Molecular mechanisms of alcohol-mediated carcinogenesis

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Abstract | Approximately 3.6% of cancers worldwide derive from chronic alcohol drinking, including those of the upper aerodigestive tract, the liver, the colorectum and the breast. Although the mechanisms for alcohol-associated carcinogenesis are not completely understood, most recent research has focused on acetaldehyde, the first and most toxic ethanol metabolite, as a cancer-causing agent. Ethanol may also stimulate carcinogenesis by inhibiting DNA methylation and by interacting with retinoid metabolism. Alcohol-related carcinogenesis may interact with other factors such as smoking, diet and comorbidities, and depends on genetic susceptibility.

Relative risk
(RR) The risk of developing a disease relative to exposure. Relative risk is a ratio of the probability of the event occurring in the exposed group versus the control (non-exposed) group. For example, if the probability of developing lung cancer was 20% among smokers and 1% among non-smokers, then the relative risk of cancer associated with smoking would be 20. Smokers would be 20 times as likely as non-smokers to develop lung cancer.

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Chronic alcohol consumption is a major health issue worldwide, and may lead to addiction and damage of almost every organ of the body. The most comprehensive estimates of death rates caused by alcohol come from the World Health Organization (WHO) Global Burden of Disease Project, which concluded that alcohol accounts for approximately 1.8 million deaths per year (3.2% of all deaths)[1]. One of the most significant diseases caused by chronic alcohol consumption is cancer.

In February 2007 an international group of epidemiologists and alcohol researchers met at the International Agency for Research on Cancer (IARC) in Lyon, France, to evaluate the role of alcohol and its first metabolite, acetaldehyde, as potential carcinogens in experimental animals and humans[2]. This Working Group has concluded from the epidemiological data available that the occurrence of malignant tumours of the oral cavity, pharynx, larynx, oesophagus, liver, colorectum and female breast are causally related to the consumption of alcoholic beverages. Thus, alcohol is considered a carcinogen for these organ sites[2,3]. Worldwide, a total of approximately 389,000 cases of cancer representing 3.6% of all cancers (5.2% in men and 1.7% in women) derive from chronic alcohol consumption[1]. For a global perspective of the role of alcohol in cancer considering various demographic factors such as age, sex and geographic region of observation, as well as other alcohol-associated diseases, readers are referred to two recent reviews[4,5]. Alcohol-related cancers are generally difficult to treat, often requiring complex and high-risk surgery as well as radiochemotherapy. Ethanol can accelerate tumour spread, as exemplified for liver metastasis of colorectal cancers, probably due to immunosuppression[6,7] and the induction of angiogenesis by the expression of vascular endothelial growth factor (VEGF)[8]. Ethanol also interacts with the metabolism of chemotherapeutic drugs, which can result in a decreased response to medication and increased side effects[9]. Thus, it seems important to elucidate the carcinogenic mechanisms associated with heavy drinking and to identify individuals at increased risk to allow prevention and early detection of alcohol-related cancers, and intervention to reduce alcohol consumption in these individuals. In this Review a brief analysis of epidemiological and experimental data will be given. However, major emphasis will be put on molecular mechanisms of alcohol-related carcinogenesis.

Epidemiology

At the beginning of the 20th century, French pathologists speculated as to whether alcohol contained in absinthe was a possible carcinogen for the oesophagus[10]. Meanwhile, countless epidemiological data from cohort and case–control studies has accumulated that identify alcohol as a major risk factor for various cancer sites[4–6,11].

Many prospective and case–control studies show a 2–3-fold increased risk for cancer of the oral cavity, pharynx, larynx and oesophagus in people who consume 50 g of alcohol a day (equal to approximately a half bottle of wine), compared with non-drinkers[2–6,11–13]. This effect is dose dependent[4]. In addition, smoking has a synergistic effect. A carefully designed French study demonstrated that an alcohol consumption of more than 80 g a day (approximately a 0.7 litre bottle of wine) is associated with a relative risk (RR) of 18 for the development of oesophageal carcinoma, which translates into

At a glance

- Together with tobacco, alcohol is the most abundantly consumed noxious compound worldwide. Within the last decade, much knowledge about the pathophysiology of alcohol-related organ damage has been gathered that draws a much clearer picture of its potential dangers.
- There is a clear association between chronic alcohol consumption and the development of cancers of the upper gastrointestinal tract, the liver, the colorectum and the female breast.
- There is convincing evidence that acetaldehyde, the first metabolite produced during alcohol degradation, is responsible for the carcinogenic effect of ethanol on the upper aerodigestive tract owing to its multiple mutagenic effects on DNA.
- Mechanisms of ethanol-induced hepatocarcinogenesis include the induction of cirrhosis of the liver, ethanol-related increase of oxidative stress, altered methylation and a reduction of retinoic acid.
- An increase in oestradiols due to alcohol may contribute to breast cancer.
- Patients with chronic hepatitis B and C; hereditary haemochromatosis and non-alcoholic fatty liver disease owing to insulin resistance; gastroesophageal reflux disease (GERD); and colorectal polyps are more susceptible to the carcinogenic properties of ethanol.
- Carriers of the inactive aldehyde dehydrogenase 2*2 (*ALDH 2*2*) allele are at increased risk for alcohol-related oesophageal cancer. Carriers of other genetic variants, such as alcohol dehydrogenase 1C*1 (*ADH1C*1*) homozygotes and methylene-tetrahydrofolate reductase (*MTHFR*) 677CT variants, should also be considered at higher risk for alcohol-related cancers.
- Lifestyle factors such as smoking, poor oral hygiene, and certain dietary deficiencies (folate, vitamin B6, methyl donors) or an excess of others (vitamin A/β-carotene), owing to unevenly composed diets or self-medication, also increase the risk for alcohol-associated tumours.

an 18-fold higher cancer risk in those exposed to this amount of alcohol compared with non-drinkers, whereas smoking more than 20 cigarettes a day resulted in an increased RR of only five. However, both factors act synergistically, resulting in an increased RR of 44 (REF. 14). The interaction between alcohol and tobacco on cancer development is complex, and it is beyond the scope of this article. Readers are therefore referred to a recent publication on this topic[15]. Asians who are deficient in aldehyde dehydrogenase 2 (ALDH2), which causes an increased accumulation of acetaldehyde following alcohol consumption, have a RR between 7.5 and 16 for oesophageal cancer compared with those with normal ALDH2 (REFS 16–20).

The RR for hepatocellular carcinoma (HCC) is between 4.5 and 7.3 when more than 80 g alcohol per day are consumed, compared with abstinence or consumption of less than 40 g per day[21]. A dose–response relationship for the amount of ethanol consumed and the risk of HCC has been shown[11]. It is noteworthy that HCC develops almost exclusively in cirrhotic livers (see below), and 1–2% of alcoholic cirrhotics develop HCC each year. Although heavy alcohol intake is strongly associated with the development of cirrhosis, data showing a direct carcinogenic effect of alcohol are limited.

Chronic alcohol consumption strikingly increases the risk of cirrhosis and HCC in patients with coexisting hepatitis B and hepatitis C virus infection, haemochromatosis or non-alcoholic steatohepatitis owing to insulin resistance[21]. According to the data available it is not possible to distinguish between increased risk for cirrhosis and increased risk for HCC in patients with alcohol use in addition to another liver disease. For example, in patients with cirrhosis owing to chronic hepatitis C, 80 g of ethanol a day increases the RR for HCC by 126, compared with 26 in patients with less than 40 g alcohol intake per day[22]. In hepatitis B, 80 g of alcohol a day has a RR of 38 compared with 32 in non-drinkers[23].

More than 100 epidemiological studies have consistently demonstrated a dose-dependent increase of the risk for breast cancer with chronic alcohol consumption[2,3]. A meta-analysis of 38 epidemiological studies found that the risk of breast cancer for one, two, or three or more drinks per day increases by 10%, 20% and 40%, respectively[24]. A pooled analysis of 53 studies on more than 58,000 women found that the risk for breast cancer increases by 7.1% for every additional 10 g of alcohol a day[25]. From these data it was concluded that approximately 4% of all newly diagnosed breast cancer cases in the US (approximately 8,000 cases per year) are attributable to alcohol[24].

