Cancer Letters 361 (2015) 164–173

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Molecular mechanisms of ethanol-associated oro-esophageal squamous cell carcinoma

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ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form 4 March 2015
Accepted 4 March 2015

Keywords:
Ethanol
Oro-esophageal squamous cell carcinoma
Molecular mechanisms

ABSTRACT

Alcohol drinking is a major etiological factor of oro-esophageal squamous cell carcinoma (OESCC). Both local and systemic effects of ethanol may promote carcinogenesis, especially among chronic alcoholics. However, molecular mechanisms of ethanol-associated OESCC are still not well understood. In this review, we summarize current understandings and propose three mechanisms of ethanol-associated OESCC: (1) Disturbance of systemic metabolism of nutrients: during ethanol metabolism in the liver, systemic metabolism of retinoids, zinc, iron and methyl groups is altered. These nutrients are known to be associated with the development of OESCC. (2) Disturbance of redox metabolism in squamous epithelial cells: when ethanol is metabolized in oro-esophageal squamous epithelial cells, reactive oxygen species are generated and produce oxidative damage. Meanwhile, ethanol may also disturb fatty-acid metabolism in these cells. (3) Disturbance of signaling pathways in squamous epithelial cells: due to its physico-chemical properties, ethanol changes cell membrane fluidity and shape, and may thus impact multiple signaling pathways. Advanced molecular techniques in genomics, epigenomics, metabolomics and microbiomics will help us elucidate how ethanol promotes OESCC.

Overview

Cancers of the upper aerodigestive tract (oral cavity, pharynx, larynx, and esophagus) represent a major public health problem worldwide, making up nearly 4.4% of all malignancies in the United States alone. Approximately 73,240 new cases were diagnosed in 2014, with an estimated mortality of 27,450 [1]. Among these cancers, the incidence rates of oro-esophageal cancers have been increasing in developed countries, especially among young males [2,3]. The most common malignancy in the oro-esophagus, oro-esophageal squamous cell carcinoma (OESCC) develops from precancerous lesions, and histopathologically follows a step-wise pattern of hyperplasia, dysplasia and squamous cell carcinoma (SCC) [4]. In the United States, the five-year survival rate has not improved significantly despite advances in radiotherapy and chemotherapy [1]. Survivors are usually left with severe functional compromise [5]. Moreover, 2% of esophageal cancer and 11% of head and neck cancer patients develop a second cancer due to field cancerization [6,7].

Many epidemiological studies have consistently shown that alcohol drinking is an etiological factor of human OESCC. OESCC has a stronger association with alcohol drinking than do cancers of other organ sites. According to a meta-analysis, strong direct trends in risk were observed for cancers of the oral cavity and pharynx (Relative Risk (RR) = 6.0 for 100 g/day of ethanol), the esophagus (RR = 4.2) and the larynx (RR = 3.9) [8]. Risk and mortality of OESCC were associated with alcohol drinking in a dose-dependent manner [8–10]. Meanwhile, the odds ratio of oral cancer patients with dysplasia increased with alcohol consumption [11]. Genetic polymorphisms of ethanol-metabolizing genes, such as acetaldehyde dehydrogenase (ALDH) and alcohol dehydrogenases (ADH), are associated with OESCC [12–14]. Several studies have identified ALDH2*1/2 heterozygotes as a high-risk group for OESCC [15–19], and ADH genotypes not including ADH3 were associated with OESCC as well [13,20,21]. Tobacco use and alcohol drinking have synergistic effects on carcinogenesis; combined use explained more than 61% of OESCC [22,23].

