The biological revolution – towards a mechanistic understanding of the impact of diet on cancer risk

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Abstract

There is strong epidemiological evidence to show that differences in diet explain a significant proportion of the variation in cancer incidence worldwide. However, because of the complex nature of eating behaviour and the chemical heterogeneity of foods, it remains very difficult to ascertain which aspects of diet, in what quantities and over what time-frames are responsible for modifying risk. In addition, there are few dietary intervention studies demonstrating reduction in cancer risk.

Much faster progress has been made in understanding the biological basis of cancer. It is now clear that damage to the genome resulting in aberrant expression of genes (principally suppression of tumour suppressor genes (TSGs) and inappropriate expression of oncogenes) is fundamental to tumorigenesis. It is also becoming clear that much of the inter-individual variation in cancer experience is due to differences in the amount of damage experienced and/or the capacity to repair that damage. Both of these processes are influenced strongly by dietary factors and by genetic predisposition (polymorphisms in the requisite genes). It is possible that understanding diet:gene interactions in DNA damage and in repair will not only explain much of the inter-individual variation in risk but also offer opportunities to design better dietary intervention studies aimed at chemoprevention.

The Human Genome maps and the SNPs databases, together with the rapid development of tools suitable for investigating genetic and epigenetic changes in small tissue biopsies provide the means to begin to test hypotheses about the mechanisms by which diet influences cancer risk directly in human subjects. This is likely to form a significant component of the emerging science of nutrigenomics.

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1. Introduction

The genome is subject to frequent insults from a variety of external agents and from normal cell functions including mitosis. This is counter balanced by a battery of defense mechanisms which (i) attempt to minimise DNA damage (such as detoxification of potential mutagens and inhibition of oxidation reactions by free radical scavenging), (ii) repair damage or (iii) shunt the damaged cell into apoptosis. All of these processes, including induction of apoptosis [1] are potentially modifiable by nutrition (Fig. 1). The challenge is to understand at a mechanistic, molecular level how food constituents or metabolites can influence the neoplastic process to provide the evidence base for the development of effective chemoprevention. The on-going biological revolution with
Fig. 1. Tumours may arise if damage to the genome is not repaired. All of these processes may be influenced by nutrition.

Fig. 2. Alternative pathways to colorectal cancer. Loss of expression of tumour suppressor genes can occur by mutation or by hyper-methylation of CpG islands in the promoter region.

2. Biology of cancer

According to classical cancer biology, a tumour occurs when a number of key cellular functions are dysregulated because of mutations in tumour suppressor genes (TSGs) and inappropriate expression of oncogenes which give the nascent tumour cell a competitive growth advantage. Knudson’s ‘two-hit’ hypothesis explains the loss of TSG function through gene mutation or chromosomal loss. More recently, it has become clear that the loss of the second allele and, therefore, TSG silencing may occur quite frequently through aberrant hyper-methylation of CpG islands in the promoter region [2] (Fig. 2). The TSG APC is the gate-keeper gene for the large bowel. Since the APC protein plays a role in many core cell functions including cell cycle regulation, signal transduction, apoptosis, cell migration, microtubule assembly and chromosome segregation [3–5] and dysfunction of these processes are hallmarks of tumour cells [6], the tumour initiating potential resulting from loss of both APC alleles and absence of a functional APC protein is readily understood.

A large majority of human cancer cells display genetic instability as a result of inherited or acquired defects in the genes that monitor genomic integrity and repair genomic damage. These include the consortia of genes responsible for:

- base-excision repair,
- nucleotide excision repair,
- DNA mismatch repair and
- maintenance of telomere function.

The extent to which genomic instability is an initiating event and/or an important driver of tumour development remains controversial [7,8] but there is no doubt that such instability allows tumour cells to acquire novel or enhanced properties which give them a competitive advantage.

Whilst a small proportion of the cases of cancers at common sites are due to inherited mutations in high-penetrance genes, e.g. retinoblastoma (the RB1 gene), some familial forms of breast cancer (BRCA1 and BRCA2) and the rare bowel cancer known as familial adenomatous polyposis (APC), most cancers are polygenic [9]. However, as is the case with cardiovascular disease, advances in understanding the origins of human cancer may emerge from recognition of the
importance of interactions between environmental factors and genetic susceptibility (illustrated in the simple model in Fig. 3). If dietary factors have a significant role in cancer prevention, and in the light of current understanding of the biology of tumorigenesis, what are the molecular mechanisms by which food components could act?

3. Epidemiology of diet and cancer

The 1981 landmark review by Doll and Peto provided quantitative estimates of the proportion of the variation in cancer incidence accounted for by a range of environmental factors including diet [10]. This established that smoking and dietary behaviour each accounted for about one third of the variation in cancer risk and emphasised that the majority of cancers in those under the age of 65 years is potentially avoidable. However, it has proved to be very difficult using conventional epidemiology to identify which aspects of food, in what amounts and over what time-frames are hazardous or protective. Given the biological and chemical complexities of foods, the absence of comprehensive food composition tables covering bioactive compounds other than nutrients and the considerable difficulty in quantifying human habitual food intake, this should not be too surprising.

