Alcohol and Cancer: Biological Basis

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Key Points

1. For decades, it has been well documented that alcohol intake increases risk for cancers of the upper aerodigestive tract (oral cavity, pharynx, larynx, and esophagus), especially at high levels of intake, as well as for breast, colon, and liver cancers.
2. Many types of cancer associations were observed for all types of alcoholic beverages, suggesting that ethanol is the main carcinogenic constituent of alcohol drinks.
3. Alcohol has multiple actions in modifying carcinogenesis, not only directly, such as disordering cell membranes, but also indirectly, such as a consequence of ethanol oxidation to acetaldehyde and other reactive intermediates.
4. The magnitude, specificity, and variability of ethanol’s actions can depend on the dose and duration of exposure and on specific biochemical and molecular characteristics of the tissues to which ethanol comes in contact.
5. The possible mechanisms underlying alcohol’s carcinogenicity include the causation of DNA damage by alcohol’s metabolic product acetaldehyde, alcohol’s effect in increasing estrogen levels, alcohol being a solvent for carcinogens, alcohol-induced generation of reactive oxygen species, alcohol-associated alterations in nutritional status, and deleterious effects of alcohol on the host immune system.

Key Words: Alcohol; ethanol; acetaldehyde; carcinogen; oxidative stress; hormones; cancer

1. INTRODUCTION

For decades, it has been well documented that alcohol intake increases the risk for cancers of the upper aerodigestive tract (oral cavity, pharynx, larynx, and esophagus), especially at high levels of intake (1–4). For instance, as early as 1977, alcohol intake was identified as a risk factor for breast cancer and resulted in numerous subsequent epidemiological reports supporting the role of alcohol in breast cancer causation (5, 6). In fact, alcohol’s interrelationship with breast cancer was listed as 1 of the top 10 greatest recent discoveries in nutrition (7). Thus, alcohol consumption can augment to varying degrees carcinogenesis at several organ sites including those in
the upper aerodigestive tract, liver, colon, and breast. The epidemiological evidence
for the role of alcohol in these and other cancers has recently been reviewed in depth
(5) and will not be discussed at length in this chapter. The evidence that alcohol
enhances cancer risk is strongest for tumors of the upper aerodigestive tract (UADT),
colon (particularly in men), and the female breast (5, 6, 8–10). There is also strong
evidence that alcohol intake is an independent risk factor for liver cancer, especially
as a consequence of cirrhosis (4). Collectively, data from various animal models
provide supportive evidence that ethanol can increase cancer when provided alone
(liver, head, neck, fore-stomach, and breast) and when co-administered with known
carcinogens (2, 11–14). In these carcinogen-induced animal tumor models, ethanol
has been observed to be a stimulator of both the initiation and the post-initiation
stages of experimental carcinogenesis (15–28). In light of the total evidence, the
IARC has concluded that there is “sufficient evidence” of ethanol’s carcinogenicity in
animals (2, 11). Likewise based on findings of epidemiological studies and preclinical
mechanistic data, the IARC indicates that alcohol drinking is “carcinogenic to humans”
(2, 11). In general, these cancer associations were observed for all types of alcoholic
beverages, suggesting that ethanol is the main carcinogenic constituent of alcohol
drinks. It also should be kept in mind that many of these alcohol–cancer associations
are most prominent at high levels of alcohol consumption. The biological basis for
this enhancing effect of alcohol intake on these various cancers remains an area of
active scientific inquiry, especially the extent to which other dietary and lifestyle factors
and genetic predisposition can modify the impact of alcohol on cancer risk (29). This
chapter, therefore, will focus on providing insights into potential mechanisms of action
whereby alcohol stimulates cancer development and highlighting areas for future
investigation.

It is readily apparent that alcohol has multiple actions in modifying carcinogenesis,
not only directly, such as disordering cell membranes, but also indirectly, such as a
consequence of ethanol oxidation to acetaldehyde and other reactive intermediates
(2, 4, 16, 29–31). The magnitude, specificity, and variability in ethanol’s actions can
depend on the dose and duration of exposure and on specific biochemical and molec-
ular characteristics of the target tissues. A recent summary of possible mechanisms
underlying alcohol’s carcinogenicity provides strong evidence that acetaldehyde causes
DNA damage and alcohol increases estrogen levels; moderate evidence that alcohol is
a solvent for carcinogens, generates reactive oxygen species (ROS), and alters folate
status; and weak evidence that alcohol causes cancer by reducing host defenses and
causing nutritional deficiencies (2).

The literature may use various terms to describe units of alcohol consumption. Gen-
erally in the United States, an alcoholic drink is recognized to contain about 14 g of
ethanol which translates to approximately 1.5 oz (44 ml) of liquor, a 5 oz (148 ml) glass
of wine, or a 12 oz (355 ml) glass of beer. For purposes of this overview, high alcohol
intake is considered ≥3 drinks/day. Furthermore, the term alcohol will be used inter-
changeably with the word ethanol, the major biologically active constituent of alcoholic
beverages.
2. ETHANOL METABOLISM: ACETALDEHYDE AND OXIDATIVE STRESS

Ingested ethanol is absorbed predominantly in the stomach and duodenum and is eliminated from the body by several metabolic processes. The liver is the main site of ethanol metabolism, although it may occur in other tissues. Individual variability in absorption, distribution, and elimination of ethanol is affected by genetic and environmental factors, which cumulatively can contribute to differences in the clinical consequences, harmful effects, and cancer risks associated with chronic ethanol consumption (4, 32–35).

