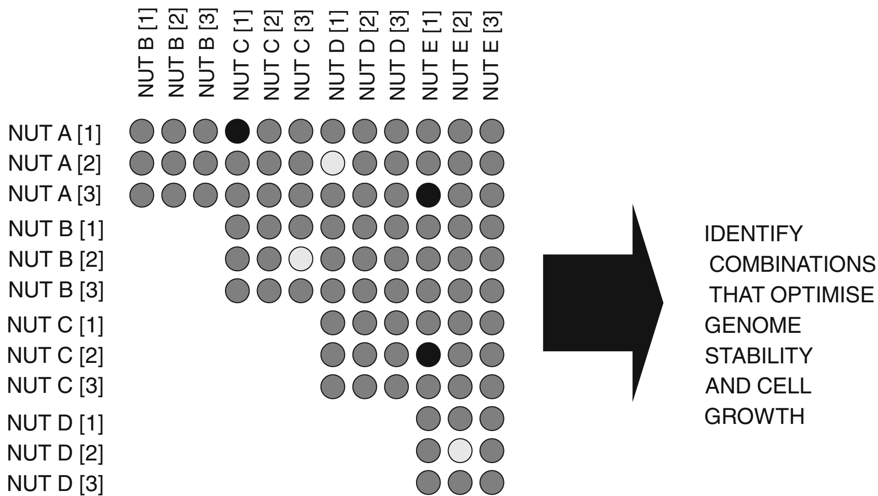

Fig. 2 Theoretical example of a simple nutrient array microculture system. NUT = single-nutrient or multiple nutrient combination; A–E = different types of nutrients or nutrient combinations; 1–3 = increasing dose levels. The different grey level colouring is simply an indication of the potential variability in cell growth, viability and genome stability that may be observed depending on the combinations used. The challenge is to identify the best combination or combinations for each individual

4 Testing Nutriomes in Nutrient Arrays

The biggest challenge in nutritional genomics is to make the quantum leap from a reductionist single-nutrient–single-gene interaction approach to studying the interaction of a complete nutrient combination (i.e. the nutriome) with the whole genome on an individual by individual basis. The ultimate goal is effectively to find for each individual the nutriome that best matches their genome so that cellular function and genome and epigenome maintenance are optimised. The "Rosetta Stone" (mechanism or code) to unravel this puzzle lies in developing nutrient arrays in microculture systems, such that multiple nutriomes can be simultaneously tested whilst taking into consideration impact of dosage in the assessment (Fig. 2). The microwell that produces cells than can proliferate adequately and viably whilst maintaining optimal genome and epigenome stability is likely to represent the best nutriome match for that individual's cells. The development of high-content automated analyses of DNA damage has already become feasible using quantitative image cytometry [57–60], such that multiple measures can be captured simultaneously in interphase cells including the number

of cells and their nuclear DNA content, multiple measures of genome stability such as telomere length and aneuploidy by FISH, oxidised guanine and DNA methylation by immunohistochemistry, chromosome damage and telomere end fusions by micronucleus cytome assays in cytokinesis-blocked binucleated cells and so on.

Such a system would also identify the deficiency and safe upper limit range for that individual for multiple micronutrients within a single scan and identify any unexpected combinations that could prove counter-intuitively cytotoxic or genotoxic. The plausibility of such a possibility is supported by our observation that genome instability increased under low folate conditions (20 nM) if riboflavin concentration was increased to replete status [29] possibly because the latter, which is the precursor of the FAD cofactor for MTHFR, increases MTHFR activity which catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate making the former folate species less bioavailable for dTMP synthesis from dUMP and thus increasing uracil in DNA. Excessive uracil in DNA causes abasic sites and DNA strand breaks when uracil glycosylases attempt to repair this highly mutagenic lesion [1, 18, 55]. Therefore, it is important to develop a nutrient array system that can efficiently interrogate multiple micronutrient combinations at different dosages. This type of approach has the added advantage that it becomes possible to identify an individual's nutriome for genome health maintenance without needing to know the person's genetic background. Furthermore, such systems could also be used to compare the response of different genotypes under the same nutriome conditions and estimate the percentage of the variance of the biomarkers measured that is explained by different genotype and different nutrients in the nutriomes tested including their interactions.

