Alcohol and cancer: genetic and nutritional aspects

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Chronic alcohol consumption is a major risk factor for cancer of upper aero-digestive tract (oro-pharynx, hypopharynx, larynx and oesophagus), the liver, the colo-rectum and the breast. Evidence has accumulated that acetaldehyde is predominantly responsible for alcohol-associated carcinogenesis. Acetaldehyde is carcinogenic and mutagenic, binds to DNA and protein, destroys the folate molecule and results in secondary cellular hyper-regeneration. Acetaldehyde is produced by mucosal and cellular alcohol dehydrogenase, cytochrome P450 2E1 and through bacterial oxidation. Its generation and/or its metabolism is modulated as a result of polymorphisms or mutations of the genes responsible for these enzymes. Acetaldehyde can also be produced by oral bacteria. Smoking, which changes the oral bacterial flora, also increases salivary acetaldehyde. Cigarette smoke and some alcoholic beverages, such as Calvados, contain acetaldehyde. In addition, chronic alcohol consumption induces cytochrome P450 2E1 enxyme activity in mucosal cells, resulting in an increased generation of reactive oxygen species and in an increased activation of various dietary and environmental carcinogens. Deficiencies of riboflavin, Zn, folate and possibly retinoic acid may further enhance alcohol-associated carcinogenesis. Finally, methyl deficiency as a result of multiple alcohol-induced changes leads to DNA hypomethylation. A depletion of lipotropes, including methionine, choline, betaine and *S*-adenosylmethionine, as well as folate, results in the hypomethylation of oncogenes and may lead to DNA strand breaks, all of which are associated with increased carcinogenesis.

Chronic alcohol consumption: Cancer: Acetaldehyde: Cytochrome P450 2E1: Alcohol–nutrient interactions

Chronic excessive alcohol consumption is a strong risk factor for cancer of the upper aero-digestive tract (oral cavity, pharynx, hypopharynx, larynx, oesophagus), the liver, the colo-rectum and the breast. A great number of epidemiological studies have demonstrated the correlation between alcohol ingestion and the occurrence of cancer in these organs (Seitz *et al*. 1998). These studies clearly show that the ingestion of all types of alcoholic beverage is associated with an increased cancer risk, which suggests that ethanol is the common ingredient that causes this effect. The exact mechanism of ethanol-associated carcinogenesis has remained obscure, since ethanol is not a carcinogen. Multiple mechanisms are involved in alcohol-associated cancer development, including the effect of acetaldehyde (AL; the first metabolite of ethanol oxidation), the induction of cytochrome P450 2E1 (CYP2E1) leading to the generation of reactive oxygen species and enhanced procarcinogen activation, modulation of cellular regeneration and nutritional deficiencies. These mechanisms have been the subject of recent reviews (Seitz *et al*. 1998, 2001).

In the present paper major emphasis is given to the most recent observations contributing to the elucidation of the mechanisms involved in alcohol-associated carcinogenesis, such as genetic factors and alcohol–nutrient interactions, and in particular the role of AL as a carcinogen. However, it is beyond the scope of the present paper to discuss all the possible mechanisms in detail.