A positive dose–response relationship between alcohol consumption and colorectal cancer was also reported in more than 50 prospective and case–control studies[26,27]. A review of 27 cohort studies reported a twofold higher risk for colorectal cancer in alcoholics[28]. Pooled results from eight cohort studies[27] and data from a recent meta-analysis[29] provide evidence for a RR of 1.4 for colorectal cancer in patients who consumed 50 g of alcohol per day compared with abstainers. According to the data by Le Marchand *et al.*, estimation of population attributable risks suggested that a comprehensive reduction in exposure to ethanol for individuals with familial predisposition for colorectal cancer may reduce tumour incidence[30]. In addition, five of six studies showed a significant correlation between colorectal polyps and alcohol consumption[26].

From these epidemiological data it can be concluded that ethanol itself and not the type of alcoholic beverage stimulates carcinogenesis.

Experimental carcinogenesis of alcohol

In the past, ethanol was not considered a carcinogen, but rather a co-carcinogen and/or tumour promoter, as, on its own, ethanol administration to animals did not induce tumours. Detailed analysis of many of these studies by the IARC Working Group revealed that they were inadequately designed and performed[3]. However, more recent animal experiments in which mice and rats received alcohol in their drinking water during their entire lifetime have clearly identified ethanol as a carcinogen[2,3,31–34] (TABLE 1).

In addition, more than 50 studies were performed to determine whether ethanol can modify chemically induced carcinogenesis, using various mouse and rat strains and various carcinogens to induce tumours. Depending on the carcinogen and the animal model used, tumour-specific target organs include the mammary gland, oesophagus, forestomach, large intestine, liver, kidney, lung, thymus and skin[2,3]. Some of the studies had to be criticized for methodological reasons.

Haemochromatosis
A genetic disorder attributable to several mutations in the haemochromatosis gene (*HFE*) leading to excessive iron storage. Clinically, affected individuals may develop liver cirrhosis, diabetes, cardiomyopathy, arthropathy and a bronze colour of the skin, which is responsible for the lay term 'bronze diabetes'.

Non-alcoholic steatohepatitis
A feature of non-alcoholic fatty liver disease. In contrast to alcoholic steatohepatitis, the accumulation of fat in the liver is mostly due to hyperinsulinemia in obese individuals. There is no difference in histomorphology between the two types of liver disease. One feature is an increase in reactive oxygen species generation, which results in lipid peroxidation.

Table 1 | **Alcohol and experimental carcinogenesis**

Animal species	Sex	No.	Exposure time	Ethanol administration	Effects	Comments	Ref
B6C3F1 mice	F and M	281	104 weeks	2.5% and 5.0% in dw	More male animals with HCA and HCC	Significant dose-related trend ($P<0.05$)	31
ICR mice	F	40	25 months	10% and 15% in dw	45% more animals with papillary and meduallary adenocarcinomas of the breast ($P=0.0012$)	No tumours in control group	32
C57/B6^{APCmin} mice	M	24	10 weeks	15% and 20% in dw	More intestinal tumours ($P=0.027$); more tumours in the distal small intestine ($P=0.01$)	C57/B6 ^{APCmin} mice respresent a genetic model that resembles that of FAP in humans.	33
SD Rats	F and M	440	Life long	10% in dw	More tumours of oral cavity, lips, tongue and forestomach ($P=0.001$)	More animals developed malignant tumours, and more tumours per animal were observed after alcohol feeding	34

dw, drinking water; F, female; FAP, familial adenomatous polyposis; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; M, male.

However, in most studies the co-administration of ethanol increased chemically induced carcinogenesis, especially in the upper aerodigestive tract (UADT)[35–37], in the mammary glands[38,39], and under certain experimental conditions in the liver[40] and large intestine[41]. In summary, it was concluded by the IARC Working Group that there is sufficient evidence for the carcinogenicity of ethanol in animals[2,3].

General mechanisms of alcohol carcinogenesis

Mechanisms of ethanol-induced carcinogenesis are closely related to the metabolism of ethanol (FIG. 1). Acetaldehyde may be the important cancer-causing agent in the upper and lower gastrointestinal tract, as acetaldehyde concentrations in saliva and the large intestine are high enough to enable it to act as a carcinogen[41–46]. Acetaldehyde concentrations in the liver are significantly lower owing to an effective acetaldehyde metabolizing system; so, in the pathogenesis of HCC, oxidative stress and cirrhosis may be the most important factors.

Acetaldehyde

Acetaldehyde, a carcinogen. Acetaldehyde is a carcinogen in animals[3,47]. Inhalation studies in rats and hamsters found that acetaldehyde resulted in the occurrence of nasal adenocarcinomas and squamous cell carcinomas[48,49]. Acetaldehyde interferes with DNA synthesis and repair, and *in vitro* studies have shown that acetaldehyde causes cytogenetic abnormalities in eukaryotic cells. Acetaldehyde causes point mutations in the hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) locus in human lymphocytes, and induces sister chromatid exchanges and gross chromosomal aberrations[50–55]. Acetaldehyde also binds to proteins, resulting in structural and functional alterations. This includes enzymes involved in DNA repair (O6 methyl guanine methyltransferase) and DNA cytosine methylation, as well as glutathione, an important anti-oxidative peptide[56,57] (FIG. 2).

Acetaldehyde binds to DNA, forming stable DNA adducts (FIG. 3)[55,58–61], and acetaldehyde DNA adducts have been found in alcohol consumers. The steady state level of DNA adducts, which can also be produced by reactive oxygen species (ROS), is influenced by various factors, including the activity of the anti-oxidative defence system, glutathione-*S*-transferase (which shows genetic polymorphism), the DNA repair system and apoptosis. Chronic ethanol ingestion may affect all of these mechanisms either directly or indirectly (FIG. 2).

The most abundant DNA adduct resulting from the reaction of acetaldehyde is N^2-ethylidene-2′-deoxyguanosine (N^2-EtidG). N^2-EtidG needs a reduction step to become a stable adduct, N^2-ethyl-2′-deoxyguanosine (N^2-EtdG). Fang and Vaca reported levels of N^2-EtdG in Swedish drinkers and controls, and found higher adduct levels in lymphocytes of alcohol consumers compared with controls[62]. They also found an increase of the same adducts in mice exposed to 10% alcohol in their drinking water[63].

α-methyl-γ-OH-propano-deoxyguanosine is another DNA adduct with acetaldehyde that has been identified. As this adduct has been observed previously in DNA treated with crotonaldehyde, it is referred to as Cr-PdG. It is important to note that levels of Cr-PdG were found to be higher compared with levels of N^2-EtdG, which were often found to be undetectable in blood[64]. This is probably due to the fact that the formation of N^2-EtdG from N^2-EtidG requires a reduction step, as mentioned above, and therefore the level of N^2-EtdG reflects not only acetaldehyde reacting with DNA, but also the efficiency of the reducing step. Therefore a better strategy to analyse total levels is to convert N^2-EtidG into N^2-EtdG during DNA isolation, as recently shown by Matsuda *et al*[65]. Although N^2-EtidG is more abundant, Cr-PdG is more mutagenic (FIG. 3). The formation of Cr-PdG adducts can be facilitated in the presence of basic amino acids, histones or polyamines[61]. Relevant polyamine concentrations are present in tissues with hyper-regeneration. Chronic alcohol

Anti-oxidative defence system
The sum of counteractive mechanisms directed to offset oxidative stress. Endogenous mechanisms include antioxidant enzymes in the cytosol and mitochondria such as glutathione peroxidase and superoxide dismutase, whereas exogenous antioxidants are usually derived from diets as antioxidant micronutrients such as tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene (provitamin A) and selenium.

Glutathione-*S*-transferase
(GST) A family of sulphur-containing enzymes deriving from four gene subfamilies (*GSTA*, *GSTM*, *GSTT* and *GSTP*), which inactivate ROS and many toxic and carcinogenic xenobiotics through conjugation with glutathione.

Hyper-regeneration
Cellular reaction of tissues with marked cell proliferation in response to a toxic or physical insult. Usually a repair mechanism, but may predispose hyperproliferating tissues to malignant transformation.