After ingestion, ethanol is rapidly absorbed through the stomach and small intestine into the bloodstream, and metabolized mainly

Abbreviations: 4NQO, 4-nitroquinoline 1-oxide; ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1; Edh, EH-domain containing gene; ELOVL, very long chain fatty acid elongase; GPCRs, G-protein coupled receptors; HNE, 4-hydroxynonenal; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; N^2-EtidG, N^2-ethylidene-2′-deoxyguanosine; NFκB, nuclear factor κB; NICD, notch intracellular domain; Nrf2, nuclear factor erythroid 2-like factor; OESCC, oro-esophageal squamous cell carcinoma; PI3K, phosphoinositide 3-kinase; RBPJ, recombining binding protein for immunoglobulin kappa j; ROS, reactive oxygen species; RR, relative risk; SCC, squamous cell carcinoma; Shh, sonic hedgehog; TGFβ, transforming growth factor beta; TLR4, Toll-like receptor 4.

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http://dx.doi.org/10.1016/j.canlet.2015.03.006
0304-3835/

in the liver before elimination. Pulmonary and urinary elimination is minimal. In the liver, ethanol is oxidized to acetaldehyde by ADH. A small amount is oxidized by cytochrome P450 2E1 (CYP2E1) and catalase. Acetaldehyde is released from the liver and metabolized into acetate by ALDH. Finally, acetate is oxidized to produce carbon dioxide, fatty acids and water. Previous studies have shown that CYP2E1 also catalyzes conversion of ethanol to acetaldehyde, and acetaldehyde to acetate [24,25].

Both ethanol and acetaldehyde may enter oro-esophageal epithelial cells through local permeation or systemic circulation. Ethanol concentration in the saliva is equal to concentration in the blood. In the saliva, ethanol is oxidized by microbes to acetaldehyde. Since further metabolism of acetaldehyde to acetate by oral bacteria is limited, acetaldehyde tends to accumulate in the saliva [26–28]. Antiseptic can significantly reduce salivary acetaldehyde concentration after alcohol drinking for this reason [27]. Tobacco changes the oral bacterial flora rapidly from Gram-negative to Gram-positive bacteria, and leads to a high concentration of acetaldehyde in saliva [29]. In agreement with these observations, alcoholics with oropharyngeal cancer had a high concentration of salivary acetaldehyde as a result of alcohol drinking, tobacco smoking and poor oral hygiene [30]. However, due to the short duration of contact and limited permeation into the epithelium, topical effects of ethanol and acetaldehyde on squamous epithelial cells are potentially weak. Presumably only superficial cells in the epithelium may be impacted by this mechanism *in vivo* [31].

Local and systemic effects of ethanol may influence carcinogenesis, especially among chronic alcoholics. However, the molecular mechanisms for ethanol-associated OESCC are still not well understood. Certain mechanisms of ethanol-associated cancer are supported by experimental studies of OESCC, but the majority of hypotheses are purely speculative or extrapolated from studies on cancers of other organ sites. Proposed mechanisms include: (1) enhanced cell proliferation and altered expression of cytokeratin suggesting inhibition of squamous cell differentiation [32]; (2) enhanced penetration of carcinogens into the squamous epithelium [31]; (3) impaired antioxidant defense and enhanced production of reactive oxygen species (ROS) in the squamous epithelium [26]; (4) interference with DNA repair machinery and DNA synthesis [33]; (5) disturbed systemic metabolism of nutrients [26]; (6) impaired immune function [34]; (7) induced chronic inflammation and enhanced angiogenesis [35].

On one hand, further experimental studies are needed to examine these mechanisms in ethanol-associated OESCC. On the other hand, these mechanisms have not been systematized to provide an overview of how ethanol promotes OESCC. In this review, we summarize current data and propose three major mechanisms of ethanol-associated OESCC: (1) disturbance of systemic metabolism of nutrients; (2) disturbance of metabolism in squamous epithelial cells; (3) disturbance of signaling pathways in squamous epithelial cells.