Of the epidemiological approaches possible, cohort studies offer some of the strongest evidence of linkage between dietary intake and cancer risk especially when the number of study participants is large and both dietary habits and disease experience are diverse as in the European Prospective Investigation into Cancer and Nutrition (EPIC) [11]. Recent papers from the EPIC study demonstrate protection against colorectal cancer among those consuming higher intakes of dietary fibre [12] whilst breast cancer risk appears to be exacerbated by high intakes of fat [13]. Amongst the strengths of the EPIC study is the collection of biological material prospectively which enables the investigation of links between biomarkers of nutritional status, e.g. plasma ascorbic acid concentration and disease outcomes [14]. However, such biobanks are potentially even more valuable because they provide material for (a) the development and testing of novel molecular markers of dietary exposure and (b) studies of diet:gene interactions in relation to cancer risk.

4. Aspirin as a model chemoprevention agent

Salicylates occur widely in many food plants where they play a central role in disease resistance [48] and in protection against consumption by herbivores [49]. More salicylates are excreted in the urine of vegetarians than non-vegetarians [50] which may be a reflection of the importance of vegetables and fruits as sources of salicylates in the human diet. Whilst amounts of bioavailable salicylates in conventional Western diets appear to be low [51,50] relative to that consumed as aspirin (acetyl salicylate), studies with aspirin provide evidence of the possible benefit of raised salicylate intake, and of potential mechanisms underlying that benefit. There is impressively consistent observational epidemiology in support of the hypothesis that frequent consumption of aspirin over a prolonged period reduces the risk of colorectal adenoma, CRC and death from CRC [15] with some
Table 1
Effects of aspirin intervention on recurrence of adenomas in the large bowel

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Aspirin dose (mg per day)</th>
<th>Relative risk mean (95% CI)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous colorectal adenoma</td>
<td>81</td>
<td>0.83 (0.70–0.98)</td>
<td>Baron et al. [18]</td>
</tr>
<tr>
<td>Previous colorectal adenoma</td>
<td>325</td>
<td>0.95 (0.80–1.12)</td>
<td>Baron et al. [18]</td>
</tr>
<tr>
<td>Previous colorectal cancer</td>
<td>325</td>
<td>0.65 (0.46–0.91)</td>
<td>Sandler et al. [17]</td>
</tr>
</tbody>
</table>

CI: confidence interval.

Evidence of ‘protection’ against cancers at other sites including the breast and ovaries (reviewed in [16]). Such evidence, combined with the relative safety of aspirin, has been sufficient to justify its use in human intervention trials. The results of the first two randomised, placebo-controlled intervention studies designed specifically to test the effectiveness of aspirin on adenoma recurrence have been published recently. In both patients with previous large bowel carcinoma [17] and those with previous colorectal adenomas [18], the adjusted relative risk of adenoma recurrence was reduced in those assigned to the aspirin intervention. However, the effects were relatively modest (Table 1) and, curiously, the lower (81 mg per day) but not the higher (325 mg per day) dose of aspirin resulted in a significant reduction in adenoma recurrence in patients with a recent history of histologically documented colonic adenomas [18]. In addition, treatment with aspirin reduced intestinal tumorigenesis in some [19,20] but not all [21] studies in the Apc\(^{Min}\)/+ mouse. This discrepancy in observations from the mouse studies may be due to the time period of aspirin exposure. Early administration of aspirin, e.g. to the mother during pregnancy and lactation may be more effective in suppression of intestinal polyp development in Apc\(^{Min}\)/+ mice [22]. This may offer clues as to the mechanism of action of aspirin. In common with other non-steroidal anti-inflammatory drugs, aspirin inhibits cyclooxygenases (COX) to some extent and upregulation of COX-2 appears to be causally involved in large bowel tumorigenesis. Whilst knockout of the COX-2 gene or administration of COX-2 selective inhibitors reduce tumour multiplicity in mouse models [23] and in people with familial adenomatous polyposis [24], it seems probable that aspirin’s anti-neoplastic action may involve additional pathways including apoptosis. Aspirin treatment enhances apoptosis in CRC cell lines [25,26] and regular aspirin use was associated with higher rates of mucosal cell apoptosis and with a lower prevalence of large bowel adenomas [27]. Further molecular targets for aspirin action including genes involved in regulation of transcription, signal transduction, cell cycle regulation, as well as apoptosis, are beginning to emerge from expression profiling studies using cDNA microarrays applied to CRC cells lines [28].