There are three metabolic pathways for the oxidation of ethanol to its primary product acetaldehyde (Fig. 1), a known carcinogen (36). Peroxisomal catalase-mediated metabolism to acetaldehyde is considered a minor pathway. A second oxidative pathway involving microsomal cytochrome P4502E1 (CYP2E1)-catalyzed conversion of ethanol to acetaldehyde may have particular importance in metabolizing ethanol in non-liver tissues, especially those lacking appreciable alcohol dehydrogenase (ADH) activity. CYP2E1-dependent ethanol oxidation (Km = 8–10 mM) plays a greater role in ethanol elimination following consumption of large amounts of alcohol that subsequently result in elevated blood alcohol content. Reactive oxygen species (ROS) such as hydroxyl radicals, superoxide anions, and hydroxyethyl radicals are also a by-product of cytochrome P450 catalysis and can contribute to cancer-promoting tissue damage. By far the major contributor to ethanol oxidation to acetaldehyde is cytosolic ADH (Km of liver

![Fig. 1. Ethanol metabolism and possible damaging outcomes.](image-url)
ADH = 0.2–2.0 mM. Acetaldehyde is subsequently converted to acetate (37) through a reaction catalyzed primarily by mitochondrial acetaldehyde dehydrogenase 2 (ALDH2). To a smaller extent acetaldehyde may also be detoxified in the liver by CYP2E1, which is referred to as the microsomal acetaldehyde oxidizing system (38). Acetate ultimately can be oxidized to CO₂ or metabolized to acetyl-CoA. Allelic variation in ADH and ALDH2 enzymes, as discussed below, may have considerable impact on risk for several alcohol-related cancers. Particularly, individuals with elevated acetaldehyde levels following drinking due to increased formation and/or impaired elimination exhibit higher rates of alcohol-related cancers and other problems. There also are nonoxidative pathways of ethanol metabolism to phosphatidyl ethanol (catalyzed by phospholipase D) and fatty acid ethyl esters that, to date, make only a small contribution to ethanol’s impact on carcinogenesis (36).

