Seminartema: Chemische Kanzerogenese


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Referat 1: Ernährungsspezifische Risikofaktoren für die Entstehung von Darmkrebs
M. Oostindjer et al.: The role of red and processed meat in colorectal cancer development: a perspective. Meat Science (2014), 97, 583-596
J. Fahrer and B. Kaina: O6-methylguanine-DNA methyltransferase in the defense against N-nitroso compounds and colorectal cancer. Carcinogenesis (2013), 34, 2435-2442

In diesem Referat sollen die Ernährungsspezifischen Risikofaktoren für die Entstehung von Darmkrebs behandelt werden, mit besonderem Fokus auf den N-Nitroso-Verbindungen und den zugrunde liegenden Mechanismen. Außerdem soll auf die Bedeutung der in diesem Zusammenhang wichtigen DNA-Reparatur-Mechanismen eingegangen werden.

Referat 2: Tabak-induzierte Kanzerogenese

Das Referat soll die mechanistischen Grundlagen der Tabak-induzierten Kanzerogenese sowie die wichtigsten kanzerogenen Inhaltsstoffe des Tabak-Rauchs erläutern und neuere Aspekte der Tabak-Kanzerogenese beleuchten.

Referat 3: Alkohol-induzierte Kanzerogenese

Innerhalb des Referates sollen die verschiedenen Mechanismen der Alkohol-assoziierten Kanzerogenese erarbeitet werden. Ebenso soll kurz auf epidemiologische Grundlagen und genetische Faktoren eingegangen werden.
Molecular mechanisms of alcohol-mediated carcinogenesis

Helmut K. Seitz* and Felix Stickel†

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.

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, 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\(^1,4-11\).

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\(^2,4-11,13\). 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 oestriadiols 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; gastrooesophageal 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.

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 hyperinsulinaemia 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.

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 day32. In hepatitis B, 80 g of alcohol a day has a RR of 38 compared with 32 in non-drinkers33.

More than 100 epidemiological studies have consistently demonstrated a dose-dependent increase of the risk for breast cancer with chronic alcohol consumption34. 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%, respectively34. 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 day35. 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 alcohol36.

A positive dose–response relationship between alcohol consumption and colorectal cancer was also reported in more than 50 prospective and case–control studies36,37. A review of 27 cohort studies reported a twofold higher risk for colorectal cancer in alcoholics38. Pooled results from eight cohort studies39 and data from a recent meta-analysis40 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 incidence8. In addition, five of six studies showed a significant correlation between colorectal polyps and alcohol consumption39.

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 performed3. 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 carcinogen23,24–26 (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 skin3. Some of the studies had to be criticized for methodological reasons.

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 hyperinsulinaemia 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.

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 topic15. 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 day41. A dose–response relationship for the amount of ethanol consumed and the risk of HCC has been shown31. 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 resistance32. According to the data available it is not possible to distinguish between increased risk...
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\(^{36,39}\), 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\(^{42}\).

**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\(^{47}\). Inhalation studies in rats and hamsters found that acetaldehyde resulted in the occurrence of nasal adenocarcinomas and squamous cell carcinomas\(^{38,39}\). 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\(^{40–45}\). 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\(^{46,57}\) (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\(^2\)-ethyldene-2′-deoxyguanosine (N\(^2\)-EtdG). N\(^2\)-EtdG needs a reduction step to become a stable adduct, N\(^2\)-ethyl-2′-deoxyguanosine (N\(^2\)-EtidG). Fang and Vaca reported levels of N\(^2\)-EtidG in Swedish drinkers and controls, and found higher adduct levels in lymphocytes of alcohol consumers compared with controls\(^{42}\). They also found an increase of the same adducts in mice exposed to 10% alcohol in their drinking water\(^{43}\).

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\(^2\)-EtidG, which were often found to be undetectable in blood\(^{44}\). This is probably due to the fact that the formation of N\(^2\)-EtidG from N\(^2\)-EtdG requires a reduction step, as mentioned above, and therefore the level of N\(^2\)-EtidG 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\(^{45}\).