It is clear that not everyone appears to benefit, in terms of protection against CRC, from aspirin exposure and it is highly probable that responsiveness to any chemoprevention agent will be influenced by individual genotype. One example is the role of ornithine decarboxylase (ODC) in the APC-regulated pathway for production of polyamines (Fig. 4). When the APC

![Ornithine decarboxylase (ODC) expression is regulated by APC](image_url)
Table 2

<table>
<thead>
<tr>
<th>ODC genotype</th>
<th>No aspirin</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>1.0</td>
<td>0.83 (0.51–1.34)</td>
</tr>
<tr>
<td>GA</td>
<td>1.05 (0.70–1.58)</td>
<td>0.64 (0.37–1.09)</td>
</tr>
<tr>
<td>AA</td>
<td>0.68 (0.30–1.51)</td>
<td>0.10 (0.02–0.66)</td>
</tr>
</tbody>
</table>

Data are odds ratios (with 95% confidence intervals) resulting from logistic regression models controlling for age, gender and number of colonoscopies. Genotype refers to a single nucleotide polymorphism at base +317 (relative to the transcription start site) in the ODC gene.

Gene is active, c-myc is switched off and Mad-1 expression is up-regulated. Mad-1 binds to an E-box within the ODC promoter and blocks ODC expression leading to lower concentrations of polyamines [29]. However, Mad-1 binds to this E-box only when an A rather than a G is found at base +317 within intron 1 of the ODC gene [30]. Those who are homozygous for the rarer (A) variant of ODC have about half the risk of adenoma recurrence as those homozygous for the common (G) variant [29] whilst regular aspirin users who are also homozygous for the A variant have only one tenth of the risk of GG non-aspirin users (Table 2). It is suggested that aspirin enhances the effect of the AA polymorphism by stimulating polyamine catabolism [29].

5. Single nucleotide polymorphisms and inter-individual variation in cancer risk

Given their key role in maintenance of genomic integrity, coding changes which modify the function of proteins encoded by DNA repair genes, i.e. functional single nucleotide polymorphism (SNPs) would be expected to alter cancer risk. Whilst several such SNPs have been reported (see, e.g. [31]) and associations between specific SNPs and cancer risk have been described, a recent review has concluded that many of these results must be considered equivocal because small sample numbers will have contributed to both false-negative and false-positive findings [32]. Further, the likelihood of a specific SNP being associated with altered cancer risk will depend on relevant environmental exposures. For example the protective effect against lung cancer of an A → G substitution in the 5’-end of the non-coding region of the XPA gene (a zinc-finger DNA binding protein which is an essential component of the nucleotide excision repair consortium) is greater in smokers than in non-smokers [33]. Interactions between dietary (and other environmental) factors and SNPs in specific genes may explain much of the inter-individual variation in cancer experience and provide a mechanistic explanation for the lack of evidence of effect of some promising dietary factors in chemoprevention studies. This provides the rationale for prospective genotyping of subjects for intervention studies and holds out the promise of individualised cancer prevention as well as treatment strategies. Whilst genomic profiling offers a rational basis for developing personalised dietary and lifestyle advice, realising this potential will require a substantial investment in well-designed epidemiological and intervention studies [34]. Such studies bring with them many challenges [35] including issues of informed consent [36]. It remains to be seen whether knowledge of one’s genotype is an effective motivator of behaviour change or whether it leads to fatalism and adoption of high risk lifestyles. Until appropriate research has been undertaken to address these questions, caution should be exercised in raising expectations among the public.

6. Diet:gene interactions and nutrigenomics

The potential for a wide range of dietary factors to modulate molecular signalling and to contribute to cancer prevention has been the subject of excellent recent reviews [37,38]. The diversity of these food constituents or metabolites and of the metabolic events that they influence is a very good example of where post-genomic technologies can provide the tools to investigate many of these events simultaneously. The emerging science of nutritional genomics or nutrigenomics is the application of high-throughput genomics tools such as transcriptomics, proteomics or metabolomics in nutritional research [39]. Such techniques are already in use to characterise tumour tissues [40] and to help identify the molecular mechanisms by which food components may exert anti-neoplastic effects [41]. Whilst some of the practical issues in the use of these approaches in vivo are being addressed, the appropriate bioinformatics tools and of the conceptual frameworks for analysis and
interpretation of the large amounts of data generated remain poorly developed.

7. Molecular markers of dietary exposure

Much nutritional epidemiology is limited by the quality of the available data on dietary exposure. All available observational methods is for recording diet prospectively or for assessing past eating habits are subject to an unknown degree of reporting bias. There has been some progress in developing biomarkers of dietary exposure, e.g. concentrations of food-derived substances in biological fluids or in tissue biopsies, but it is usually not possible to distinguish long term from recent exposure. There is growing recogni- tion that epigenetic changes, e.g. patterns of DNA methylation and of histone decoration, are influenced by dietary and other environmental factors [42–44]. Further, because such epigenetic marking is copied faithfully from one cell generation to the next, it may provide a long term record of environmental exposure [45,46]. Making the most of the opportunity to de-

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