Acetaldehyde, the primary oxidative metabolite of ethanol, is considered to be an important mediator of ethanol’s cancer-promoting actions, particularly in the upper aerodigestive tract (UADT) and colon (39–41). Actually, exposure of tissues to acetaldehyde may come from several sources. First, alcoholic beverages, especially whiskey and beer, may contain high levels of acetaldehyde (4). Second, as discussed above, acetaldehyde can be formed from ethanol by ADH in a variety of tissues such as liver, UADT mucosa, colon, and the parotid glands. An under-appreciated third source of acetaldehyde results from microbial metabolism of ethanol in the oral cavity and the colon (4, 42). Salivary acetaldehyde concentrations measured after ethanol intake have been detected at levels many-fold higher than those in corresponding blood samples, a response that can be ameliorated by using an antibiotic rinse prior to consuming alcohol (43). In alcoholics it has been also noted that smoking and poor oral hygiene can further increase acetaldehyde levels (44). Likewise, in the colon, high acetaldehyde levels may be a consequence of microbial oxidation of ethanol (45). Depending on the organ, Neisseria species, Streptococcus (viridans group), Rothia species, and the yeast Candida albicans are micro-organisms identified as being potential participants in this conversion (46, 47). High levels of acetaldehyde in the oral cavity also may be explained by the fact that the ALDH activity of microbes and oral mucosa is low. Dissimilarities in relative activities of ADH and ALDH have been observed at several sites along the GI tract. For example, Yin et al. (48, 49) reported that esophageal ADH activity was 4-fold higher than ADH activity in the gastric mucosa, whereas esophageal ALDH activity was 20% stomach ALDH activity. This would then account for selective tissue accumulation of acetaldehyde during alcohol ingestion. Of interest, alcohol consumers with the less active aldehyde dehydrogenase enzyme encoded by the ALDH2*2 allele exhibited 2–3-fold higher concentrations of acetaldehyde in the mouth compared to those with normal ALDH2, and salivary acetaldehyde concentrations 9-fold higher than blood levels (50). Tissue accumulation and toxicity of acetaldehyde also may be a concern in other sites beyond the UADT. Recently, it was reported that acute oral administration of ethanol to female rats (0.6 g–6.3 g/kg body weight) resulted in prolonged accumulation of acetaldehyde in mammary tissue to levels higher than those measured in blood (51). These researchers also have suggested that, at least in mammary tissue, xanthine oxidoreductase and CYP2E1 may be additional enzymes contributing to steady-state acetaldehyde levels (52–54).
Acetaldehyde is considered by the International Agency for Research on Cancer (IARC) as a human carcinogen, and is classified as an animal carcinogen, too. In rodents acetaldehyde inhalation leads to nasal and laryngeal cancers and its consumption leads to hyperproliferation and inflammation of UADT mucosa (2). In humans, those populations having allelic variants that lead to higher tissue exposure to acetaldehyde have higher cancer risks (4, 50, 55). Acetaldehyde’s action as a carcinogen is due, in part, to its genotoxicity and the consequent generation of genetic abnormalities (56–59). Both stable and unstable adducts can be formed not only with specific amino acid residues of proteins but also with nucleic acids (such as that formed by reaction with deoxyguanosine, N2-ethyl-dG). Covalent DNA adduct formation is considered to be a critical initiating event in the process of chemically induced cancer. This DNA adduct and acetaldehyde can be detected in human urine, although the extent of its mutagenicity in cancer target tissues is not well characterized (60, 61). In Aldh2−/− mice, having minimal capacity to metabolize away acetaldehyde, consumption of ethanol led to higher covalent binding of ethanol metabolites to DNA in several organs compared to Aldh 2+/+ mice with normal acetaldehyde oxidizing capacity (62). In human alcohol abusers, white blood cell (WBC) acetaldehyde–DNA adducts may reach levels 7-fold greater than those in non-consumers (63). Habitual or moderate drinkers with polymorphisms in alcohol metabolizing enzymes that lead to elevated circulating acetaldehyde have significantly higher frequencies of sister chromatid exchanges and micronuclei frequency in peripheral lymphocytes, an established biomarker of genomic instability (64–67). DNA–acetaldehyde adducts can be formed in a dose-dependent manner even at low concentrations that are relatively nontoxic to human buccal epithelial cells (57). Moreover, acetaldehyde in combination with other biological molecules (such as polyamines) that accumulate in tissues damaged by ethanol exposure may generate other forms of stable DNA damage such as crotonaldehyde–DNA and N2-propano-dG–DNA adducts that can lead to particularly damaging DNA–DNA and DNA–protein cross-links (56). Again, habitual drinkers with polymorphisms leading to inactive forms of ALDH2 evidence a greater frequency of chromosomal aberrations and other evidence of genetic damage in blood samples (68). What may compound this carcinogenic and genotoxic action of acetaldehyde is that it also may bind to and compromise the function of cellular proteins involved in DNA synthesis and repair, in maintaining normal DNA cytosine methylation and in mounting antioxidant defenses (69–71). Furthermore, ethanol metabolism may disrupt critical intracellular signaling events that contribute to maintaining genomic stability (72, 73). Recently, for example, ethanol-treated hepatic cells exhibited more highly acetylated microtubules, presumably due to acetaldehyde, that substantially disrupted microtubule integrity (74). Acetaldehyde–protein adducts and malondialdehyde adducts (generated from lipid peroxidation) also may stimulate an immune response and induce inflammatory processes particularly in certain types of liver cells, all of which can further exacerbate local tissue damage and elevate disease risk (58, 75–77). Acetaldehyde may contribute to precancerous lesion formation, as has been reported in the colon (accelerated crypt cell formation, polyp formation, and hyper-regeneration) and in the oral cavity (leukoplakia) (78–81).
There is another consequence of ethanol metabolism that may be important in contributing to cancer particularly in the liver and that is the generation of oxidative stress (32, 58, 82–85). ROS may be produced by multiple pathways that are triggered following consumption of ethanol (Fig. 1). For example, ethanol oxidation via P4502E1 and oxidation in the mitochondria of NADH (that is generated by ADH as ethanol is oxidized) produce ROS. Ethanol-associated inflammation also can produce ROS. Besides contributing to liver cirrhosis and cancer, ROS generation may also be associated with extrahepatic cancers. For example, ethanol-induced oxidative stress and acetaldehyde formation have been detected in rat mammary tissue that was accompanied by ultra-cellular aberrations in epithelial cell structure (52, 54). The magnitude of ROS generated depends on the amounts of ethanol consumed as well as the individual’s genotype encoding for ethanol metabolizing enzymes. Whatever the source of increased ROS, the consequences can include increased direct DNA damage (such as formation of 8-oxo-dG) or indirect DNA damage following ROS-induced lipid peroxidation. Lipid peroxidation produces malondialdehyde, 4-OH nonenal, and other metabolites, all of which subsequently can form adducts and modify cellular proteins (75, 86). In fact, exocyclic etheno adducts, generated by reaction of DNA bases with lipid peroxidation products such as trans-4-hydroxy-2-nonenal, can be detected in the urine of alcoholics with fatty liver. There are other damaging cellular consequences of ROS formation. For example, in mouse liver the oxidation of cytosolic proteins, such as those participating in stress responses, intermediary metabolism, and antioxidant defense, leads to increased protein degradation (87). This may certainly create cellular dysfunction and susceptibility to toxic agents that are contributors to cancer development. Moreover, oxidative stress may contribute to redox imbalance and disturbances in signaling cascades within liver cells, such as those described for mitogen-activated protein (MAP) kinases, NFkappaB, and AP-1 (88). The extent to which these ROS-induced processes of protein degradation and signaling disruption occur in tissues other than the liver deserves further study and is likely to depend on the dose of ethanol.