Disturbance of systemic metabolism of nutrients

Inhibition of retinol metabolism

When metabolized, ethanol impairs retinoid metabolism by inhibiting retinol metabolism to retinoic acid via competing with retinol for ADH and ALDH active sites, and by accelerating catabolism of vitamin A through induction of CYP2E1 [36]. A recent study showed that acetaldehyde inhibited formation of retinoic acid from retinal in rat esophagus *ex vivo* [37]. Lecithin:retinol acyltransferase, which regulates retinol metabolism by esterifying retinol, is down-regulated in human head and neck SCC cells. In a study using knockout mice, partial retinol deficiency during carcinogen treatment promoted cell proliferation and carcinogenesis in tongue epithelium [38]. Retinoic acid is known to exert profound effects on cellular growth, differentiation, and cancer development in the oro-esophageal epithelium through its interaction with receptors [36,39]. These studies suggest that ethanol promotes OESCC through inhibition of retinoic acid signaling. In fact, a retinoid X receptor agonist and a retinoic acid receptor γ selective agonist inhibited 4-nitroquinoline 1-oxide (4NQO)-induced oral carcinogenesis in mice [40].

Zinc deficiency

Alcohol abuse has long been associated with zinc deficiency [41]. Ethanol treatment down-regulates the expression of zinc transporters 1 and 4, as well as the zinc storage protein metallothionein 1 in alveolar macrophages, disrupting zinc bioavailability [42]. ADH is a zinc metalloenzyme, and removal of zinc from ADH leads to a complete loss of its catalytic activity [43]. While zinc supplementation prevents alcoholic liver injury through attenuation of oxidative stress [43], zinc depletion is known to enhance oro-esophageal carcinogenesis in rats and mice [44,45]. Mechanistically, zinc deficiency causes extensive alterations in gene expression in mouse and rat esophageal epithelia [46–48]. In particular, a group of cancer-related pro-inflammatory genes was up-regulated (CXC and CC chemokines, chemokine receptors, cytokines and cyclooxygenase 2, S100A8/A9, and nuclear factor κB (NFκB)), suggesting that multiple inflammatory pathways participate in zinc deficiency-related OESCC. Consistent with this observation, zinc supplementation caused a shift to a less proliferative cancer phenotype by normalizing the inflammatory gene signature, inhibiting cell proliferation, and stimulating apoptosis [49,50].

Iron overload

Alcohol drinking has been shown to cause iron overload in the liver [51]. Ethanol increases total iron content via overexpression of genes involved in iron transport (divalent metal transporter 1, transferrin receptor 1, ferroportin, ceruloplasmin) and iron storage (L-ferritin) [52]. We have shown that iron accumulation in the esophagus promoted inflammation-associated carcinogenesis [53]. Mechanistically, iron overload may initiate and promote carcinogenesis through oxidative damage [53] and modification of the immune reaction [54]. It is expected that oxidative stress affects carcinogenesis through redox signaling pathways inside cells [55,56].

Increased requirements for methyl groups

Chronic alcoholism increases the requirements for methyl groups and causes dietary methyl group deficiency [26]. Deficiency of S-adenosylmethionine, folate and betaine, primarily due to low intake and destruction by acetaldehyde, is common in alcoholics. Inhibition of methyl group transfer regulates expression of genes involved in carcinogenesis [57,58]. DNA hypomethylation of oncogenes (e.g., c-Ha-ras, c-Ki-ras and c-fos) is associated with an increased incidence of liver cancer in rats [59,60]. These data suggest that ethanol may contribute to OESCC through aberrant gene methylation [61,62]. In addition, aberrant gene methylation may impact signaling pathways through critical pathway genes, such as Notch4 of the Notch signaling pathway [63], PTEN of the phospoinositide 3-kinase (PI3K)/Akt pathway [64], and Wnt inhibitory factor 1 (WIF1) of the Wnt signaling pathway [65]. Recent studies have also showed that aberrant methylation of histones and non-histone proteins also modulates multiple signaling pathways [66].

Disturbance of redox metabolism in squamous epithelial cells

After ethanol and acetaldehyde get into epithelial cells through systemic circulation, they undergo intracellular metabolism [67]. It should be noted that ethanol metabolism in the oro-esophageal epithelial cells is different from that in the liver due to different