The initiation of breast and prostate cancer

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The agents responsible for the initiation of human mammary and prostatic cancers remain unidentified. Population migration studies on breast and prostate cancer risk have revealed that incidence rates in migrants from low-risk to high-risk ‘Westernized’ countries rise over time to match those of the host populations. The parallels suggest that the two diseases may share a common aetiology, with changes in diet, rather than in environment, being responsible for the migration-related increases in cancer incidence. Genotoxins, such as polycyclic aromatic hydrocarbons and heterocyclic aromatic amines, are formed when foodstuffs are cooked at elevated temperatures and can be extracted with solvents: other genotoxins may only be released from cooked proteins when digestion occurs in the gastrointestinal tract. Human mammary and prostatic epithelial cells are known to be capable of metabolically activating members of different classes of chemical carcinogens to DNA-reactive species and, in rodents, five out of six mammary carcinogens can also induce prostatic neoplasms. Genotoxins have been detected in some 40% of breast lipid and milk samples donated by UK-resident women but the agents, currently thought to be of dietary origin, have not been characterized or identified as yet. Reduction mammoplasty and lactation both reduce breast cancer risk and the reduction is proportional either to the amount of tissue removed or to the total duration of lactation. As DNA damage has been detected in otherwise untreated mammary epithelial cells isolated both from breast tissue and from breast milk, we have proposed that reduction mammoplasty and lactation reduce risk through a common mechanism, i.e. the loss of pre-malignant cells. Further research, perhaps aimed particularly at the characterization of all the carcinogens formed when different dietary components are cooked in different ways, should succeed in identifying the agents that initiate breast and prostate cancer.

Introduction

Breast and prostate cancers occur with higher frequency in Western societies where, in ageing populations they account for an increasing proportion of cancer morbidity and mortality (1). There are quite marked international variations in the incidence of these two cancers (2). However, the factors that initiate breast and prostate cancer still remain to be identified as an essential prelude to strategies designed to reduce disease occurrence. Commonalities between breast and prostate cancer suggest similar aetiological risk factors and the involvement of the same causative agents.

Increased incidence rates for both cancers are associated with the development and adoption of a Western diet as typified by high intake of cooked proteins and fat that, in terms of mankind’s evolution, has been a dramatic departure from our prehistoric diet (3). Gene alterations, i.e. mutations, undoubtedly play a significant role in the ‘multi-stage’ process of malignant transformation. Cancers of the breast and prostate are associated with very low frequencies of familial predisposition (4,5). The non-hereditary cancers arise following at least two random mutational events in somatic cells (6) and, even in cases of familial predisposition, other subsequent somatic mutations are still thought to be required (7).

The implications are that dietary/environmental factors are important determinants in the aetiology of both non-hereditary and hereditary cancer. However, the occurrence of two successive hits or mutational events becomes more likely as individuals age. Non-hereditary breast and prostate cancers are commonly described as ‘idiopathic’ or ‘sporadic’, terms that half-imply the lack of causative agents and, therefore, the absence of any need to identify them.

This commentary discusses factors that may influence the incidence of breast and prostate cancer, why these organs might be particularly susceptible to DNA-damaging agents and the possible sources of such agents. It also puts forward future avenues of research that may help to identify the cause. We have proposed that breast and prostate cancer have a ‘shared’ aetiology and that dietary factors play a significant role in the causation of both.

Breast cancer aetiology

Breast cancer currently accounts for 20% of all female cancers worldwide and is the most frequent malignancy occurring in women (4): in Western Europe and North America, one in every eight or nine women will develop the disease. Inheritance of high penetrance susceptibility genes, such as BRCA1 and BRCA2, account for only 5% of breast cancer cases and the factors responsible for the other 95% remain obscure (4). A significant proportion of sporadic breast cancers contain point mutations clustered within exons 5–8 of the TP53 gene, and this mutational spectrum suggests the occurrence of xenobiotic-induced mutagenic events in some cases (8,9).

The only environmental exposure proven to induce breast cancer is ionizing radiation (10). Total cumulative exposure to oestrogen may play a role in breast cancer incidence and is consistent with nulliparity, late age at first pregnancy, early menarche and late menopause as weak risk factors (11).
However, oestrogens probably act as tumour- and growth-promoters rather than as complete carcinogens (4) but this is not a view that is universally accepted (12). A correlation between alcohol intake and breast cancer incidence amongst individuals lacking both the glutathione S-transferase (GST)M1 and GSTT1 genes has been reported (13). However, different patterns of alcohol intake make it difficult to quantify the importance of this risk factor (14). Finally, it is also possible that consumption of well-done meats may correlate with an increased risk of breast cancer (15). So far, classic epidemiological studies have failed to highlight a dominant risk factor that could account for sporadic breast cancer incidence and investigations into the relationship of risk to gene–environment interactions are still in their infancy (16).