Lastly, exposure of tissues to ethanol-generated ROS and acetaldehyde can lead to enhanced cell proliferation and damage-induced hyper-regeneration (81), at least in the gastrointestinal tract. Such consequences of acetaldehyde and ROS formation combined with their genotoxic effects and their impairment of cellular repair capacities likely contribute to a tissue environment conducive to cancer promotion.

3. ALCOHOL AND CARCINOGEN BIOACTIVATION

Direct exposure of tissues to ethanol such as occurs in the UADT may lead to solvent effects of ethanol on membranes that may enhance the bioavailability of tobacco-related and other carcinogens. Carcinogens and xenobiotics normally can be metabolized and detoxified to excretable forms by numerous enzymes (84, 89). However, in numerous tissues ethanol may stimulate carcinogenesis by inducing enzymes involved in carcinogen bioactivation (84, 89–93). Specifically, ethanol may induce enzymes that metabolically activate inert procarcinogens to DNA-reactive intermediates. Decades ago it was discovered that chronic exposure of the liver to ethanol resulted in increased activity of the cytochrome P450s of the microsomal ethanol oxidizing system, especially CYP2E1.
There is substantial human variability in CYP2E1 induction but, nonetheless, its induction can increase the metabolic activation of such carcinogens as hydrazines and nitrosamines. Likewise, induction of CYP3A4 and possibly CYP1A2 by ethanol increases activation of such procarcinogens as aflatoxin and heterocyclic amines such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), which in animals are known to cause tumor formation in several tissues. The induction of CYP 3A4 activity was due to multiple mechanisms including stabilization of mRNA and protein. It is of interest to note that exposure of human breast epithelial cells to ethanol and acetaldehyde increased adduct formation by the polycyclic aromatic hydrocarbon benzo[a]pyrene (BP). This occurred at physiologically relevant concentrations of ethanol and acetaldehyde, and in part, was the result of an ethanol-induced decrease in expression of the phase II detoxification enzyme glutathione-S-transferase. Furthermore, this effect of ethanol on these human breast epithelial cells led to formation of 8-oxo-dG adducts and the inhibition of the BP–DNA adduct removal. These data suggest, therefore, that ethanol may impact the steady-state levels of DNA-reactive metabolites by affecting both activation and detoxification of suspected carcinogens. With regard to the latter, ethanol-induced CYP2E1 metabolism may not only stimulate carcinogen bioactivation but also stimulate the release of ROS that in turn could deplete the cell of reduced glutathione and other thiol substrates that are important for carcinogen detoxification pathways.

Another consequence and concern related to ethanol-induced enzyme activity, specifically for CYP3A4 is that sex hormone metabolite profiles may be altered. CYP3A4 is the most abundant enzyme in human liver and, due to its role in steroid hormone interconversions, may play a role in breast and prostate carcinogenesis. It should be noted that the consequences of ethanol-associated induction of carcinogen and hormone metabolizing enzymes are complex and depend in large part on the structure of the carcinogen as well as whether ethanol exposure is acute or chronic. In contrast to the stimulating effects of chronic ethanol consumption, acute ethanol exposure may inhibit the metabolism of drugs and carcinogens. For those enzymes capable of reacting with ethanol (and/or acetaldehyde) following acute alcohol dosing, the reaction with ethanol can occur at the expense of another substrate, such as a carcinogen, leading to competitive inhibition of carcinogen bioactivation. In mice, for example, concomitant dosing with alcohol and the lung carcinogen N-nitrosodimethylamine (NDMA) actually resulted in competitive inhibition of NDMA metabolism in the liver, which led to enhanced exposure of the lungs to NDMA in the circulation and subsequently to increased lung tumorigenesis.

4. ALCOHOL AND GENE INTERACTIONS

The primary enzymes catalyzing alcohol metabolism are ADH and ALDH. There is considerable ethnic variation in the distribution of these forms and their biological characteristics. Augmented formation of acetaldehyde and/or its deficient detoxification can lead to elevated tissue exposure and resultant deleterious consequences. Thus, variant alleles encoding ADH and ALDH2 enzymes can play particularly important roles in determining peak blood acetaldehyde and ROS concentrations that can ultimately
impact the magnitude of acetaldehyde- and ROS-mediated damage to cellular macromolecules (4, 33, 34). Several examples of these polymorphisms and their impact on risk of certain cancers will be illustrated. A more detailed description of the genetics of alcohol metabolism can be found in the review by Edenberg (34).