Acetaldehyde and ethanol-associated carcinogenesis

AL is a carcinogen. There is increasing evidence that AL rather than alcohol is responsible for the co-carcinogenic effect of alcohol (Seitz *et al.* 2001). In the gastrointestinal tract AL can be generated from ethanol through the action of mucosal and/or bacterial alcohol dehydrogenase (ADH; Seitz & Oneta, 1998). AL is highly toxic, mutagenic and carcinogenic. AL interferes with DNA synthesis and repair at many sites and can consequently result in tumour development (Anonymous, 1985). Numerous *in vitro* and *in vivo* experiments in prokaryotic and eukaryotic cell cultures and in animal models have shown that AL has direct mutagenic and carcinogenic effects. It causes point mutations in the hypoxanthine-guanine-phosphoribosyl transferase locus in human lymphocytes, induces sister chromatid exchanges and cross-chromosomal aberrations (Dellarco, 1988; Helander & Lindahl-Kiessling, 1991). It induces inflammation and metaplasia of the tracheal epithelium, delays cell cycle progression and enhances cell injury associated with hyper-regeneration (Seitz *et al.* 2001). Thus, when AL was administered in drinking water to rodents the mucosa lesions that were observed (Homann *et al.* 1997*b*) resembled those that resulted from chronic alcohol ingestion (Simanowski *et al.* 1993). It has also been shown that AL interferes with the DNA repair machinery. AL directly inhibits O^6-methyl-guanyltransferase, an enzyme important for the repair of adducts caused by alkylating agents (Espina *et al.* 1988). Moreover, when AL is inhaled it causes naso-pharyngeal and laryngeal carcinoma (Woutersen *et al.* 1986). According to the International Agency for Research on Cancer there is sufficient evidence to identify AL as a carcinogen in animals (Anonymous, 1985). AL also binds rapidly to cellular proteins and DNA, which results in morphological and functional impairment of the cell and an immunological cascade reaction. The binding to DNA and the formation of stable adducts represent one mechanism by which AL could trigger the occurrence of replication errors and/or mutations in oncogenes or tumour suppressor genes (Fang & Vaca, 1995). The occurrence of stable DNA adducts has been shown in different organs of alcohol-fed rodents and in leucocytes of alcoholics (Fang & Vaca, 1997). Moreover, it has been shown recently that the major stable DNA adduct N^2-ethyldeoxyguanosine can indeed be used efficiently by eukaryotic DNA polymerase (Matsuda *et al.* 1999). These AL-associated effects occur at AL concentrations of 40–1000 μM, similar to concentrations observed in human saliva following alcohol ingestion (Homann *et al.* 1997*a*).

Recent evidence of the causal role of AL in ethanol-associated upper aero-digestive tract carcinogenesis derives from genetic linkage studies in alcoholics. Individuals who accumulate AL, as a result of polymorphism and/or mutation in the gene coding for enzymes responsible for AL generation and detoxification, have been shown to have an increased cancer risk. In Japan, as in other Asian countries, a high percentage of individuals have a mutation of the acetaldehyde dehydrogenase (*ALDH*)-2 gene. In man there are at least four to five ALDH isoenzyme classes. Mitochondrial ALDH2 is primarily responsible for AL oxidation. The human ALDH2 enzyme is polymorphic, having two allelic forms, *ALDH2*1* and *ALDH2*2*. *ALDH2*2* is a result of a single point mutation in chromosome 6 coding the normal *ALDH2*1* allele. Individuals homozygous for the mutated *ALDH2*2* allele have no ALDH2 activity, whereas heterozygous individuals with the *ALDH2*1,2* genotype have 30–50% of normal ALDH2 activity only. Blood AL levels of *ALDH2*2* homozygous individuals are six to twenty times higher than those of *ALDH2*1* individuals in whom AL is hardly detectable after alcohol consumption. The elevated AL concentrations cause unpleasant side effects (flush syndrome), which protect those individuals from alcoholism. However, heterozygous individuals may become heavy drinkers or even alcoholics (Seitz *et al.* 2001).

In a series of publications Yokoyama *et al.* (1996, 1999) were the first to report that in Japanese subjects the heterozygous mutation of the *ALDH2* gene (*ALDH2*1,2*), which leads to elevated serum AL levels, is a strong risk factor for oesophageal cancer in both every-day drinkers and alcoholics. A comprehensive study of the *ALDH2* genotype and cancer prevalence in Japanese alcoholics showed that the frequency of inactive ALDH2 increased remarkably among alcoholics with cancer of the oral cavity, oro-pharynx, hypopharynx, larynx, oesophagus and colo-rectum (Yokoyama *et al.* 1998; Seitz *et al.* 2001). It is important to note that these individuals also had high AL levels in their saliva (Vakevainen *et al.* 2000).

In addition to the mutation of the *ALDH2* gene, polymorphisms of *ADH2* and *ADH3* also result in elevated AL levels. While the *ADH2*2* allele encodes for an enzyme that is approximately forty times more active than that encoded by the *ADH2*1* allele, the *ADH3*1* allele encodes for an ADH 2·5 times more active than that encoded by the *ADH3*2*. *ADH2*2* allele frequency is high in Asians but low in Caucasians. It protects from alcoholism because of the high levels of AL produced and its toxic side effects (Borras *et al.* 2000; Seitz *et al.* 2001). As Caucasians have a low *ADH2*2* allele frequency and a lack of *ALDH2* mutations, it is possible to investigate *ADH3* polymorphism and its role in alcohol-associated carcinogenesis in this population.