Prostate cancer aetiology
Pathological abnormalities occur more frequently within the prostate gland than anywhere else in the human male (17). Such changes increase in prevalence with increasing age and include benign prostatic hyperplasia, which tends to occur in the transitional zone, and adenocarcinoma, which arises mainly in the peripheral zone (17). Although prostate cancer is the second most common cause of cancer mortality in men in the UK and in the US (18,19), the mortality rate from prostate cancer is ranked only eleventh amongst all types of cancer in males in Japan (20). Thus, prostate cancer is characterized by marked differences in both its ethnic incidence (21) and in its worldwide distribution (22). Susceptibility genes appear to account for no more than a small proportion of cases (5). It has been proposed that androgens play a significant role in prostate cancer development (23) but this hypothesis remains controversial (24). Ageing is the most significant risk factor identified to date (25) and, although the World Health Organization lists meat and animal fat intake as other risk factors (26), the aetiology of prostate cancer is still poorly understood.

Migration
The best evidence that diet and/or environment play a significant role in the aetiology of both breast and prostate cancer comes from studies in which incidence rates or mortality have been determined for migrants moving from lower risk to higher risk countries. For example, data from 1962–1971 showed that, although breast cancer mortality in recently arrived Italian migrants to Australia was low, it had risen to match that of the Australian-born population in immigrants resident for more than 17 years (27). Similarly, breast cancer risk in females of Chinese, Japanese or Filipino origin migrating to the US increased by 80% in those resident for > 10 years (28).

Prostate cancer incidence varies widely (29). Whilst the prostate cancer incidence rate for men in mainland China is very low (1.3 per 100 000) it is 5-fold higher for Chinese men in Hong Kong and 16-fold higher for Chinese men living in the USA (30). Japanese migrants to Hawaii or the US mainland and Japanese living in Brazil have higher prostate cancer incidence rates than Japanese men in Japan (31). However, within the USA, no differences in diet and/or lifestyle have been found that would account for the higher prostate cancer incidence in Afro-American males compared with Caucasians (21).

The effect of age at migration on both breast and prostate cancer incidence has been examined in Japanese migrants to the west coast of the USA. Prostate cancer incidence rates for ‘early-in-life’ migrants and for US-born Japanese were 4-fold higher than for Japanese men in Japan (32). The same study also showed that breast cancer incidence rates in US-born Japanese females were 2.6-fold higher than for Japanese women living in Japan and that, for ‘early-in-life’ Japanese migrants to the USA, the incidence was 2.4-fold higher.

As increases in breast and prostate cancer incidence in migrants cannot be put down to ethnic differences, changes in other factors, including lifestyle, environment, diet and cooking practices, that occur following migration need to be examined. Almost 30 years ago McMahon predicted that breast cancer risk in Asian-American women would continue to rise due to the assimilation of Western lifestyles (33). Increases in incidence with time in the West have been detected with rates up to, and even exceeding, those in US Caucasians (28).

Thus, accumulating evidence from migration studies indicates that changes in diet and/or environment can, over time, markedly affect both breast and prostate cancer incidence rates. Although the dietary or environmental agents responsible for the initiation of the two diseases remain unidentified, the parallels in migration patterns strongly suggest that they share a common aetiology.

Diet or environment?
Although changes in both the diet and the environment clearly do occur following migration from countries with low to countries with higher breast and prostate cancer incidence rates, the main factor responsible has not been determined. There is no published evidence that points the finger at environmental change. Indeed, one might suppose that moving from, say, Japan to Hawaii would not result in a ‘worse’ living environment. On the other hand, there is some evidence, albeit circumstantial, that implicates dietary changes. The diets and the cooking practices of migrants are known to become progressively more ‘Westernized’ following migration (34,35) and, in Japan itself, the ‘Westernization’ of diet has been found to correlate with recent increases in breast cancer incidence (2,36,37). Anecdotal evidence suggests that, whilst Japanese expatriates living temporarily in the UK make every effort to adhere to their oriental diet, prepared in the traditional manner, their offspring are keen to abandon, for example, steamed rice and vegetables and raw fish in favour of a more ‘Western’ cuisine. A simultaneous fall in the consumption of protective dietary elements such as soy may also occur (38).