**ADH polymorphisms.** There are seven ADH enzymes encoded on genes in chromosome 4q that are capable of oxidizing ethanol. Polymorphic forms of at least two genes have been studied for their impacts on alcohol drinking and cancer risk. The polymorphisms of ADH1B and ADH1C are ADH1B*1, ADH1B*2, ADH1C*1, and ADH1C*2. ADH1B genes encode β subunits and ADH1C genes encode γ subunits of ADH that are capable of forming homo- or heterodimers (33). ADH1B*2 exhibits considerably more (about 40-fold) activity compared to the reference allele ADH1B*1 (Fig. 2). The frequency of ADH1B*2 allele is higher in Asians and less prevalent in individuals of Caucasian and African descent. Those East Asian heavy drinkers heterozygous for the ADH1B alleles (ADH1B*1/2) are reported to have higher levels of ethanol persisting in the blood for longer periods as compared to those homozygous for the ADH1B*2 allele (99). Furthermore, acetaldehyde levels in saliva of ADH1B*1 carriers were higher than in their corresponding blood samples and were about 30-fold higher than salivary levels in those ADH1B*2 carriers. The higher salivary acetaldehyde levels in the ADH1B*1 carriers were associated with considerable oral micro-organism overgrowth. The less active ADH1B*1 form is associated with the higher risk for UADT cancer in East Asian drinkers and also may confer higher risk in Central Europeans as well (4, 55, 99–102). Its presence has a dramatic multiplicative effect on those consuming both alcohol and tobacco (103), and there also appears to be significant gene–gene interaction with ALDH2 (55).

![Fig. 2. Polymorphisms in alcohol and aldehyde dehydrogenases that may influence the alcohol–cancer relationship.](image)

Among Western populations, ADH1C is a rate-limiting factor in acetaldehyde metabolism (55). The ADH1C*1 form has been estimated to have about 2.5-fold the activity in metabolizing ethanol compared to the reference ADH1C*2. In this regard, it has been reported that individuals homozygous for ADH1C*1 have nearly twice the salivary acetaldehyde concentration following alcohol intake compared to heterozygous populations (104). The prevalence of the ADH1C*1 is more consistently associated with
increased risk for cancers of the head, neck, and esophagus for populations consuming high amounts of ethanol (>40 g/day). On the other hand, at low levels of ethanol consumption, evidence for this relationship is inconsistent (4, 105). Similarly, the relationship of ADH1C*1 to alcohol and colon cancer is inconsistent (4). On the other hand, a majority of studies of alcohol and breast cancer that include examination of the role of ADH polymorphisms report findings that ADH1C*1 homozygosity increases risk (4, 106, 107).

As far as other ethanol oxidizing enzymes are concerned, no associations between cytochrome P4502E1 and liver and esophageal cancers have been reported (4).

**ALDH polymorphisms.** Two main ALDH enzymes metabolize acetaldehyde to acetate, the cytosolic ALDH1 and mitochondrial ALDH2 (33, 34) (Fig. 2). The low Km homotetrameric mitochondrial ALDH2*1 is most active in metabolizing acetaldehyde. The variant of ALDH2 that codes for a nearly inactive form of ALDH2 is ALDH2*2. This inactive allele is much more common in Chinese, Japanese, and Koreans compared to those of European or African descent and is responsible for the acetaldehyde-induced alcohol flushing reaction that usually mitigates high alcohol consumption (33, 34). Those that are heterozygous or homozygous for ALDH2*2 exhibit nearly undetectable acetaldehyde metabolizing activity. This explains why alcohol-consuming populations, homozygous or heterozygous for the inactive allele, have about 18-fold and 5-fold higher concentrations of peak blood and saliva acetaldehyde, respectively, compared to those with ALDH2*1/1 (108). Moreover, those with the ALDH2*1/2 genotype who consume moderate doses of alcohol exhibit salivary acetaldehyde levels 2–3-fold higher than those levels measured in individuals with the ALDH2*1/1 genotype (109). This is consistent with the observation that those males with low-activity ALDH2 alleles who consume high amounts of alcohol have greater than a 10-fold elevation in oral, throat, laryngeal, and esophageal cancer risks (55, 101, 102, 110). A particularly compelling demonstration of the impact of ALDH genetics on cancer risk was reported in a case–control study of Japanese men by Yokoyama et al. (101). These investigators observed that light-to-moderate drinkers with inactive ALDH2 evidenced a 5–10-fold increase in esophageal cancer risk compared to those with the active allele. Of note, they detected that the risk for light drinkers with the inactive allele was comparable to the risk for moderate drinkers with the active allele. Likewise, risk for moderate drinkers with the inactive allele was similar to heavy drinkers with the active allele. Furthermore, those with the inactive allele were at increased risk for a second tumor. Much less information has been gathered regarding increased cancer risks in women harboring these inactive alleles, and current observations suggest that male–female disparities do exist among lifestyle-associated risk factors for UADT cancers (55). There is also disturbing evidence that alcohol consumption is increasing for those Japanese with the ALDH2*2 polymorphism, a group previously thought to be protected from alcoholism (due to aversive effects of the flushing response to drinking) and consequent high UADT cancer risk (34). It has been reported that low vegetable and fruit intake also contributed to higher UADT risk among high alcohol consumers (101, 102, 111).