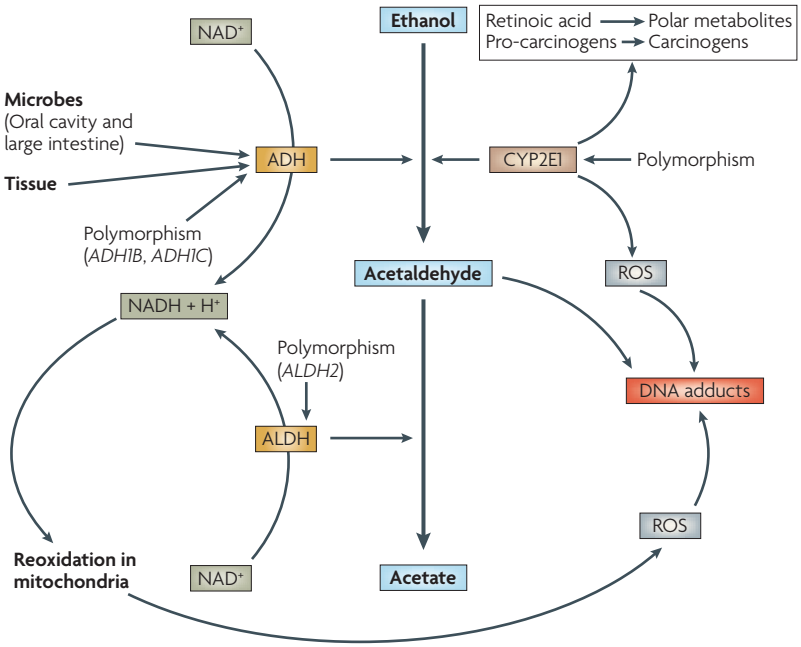

Figure 1 | **Ethanol metabolism and its role in carcinogenesis.** Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase (ADH), cytochrome P450 2E1 (CYP2E1) and, to a much lesser extent by catalase (not shown), and is further oxidized to acetate by acetaldehyde dehydrogenase (ALDH). ADH-mediated ethanol metabolism results in the generation of reducing equivalents in the form of reduced nicotinamide adenine dinucleotide (NADH) and acetaldehyde, whereas ethanol oxidation by CYP2E1 leads to the production of acetaldehyde, but also to the generation of reactive oxygen species (ROS). Single nucleotide polymorphisms (SNPs) of *ADH1B*, *ADH1C* and *ALDH2* cause the amount of production and/or oxidation of acetaldehyde to vary between individuals (see text). *CYP2E1* also has SNPs that affect enzyme activity, and is inducible by chronic ethanol ingestion. Increased CYP2E1 activity not only leads to increased generation of ROS, but also to an increased activation of various environmental pro-carcinogens present in tobacco smoke and certain diets such as polycyclic hydrocarbons, hydrazines and nitrosamines that require CYP2E1 to be activated. CYP2E1 also decreases tissue levels of retinol and retinoic acid, which have important functions in the regulation of cell growth and transdifferentiation. NADH is reoxidized to NAD^+ in the mitochondria, which may further increase the generation of ROS (see text). Acetaldehyde can bind to DNA, forming stable adducts, and ROS results in lipidperoxidation and lipidperoxidation products such as malondialdehyde and trans-4-hydroxy-2-nonenal that also bind to DNA forming exocyclic DNA etheno adducts. Ethanol oxidation by catalase seems to be of secondary importance.

consumption results in mucosal hyperproliferation of the UADT[66], as well of the large intestine[67], probably owing to the local toxic effect of highly concentrated acetaldehyde[68,69]. In addition, high acetaldehyde concentrations are found in the saliva and colonic content following moderate alcohol consumption owing to the bacterial oxidation of ethanol[42–46]. As a consequence of high acetaldehyde concentrations in a hyper-regenerative environment, the generation of the highly-mutagenic Cr-PdG may be facilitated in these tissues.

In this context, gastroesophageal reflux disease (GERD) is of clinical interest. It is characterized by the reflux of gastric juice rich in gastric acid into the oesophagus, leading to inflammation and hyper-regeneration of the mucosa. This reflux is increased by ethanol owing to its relaxing effect on oesophageal motility and the tonus of the lower oesophageal–gastric junction. Thus, GERD is an additional risk factor for cancer development in people who consume large quantities of alcohol[70].

Genetic modification of acetaldehyde levels following ethanol ingestion. The amount of acetaldehyde present in various tissues following ethanol ingestion may not only depend on the amount of ethanol consumed but also on the genotype coding for ethanol-metabolizing enzymes. As shown in FIG. 1, the activity of alcohol dehydrogenase (ADH) and ALDH is primarily responsible for the amount of acetaldehyde generated. In 40–50% of Asians, ALDH2 has extremely low activity owing to an amino acid substitution of lysine for glutamine at position 487 of the protein following a single nucleotide polymorphism (SNP) G–A within the coding region of the *ALDH2* gene. The normal allele is termed *ALDH2*1*, whereas the inactive variant is designated *ALDH2*2*. People homozygous for *ALDH2*2* are unable to oxidize acetaldehyde, whereas heterozygotes have markedly reduced but still detectable ALDH2 activity. As the ALDH2 isoenzyme is a tetramer, only one of every 16 ALDH2 enzymes is functional in heterozygous individuals, so they can metabolize only small amounts of acetaldehyde. Homozygotes cannot tolerate alcohol at all owing to a flush syndrome that includes nausea, vomiting and facial flushing following a small amount of alcohol ingestion; heterozygotes may tolerate alcohol despite the flush reaction. Individuals with a heterozygous *ALDH2* genetic background who drink alcohol generate threefold higher concentrations of acetaldehyde in serum and saliva than individuals with homozygosity for *ALDH2*1* (REF. 44). Other members of the ALDH family are either not polymorphic (ALDH1) or have a low affinity to acetaldehyde with a high Michaelis-Menten constant (Km), and therefore do not participate substantially in the degradation of acetaldehyde, so their role in explaining alcohol-related tumorigenesis is negligible[71].

Landmark studies from Japan by Yokoyama and colleagues have identified *ALDH2*1/2* heterozygotes who consume ethanol as a high-risk group to develop UADT cancer, in particular oesophageal cancer[16–20,72]. The RR for oesophageal cancer in this high-risk group compared with *ALDH2*1* homozygotes was reported to be 10–15, and the RR for multiple oesophageal carcinomas in one study was found to be as high as 54 (REF. 16). This might be because these individuals have increased levels of acetaldehyde DNA adducts. Matsuda analysed the levels of acetaldehyde-derived DNA adducts in peripheral lymphocytes from Japanese alcoholics with the *ALDH2*1/1* and *ALDH2*1/2* genotypes[64]. Levels of N^2-EtdG and Cr-PdG were significantly higher in patients with *ALDH2*1/2* compared with *ALDH2*1/1*. In addition, sister chromatid exchanges and micronuclei are more frequently found in lymphocytes of habitual drinkers with *ALDH2*1/2* than in lymphocytes of drinkers with fully active ALDH2 (REFS 73,74). The data from Japan gave sufficient evidence for the IARC to conclude that acetaldehyde has a causal role in ethanol-related oesophageal carcinogenesis[2,3].

Nitrosamines
A group of chemicals with carcinogenic potential generated from nitrate and biogenic amines. Nitrosamines are contained in preserved food such as smoked ham, sausages, cheese, some alcoholic beverages such as beer, and tobacco smoke.