Studies of *ADH3* polymorphism and upper aero-digestive tract cancer in Caucasians have shown contradictory results. Although an increased risk of oro-pharyngeal and laryngeal cancer in individuals with the *ADH3*1* allele has been reported (Coutelle *et al.* 1997; Harty *et al.* 1997), other case–control epidemiological studies have not been able to confirm such an association (Olshan *et al.* 2001; Sturgis *et al.* 2001). In a study of 187 alcoholic patients with oro-pharyngeal, laryngeal, hypopharyngeal and oesophageal cancer, in which their *ADH3* genotype was compared with that of age-matched alcoholics without cancer an increased cancer risk was found in individuals with the *ADH3*1* allele (Stickel *et al.* 2003). Furthermore, individuals homozygous for *ADH3*1* were found to have elevated salivary AL levels (Li *et al.* 2001), which may explain why they have an increased cancer risk, since AL comes into direct contact with the mucosa and may act as described earlier. Also, it is interesting to note that AL-fed rats showed a severe hyper-regeneration of the upper gastrointestinal mucosa (Homann *et al.* 1997*b*), which is

very similar to the morphological changes observed after chronic alcohol consumption (Simanowski *et al*. 1993). These changes were observed only when the animals had intact salivary glands (Simanowski *et al*. 1993). After sialo-adenectomy this proliferation disappeared, which supports the hypothesis that salivary AL is involved in carcinogenesis. Furthermore, it has been shown that chronic alcohol consumption alters salivary morphology and function (Maier *et al*. 1986).

AL can also be produced by oral bacteria. AL can be detected in the saliva of healthy volunteers after ingestion of a moderate dose of alcohol, and the levels are ten to twenty times higher than those in systemic blood, even at higher alcohol intakes (Homann *et al*. 1997a). Salivary AL concentrations following ethanol ingestion can be considerably reduced by using an antiseptic mouthwash such as chlorhexidine before alcohol intake, which emphasizes the important role of oral bacteria in AL production (Homann *et al*. 1997a). It has been shown that alcoholics with oropharyngeal cancer have very high salivary AL concentrations (Jokelainen *et al*. 1996). These high levels may be present as a result of bacterial AL production associated with smoking (Homann *et al*. 2000) and poor oral hygiene (Homann *et al*. 2001), both of which are frequently observed in alcoholics. Very recently it was shown that smoking changes the oral bacterial flora rapidly from Gram-negative to Gram-positive bacteria, which leads to AL concentrations that are 50–60 % higher than those observed in non-smokers (Salaspuro, 2003). Indeed, Gram-positive bacteria are capable of producing much higher levels of AL than Gram-negative bacteria. In addition, *Candida albicans*, which is also frequently present in smokers, converts alcohol into AL (Salaspuro, 2003). The data imply that after moderate alcohol intake smokers produce higher AL concentrations compared with non-smokers. In addition, poor oral hygiene is associated with bacterial overgrowth, parodontitis and caries and also increases salivary AL concentrations, very frequently associated with *Candida albicans*. Moreover, the non-pathogenic Neiseria species isolated from oral cavity also produce AL (Salaspuro, 2003).

Induction of cytochrome P450 2E1 and ethanol-associated carcinogenesis

Chronic alcohol consumption leads to the induction of CYP2E1 enzyme activity, which leads to the conversion of ethanol to AL. This cytochrome is also involved in the metabolism of various xenobiotics, including procarcinogens. It has been shown that in the liver the concentration of CYP2E1 can be correlated with the generation of hydroxyethyl radicals and thus with lipid oxidation (Albano & Clot, 1996). The induction of CYP2E1 results in enhanced hepatic injury and the inhibition of CYP2E1 is associated with an improvement in these lesions (Gouillon *et al*. 2000), These effects have been attributed mainly to stimulation and inhibition respectively of free radical formation. The role of CYP2E1 induction in cellular injury has been studied in detail in the liver. It has also been shown that chemically-induced oesophageal carcinogenesis is stimulated by concomitant chronic alcohol administration