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\(^{46}\). Relevant polyamine concentrations are present in tissues with hyper-regeneration. Chronic alcohol

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**Table 1 | Alcohol and experimental carcinogenesis**

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

*dw, drinking water; F, female; FAP, familial adenomatous polyposis; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; M, male.*
DNA forming exocyclic DNA etheno adducts. Ethanol oxidation by catalase seems to further increase the generation of ROS (see text). Acetaldehyde can bind to DNA, beer, and tobacco smoke.

A group of chemicals with nitrosamines activation of various environmental pro-carcinogens present in tobacco smoke and activity not only leads to increased generation of ROS, but also to an increased requirement of acetaldehyde to be activated. CYP2E1 also decreases tissue levels of retinol and retinoic acid, which have important functions in the regulation of cell growth and differentiation. NADH, a high acetaldehyde concentration is 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.

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 lipid peroxidation and lipid peroxidation 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.

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. Of the lower oesophageal–gastric junction. Thus, GERD is an additional risk factor for cancer development in people who consume large quantities of alcohol.

**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.

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. 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. Levels of N²-EtGc 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. The data from Japan gave sufficient evidence for the IARC to conclude that acetaldehyde has a causal role in ethanol-related oesophageal carcinogenesis.

![Diagram](https://via.placeholder.com/150)
Acetaldehyde

Mitochondrial damage may initiate a cascade of reactions, including the production of ROS through the ADH reaction. Mitochondrial damage is associated with an increase in ROS production, which can damage DNA, leading to the formation of oxidative DNA lesions. In addition, acetaldehyde also increases DNA damage indirectly by upregulating anti-apoptotic genes, such as MCL1, which inhibit DNA repair and apoptosis.

ROS-induced lipid peroxidation leads to the production of lipid peroxidation products, such as 4HNE, which can be converted to 2,3-epoxy-4-hydroxynonenal (4HNE). This compound is a substrate for the enzyme 4HNE dioxygenase (4HNE-D) and can be neutralized by an effective antioxidative defence system (AODS). As the ROS/RNS generation increases, the production of acetaldehyde also increases, resulting in an increase in DNA damage.

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

In addition, SNPs exist for the alcohol dehydrogenase genes ADH1B*2 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 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 also have 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. 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 Caucasians have shown contradictory results, and are still inconclusive (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. For breast cancer, three out of four 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 (BOX 2). Similar to individuals with ALDH2 deficiency, 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).

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 mucous 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.

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 that is very similar to the morphological changes observed after chronic alcohol administration. These changes were only observed when the animals had functionally intact salivary glands, which supports the hypothesis that salivary acetaldehyde is involved in carcinogenesis.

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. Salivary acetaldehyde concentrations are decreased after an antiseptic mouthwash by approximately 30–50%, underlining the importance of oral bacteria in acetaldehyde generation. 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.
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 \textit{in vitro} acetaldehyde production 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 \(\mu\)M\(^110\). The colon contains an enormous amount of bacteria, and colonic acetaldehyde concentrations following ethanol administration to piglets and rats exceed 250 \(\mu\)M\(^45\) and 500 \(\mu\)M\(^39\), 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\(^68\). 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
The radical also binds to proteins, resulting in a neo-antigen formation, which may induce an immune reaction.

**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. Other studies could not confirm this observation. 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. 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. Those studies that incorporated patients with higher daily alcohol intake found a significant effect of ADH1C polymorphism on UADT and colonic cancer, whereas those on patients with small daily alcohol ingestion did not.

ROS produced by CYP2E1 result in the generation of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal (4-HNE). Chronic ethanol consumption results in a 10–20 fold increase in hepatic CYP2E1 in animals and humans. 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. 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 chlorimethiazole. In addition, oxidized DNA products have been found to be lower in Cyp2e1 knockout mice compared with wild-type mice, and hepatic injury was strikingly increased in transgenic mice that overexpressed CYP2E1. CYP2E1 has a high rate of NADPH oxidase activity, resulting in the generation of large quantities of O$_2^-$ and H$_2$O$_2$.