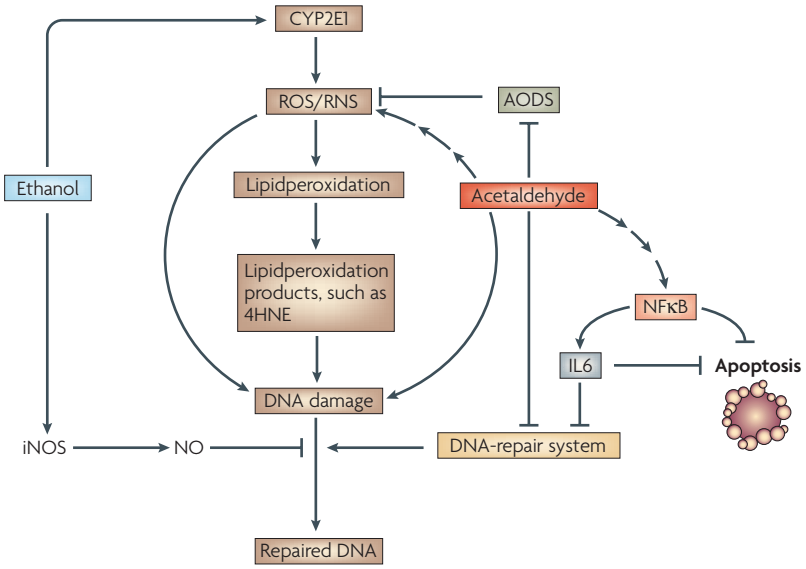

Figure 2 | **Effect of CYP2E1 and acetaldehyde on DNA damage and repair.** Chronic ethanol consumption induces cytochrome P450 2E1 (CYP2E1), which leads to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can be neutralized by an effective anti-oxidative defence system (AODS). As the system is overloaded by an increased burden of ROS and RNS, DNA damage may occur. ROS-induced lipidperoxidation leads to lipidperoxidation products such trans-4-hydroxy-2-nonenal (4HNE), which can be converted to 2,3-epoxy-4-hydroxynonenal before reacting with deoxyadenosine or deoxycytidine to form exocyclic etheno-DNA adducts such as 1,N^6-ethenoadenine or 3,N^4-ethenocytosine. An adequate DNA repair does not take place, as acetaldehyde and nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS), which is induced by ethanol, inhibit the DNA repair system (inhibition of O^6-guanine-methyltransferase and 8-oxo-guanine-DNA-glycosylase). In addition, acetaldehyde also increases the burden of ROS indirectly by injuring mitochondria, resulting in an inadequate reoxidation of the large quantities of nicotinamide adenine dinucleotide (NADH) that are produced through the alcohol dehydrogenase (ADH) reaction. Mitochondrial damage may initiate a cascade of events leading to apoptosis, which is counteracted by an activation of the survival factor nuclear factor κB (NFκB). In addition, interleukin 6 (IL6) released in alcoholic hepatitis, for example, and induced by NFκB, also inhibits DNA repair and apoptosis through the upregulation of an anti-apoptotic gene, *MCL1*, thus retaining more oxidative DNA lesions[160]. Indirect effects are indicated by broken arrows.

Seven Japanese studies and one Chinese study provided inconclusive data for an association between the *ALDH2*1/2* genotype and HCC[3]. With respect to colorectal cancer, Yokoyama and collegues[16] found a 3.4-fold increased risk, but this was not confirmed by other studies.

In addition, SNPs exist for the alcohol dehydrogenases *ADH1B* and *ADH1C*. The *ADH1B*2* allele codes for an enzyme 40-fold more active than the enzyme encoded by the *ADH1B*1* allele. The frequency of the *ADH1B*2* allele is low among Caucasians, but high among Asians. The presence of the *ADH1B*2* allele is also associated with protection against alcoholism owing to the large production of acetaldehyde and corresponding flush syndrome described above, which deters carriers from drinking alcohol. SNPs have also been identified within the *ADH1C* gene at a frequency of approximately 40–50%. The functionally important variation within the ADH1C protein is a substitution of isoleucine for valine at position 350; this is the *ADH1C*1* variant. *ADH1C*1* increases ethanol metabolism by about 2.5 times compared with *ADH1C*2*. As *ADH1C* and *ADH1B* are closely located on chromosome 4 q21–q23, linkage disequilibrium between the two genes has been shown in several populations[75]. This might be a limitation of epidemiological studies of these genes, as adequate studies controlling for an effect of one gene versus the other are lacking. It was proposed that the different kinetics of polymorphic ADH enzymes may modulate the development of alcohol-related cancer. However, studies on the effect of *ADH1C* polymorphism on UADT cancer in Causians have shown contradictory results, and are still inconclusive[76–89] (BOX 1). With respect to colorectal adenomas, a Dutch study reported an increased RR for individuals with *ADH1C*1* homozygosity who drank more than 10 drinks a day[90]. For breast cancer, three[91–93] out of four[94] studies found a correlation between *ADH1C*1* homozygosity and cancer. In this context the relationship between ethanol, acetaldehyde and oestrogens may be of pathogenetic importance[95,96] (BOX 2). Similar to individuals with ALDH2 deficiency[44], *ADH1C*1* homozygotes have increased acetaldehyde levels in their saliva (see below) compared with heterozygotes or *ADH1C*2* homozygotes after ethanol ingestion (approximately twofold more)[88].

Bacterial production of acetaldehyde from ethanol. After its absorption from the stomach and duodenum, ethanol is circulated by the blood to other organs, including the salivary glands and mucus membranes of the upper gastrointestinal tract. Ethanol concentrations in the saliva as well as in the intestine and colon are equal to concentrations present in the blood, as long as ethanol is detectable in the body. In the saliva, ethanol is oxidized by microbes to acetaldehyde. As further metabolism of acetaldehyde to acetate by oral bacteria is limited, acetaldehyde concentrations in the saliva are 10–100 times higher than in the blood[42,97].

Salivary acetaldehyde comes into direct contact with the mucosa of the UADT. It is interesting to note that acetaldehyde-fed rats show a severe hyper-regeneration of the upper gastrointestinal mucosa[69] that is very similar to the morphological changes observed after chronic alcohol administration[66]. These changes were only observed when the animals had functionally intact salivary glands, which supports the hypothesis that salivary acetaldehyde is involved in carcinogenesis[66].

Increasing ethanol intake results in increasing acetaldehyde concentrations in the saliva. Acetaldehyde concentrations of 50–100 μM, which are known to be mutagenic, can already be detected following the intake of 0.5 g alcohol per kg of body weight, equalling approximately half a bottle of wine[42,43]. Salivary acetaldehyde concentrations are decreased after an antiseptic mouthwash by approximately 30–50%, underlining the importance of oral bacteria in acetaldehyde generation[42]. A Finnish study clearly showed the effect of poor dental hygiene (a risk factor for oral cancer) on salivary acetaldehyde levels due to the larger abundance of aerobic bacteria and yeasts highly capable of generating acetaldehyde from ethanol[98].

Linkage disequilibrium
When alleles at two distinctive loci occur in gametes more frequently than expected given the known allele frequencies and recombination fraction between the two loci, the alleles are said to be in linkage disequilibrium.