Prototypes of this approach have been designed by our group and others to investigate the following interactive effects on DNA damage, cell death and cell growth:

1. different ratios of sulphur- and seleno-methionine at constant physiological methionine concentration [54].
2. folate concentration with alcohol [61].
3. alcohol/acetaldehyde concentration and ADH1 or ALDH2 genotype [62, 63].
4. folate concentration with BRCA1 or BRCA2 genotype [64, 65].
5. folate concentration with riboflavin concentration with MTHFR C677T genotype [29].

In these studies, the CBMN Cyt assay was used to obtain multiple measures of chromosomal instability, cell death and cell division [66, 67]. The results of this approach are very promising because not only can they readily define the percentage variation in genotoxicity, cytotoxicity, metabolite and cell growth biomarkers which is attributable to a specific micronutrient, genotype and interactions between these parameters but they can also define the shape of the nutrient/DNA damage dose-response curve for genetically defined cell types. The use of the CBMN Cyt assay is particularly relevant for this purpose because the relative incidence of DNA damage, cell death events and cytostasis varies as

micronutrients and their concentrations within a nutriome are increased or decreased in multiple combinations. The relevant nutriomes within a single metabolic pathway may involve more than just two micronutrients; for example, the folate-methionine cycle requires folate in various forms as a substrate and betaine, vitamin B12, vitamin B6 and vitamin B2 as cofactors. Therefore, the nutrient array should also be designed to interrogate combinations of multiple micronutrients simultaneously in a dose-related manner and at different or contrasting dosage levels for each micronutrient relative to the others.

The in vitro nutrient array system would also be an ideal mechanism to test whether the predictions of emerging nutrigenomic mathematical models in specific key metabolic pathways [68, 69] actually hold true because this system is less likely than in vivo human models to be affected by problems relating to compliance to dietary intervention and unexpected lifestyle and exposure variables such as stress and recreational drug consumption as well as environmental genotoxicants which can impact on the genome damage indices measured. Furthermore, it is financially prohibitive to test multiple micronutrient combinations in vivo.

5 Nutrient Restriction for Cancer Growth Control

One of the greatest challenges in ageing populations is the need to prevent the proliferation of cancers which accumulate with age. Currently, there is no validated advice on the appropriate diet to adopt once a person is diagnosed with cancer because our knowledge on nutrient-gene interaction with respect to cancers is rudimentary. Furthermore, there is concern that supplementation with certain nutrients that are required for genome maintenance and cell growth (e.g. folate, methionine) may stimulate the cancer growth. Is it possible to identify the nutriome that prevents the growth of each cancer?

Ideally, nutriomes in nutrient array systems will be not only able to interrogate the optimal nutritional requirements for growth and genome maintenance of normal cells from an individual but also to verify that such a nutriome does not stimulate growth of cancer cells that the individual might have. Cancer cells are likely to have a markedly different genotype to that of the host's normal cells and could respond differently to the same nutriome environment. For example, some cancer cells amplify the high affinity folic acid receptor [68] giving them a distinct potential advantage over normal cells, when folate is limiting, in accessing folate from the surrounding fluid. The ideal nutriome for an ageing or cancer-prone individual would be the combination that not only sustains the replenishment of normal cells in a genetically integral manner but also inhibits the growth of cancer cells. It is conceivable that both normal cells and cancer cells from an individual could be simultaneously tested within a single-nutrient array system.