The authors’ view is therefore, that it is changes in diet rather than the environment that are responsible for the increases in the incidence of breast and prostate cancer in migrants referred to above. If this is correct, then logic dictates that the many thousands of new cases of breast and prostate cancer currently labelled as ‘sporadic’ that are seen every year in developed countries are also caused by tumour-initiating agents that are of dietary origin.

Animal models
Mammary cancer
The chemical carcinogens that have induced mammary tumours in rodents include the polycyclic aromatic hydrocarbons (PAHs) benzo[a]pyrene (BaP), 7,12-dimethylbenz[a]anthracene (DMBA) and dibenz[a,l]pyrene, the nitropolycyclics (NO2-PAHs) 2-amino-1-methyl-6-phenylimidazo[4,5-b]
rodent prostate carcinogens are fat-soluble compounds that require metabolic activation and perhaps it is significant that five out of the six also induce rodent mammary tumours.

**Dietary carcinogens**

**HAAs identified to date**

In 1939 roasted food was shown to possess cancer-producing substances (47) and, more recently, well-done meat was found to contain a level of mutagenicity that could not be attributed to B[α]P content alone (48) and which was possibly due to protein-derived mutagens (49). Since then much effort has been invested into determining the factors affecting the formation, yield and structures of HAAs (for recent reviews see refs 50–53). These compounds are formed at parts-per-billion (p.p.b.) levels in, for example, fried or grilled meats as the products of protein pyrolysis or Maillard reactions (50) and have been produced in modelling reactions in which amino acids such as glycine or alanine are heated, either dry or in solution, in the presence of creatinine or creatine (51,52). Because of their ability to induce tumours in rodent bioassays (53), suspicions that HAAs may contribute to human cancer risk have been aroused (54). However, there is as yet no definitive proof of their involvement in the initiation of human cancer (55). Their characteristic structure includes one or two heterocyclic rings fused to an aminimidazol ring. These imidazole mutagens include the aminimidazo-quinoline derivatives, e.g. 2-amino-3,8-dimethylimidazo[4,5-f]quinoline, the aminimidazo-quinoline derivatives, e.g. IQ, and the pyrido-indole derivatives, e.g. PhIP. The quinolines and quinoxalines are strongly mutagenic to bacteria whereas the pyridine derivatives are much less potent (56,57). However, the pyridines seem to possess greater genotoxicity in test systems involving mammalian cells (56,57). HAAs require a two-step metabolism via oxidation of the exocyclic amino group, a reaction mediated mainly by the cytochrome P-450 isoenzyme CYP1A2 (58), followed by esterification with acetyl or sulphate groups, catalysed by O- or N-acetyltransferases or sulphotransferases, respectively (54). Covalent DNA adducts are formed within the exocyclic amino group binding preferentially to the C8 position of guanine (59,60). Although HAAs are generally perceived as being products of high-temperature cooking practices, IQ, 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) are also formed when aqueous homogenates of beef are heated at 100°C for 30 min (61). Although some 24 HAAs have either been identified or partially identified (62), >70% of the mutagenic activity extractable from grilled, fried or broiled meat samples remains unaccounted for (63).

**Unidentified HAAs**

Recent work has shown that, within a series of HAAs, there is a rough inverse correlation between mutagenic potency in a *Salmonella typhimurium* assay and activity in inducing the morphological transformation of mouse prostate cells (56) (see Figure 2). Mutagenicity in *S. typhimurium*-based assays has been used almost universally in the purification and isolation of HAAs from complex mixtures (63). The fact that the most active HAAs in the mouse cell morphological transformation assay were the least mutagenic in *S. typhimurium* raises an interesting question. Do the diet-derived complex mixtures from which known HAAs have been isolated (64,65) also contain other similar compounds which are biologically active?
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Fig. 2. Comparative biological activities of HAAs in each of three genotoxicity assays and the morphological transformation assay. Values are normalized to the most active HAA in each assay, assigned an arbitrary value of 100 and shown on the vertical axis (taken from ref. 56). The abbreviations used are: PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; MeA(a)C, 2-amino-3-methyl-9H-pyrido[2,3-b]indole; A(a)C, 2-amino-9H-pyrido[2,3-b]indole; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline; 8-MeIQx, 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline.

in, say, a cell transformation or a Comet assay system but which have not been detected and isolated solely because they are not active in the standard S.typhimurium-based mutagenicity assay which has been universally applied in the isolation of HAAs? Such genotoxins, inactive in the S.typhimurium assay but active in other genotoxicity test systems, would thus be present in addition to the total detectable (S.typhimurium) mutagenic activity referred to above. This possibility may merit further examination especially if reliable total daily intake data are required.