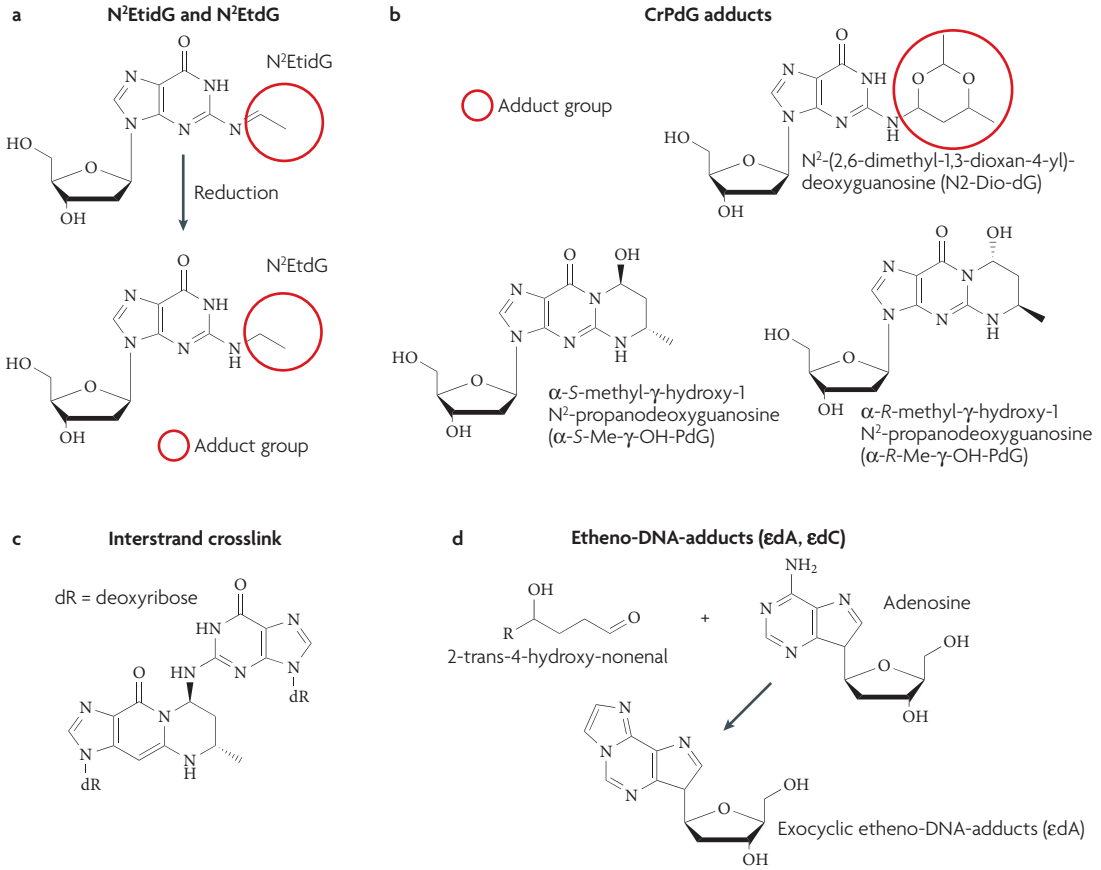

Figure 3 | **Structures of acetaldehyde-derived DNA adducts and adducts from lipid peroxidation products**[61]. Adducts are marked with red circles. **a** | The most abundant DNA adduct resulting from the reaction of acetaldehyde with deoxyguanosine (dG) is N^2-ethylidene-dG (N^2-EtidG), which can be converted to a stable adduct, N^2-ethyl-2′-deoxyguanosine (N^2-EtdG), in the presence of a reducing agent such as glutathione or ascorbic acid. **b** | N^2-dimethyldioxane-dG (N^2-Dio-dG), an interstrand crosslinker, and two diasteroemers of α-methyl-γ-OH-propano-dG have also been identified. As the latter adduct has been observed previously in DNA treated with crotonaldehyde, it is referred to as Cr-PdG. **c** | PdG adducts can undergo a ring-opening reaction when located in double-stranded DNA, allowing DNA–protein crosslinks or DNA interstrand crosslinks (ICLs) to be generated from Cr-PdG and deoxyguanosine. DNA–protein crosslinks are precursor lesions to sister chromatid exchanges (observed in alcoholics), and both DNA–protein crosslinks and DNA ICLs are mechanistically consistent with the generation of chromosomal aberrations (also observed in alcoholics). Acetaldehyde can also react with malondialdehyde, and the resulting conjugate can form DNA adducts *in vitro*. **d** | The lipidperoxidation product 4-hydroxynonenal (4HNE) reacts with deoxyadenosine or deoxycytidine to form stable exocyclic etheno-DNA adducts (εdA and εdC).

In alcoholic patients with head and neck cancer, salivary acetaldehyde concentrations were found to be increased following ethanol ingestion compared with controls[99].

Salivary acetaldehyde levels in smokers are found to be twice as high as in non-smokers, so smoking seems to shift oral microflora more towards colonization with yeasts and gram-positive bacteria, which create more acetaldehyde owing to a higher bacterial ADH activity[100]. Indeed, saliva from people who smoked 20 cigarettes a day had a 50% increase in *in vitro* acetaldehyde production from ethanol versus non-smokers[100]. In addition, tobacco smoke also contains high concentrations of acetaldehyde itself. During cigarette smoking salivary acetaldehyde concentrations increase by up to 400 μM[100].

The colon contains an enormous amount of bacteria, and colonic acetaldehyde concentrations following ethanol administration to piglets and rats exceed 250 μM[45] and 500 μM[101], respectively. Increased acetaldehyde concentrations have been convincingly shown in the colonic mucosa of conventional rats compared with germ-free animals[41], and this was associated with more pronounced mucosal injury and cellular hyperregeneration[41,68]. When acetaldehyde concentrations were increased in these animals by inhibiting ALDH activity with cyanamide, chemically induced carcinogenesis was strikingly accelerated, emphasizing the carcinogenic role of acetaldehyde in the colon[41].

Oxidative stress

As mentioned earlier, acetaldehyde is probably less important in hepatocarcinogenesis, as hepatic concentrations are relatively low following ethanol consumption, owing to an effective hepatic acetaldehyde metabolism. In the liver, experimental data support the theory that

Box 1 | *ADH1C* polymorphisms and risk of alcohol related cancer

Polymorphisms in alcohol dehydrogenase 1C (*ADH1C*) have been investigated with respect to the risk of upper aerodigestive tract (UADT) cancer. The first study from Puerto Rico demonstrated that people homozygous for the *ADH1C*1* allele, which encodes a 2.5-fold more active enzyme than *ADH1C*2*, have an increased alcohol-related cancer risk, mainly in patients with high alcohol intake. In this study oral cancer risk with eight drinks a day was increased approximately 40-fold in *ADH1C*1* homozygotes compared with a fourfold increase in *ADH1C*2* homozygotes[76]. Other studies could not confirm this observation[78–86]. When data from seven population-based studies including a total of 1,325 cases and 1,760 controls were analysed, it was concluded that the *ADH1C*1* allele does not confer an increased risk for head and neck cancer[87]. In more recent studies with heavy alcohol consumers (>40 g a day for more than 10 years), a significantly increased alcohol-related cancer risk for head and neck, oesophagus and liver was noted for individuals homozygous for the *ADH1C*1* allele[88,89]. Those studies that incorporated patients with higher daily alcohol intake found a significant effect of *ADH1C* polymorphism on UADT and colonic cancer[90], whereas those on patients with small daily alcohol ingestion did not. In the colon, most of the acetaldehyde is produced by bacteria, and therefore a genetic contribution to acetaldehyde levels could only be seen when high doses of alcohol were consumed. Three studies reported a positive correlation between *ADH1C*1* homozygosity and breast cancer in premenopausal women[91–93]. The relative risk values were between 1.8 and 3.6. Terry and colleagues found a twofold increase in risk in women with a lifetime consumption of 15–30 g of alcohol a day. By contrast, Hines and colleagues did not find any effect of *ADH1C* polymorphism on breast cancer risk in a case–control study[94].

oxidative stress together with cirrhosis are important factors in ethanol-related carcinogenesis[56].

The formation of ROS such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) causes oxidative injury leading to various diseases, including cancer. Several enzyme systems are capable of producing ROS, including the cytochrome P450 2E1 (CYP2E1)-dependent microsomal mono-oxygenase system, the mitochondrial respiratory chain and the cytosolic enzymes xanthine oxidase and aldehyde oxidase[102]. Ethanol-mediated ROS formation may be due to various factors. Increased electron leakage from the mitochondrial respiratory chain associated with the stimulation of reduced nicotinamide adenine dinucleotide (NADH) shuttling into mitochondria[103] can create ROS, as can the interaction between N-acetylsphingosine (from tumour necrosis factor-α (TNFα)) and mitochondria[104]. The induction of sphingomyelinase by TNFα increases levels of ceramide, an inhibitor of the activity of the mitochondrial electron-transport chain, leading to increased mitochondrial production of ROS.

Inflammation-driven oxidative stress, including activated hepatic phagocytes as constantly observed in alcoholic hepatitis, is predominantly responsible for the generation of ROS[105]. Furthermore, hepatic iron overload (increased by chronic ethanol ingestion) increases ROS[56]. Nitric oxide production is increased by the effect of ethanol on inducible nitric oxide synthase, leading to the formation of the highly reactive peroxynitrite ($ONOO^-$)[106]. Most importantly, the induction of CYP2E1 by ethanol causes ROS formation, including hydroxyethyl radicals (HER)[56,102].

Animal experiments have convincingly shown the important role of CYP2E1 in the generation of ROS. As for ADH, polymorphisms of CYP2E1 are associated with different levels of enzyme activity, but a meta-analysis found no association between CYP2E1 polymorphisms and cancer of the oesophagus or liver[107,108]. Chronic ethanol consumption results in a 10–20 fold increase in hepatic CYP2E1 in animals and humans[56]. In humans, this induction was observed following the daily ingestion of 40 g of ethanol (approximately 400 ml of 12.5% alcohol wine) for just 1 week[109]. CYP2E1 further increased after 4 weeks of daily alcohol consumption, but this increase varies among individuals, giving evidence for genetically controlled mechanisms. In animal experiments, the induction of CYP2E1 correlates with NAD phosphate (NADPH) oxidase activity, the generation of HER, lipid peroxidation and the severity of hepatic injury, all of which could be prevented by the CYP2E1 inhibitor chlormethiazole[110]. In addition, oxidized DNA products have been found to be lower in *Cyp2e1* knockout mice compared with wild-type mice[111], and hepatic injury was strikingly increased in transgenic mice that overexpressed CYP2E1 (REF. 112). CYP2E1 has a high rate of NADPH oxidase activity, resulting in the generation of large quantities of O_2^- and H_2O_2.

ROS produced by CYP2E1 result in the generation of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal (4-HNE) [113] (FIG. 3). 4-HNE can react with DNA bases such as deoxyadenosine and deoxycytidine to form the exocyclic DNA adducts 1,N^6-ethenodeoxyadenosine (εdA) and 3,N^4-ethenodeoxycytidine (εdC)[114]. These adducts are highly mutagenic; for example one leads to a mutation at codon 249 of *TP53* (which encodes p53) that makes cells more resistant to apoptosis and gives them some growth advantage[115]. εdA and εdC adducts can be measured in the urine by immuno-enriched high-pressure liquid chromatography (HPLC) fluorescence and by immunohistochemistry in the liver[116]. It has been shown that these adducts already occur at the fatty liver stage of alcoholic liver disease (ALD), but they are more frequently observed in advanced ALD[116]. Increased ROS production has also been found in HepG2 hepatoma cells transfected with *CYP2E1*. A correlation between CYP2E1 levels and DNA adduct formation was found in HepG2 cells, which could be prevented by the addition of chlormethiazole, a specific inhibitor of CYP2E1 (H.K.S. and J. Nair, unpublished data).