**Other polymorphisms.** Polymorphisms for glutathione-S-transferase (GST), a phase II enzyme involved in carcinogen detoxification, have been studied. Women who are drinking alcohol and null for GSTM1 or GSTT1 have been reported to have either increased
breast cancer risk or have had increased levels of carcinogen–DNA adducts detected in their breast tissue (112–114). This relationship deserves further clarification. No association of GSTM1 genotype and lifestyle factors with esophageal cancer in Japanese was reported (101). Data regarding alcohol intake and cancer risk in those with polymorphisms for the CYP2E1 gene, genes encoding enzymes for folate metabolism, and for DNA repair genes are inconclusive (2, 115, 116). There is some evidence that CYP2E1 expression may interact with certain ADH and ALDH alleles in modifying an alcohol–cancer interaction (81, 117). Also related to this topic, it will be informative to confirm in multiple cancer target tissues how loss of tumor suppressor gene function (such as p53) exacerbates the carcinogenic effects of alcohol in mice (118).

5. ALCOHOL, HORMONES, AND GROWTH FACTORS

Alcohol intake, particularly at high levels, impairs normal functioning of most endocrine systems and can affect the hormone sensitivity of endocrine target tissues (119, 120). The magnitude and direction of change in hormone levels depends on numerous factors including the specific hormonal system, target tissue, gender, lifestyle factors, age, and alcohol drinking pattern. Epidemiological, clinical, and preclinical studies point to several ways by which ethanol may affect the hormonal environment of normal and neoplastic cells, especially as it relates to estrogen and breast cancer (16, 30, 31) (Fig. 3). This is especially important, since it is considered that lifetime exposure to estrogens directly contributes to breast cancer risk (121). Circulating estrogens in women may be generated from ovarian synthesis or from peripheral conversion (aromatization) of other steroid substrates such as testosterone, androstenedione, and hydroepiandrosterone sulfate. Alcohol-associated changes in the hormonal milieu have been observed in both premenopausal and postmenopausal women. For example, in a cross-sectional study of premenopausal women, alcoholic beverage intake was associated with significantly higher levels of estradiol, androstenedione, and testosterone averaged throughout the menstrual cycle, as well as higher progesterone levels during the luteal phase (122). The literature collectively suggests that there is a positive association between chronic and acute ethanol intake and circulating estrogens (123–131). The effect of alcohol may also include derangements of menstrual cycle and reproductive hormone function. The relationship between alcohol intake and levels of androgens and progesterone appears to be less consistent. In postmenopausal women, observational studies are inconsistent (132–134), although in a recent large cross-sectional study, a significant positive relationship between alcohol drinking and sex steroids in the blood of both pre- and postmenopausal women was detected (123). Of particular interest are reports that postmenopausal women who consume alcohol and use exogenous estrogen have substantial elevations in serum estradiol concentrations (128, 133), although a recent large study found no significant interactions (123). It is likely that the impact of ethanol on circulating estrogens is, in part, a result of decreased metabolic clearance and/or increased production (119, 120, 133). This cancer-promoting environment due to higher estrogen exposure may be further exacerbated by the fact that blood levels of acetaldehyde are significantly increased during the peak estradiol phase of the menstrual cycle of women who drink and for those female alcohol consumers using synthetic
estrogens (135). The interaction of alcohol and oral estradiol needs to be more carefully characterized for their combined impact on circulating estrogen and ultimately cancer risk (134).

As with other cancers, genetic variability may contribute to this hormonal mechanism of alcohol and breast cancer. Coutelle et al. (136) reported that those women with the ADH1C*1 allele consuming small amounts of alcohol (0.225 g ethanol/kg body weight) exhibited both an increase (27–38%) in circulating estradiol levels and an increase (1.8-fold) in breast cancer risk. In another study, however, ADH1C genotype was not found to influence the relationship between alcohol and breast cancer (137).

An additional hormone-related issue to consider is that there is some evidence in cell cultures that ethanol exposure may stimulate androgen aromatization in breast cancer cells (138, 139).

The effect of a hormone on a target cell is in large part determined by binding of the hormone ligand to its cognate receptor. Therefore, it is important to consider how alcohol may affect hormone receptor expression or ligand–receptor dynamics. For example, a well-characterized response of some cells to estradiol binding to the estrogen receptor-alpha (ERα) is upregulation of a number of estrogen-responsive genes including that for the progesterone receptor (PR). Therefore, ethanol intake may stimulate cancer cell proliferation not only by increasing circulating levels of estrogens but also by increasing cancer cell hormone receptor expression and hormone responsiveness. The association of alcohol intake with specific steroid hormone receptor tumor subtypes in breast cancer
has been the subject of numerous investigations with inconsistent results. Recently, a meta-analysis of such epidemiological studies found support for a positive relationship between alcohol drinking and the development of all ER-expressing (ER+) tumors, including ER+PR+ and ER+PR− tumors (140). The mechanisms responsible for this finding are unknown, but, in light of the observation that both PR+ and PR− subtypes of tumors were increased, biological explanations would need to include both classical ER-mediated estrogenic modes of action as well as hormone-independent pathways.