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 can create ROS, as can the interaction between N-acetylsphingosine (from tumour necrosis factor-α (TNFα)) and mitochondria. 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. Furthermore, hepatic iron overload (increased by chronic ethanol ingestion) increases ROS. 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$^-$). Most importantly, the induction of CYP2E1 by ethanol causes ROS formation, including hydroxyethyl radicals (HER). 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. Chronic ethanol consumption results in a 10–20 fold increase in hepatic CYP2E1 in animals and humans. 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. 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 chlorimethiazole. In addition, oxidized DNA products have been found to be lower in Cyp2e1 knockout mice compared with wild-type mice, and hepatic injury was strikingly increased in transgenic mice that overexpressed CYP2E1. CYP2E1 has a high rate of NADPH oxidase activity, resulting in the generation of large quantities of O$_2^-$ and H$_2$O$_2$.

ROS produced by CYP2E1 result in the generation of lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal (4-HNE) FIG. 5. 4-HNE can react with DNA bases such as deoxyadenosine and deoxyctydine to form the exocyclic DNA adducts 1,N$^6$-ethenodeoxycytidines (εdA) and 3,N$^4$-ethenodeoxycytidine (εdC). 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. 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. 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. 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 chlorimethiazole, 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. 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.

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. Such interaction has been shown for nitrosamines in experiments with rats in which the alternating administration of alcohol and dimethylnitrosamine caused liver cancer. On the other hand, ethanol may competitively...
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.

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 tumours6,119,121.

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 HCC121. 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 pretreatment121. 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 observed124. Oval cells are considered to be hepatic stem cells harbouring pluripotency, and it has been shown that their appearance precedes the development of some HCCs125. 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 cells126. 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 gut127. After binding to its cellular receptors, TNFα may precipitate an array of distinct downstream biological effects depending on the extent of TNFα upregulation127. 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 supply128. However, the role of NFκB in hepatocarcinogenesis has been challenged. Although Pikarsky et al. showed the carcinogenic action of NFκB129, experiments from Maeda et al. contradicted this130. These authors used the conditional hepatocyte-specific 1κB kinase 2 (IKK2) knockout mouse (IKKΔ2hep) 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 ethanol131, 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 EMT133.

Recent data indicate that EMT requires certain signals for initiation, and TGFβ1 has been implicated as a key inducer of this event134. 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 without135. Recent data confirms the assumption that Mallory bodies represent a pre-neoplastic phenotype in the malignant transformation of hepatocytes136.

### 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...
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 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 dysfunction135. In addition, a strong inverse relationship between serum concentrations of vitamin A and later development of HCC in humans has been observed137, and disruption in retinoid metabolism and signalling may have a key role in carcinogenesis139. 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 apoptosis141–143. 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 observed142,143.

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
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. 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 methylation reactions. 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 $MAT2A$. $MAT1A$ 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.

$\beta$-carotene

Synonym for provitamin A. Results in the generation of retinoids after centric or eccentric cleavage. Contained in carrots and other vegetables and has antioxidant activity.
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 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. Experimental data show that Mat1a knockout mice develop marked SAMe deficiency, hepatomegaly, fatty liver and eventually HCC.

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.

Rats chronically fed alcohol had global hepatic DNA hypomethylation but a normal pattern of methylation of Trp53, which encodes p53. 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. 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 ‘lipoproteins’. 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 lipoproteins. In addition to poor intake of these nutrients, unfavourable interactions of alcohol with their metabolism causes impaired methylation capacity in alcoholics. 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. Similar data have been reported for vitamin B6 (Ref. 156). Methyle tetrahydrofolate 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.