leading to an increase of free radicals. This process is inhibited by α-tocopherol, demonstrating that antioxidant treatment is effective in experimentally-induced carcinogenesis (Eskelson *et al*. 1993). Induction of CYP2E1 also increases the conversion of various xenobiotics, including procarcinogens (nitrosamines, aflatoxin, vinylchloride, polycyclic hydrocarbons, hydrazines), to their corresponding carcinogens (Seitz & Oswald, 1992). It is believed that this induction process plays a major role in experimentally-induced chemical carcinogenesis. Although polymorphism of CYP2E1 has been documented, it does not seem to play an important role in cancer development in the alcoholic. However, the induction of CYP2E1 activity occurs at relatively low levels of alcohol (40 g/d), and at these levels of intake induction is already apparent after 1 week, although the extent varies inter-individually. Some individuals exhibit a very low extent of induction of CYP2E1 activity, whereas others show a high extent of induction (Oneta *et al*. 2002). Thus, it could well be that the variation in the extent of induction of CYP2E1 activity may modulate alcohol-associated carcinogenesis in man.

Nutritional deficiencies and their role in ethanol-associated carcinogenesis

Retinoids

Interference with vitamin A metabolism and its nutritional status is one of the major alterations caused by alcohol (Crabb *et al*. 2001; Wang, 2001). Lower hepatic vitamin A levels in alcoholics have been well documented (Leo & Lieber, 1982). Several mechanisms have been proposed to explain how ethanol might interfere with retinoid metabolism in the liver. Ethanol lowers hepatic retinoid levels through increased catabolism of retinol (ROL) and retinoic acid (RA) into more polar metabolites (Leo & Lieber, 1982; Sato & Lieber, 1982; Wang *et al*. 1998; Liu *et al*. 2001). Ethanol increases vitamin A mobilization from the liver to other organs, as indicated by increased vitamin A concentration in extrahepatic tissues after chronic alcohol consumption (Leo *et al*. 1986; Mobarhan *et al*. 1991). In addition, alcohol acts as a direct competitive inhibitor of ROL oxidation to RA in the liver and other tissues. These alcohol-induced changes result in decreased hepatic levels of ROL and retinyl esters, which are precursors of RA, the most active form of vitamin A and a ligand for retinoid receptors. RA plays an important role in controlling cell growth, differentiation and apoptosis, and is of potential clinical interest in cancer chemo-prevention and treatment (Lippman & Lotan, 2000). Thus, interference by ethanol in RA metabolism has an important impact on the aetiology, prevention and treatment of alcohol-related disease.

As already mentioned, chronic alcohol consumption induces the activity of hepatic cytochrome P450 enzymes (Lieber, 1994b), and these enzymes, predominantly CYP2E1, are involved in ROL and RA metabolism. In a recent study by Liu *et al*. (2002) treatment of rats with high-doses of ethanol led to a reduction in hepatic ROL and retinyl ester concentrations and the occurrence of several polar retinoid metabolites, when compared with rats pair-fed with an isoenergic control diet containing the

same amount of vitamin A. Chlormethiazole, an efficient inhibitor of CYP2E1, can prevent this ethanol-enhanced metabolism of ROL and RA in rats (Liu *et al.* 2001, 2002). Chlormethiazole also reduces the formation of oxidative polar metabolites of ROL (Liu *et al.* 2002). These data support the fact that both ROL and RA are metabolized by CYP2E1, at least in the liver. CYP2E1 is the key mechanism for the ethanol-enhanced catabolism of retinoids in hepatic tissue that occurs after treatment with alcohol.

Since in man induction of CYP2E1 activity occurs 1 week after the ingestion of ethanol and the disappearance of CYP2E1 occurs 3–8 d after ethanol withdrawal (Oneta *et al.* 2002), it is possible that induction of CYP2E1 enzyme activity in chronic intermittent drinking could continue to be a factor in the metabolism of ROL and RA, even after alcohol is cleared. This process may provide a possible explanation for why chronic and excessive alcohol intake is a risk not only for hepatic, but also for extra-hepatic, cell proliferation and carcinogenesis, since it has been reported that CYP2E1 is also present and inducible by alcohol in the oesophagus, stomach and surface epithelium of the proximal colon (Shimizu *et al.* 1990). The restoration of vitamin A levels by chlormethiazole also provides a possible mechanism for the protective effect of chlormethiazole in ethanol-induced liver injury (Gouillon *et al.* 2000).