Cell culture experiments point to the capacity of ethanol to increase the content of ER in human breast cancer cells (138, 141). These changes in ER content were associated with an increase in cancer cell proliferation particularly in ER+ cells (141). Moreover, treatment of breast cancer cells in culture with ethanol-induced ligand-independent activation of the ERα that in part involved signaling by cyclic AMP and protein kinase A pathways (142). Fan et al. (143) reported an ethanol-induced downregulation of the tumor suppressor BRCA1 along with an upregulation of ERα expression and transcriptional activity in human breast cancer cells in vitro. Others have also reported a decreased BRCA1 expression that occurred along with decreased expression of proteins maintaining tissue organization, such as E-cadherin and α- and β-catenin (144, 145). These findings are consistent with other studies using a variety of cancer cell lines, which show that ethanol exposure can affect cell adhesion and substantially enhance invasiveness. This action of ethanol was due in part to its action on a number of biological intermediates including vascular endothelial growth factor (VEGF), matrix metalloproteinases, NFκB, transforming growth factor-beta (TGFβ), and ErbB2/Her2 (146–152). Taken together, these data suggest that breast and other neoplasms may be affected by ethanol in multiple ways leading to increased exposure to estrogens and other hormones, to heightened sensitivity to hormonal stimulation, and to aberrations in hormone-associated signaling cascades. Thus, ethanol may have a variety of actions that could lead to greater tumor development, including increased tumor cell proliferation, suppression of tumor suppressor gene-mediated genomic stability, and stimulation of tumor invasiveness and metastases. These in vitro observations warrant confirmation in appropriate animal models.

Alcohol drinking may affect carcinogenesis by mechanisms other than those involving sex steroid hormone levels and responsiveness. For example, insulin-like growth factor-1 (IGF-1) is a peptide hormone that exhibits multiple actions in regulating cell proliferation and apoptosis, largely mediated through the IGF-1 receptor (153, 154). IGF-1 bound to its binding proteins (BP), predominantly IGFBP-3, makes IGF-1 unavailable for binding to its receptor. Thus, the expression of IGF-1 and its BP contributes to regulation of breast cancer proliferation. There is evidence that increasing blood concentrations of IGF-1 are associated with increased risk not only for pre and postmenopausal breast cancer but also for prostate and colorectal cancers (155, 156). It has been suggested that the promotion of breast cancer by alcohol also may be a consequence of perturbations in growth factor dynamics (157, 158). Both controlled and cross-sectional studies in women have examined the relationship of alcohol to circulating IGF-1 levels and some to IGFBP status, but the results are mixed (159–162). Cell culture and animal studies have reported inconsistent effects of ethanol on IGF-1 levels, cell division, and intracellular signaling pathways. Some of these inconsistent
findings may be due to variability in ethanol doses used and tissue-specific differences in responses to ethanol (163–171). Because of the importance of IGF-I in chronic disease risk, the dose–response relationship and the factors contributing to variability in IGF-I and IGFBP responses to alcohol drinking need to be better characterized, particularly for breast cancer. Furthermore, the effects of ethanol on other growth factor and hormonal systems as well as diverse signaling pathways that can influence numerous cancers, such as AMPK, PPAR, and G-proteins, deserve more attention (172–178).

In regard to other hormones, there is inconsistent evidence linking circulating prolactin levels with breast cancer risk. In humans, it is unclear how alcohol intake affects this relationship. In preclinical studies, ethanol influenced prolactin homeostasis and in a short-term feeding study alcohol intake (0.4 g/kg body weight) increased blood concentrations of prolactin (179–181). Characterizing the effects of alcohol on prolactin status is important since locally produced prolactin and stimulation of the prolactin receptor in mammary tissue can affect breast carcinogenesis (182).

A phenomenon related to the alcohol–breast cancer interaction is that alcohol intake is associated with the development of high-risk breast characteristics (mammographic parenchymal or fibroglandular densities) that are influenced by sex hormones and growth factors. The number of breast parenchymal cells and the integrity of the surrounding collagen matrix are reflected in the amount of radiologically dense breast tissue. Mammographic percent density has been identified as a strong and independent risk factor for breast cancer (183, 184). In fact, women with mammographic densities occupying over 60–70% of the breast have 4–6 times higher risk for breast cancer than those women with breast densities occupying <10% of the breast. These dense patterns are associated with atypical hyperplasia, carcinoma in situ, with atypical cytology of nipple aspirates, and may be the consequence of enhanced mitogenesis and mutagenesis in the breast (183–185). Estrogens and growth factors are associated with the prevalence of these breast structures as are dietary factors (183, 186–189), such as alcohol consumption. In regard to alcohol intake, most but not all investigations report a positive association between alcohol drinking and the percentage of the breast occupied by mammographic densities (189–198). These human data are supported by preclinical experiments in rodents. In the rat mammary gland of young, virgin females, immature terminal end bud (TEB) structures are highly susceptible to carcinogen-induced DNA damage and tumorigenesis. With ageing, TEB mature into more differentiated alveolar bud (AVB) structures that are less sensitive to carcinogen-induced damage. Compared to control rats, it was observed that the intake of ethanol by young, virgin female rats was associated with an increased ratio of TEB to AVB structures, a change indicative of less differentiation and maturation of the TEB to AVB (199, 200). This action was associated with a small decrease in estradiol and more marked suppression of circulating progesterone levels (199). These changes would suggest that ethanol-fed rats would have greater mammary gland susceptibility to carcinogen-induced tumorigenesis. This contention is supported by other studies in which female rats consuming ethanol exhibited enhanced initiation of chemically induced breast carcinogenesis (16). The molecular mechanisms responsible for these effects of ethanol on maturation and differentiation of breast structures and, potentially, on development of preneoplastic breast lesions warrants further scrutiny.
6. OTHER BIOLOGICAL ACTIONS/INTERACTIONS