Proteolytic release of genotoxins

As noted above, there is circumstantial evidence that implicates dietary factors in the initiation of breast and prostate cancer. Although neither the agents responsible nor the manner in which they arise have so far been identified, some progress, in a negative sense, has been made. Analytical comparisons between genotoxic and inactive extracts of breast lipid and/or milk (see below) have enabled several of the most obvious classes of chemical carcinogens to be ruled out (unpublished data), including 13 HAAs that were searched for using GC/MS with selective ion monitoring (66).

Are there, therefore, other genotoxins present in the diet and that have so far avoided detection and identification? When proteinaceous foods are heated, free HAAs are formed that are extractable by well-documented methods (67–69). Higher yields can be produced if the protein is hydrolysed to its amino acid constituents prior to heating (70,71), in agreement with the results obtained in modelling reactions (72).

What does not appear to have been examined previously is the notion that genotoxins are formed in heated proteins that remain integral with and covalently bound to the protein until hydrolysis occurs in the gastrointestinal tract. Condensation reactions between, for example, C- or N-terminal aromatic amino acid residues and creatine or creatinine might be expected to yield HAA-like compounds. The products would not be extractable prior to proteolysis and would therefore remain undetected: they would not necessarily be identical to known HAAs as either the carboxylic acid or the amino group will have been part of a peptide bond when, or if, condensation reactions occurred (with ‘internal’ amino acid residues both the carboxylic and the amino groups would be unavailable for condensation reactions).

Results from a preliminary experiment have lent some support to this idea. Thus, when an aqueous homogenate of ‘well-done’ hamburger was proteinase K-digested, genotoxic (Comet assay employing MCL-5 cells) and mutagenic (S.typhimurium TA1538 and YG1019) material became extractable that was not apparently solvent-soluble before proteolysis (F.L.Martin, K.J.Cole, D.H.Phillips and P.L.Grover manuscript in preparation). Further work is required to (i) identify the
genotoxins released by hydrolysis of heat-treated proteins and (ii) to assess the contribution that such putative ‘closet carcinogens’ may make to the levels of genotoxins that are ingested in the diet.

Metabolic activation in breast and prostate

The wide substrate specificity and inducibility of mammalian enzyme systems is required for endogenous compound synthesis and metabolism (73,74). However, such enzymes may also activate carcinogens to DNA-damaging species.

Human mammary epithelial cells (HMECs) possess a complex metabolic machinery that, dependent on different variables such as genotype, can activate or deactivate potential carcinogens (for a recent review see ref. 16). Metabolizing enzymes include CYP enzymes that may catalyse the C- or N-hydroxylation of carcinogens such as PAHs or HAAs, epoxide hydrolase which is involved in PAH activation, N-acetyltransferase (NAT) that may further metabolically activate N-hydroxylated HAAs or aromatic amines by O-acetylation, and sulfo transferases that may activate metabolites of PAHs, aromatic amines or HAAs (15).

In human prostate, expression of mRNA transcripts for phase I-activating enzymes such as CYP1A2, CYP1A1 and CYP1B1 has been demonstrated (75). mRNA transcripts for phase II-conjugating enzymes such as NAT1 and NAT2 are expressed in human prostatic epithelium (76), and SULT2B1 mRNA transcripts have also been detected in human prostate (77). Conversely, loss of GSTP1 expression in human prostate appears to enhance its susceptibility to carcinogenic insult by compounds such as N-OH-PhIP (78). However, a lot more research is required to raise our current level of understanding of the metabolizing capabilities of the prostate to that of our understanding of enzyme expression in the breast.

Breast cell initiation—genotoxins in lipid and milk

In structural terms the breast is a unique organ in that it consists of 70–90%, by weight, of a fatty stroma. Dispersed in this lipid matrix are the functional elements lined with epithelial cells and it is from these cuboidal or low columnar cells that carcinomas of the breast arise. Compounds present in the human diet and/or environment that are capable of inducing mammary cancer in rodents include PAHs, nitro-PAHs and HAAs (34). Many are fat-soluble and it may be that this physicochemical property enhances the exposure of mammary epithelia to such carcinogens. The detection of low (p.p.b.) concentrations of aromatic amines, one of which is known to be a rodent mammary carcinogen, in breast milk has been reported (79). Organochlorines such as lindane have also been found in both breast tissue and breast milk (80,81).