A nutrigenetic dietary restriction strategy for cancer growth control may be feasible. The strategy I propose is based on the following steps:

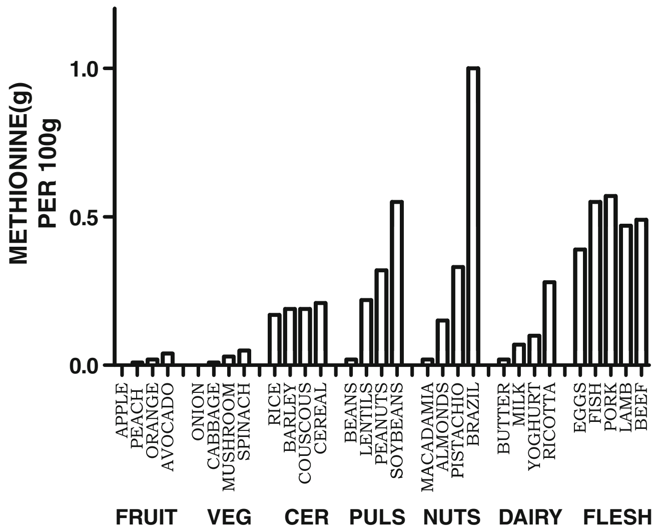

Fig. 3 The methionine content of typical fruits, vegetables (VEG), cereals (CER), pulses (PULS), nuts, dairy and flesh foods in grams per 100 grams. The methionine content was derived from tables such as the USDA Nutrient Database (http://www.nal.usda.gov/fnic/foodcomp/search/) and McCance and Widdowson's Composition of Foods Sixth Summary Edition

1. Identify the genetic defect that causes the cancer to be specifically and strongly dependent for a micronutrient required for DNA repair or growth.
2. Test susceptibility to micronutrient restriction in vitro using a nutriome-nutrient array system.
3. (1) Design personalised dietary restriction treatment to improve therapeutic ratio of therapy with genotoxic drugs or (2) design personalised dietary restriction therapy to inhibit growth or induce cell death of residual cancers post-therapy.

Recently, considerable interest has emerged regarding the use of methionine restriction to prolong healthy lifespan and to control growth of cancers (recently reviewed by Cavuoto and Fenech [70]). The reason for this possibility is that methionine dependency phenotype is a common feature of cancers and appears to be caused by mutations in methionine metabolism genes in either the salvage or de novo pathways. The best characterised and most common of these mutations is the deletion of the MTAP gene in the salvage pathway which often occurs together with the common CDKN2A (p16INK4) deletion found in 8–60 % of cancers depending on the cancer site. The coincidence of these two important genetic events occurs because these two genes are in very close proximity on the p arm of chromosome 9. A high level of methionine tends to fuel the growth of cells because it is required for polyamine synthesis. When MTAP is deleted in cancer cells, they are unable to regenerate methionine and thus are completely dependent

on dietary supply. Knowing that a cancer is methionine dependent therefore brings forward the possibility to give targeted nutritional methionine restriction advice to control a person's cancer should it have this critical mutation. The feasibility of this approach is currently being tested in our laboratory. Should methionine restriction prove to be a viable modality for personalised nutrition for cancer growth control, particularly in those individuals with cancers with the MTAP deletion, it will be necessary to give greater attention to the dietary sources of methionine.

A close inspection of Fig. 3 shows that the richest sources of methionine tend to be flesh foods, eggs, certain dairy foods (e.g. cheese), certain nuts (e.g. Brazil nuts) and certain legumes (e.g. soya beans). The poorest sources are fruits and vegetables. It is evident that a carefully constructed vegan diet rich in fruits and vegetables would be required to achieve a strong restriction of methionine intake.

6 Conclusions

In conclusion, the investigation of nutriomes and the use of nutrient array systems to interrogate genomic responses to multiple nutrient doses and combinations are in principle feasible and hold great promise to define the complete nutritional requirements, including supplementations and restrictions, of any cell type to either optimally sustain its growth and reproduction in a genetically stable manner in the case of normal differentiated progenitor cells and stem cells or suppress its growth and cause its death in the case of cancer cells.

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