It has been shown that hepatocytes become hyperproliferative after chronic ethanol treatment (Halsted *et al.* 1996; Chung *et al.* 2001, 2002), which may contribute to ethanol-induced disease. Recent studies have demonstrated that the restoration of ethanol-lowered RA levels to normal levels alters hepatocyte proliferation and apoptosis in alcohol-fed rats (Chung *et al.* 2001, 2002), indicating that retinoids could protect against alcohol-induced diseases.

The interaction between ethanol and retinoid signalling may involve several mechanisms. First, retinoid receptors function as ligand-dependent transcription factors, thereby activating the transcription of a series of genes with distinct anti-proliferative activity and tumour suppressor function. The expression of the retinoid receptor β gene, a tumour suppressor, is down regulated by alcohol (Grummer & Zachman, 2000) and RA supplementation increases the level of retinoid-responsive mitogen-activated kinase phosphatase-1 in the liver of ethanol-fed rats (Chung *et al.* 2002). This induction of mitogen-activated kinase phosphatase-1 attenuated the ethanol-induced phosphorylation of Jun N-terminal kinases, which have been shown, using a multi-step carcinogenesis model in mice lacking the Jun N-terminal kinase 2 gene, to be required for tumorigenesis (Chen *et al.* 2001). Thus, the down-regulation of specific retinoid acid receptors or a lack of retinoid acid as a result of excessive alcohol intake could interfere with retinoid signal transduction, resulting in enhanced cell proliferation and potentially malignant transformation (Lippman & Lotan, 2000).

Second, it has been shown that the low levels of RA in the liver associated with chronic alcohol consumption lead to a 10-fold increase in activator protein-1 gene expression. The administration of RA to alcohol-fed animals dramatically suppresses ethanol-induced overexpression of c-Jun, activator protein-1 DNA-binding activities, levels of cyclin

D1 and ethanol-induced proliferation of cellular nuclear antigen-positive hepatocytes (Chung *et al.* 2001, 2002). Since the transactivation of activator protein-1-dependent genes is required for tumour promotion (Young *et al.* 1999) and cyclin D1 plays an important role in tumorigenesis and tumour progression in hepatocellular carcinoma (Uto *et al.* 2001), the identification of c-Jun and cyclin D1 as two potential targets of RA action in ethanol-fed rats indicates that retinoids may play an important role in preventing certain types of ethanol-promoted cancer.

Third, retinoids have been implicated in the induction of cell death in many tumour-derived cultured-cell systems through retinoid receptor-dependent or -independent mechanisms (Simoni & Tolomeo, 2001). Recently, it has been shown that hepatocellular apoptosis can be regulated by either ethanol feeding or RA supplementation. Feeding rats alcohol increases apoptosis. However, after 6 months hepatic apoptosis decreases relative to that of the controls (Chung *et al.* 2002) and RA supplementation increases apoptosis by 4-fold in ethanol-fed rats as compared with ethanol alone (Chung *et al.* 2002). Although the mechanism is not well defined, these data indicate that induction of apoptosis by RA plays an important role in preventing alcohol-promoted carcinogenesis by eliminating cells with irreparable alterations in the genome or killing neoplastic cells.

Methyl deficiency

Changes in the extent of methylation of cytosine are frequently encountered in human cancers, but their relevance as an epigenetic factor in carcinogenesis is only partially understood (Counts & Goodman, 1995). However, DNA methylation is an important determinant in controlling gene expression, whereby hypermethylation has a silencing effect on genes and hypomethylation may lead to increased gene expression. In hepatocarcinogenesis general hypomethylation may be coupled with areas of regional hypermethylation. Thus, hypermethylation of tumour suppressor genes can result in decreased gene transcription of p53 and HIC-1 (Kanai *et al.* 1999), and hypomethylation of certain oncogenes such as *c-myc* and *C-N-ras* may lead to dedifferentiation and proliferation (Wainfan *et al.* 1989; Shen *et al.* 1998). Recently, it has been suggested that aberrant DNA hypermethylation may be associated with genetic instability, as determined by loss of heterozygosity and microsatellite instability in human hepatocellular carcinoma resulting from chronic viral hepatitis (Kanai *et al.* 2000; Kondo *et al.* 2000). Iwata *et al.* (2000) detected hypermethylation of the *14-3-3 sigma* gene, which has been implicated as a key inducer of cell cycle arrest associated with p53 in 89 % of investigated human hepatocellular carcinoma. However, the genetic alterations in animal models and those in human hepatocarcinogenesis differ substantially. Thus, it was shown that activation of *N-myc* and *c-myc* oncogenes is frequent in hepatitis virus-associated hepatocellular carcinoma in the woodchuck (*Marmota monax*), while no p53 mutations were found. This mutational pattern is reversed in man, i.e. p53 mutations are frequent and oncogene activation seems to play only a minor role (Hui & Makuuchi, 1999).