Human mammary lipid can be obtained following collagenase digestion of tissues removed at elective reduction mammoplasty (82) and breast milk is a natural, lipid-containing medium that has been used as a source of mammary epithelial cells (83). When 20 breast milk extracts were tested in S.typhimurium, six produced a positive mutagenic response (84). Six samples (four of which were positive for bacterial mutagenicity) also induced significant micronucleus formation in MCL-5 cells.

Extracts of human mammary lipid and of breast milk induced comet formation in MCL-5 cells in the presence of the DNA-repair inhibitors, hydroxyurea and cytosine arabinoside (84,85). In addition, it was found that HMECs, from several donors, obtained either following collagenase digestion of elective reduction mammoplasty tissues or recovered as exfoliated breast milk cells, contained ‘pre-existing’ DNA damage. It was also noticed that the most active extracts, either of lipid or of milk, tended to come from those donors whose cells also contained the most pre-existing DNA single-strand breaks (85–87).

Interindividual variations were observed in the ability of mammary lipid and breast milk extracts to induce morphological transformation of C3H/M2 mouse fibroblasts. Morphologically transformed foci of C3H/M2 mouse fibroblasts have formed tumours that metastasize in nude mice (88). The ability of mammary lipid extracts and breast milk extracts to induce the morphological transformation of C3H/M2 mouse fibroblasts certainly heightens the suspicion that genotoxins detected in breast lipid and breast milk (66,89) may be involved in mammary tumour initiation but does not, of course, prove it. There is no evidence that these agents accumulate in mammary lipid and the fluctuations over time in breast milk genotoxin levels that occur during lactation (84) are more suggestive of variations in dietary intake than of clearance.

Initiation in prostate

Epidemiological evidence linking high meat consumption with an elevated risk of prostate cancer (26) suggests that prostatic epithelial cells possess the metabolic machinery required to activate dietary carcinogens. F344 rats that had received 400 p.p.m. of PhIP in the diet for 52 weeks were found to have developed prostate carcinomas that were limited to the ventral lobe in 18 out of 27 animals (42). It is now known that PhIP causes G:C → T:A transversions and deletions of G:C base pairs thus confirming its mutagenicity in rat prostate (90). Recent work suggests that human prostate epithelial cells may be particularly susceptible to the DNA-damaging effects of PhIP, its N-hydroxy metabolite (N-OH-PhIP) and B[a]P (91,92). Differences in the p53 gene mutational spectra of prostate cancers between different populations suggests that different agents have acted as initiators (93). Thus, the human prostate may well be a target for exogenous carcinogens that require metabolic activation.

Does lactation protect through breast cell loss?

Breastfeeding is one factor that is known to reduce breast cancer risk (94–96). A recent study in Chinese women indicates that the cumulative duration of lactation is proportional to the reduction in risk (96) and an Icelandic cohort study reached a similar conclusion (97). Changes in hormone levels have been suggested as a reason for the reduced risk (94).

Reduction mammoplasty also reduces breast cancer risk and the reduction in risk is related to the amount of tissue removed (98). However, this relationship did not hold in one experiment in rats that had been pre-treated with the mammary carcinogen DMBA prior to surgical removal of mammary fat pads (99).

No satisfactory biological explanation for the protective effect of lactation that would also apply to reduction mammoplasty has so far been advanced (94). One possible mechanism would involve the exfoliation of initiated cells. Breast milk contains $1 \times 10^4$ to $1 \times 10^5$ cells/ml (84), a high proportion of
which are epithelial in origin (100) and are known to contain DNA damage (86). To take an extreme example, lactation for up to a total of 109 months, as noted in the recent Chinese study (96) at a rate of 750 ml milk/day (101), could involve the loss of some \(10^{11}\) breast cells. With shorter periods of lactation, cell loss may still be significant, in terms of risk, even if only a small proportion of the cells shed were pre-malignant. Reduction mammoplasty would also involve the loss of initiated cells and would be expected to affect risk in a similar manner.

Concluding remarks

The agents that initiate breast and prostate cancer remain unknown. If, as seems most likely, changes in diet are responsible for the increases in breast and prostate cancer incidence that occur in migrants, then the dietary components responsible need to be identified because the same compounds will almost certainly be involved in the initiation of these diseases in the much larger host populations. Such studies must include the detection of the genotoxins formed when foodstuffs are cooked in different ways. The use of more than one biological endpoint will be required since some substances that are weak or inactive as mutagens in the Ames test are one biological endpoint will be required since some substances may be easier than persuading damaging agents in the diet may be easier than persuading people to change the cooking procedures in which they are formed.

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