There are additional actions of alcohol that have been identified as potential mediators of its procarcinogenic influence. For example, alcohol drinking, especially at high levels, may affect the risk of cancer by compromising the status and function of nutrients that participate in maintenance of normal cell proliferation, differentiation, and routine functions. Evidence from preclinical studies suggest that ethanol compromises the bioavailability of dietary folate and interferes with folate-mediated methionine synthesis (201–205). Thus, low folate intake along with exposure to ethanol and/or acetaldehyde could lead to inhibition of important methylation reactions catalyzed by S-adenosylmethionine, and consequently affect DNA repair processes, DNA stability, and the epigenetic control of gene expression through hypomethylation of DNA. This alcohol–folate interaction may in part explain epidemiological observations that ethanol consumption and low dietary folate increase risk for several cancers (115, 201, 206–214), although the results are not entirely consistent. The interrelationships among alcohol, one-carbon metabolism, and carcinogenesis are complex and depend not only on folate status but also on the activity of other dietary factors such as vitamins B12, B6, and riboflavin, and the lipotropes choline and betaine (206, 215). Alcohol consumption also has been associated with changes in the status of nutrients and biologically active dietary constituents such as beta-carotene, lutein, zeaxanthin, and vitamins A, B12, C, and alpha-tocopherol (216–221). Taken together these findings suggest that alcohol intake, especially at high levels, may contribute to increased cancer risks by disrupting the disposition or biological functions of cancer preventive dietary factors. Thus, considerable additional research is needed to better understand the overall impact of alcohol on these dietary constituents and their molecular/epigenetic consequences before a public health recommendation on alcohol, vitamin nutrition, and cancer can be made.

Alcohol has distinctly different effects on the immune system depending on dose and frequency of exposure. A biphasic influence has been reported in that high doses of alcohol result in broad suppression of immune system activities that are associated with greater susceptibility to infectious diseases. On the other hand, moderate intake appears to have a beneficial effect on the immune system and inflammatory processes when compared to heavy drinkers and abstainers (222–227). Based on observations from numerous preclinical studies, the deleterious effects of alcohol exposure on immunity are due to its actions in compromising humoral competence of the host, delaying activation of adaptive immunity, and altering inflammatory cytokine responses and neuroendocrine functions, to name a few (228–235). Some of these actions may be due to ethanol-induced generation of ROS or lipid peroxidation products (236, 237). However, there are fewer studies that have characterized the interplay of alcohol and immunity in affecting the development at several sites. Some studies indicate that ethanol intake by mice can suppress host resistance to metastatic spread of implanted tumors (especially melanomas) in rodents, in part due to the effect of alcohol in decreasing natural killer (NK) cell activity and compromising associated signaling pathways (237–249). This aspect of alcohol’s impact on cancer deserves further attention in both preclinical and clinical studies.
7. CONCLUSIONS AND FUTURE RESEARCH OPPORTUNITIES

Overall, there is clearly a need for better characterization of individual risk factors affecting the alcohol–cancer relationship so that prevention and early detection strategies can be enhanced. Some of these issues are especially important in situations where an alcohol-associated cancer risk may be modest in a general population, but greater in subgroups with biological characteristics that make them at substantially higher risk for cancers due to alcohol intake. Several research questions still need to be addressed:

What is the dose–response relationship between alcoholic beverage intake or ethanol exposure and the mechanisms associated with cancer promotion? Increased cancer risk is most evident at high intakes of ethanol, but what is the magnitude of the effect at lower levels of ethanol exposure? Furthermore, particularly in extrahepatic and nonUADT tissues, the local concentrations of ethanol, acetaldehyde, and other reactive metabolites (e.g., ROS) need to be better quantified, since these local metabolite levels may be considerably different in quality and quantity compared to those in the circulation.

What are other contributors to genetic variability in alcohol-associated cancer risks? Attention has been given to ADH and ALDH polymorphisms as modifiers of the alcohol–cancer relationship. Yet, further insights into the impact of gene variants for enzymes involved in one-carbon metabolism, in DNA methylation, in DNA repair, and in phase I and II metabolism of hormones and xenobiotics are warranted. For example, the interactive effects of methyl group diet, alcohol intake, and specific MTHFR polymorphisms need to be better understood (215). Related to this is the issue as to whether and with what magnitude other lifestyle factors modify the alcohol–gene–cancer interaction.

What is the impact of ethanol intake on endocrine-related and growth factor-associated signaling pathways? And how might subtle changes in immune system efficacy affect susceptibility to cancer? The dose-, gender-, and cancer-specific differences in the influence of ethanol on these signaling pathways, physiological control of cell growth and differentiation, and even immune surveillance should be better characterized. This hormone and growth factor issue is likely to be of particular importance for breast cancer, but may have implications for other cancers as well.

REFERENCES