Importantly, modifications of the extent of hepatic DNA methylation have also been observed in experimental models of chronic alcoholism (Garro *et al.* 1991; Choi *et al.* 1999). Hypomethylation is a plausible consequence of metabolic alterations associated with ethanol consumption. In fact, alcohol has a marked impact on hepatic methylation capacity, as reflected by decreased levels of *S*-adenosylmethionine, an important methyl group donor, and increased levels of *S*-adenosylhomocysteine, resulting in a ≤2·5-fold decrease in the *S*-adenosylmethionine : *S*-adenosylhomocysteine (Lieber *et al.* 1990; Trimble *et al.* 1993; Stickel *et al.* 2000). Several mechanisms have been suggested by which ethanol could interact with C metabolism and DNA methylation and thereby enhance carcinogenesis: (1) chronic alcohol consumption affects the intake, absorption and subsequent metabolism of B-vitamins involved in hepatic transmethylation reactions, i.e. folate and pyridoxal-5′-phosphate, resulting in impaired methyl group synthesis and transfer (Lumeng & Li, 1974; Labadarios *et al.* 1977; Savage & Lindenbaum, 1986; Gloria *et al.* 1997; Stickel *et al.* 2000); (2) ethanol reduces the activity of methionine synthase, which remethylates homocysteine to methionine with methyltetrahydrofolate as a methyl donor (Barak *et al.* 1993; Lieber, 1994*a*); (3) chronic alcohol consumption decreases levels of glutathione, a reductive tripeptide that is synthesized from homocysteine via trans-sulfuration in the liver, and thereby enhances the susceptibility of the liver to alcohol-related peroxidative damage (Speisky *et al.* 1985; Lieber 1994*a*); (4) in rats alcohol can inhibit the activity of DNA methylase, which transfers methyl groups to DNA (Garro *et al.* 1991), a finding that could not be confirmed in human subjects (Miyakawa *et al.* 1996).

To date, it is well established that dietary depletion of lipotropes, including methionine, choline, betaine, *S*-adenosylmethionine and folate, leads to DNA hypomethylation of oncogenes (i.e. *c-Ha-ras*, *c-Ki-ras* and *c-fos*) and to DNA strand breaks, all of which are associated with an increased incidence of hepatocellular carcinoma in rats (Zapisek *et al.* 1992; Pogribny *et al.* 1995). Whether chronic alcohol consumption alone is capable of inducing a lack of methylation capacity sufficient to cause hypomethylation of DNA and the genes involved in hepatocarcinogenesis is not yet known.

Conclusions and future perspectives

Chronic alcohol consumption is a major risk factor for cancer of the upper aero-digestive tract, liver, colo-rectum and breast. Animal experiments and genetic linkage studies in man have identified AL as a carcinogenic factor in ethanol-associated carcinogenesis. Polymorphisms and mutations of genes coding for enzymes involved in alcohol and AL metabolism may determine, among other factors, the predisposition of an individual to develop alcohol-associated cancer. Thus, ADH genotyping may identify high-risk individuals. Since AL occurs in the saliva after alcohol consumption its detoxification by the oral ingestion of compounds binding AL may be an interesting approach for prevention. In addition, there is some evidence that reactive oxygen species produced by CYP2E1 initiate

alcohol-associated carcinogenesis. Since the ability to induce CYP2E1 activity differs between individuals it would be important to note whether individuals with a maximal CYP2E1 induction also have a high risk of developing cancer. Most of the data have been obtained in animal studies and it would be important to undertake more studies with human subjects to investigate whether RA deficiency occurs in hepatocytes and mucosal cells following chronic alcohol consumption. Thus, future research should identify the individual risk factors for alcohol-associated carcinogenesis.

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