In experimental animals, CYP2E1 induction has also been observed in the gastrointestinal mucosa[117]. Some studies have shown that chemically induced carcinogenesis or ethanol-associated mucosal hyper-regeneration could be counteracted by the concomitant administration of radical scavengers such as α-tocopherol, supporting evidence for a role of ethanol-induced oxidative stress in carcinogenesis[36,118].

Another mechanism by which alcohol may be oncogenic relates to the metabolism of certain pro-carcinogens, including nitrosamines, polycyclic hydrocarbons and aflatoxins, by alcohol-induced CYP2E1 (REF. 119). Such interaction has been shown for nitrosamines in experiments with rats in which the alternating administration of alcohol and dimethylnitrosamine caused liver cancer[120]. On the other hand, ethanol may competitively

Microsomal mono-oxygenase system
An enzymatic system located in microsomes that depends on cytochrome P450s, and metabolizes drugs, xenobiotics (including toxins and carcinogens) and some intermediary metabolites, detoxifies them and makes them more hydrophilic. Further reactions (such as glucoronidation and sulphation) then render them water soluble.

Hydroxyethyl radicals
A radical generated through the CYP2E1-dependent microsomal ethanol oxidation. The radical also binds to proteins, resulting in a neo-antigen formation, which may induce an immune reaction.

Box 2 | **Ethanol, acetaldehyde and oestrogens**

Alcohol ingestion has been associated with higher blood oestrogen concentrations in premenopausal women, although some studies observed this effect only in women taking oral contraceptives[95]. Such an increase was even seen at relatively low ethanol blood concentrations of 25 mg per 100 ml, corresponding to an intake of approximately one drink, and was noted especially at the mid-phase of the menstrual cycle[93]. The mechanism for this increase is not known. However, steroid hormones including oestrogens can also be metabolized by alcohol dehydrogenase (ADH), and a competition between the metabolism of oestrogens and ethanol may occur. The fact that blood concentrations of acetaldehyde were found to be particularly high when oestradiol levels were highest during the menstrual cycle may support this hypothesis[96]. This could have an impact on breast cancer risk, as in the mid-phase of the menstrual cycle relatively high serum oestradiol concentrations may be further increased by ethanol, and may result in even higher acetaldehyde concentrations. Thus, in this situation two risk factors would act together.

inhibit the hepatic activation of nitrosamines to their corresponding carcinogens if ethanol and nitrosamines are administered simultaneously (FIG. 1). In this situation nitrosamines may induce extrahepatic tumours[6,119,121].

Cirrhosis of the liver

Long-term alcohol consumption is one of the major causes of liver cirrhosis. In industrialized countries, nearly all HCCs develop in cirrhotic livers, and cirrhosis itself is a well-recognized pre-neoplastic lesion. Among the pre-neoplastic alterations typically found in the liver are enzyme-altered foci and dysplastic nodules, which precede the evolution of HCC[122]. In this setting, fibrosis and cirrhosis, along with an altered cytokine and growth factor milieu have an important role in triggering malignant growth. Such small enzyme-altered hepatic foci are inducible by choline-deficient, ethionine-supplemented diets in rats, but their number and size further increase with concomitant alcohol pretreatment[123]. In enzyme-altered foci induced in rodents after long-term alcohol administration, the appearance of initially quiescent hepatic progenitor cells, termed 'oval cells', has been observed[124]. Oval cells are considered to be hepatic stem cells harbouring pluripotency, and it has been shown that their appearance precedes the development of some HCCs[125]. Furthermore, recent work has convincingly shown that HCCs may arise directly from oval cells and exhibit a gene-expression pattern distinct from other types of HCC but typical for oval cells[126]. Importantly, oval cells reveal an unusual reciprocal relationship with hepatocyte proliferation in response to exposure to hepatotoxins — oval cells proliferate whereas hepatocyte proliferation is usually inhibited. By contrast, oval cell proliferation is nearly absent after partial hepatectomy, but hepatocytes are highly proliferative. Important stimulators of oval cell proliferation are the cytokines TNFα and transforming growth factor β1 (TGFβ1), both of which are markedly upregulated in ALD. In heavy drinkers, high levels of TNFα in particular are secreted from Kupffer cells after stimulation by bacterial endotoxins derived from the gut[127]. After binding to its cellular receptors, TNFα may precipitate an array of distinct down-stream biological effects depending on the extent of TNFα upregulation[127]. Thus, TNFα can either trigger JUN N-terminal kinase 1 (JNK1) and thereby promote proliferation in synergy with other growth factors, including epidermal growth factor, or induce apoptosis and/or necrosis through the caspase cascade or through mitochondrial damage. TNFα may dose-dependently cause cell death (apoptosis and/or necrosis) or improved cellular survival. Cell survival may occur when cells are exposed to a TNFα stimulus below the lethal dose, and at this point may be rendered more susceptible to transdifferentiation caused by carcinogens or acetaldehyde. In addition, increased levels of TNFα elicit the activation of nuclear factor κB (NFκB), which activates cell-survival machinery involving anti-apoptotic mitochondrial proteins such as BCL2 and manganese superoxide dismutase, which maintain mitochondrial integrity and ongoing cellular energy supply[128]. However, the role of NFκB in hepatocarcinogenesis has been challenged. Although Pikarsky *et al.* showed the carcinogenic action of NFκB[129], experiments from Maeda *et al.* contradicted this[130]. These authors used the conditional hepatocyte-specific IκB kinase 2 (IKK2) knockout mouse (IKK2Δhep) to reduce NFκB activation in a chemical carcinogenesis model, and found an increase in carcinogenesis. However, when the authors used an IKK2 knockout mouse that lacked the gene in hepatocytes and non-parenchymal liver cells, such as the cytokine-producing Kupffer cells, these mice were protected from liver carcinogenesis. The function of NFκB in the outcomes of the above-mentioned studies may be explained by the different natures of the tumour models that were used.

TGFβ1, a pro-fibrotic cytokine that is stimulated by ethanol[131], can also act as a growth inhibitor for hepatocytes and immune cells, but not for oval cells, which are less sensitive to TGFβ1 (REF. 132). In addition, TGFβ1 was identified as a crucial participant in an important process in tumorigenesis termed epithelial–mesenchymal transition (EMT). TGFβ1 can switch from an early tumour suppressor to a stimulator of growth and invasion during colon carcinoma progression, possibly based on its ability to regulate EMT[133]. Recent data indicate that EMT requires certain signals for initiation, and TGFβ1 has been implicated as a key inducer of this event[134]. This seems particularly noteworthy as TGFβ1 is activated, at least in part, by acetaldehyde and ROS. Thus, it can be proposed that the alcohol-driven increase of TNFα and TGFβ1 expression and activation creates a cytokine pattern that promotes carcinogenesis.

A typical histological feature of alcoholic liver damage is the occurrence of Mallory bodies, and the risk of developing HCC is significantly higher in cirrhosis with Mallory bodies than without[135]. Recent data confirms the assumption that Mallory bodies represent a pre-neoplastic phenotype in the malignant transformation of hepatocytes[136].

Other mechanisms: nutritional factors

Ethanol and retinoid metabolism. Retinoids are fat-soluble compounds with vitamin A activity. Retinoic acid is particularly important because of its profound

Enzyme-altered foci
(EAF) Hepatocyte conglomerates with altered protein expression as reflected by immunohistochemistry, typically of glutathione-*S*-transferase P1 and transforming growth factor-β. EAF are typically found in chemically-induced hepatocarcinogenesis, and indicate early malignant transformation.

Hepatectomy
Partial or complete surgical removal of the liver. Usually performed to resect malignant or benign liver tumours.

Kupffer cells
These are specialized macrophages located in the liver. The activation of these cells by various insults (such as exposure to bacterial endotoxin) results in the release of various cytokines in the liver that might lead to hepatocyte death or damage.

Epithelial–mesenchymal transition
Conversion from an epithelial to a mesenchymal phenotype, which is a normal component of embryonic development. In carcinomas, this transformation results in altered cell morphology, the expression of mesenchymal proteins and increased invasiveness.

