Free Radical Biology and Medicine (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

HNE and cholesterol oxidation products in colorectal inflammation and carcinogenesis

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ARTICLE INFO

Keywords:
4-Hydroxynonenal
Oxysterols
IBD
CRC
Colon
Intestinal
Antioxidant response
Survival

ABSTRACT

Consistent experimental data suggest the importance of inflammation-associated oxidative stress in colorectal cancer (CRC) pathogenesis. Inflammatory bowel disease with chronic intestinal inflammation is now considered a precancerous condition. Oxidative stress is an essential feature of inflammation. Activation of redox-sensitive pro-inflammatory cell signals and inflammatory mediators concur to establish a pro-tumoral environment. In this frame, lipid oxidation products, namely 4-hydroxynonenal and oxysterols, can be produced in big quantity so as to be able to exert their function as inducers of cell signaling pathways of proliferation and survival. Notably, an important source of these two compounds is represented by a high fat diet, which is undoubtedly a risk factor for inflammation and CRC development. Current evidence for the emerging implication of these two oxidized lipids in inflammation and CRC development is discussed in this review.

1. Introduction

Chronic inflammation contributes to the pathogenesis of the majority of diseases considered as leading causes of mortality in Western countries. Its influence in the development and progression of different types of cancer has been widely accepted, especially in those tissues that are easily exposed to injury by environmental agents such as intestinal mucosa. Inflammation can be triggered by cellular stress and dysfunction caused by excessive calorie consumption, with high production and storage of lipids, and elevated blood glucose levels involved in oxidative metabolic pathways [1].

On the basis of epidemiologic studies, colorectal cancer (CRC) risk is strongly associated with red and processed meat intake resulting in high quantity of fats [2]. World red meat consumption has increased from 1990 to 2010 especially in emerging economies in relation with rising income and rapid urbanization [3]. Diet with high animal fat and low in fruits and vegetables is the most common pattern associated with an increased risk of developing Inflammatory Bowel Disease (IBD), a group of intestinal diseases characterized by chronic intestinal inflammation that includes Ulcerative Colitis (UC) and Crohn's Disease (CD) [4]. Therefore, policy efforts should be especially focused on limiting the consumption of red meat and avoiding the consumption of processed meat as recommended by worldwide nutritional organizations [5].

Cholesterol and fatty acids, which are the major constituents of cellular membranes, are crucial for the maintenance of their structure and normal functioning, and represent an important part of diet. Unfortunately, excess dietary fats of animal origin can induce the formation of large quantity of oxidized molecules that initiate lipid oxidation processes able to generate different end-products that could contribute to the loss of intestinal epithelial barrier function and the production of pro-inflammatory molecules. Vice versa, during inflammation activated leukocytes can contribute to maintain an oxidative microenvironment which, in all likelihood, leads to functional impairment and dysplasia of the enteric mucosa.

Among various secondary non-enzymatic lipid oxidation compounds, 4-hydroxynonenal (HNE), derived by peroxidative breakdown

Abbreviations: 7K, 7-ketocholesterol; 7βOH, 7β-hydroxycholesterol; 22OH, 22-hydroxycholesterol; 24OH, 24-hydroxycholesterol; 25OH, 25-hydroxycholesterol; 27OH, 27-hydroxycholesterol; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; Akt, AKT serine/threonine kinase 1; AP-1, activator protein-1; CAC, colitis-associated cancer; CCR7, chemokine receptor-7; CD, Crohn's disease; COX, ciclooxygenase; CRC, colorectal cancer; DSS, dextran sulfate sodium; ERK, extracellular signal-regulated kinase; FOXO, forkhead box protein O1; GSH, glutathione; GSSG, glutathione disulfide; GSTA4, glutathione S-transferase alpha 4; HNE, 4-hydroxynonenal; HNE-dG, HNE-deoxyguanosine; HO-1, heme oxygenase-1; IBD, inflammatory bowel disease; Iκβ, inhibitor of κB; IKK, IκB kinase; IL, interleukin; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; LOX, lipooxygenase; LT, leukotriene; LXR, liver X receptor; MAPK, mitogen activated protein kinase; MCP-1, Monocyte Chemoattractant Protein-1; MMP, matrix metalloproteinase; NADPH, nicotinamide adenine dinucleotide phosphate; NF-κB, nuclear factor-κB; NLRP3, NLR family pyrin domain containing 3; NOX, NADPH oxidase; NRF2, nuclear factor-erythroid 2-related facyor 2; p38, protein 38; PG, prostaglandin; PI3K, phosphoinositide 3-kinase; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; RNS, reactive nitrogen species; STAT, signal transducer and activator of transcription; TGFβ1, tumor growth factor β1; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF, tumor necrosis factor; TRL, toll like receptor; TrxR1, thioredoxin reductase 1; UC, ulcerative colitis

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http://dx.doi.org/10.1016/j.freeradbiomed.2017.01.017
Received 17 November 2016; Received in revised form 9 January 2017; Accepted 10 January 2017

of ω6 polyunsaturated fatty acids (PUFAs) in biological membranes, and oxysterols, which are cholesterol oxidation products, have received particular attention for their potential involvement in the pathogenesis of different human diseases, including cancer.

In this review, we summarize the recent progress on understanding the role of HNE and oxysterols in CRC pathogenesis, and focus on their involvement in inflammatory signaling pathways activated during carcinogenesis.

2. Inflammation and oxidative tissue reactions in colorectal cancer

The mechanism underlying CRC pathogenesis continues to require extensive investigation in the field of cancer research. In order to understand what the role of specific lipid oxidation compounds is in colorectal carcinogenesis, it is important to point out what the possible events triggering the formation of such compounds are in the tumor microenvironment.