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. 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. 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.
**Gastrointestinal tract, liver, colorectum and female genital organs**


**Landmark study identifying the mutant ALDH2*2 allele as a genetic risk factor for the development of upper aerodigestive tract cancer in regular alcohol drinkers from Japan.**


**An excellent summary focusing on the role of alcohol in the development of hepatocellular carcinoma.**


**An extensive reanalysis of 53 studies including 58,515 women with invasive breast cancer and 95,067 controls estimating the relative risks for development of breast cancer after stratification for alcohol and tobacco consumption.**


A pooled analysis of eight studies from North America and Europe assessing the contribution of alcohol consumption to the risk of colorectal cancer showing no evidence of an increased risk of the colorectal cancer rate at daily alcohol consumption of 45 g and more...


**An important study that showed the carcinogenicity of alcohol in animals.**


The first study in animals to identify acetaldehyde as a carcinogen and to demonstrate the role of gastrointestinal bacteria in acetaldehyde...


Bestor, T. H. & Tycko, B. Creation of genomic

Hepatology
AP-1 (c-jun and c-fos) expression in rat liver.
reduces retinoic acid concentration and enhances
Wang, D., Liu, C. & Chung, J. Chronic alcohol intake
polar metabolites in rat liver via induction of
Ethanol enhances retinoic acid metabolism into
Liu, C., Russell, R. M., Seitz, H. K. & Wang, X. D.
35 rat liver.
overexpression and hepatocyte hyperproliferation in
concentration suppresses ethanol induced c-jun
proliferation owing to an upregulation of AP1.
Kass, S., Pruss, D. & Wolffe, A. P. How does DNA
Kam, S., Pruss, D. & Wolffe, A. P. How does DNA
expression of c-myc, c-fos and c-jun in rat liver
Kohn, K. A., Torrance, J. & Weitzman, E. Development
mice: implications for the fetal alcohol syndrome.
donates to alcohol metabolism and hepatocyte
apoptosis in rat liver. Alcoholism Clin. Exp. Res.
Larsson, S. C., Giovannucci, E. & Wolk, A. Vitamin B6,
methylation patterns in the evolution of liver disease.
A detailed review on the role of impaired
methylation patterns in the evolution of liver disease.
Torres, L. et al. Liver-specific methionine
adenosyltransferase MAT1A gene expression is
associated with a specific pattern of promoter
methylation and histone acetylation: implications for
MAT1A silencing during transformation. FASEB J.
Santamaria, E. et al. Molecular profiling of
hepatocellular carcinoma in mice with a chronic
deficiency of hepatic s-adenosylmethionine: relevance
in human liver diseases. J. Proteome Res. 5, 944–953
Ethanol consumption inhibits fetal DNA methylation in
mice: implications for the fetal alcohol syndrome. Alc.
This study in rats shows the induction of global
dNA hypomethylation after chronic alcohol
feeding.
Choi, S. W. Chronic alcohol consumption induces
genomic but not p53-specific DNA hypomethylation in
Giovannucci, E. et al. Alcohol, low-methionine-low
folate diets and risk of colon cancer in men. J. Natl
Larsson, S. C., Giovannucci, E. & Wolk, A. Vitamin B6,
alcohol consumption, and colorectal cancer: a
longitudinal population-based cohort of women.
Sharp, L. & Little, J. Polymorphisms in genes involved
in folate metabolism and colorectal neoplasia: a
HuGE review. Am. J. Epidemiol. 159, 425–443
(2004).

Boyle, P et al. European code against cancer and

International Center for Alcohol Policies. International
Drinking Guidelines. ICAP [online], http://www.icap.
org/PolicyIssues/DrinkingGuidelines/GuidelinesTable/

Lin, M. T., Juan, C. Y., Chang, K. J., Chen, W. J. & Kuo,
M. L. IL-6 inhibits apoptosis and retains oxidative
DNA lesions in human gastric cancer AGS cells
through up-regulation of anti-apoptotic gene mcl-1.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
The following terms in this article are linked online to:
ADH1B | ADH1C | ADH4 | ADH5 | ALDH1 | ALDH2 | BCL2 | CYP2E1 | HPR1 |
JNK1 | MAT1A | MAT2A | MTHR | MTHFR | MTHFD1 | TGFβ1 | TNFα | VEGF

FURTHER INFORMATION
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