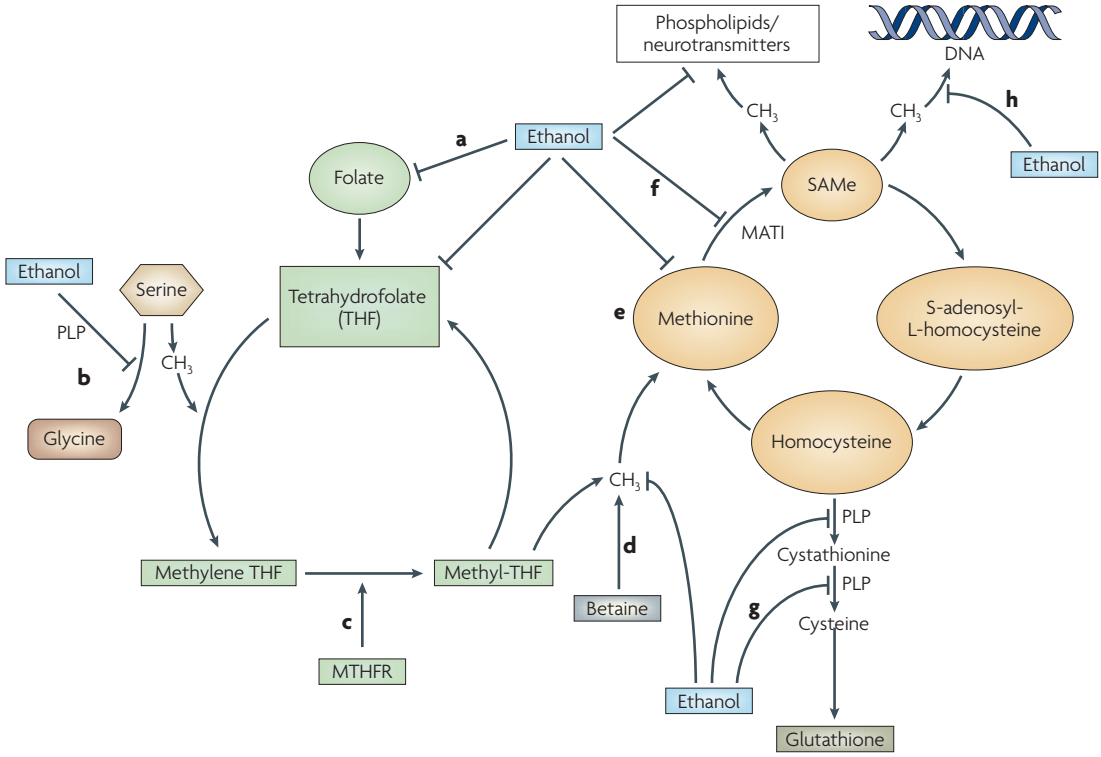

Figure 4 | **Effect of ethanol and acetaldehyde on methyl transfer.** Alcohol and/or acetaldehyde interact at several steps with methyl transfer. **a** | Inhibition of folate absorption. **b** | Interaction with pyridoxal-5′ phosphate (PLP) and interruption of methyl group generation. **c** | Polymorphism of methylene tetrahydrofolate reductase (*MTHFR*) modulates the availability of tetrahydrofolate (THF). **d** | Interaction with methyl group transfer from betaine to homocysteine through the inhibition of betaine-homocysteine methyltransferase. **e** | Inhibition of methionine synthase. **f** | Inhibition of methionine adenosyltransferase I (MATI) and thus of the synthesis of S-adenosyl-L-methionine (SAMe). Two additional levels of interaction probably confer a risk of malignant transformation. **g** | First, the coordinate disposal of homocysteine is disrupted by its trans-sulphuration to cystathionine through inactivating cystathionine-β-synthase. Cystathionine is further hydrolysed to cysteine, which is a substrate for the generation of glutathione. Glutathione, in turn, is then reduced to counteract the increased oxidative stress generated during alcohol metabolism. **h** | Second, methyl group transfer onto DNA cytosine residues is impaired.

effects on cellular growth and differentiation. Retinoic acid regulates gene transcription of various regulators of cell proliferation and migration by signalling through its nuclear retinoic acid receptors (RARα, RARβ and RARγ, and RXRα, RXRβ, and RXRγ). So, depletion of systemic and tissue-specific retinoic acid levels may have important consequences for cell proliferation, differentiation and possibly malignant transformation. Chronic alcohol consumption decreases vitamin A and retinoic acid concentrations in the liver, and is associated with clinical signs of vitamin A deficiency, such as night blindness and sexual dysfunction[137]. In addition, a strong inverse relationship between serum concentrations of vitamin A and later development of HCC in humans has been observed[138], and disruption in retinoid metabolism and signalling may have a key role in carcinogenesis[139]. The main reason for the substantial decrease in hepatic retinoic acid following alcohol consumption is increased catabolism by ethanol-induced CYP2E1 (REF. 140) (FIG. 1).

The decrease in retinoic acid levels following chronic ethanol administration in rats was associated with a decrease in mitogen-activated protein kinase (MAPK) and an increase in levels of phosphorylated JNK. This was further associated with a functional downregulation of retinoic acid receptors and up to an eightfold higher expression of the AP1 (JUN and FOS) transcriptional complex, resulting in hepatic cell hyperproliferation and a decrease in apoptosis[141–143]. Hence, increased AP1 expression favours the proliferation and survival of cells undergoing malignant transformation. In this context retinoic acid is of importance, as it might act as a negative regulator of AP1-responsive genes through protein–protein interactive inhibition or 'cross talk' inhibition with the JNK signalling pathway. These findings were almost completely normalized by supplementation with retinoic acid and/or by the administration of chlormethiazole, a specific CYP2E1 inhibitor, supporting the hypothesis that alcohol-associated loss of hepatic retinoic acid is CYP2E1 dependent and is responsible for the changes observed[142,143].

More recently, nitrosamine-induced hepatic carcinogenesis in rats resulted in the production of nodular regenerative hyperplasia and even hepatic adenoma following chronic alcohol consumption, but not in control animals. Administration of chlormethiazole

Mallory bodies
Mallory body inclusions are a characteristic feature of alcoholic and non-alcoholic steatohepatitis, but may also be found in chronic cholestatic and metabolic diseases and hepatocellular neoplasms, particularly hepatocellular carcinomas. Mallory bodies share similarities with cytoplasmic inclusions observed in neural diseases and myopathies, and primarily consist of cytokeratins.

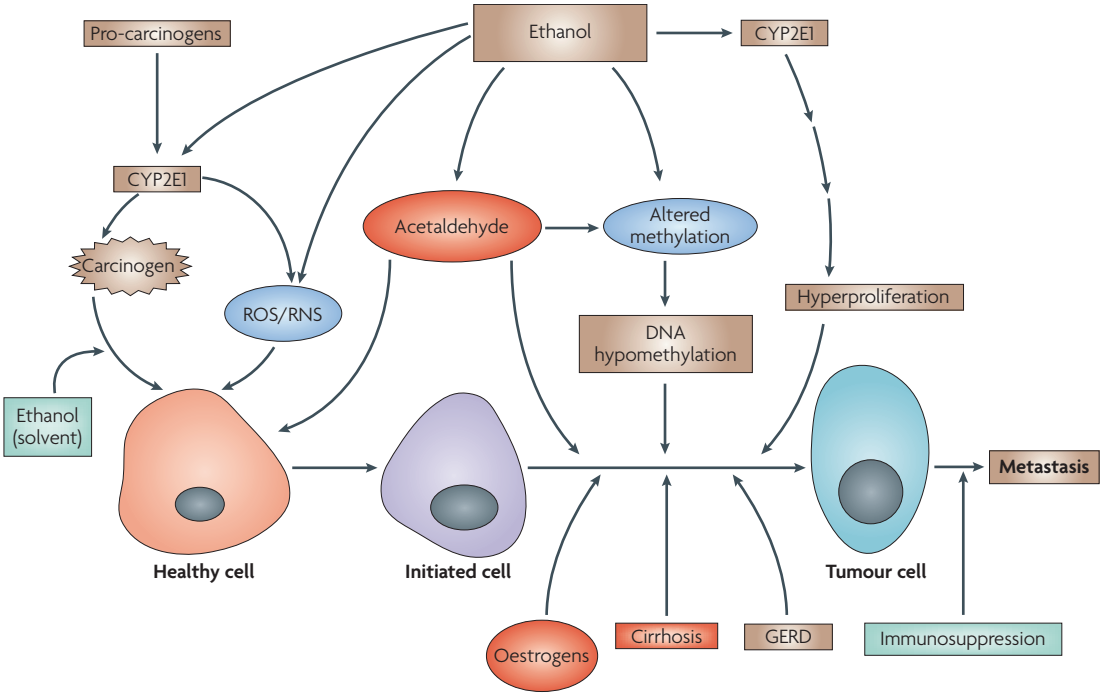

Figure 5 | **A simplified scheme of the mechanisms by which alcohol may affect carcinogenesis.** Mechanisms with strong evidence are shown in red, with moderate evidence in blue and with weak evidence in green. During cancer initiation, ethanol increases the activation of various pro-carcinogens present in alcoholic beverages, tobacco smoke, diets and industrial chemicals to carcinogens through the induction of cytochrome P450 2E1 (CYP2E1). Ethanol may further act as a solvent for these carcinogens to enter the cell, especially into the mucosa of the upper aerodigestive tract (UADT). Ethanol is metabolized by alcohol dehydrogenase (ADH) to acetaldehyde, which is a carcinogen and binds to DNA. This metabolism is modified by polymorphisms in the genes that encode ADH and acetaldehyde dehydrogenase (ALDH), yielding various amounts of acetaldehyde. In addition, ethanol is also oxidized by CYP2E1, again producing acetaldehyde but also reactive oxygen species (ROS). ROS lead to lipid peroxidation and lipid peroxidation products such as 4-hydroxynonenal (4HNE), which binds to DNA to form mutagenic adducts. During cancer promotion, ethanol and acetaldehyde alter methyl transfer, leading to DNA hypomethylation that could change the expression of oncogenes and tumour-suppressor genes. Ethanol also decreases levels of retinoic acid owing to increased CYP2E1-mediated metabolism, leading to the generation of toxic metabolites that are associated with changes in cell-cycle behaviour and cellular hyper-regeneration. Ethanol also increases oestrogen levels, an important mechanism in breast cancer. Other ethanol-mediated toxic effects associated with cancer development are cirrhosis of the liver and gastroesophageal reflux disease (GERD), resulting in hyperproliferation of the oesophageal mucosa. Finally, ethanol-associated immune suppression may facilitate tumour cell spread.

restored hepatic retinoic acid levels to normal, and most importantly prevented hepatic tumorigenesis completely (H.K.S and X.D. Wang, unpublished data). In summary, chronic alcohol administration alters both MAPK and retinoid signalling pathways owing to a decrease in hepatic retinoic acid concentration, which augments cell proliferation as well as apoptosis and may contribute to carcinogenesis. Chronic alcohol consumption can also increase the toxicity of retinoids. In the α-tocopherol, β-carotene cancer prevention study (ATBC trial) study, in which β-carotene was given to smokers to prevent lung cancer, drinking 11 or more grams of ethanol a day resulted in a significantly increased occurrence of lung cancer[144], possibly owing to the toxicity of retinoic acid metabolites generated through CYP2E1 (REF. 145).

β-carotene
Synonym for provitamin A. Results in the generation of retinoids after centric or excentric cleavage. Contained in carrots and other vegetables and has antioxidant activity.

Ethanol and altered methyl group transfer. Methylation of genes is an important tool to control gene expression, whereby hypermethylation has a silencing effect on gene transcription and hypomethylation results in increased gene expression. Therefore, DNA methylation or demethylation is an effective mechanism to suppress or activate gene transcription[146–148]. This obviously has important implications for tumorigenesis, in which the activation of oncogenes or the silencing of tumour-suppressor genes is a pivotal step in the evolution of a malignant cell clone. Aberrant methyl transfer may be important for alcohol-mediated carcinogenesis, and the evidence is most compelling for liver and colorectal carcinogenesis.

Particularly important is the alcohol-mediated inhibition of *S*-adenosyl-L-methionine (SAMe) synthesis, as SAMe is the universal methyl group donor and enzyme activator in methyl transfer reactions[149,150] (FIG. 4). SAMe is generated predominantly in the liver from L-methionine and ATP by the enzyme methionine adenosyltransferase (MAT), encoded by two different genes, *MAT1A* and *MAT2A*[151]. *MAT1A* encodes the isoenzymes MATI and MATIII; *MAT2A* encodes the isoenzyme MATII. MATI and MATIII are capable of maintaining high intracellular SAMe levels, and are predominantly transcribed in adult liver, whereas MATII is active in fetal and regenerating

liver tissue. Therefore, *MAT1A* is crucial for providing MAT activity and the sufficient production of SAMe required for adaptive gene silencing. An important finding was that *MAT1A*[151] is almost completely silenced in liver injury and hepatocarcinogenesis, mainly due to hypermethylation, which may explain the decreased MATI and MATIII activity as well as the resulting reduced SAMe levels in ALD[150]. Experimental data show that *Mat1a* knockout mice develop marked SAMe deficiency, hepatomegaly, fatty liver and eventually HCC[152].

With regard to carcinogenesis, a major function of SAMe is donating methyl groups for gene methylation. Approximately 1% of DNA is methylated by the replacement of a hydrogen atom attached to the C5 of cytosine by a methyl group, mediated through the activity of DNA methyltransferases (DNMTs), of which four isoforms are identified with distinct patterns of activity: *de novo* DNMT and DNMT. The former is responsible for the addition of methyl groups to a target sequence devoid of pre-existing methylation, whereas the latter restores partially methylated DNA substrates. Acetaldehyde inhibits DNMT activity, but so far this has only been observed in rats[153].

Rats chronically fed alcohol had global hepatic DNA hypomethylation but a normal pattern of methylation of *Trp53*, which encodes p53 (REF. 154). This implies possible hypomethylation, that is, the upregulation of oncogenes, in the absence of the potentially protective higher expression of tumour-suppressive p53.

Ethanol also interferes with the disposal of homocysteine and the generation of glutathione (FIG. 4). Glutathione is the main reductive compound that counteracts the increased oxidative stress generated during alcohol metabolism. To maintain a sufficient methylation capacity, the organism needs to be supplied with nutritional factors, so called 'lipotropes'. This group of micronutrients includes choline, betaine and methionine, all of which are essential in the formation, transport and transfer of methyl groups to target molecules. A large body of literature convincingly shows that malnutrition as a result of chronic alcohol consumption depletes all of these lipotropes. In addition to poor intake of these nutrients, unfavourable interactions of alcohol with their metabolism (FIG. 4) causes impaired methylation capacity in alcoholics[149]. In addition, alcoholics are frequently severely deficient in cofactors of methyl group transfer such as folate, vitamin B6 and vitamin B12, largely due to malnutrition. Indeed, epidemiological studies have noted a RR of 7.4 for distal colorectal cancer in individuals who consume more than 20 g of ethanol a day and, consequently, have low methionine and folate levels compared with occasional drinkers who have a normal methionine and folate intake[155]. Similar data have been reported for vitamin B6 (REF. 156). Methyle netetrahydrofolate reductase (MTHFR) is important to restore folate levels. The gene that encodes this enzyme is polymorphic, and individuals with the 677CT variant associated with reduced enzyme activity may have an increased risk for colorectal cancer when they drink alcohol[157].

Conclusion

The aim of this Review was to summarize the current evidence for a contributory role of chronic alcohol consumption to worldwide cancer burden and the mechanisms involved (FIG. 5). The evidence includes data from animal and human studies, which show a causal relationship between chronic alcohol consumption and cancers of the upper gastrointestinal tract, the liver, the colorectum and the female breast.

Considering the high frequencies of these cancers and the persistently high alcohol consumption of the general population, the link between alcohol and certain tumours has important consequences for prevention and early detection. So far, very little is known about safe margins of alcohol consumption, and even less about an individual's risk of developing alcohol-related malignancies. More accurate assessment of this risk may become available in the future through the identification of additional risk factors, particularly through exploiting the potential of human genomic and proteomic research.

Although difficult to implement in practice, health authorities should introduce more effective measures in order to educate the public about the potential hazards of regular and excessive alcohol consumption, not only with regard to widely known alcohol-induced diseases, but also with regard to certain cancers. As a dose–response relationship between alcohol consumption and cancer risk exists, one of the most important aspects is the control of heavy drinking. The European Code Against Cancer recommends a daily alcohol intake of 20–30 g (approximately 250 ml wine or 500 ml beer) in healthy men, and half of that in healthy women to avoid alcohol-associated diseases, including cancer[158]. Similar guidelines from the US Departments of Agriculture, and Health and Human Services suggest a maximum of 28 g of alcohol a day in men and half of this in women[159].

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