The opinion that CRC is a consequence of specific sequences of mutated genes (*apc/β*-catenin pathway, *ras/raf, p53*), and epigenetic modifications has been widely accepted [6]. Of all CRCs, 5–6% accounts for hereditary types, 20–25% has a positive family history or genetic predisposition, while the majority of cases occurs sporadically as the result of somatic mutations in response to environmental factors [7].

A large number of environmental factors is strictly involved in the induction of inflammation, which, in turn, is well recognized as a major driving force in CRC initiation and promotion, as well as progression. It is, in fact, a key event in the induction of cancer genetic instability [8].

Colitis-Associated Cancer (CAC) is a subtype of CRC, which is associated with IBD. This association has been found to be responsible of deaths in up to 15% of IBD patients [9]. IBD patients with active disease show hyper-responsiveness of the host mucosa against intestinal flora with exaggerate immune and inflammatory responses. This can be caused by altered functions of pathogen recognition receptors and can lead to mucosal barrier defect with corresponding uncontrolled induction of proliferative and survival cell signals of neoplastic transformation [10,11]. The increased risk in CAC development in IBD depends on the persistence of inflammation, actually linked to longer colitis duration and the extent of inflamed colonic mucosa [12]. The hallmark of IBD is massive infiltration into lamina propria of innate and adaptive immune cells, which generate many inflammatory cytokines included IL-1β, IL-6 and TNFα, which play a crucial role in colorectal carcinogenesis [13].

The promoting role of inflammation in CAC has been strongly proved in different animal models in which colitis-associated neoplasia is induced chemically [14]. The most widely used mice model consists of the administration of inflammatory promoting agent dextran sulfate sodium (DSS) in association with a single initiating dose of carcinogen azoxymethane [15]. This experimental model shows IBD similar intestinal inflammatory infiltrate with multiple colonic tumors [14].

Sporadic CRC and CAC share inflammation as pathogenetic process involved in different phases of colorectal carcinogenesis. While inflammation in CAC has been considered to be involved in tumor initiation and promotion, inflammatory microenvironment in sporadic CRC can contribute to tumor progression by producing specific cytokines and chemokines, which increase aggressiveness of tumor by promoting survival and angiogenesis.

A major link between oxidized lipids production and CRC development is represented by activated inflammatory cells, which are critical actors to start and maintain the oxidative environment through repeated generation of inflammatory mediators and high levels of reactive oxygen (ROS) and nitrogen species (RNS) produced during oxidative burst. Increased levels of ROS and RNS have been found in inflamed tissues from patients with active IBD [16]. All these species participate to the different phases of colorectal carcinogenesis, being the cause of initial and additional cell mutations that can initiate CAC or favor CRC progression. Reactive radicals such as $O2^{\cdot-}$ and $HO^{\cdot}$ can act indirectly by non-enzymatic breakdown of membranes PUFAs leading to reactive aldehyde-end products such as HNE involved in CRC. On the other hand, H_2O_2, $O2^{\cdot-}$ and $HO^{\cdot}$ can function as signaling molecules of proliferation and survival [17] or can cause massive oxidative DNA lesions such as 8-hydroxy-2′-deoxyguanosine (8-OHdG); the concentration of 8-OHdG, which is considered a marker of oxidative DNA damage relevant for mutagenesis, increases in IBD patients [18].

Cell signals involved in inflammation and CRC are jointed by the induction of the common redox sensitive nuclear factor κB (NF-κB), which is frequently activated in the carcinogenic process [19,20]. NF-κB activation represents the rate limiting event that concurs to the induction of survival and the inhibition of apoptosis in dysplastic cells turning into tumor phenotype. Chronic activation of NF-κB in IBD intestinal tissue has been associated with oxidative stress. Increased H_2O_2 levels counteract the activity of thioredoxin1, an antioxidant small protein that can inhibit NF-κB activation by interacting with IκBα inhibitor and thereby preventing its phosphorylation by IκB kinase (IKK); $O2^{-\cdot}$ production, and consequently NF-κB activation, has been also ascribed to the TNFα-mediated induction of NADPH oxidase enzyme, which is actively involved in phagocytic function [21].

The activity of NF-κB itself is regulated by other transcription factors, in particular signal transducer and activator of transcription (STAT) 3, whose activity has been strongly associated to oncogenesis [22] NF-κB, IL-6 and STAT3 have been shown to represent the signaling axis, which can regulate proliferation and survival of tumor initiating intestinal epithelial cells [23]. Persistent activation of STAT3 in UC colonic tissue involves cytokine production such as IL-22, IL-6 and TNFα enabling progression towards CRC [24]. TNFα activity is also strongly involved in IBD pathogenesis for its pro-inflammatory and pro-apoptotic properties; in fact, TNFα blockers have become a mainstay in the therapy of IBD [25]. NF-κB and STAT3 have been shown to improve β-catenin transcription, whose hyperactivation is present in the majority of sporadic and familial CRCs [26]. Targeting NF-κB signaling pathway by using antioxidants such as polyphenols has been now considered as an emerging approach to prevent CRC development [27,28].

The discovery of redox-sensitive Nuclear Factor-Erythroid 2-Related Factor 2 (NRF2) has been very interesting. This transcription factor plays a key role in the maintenance of redox balance in intestinal mucosa, being activated by increased oxidative cell conditions to transcribe several genes coding for antioxidant enzymes [29]. NRF2 has been suggested protecting against CRC-associated inflammation in colitis animal model [30]. However, NRF2 plays a contradictory role in carcinogenesis depending on different phases of cancer. It suppresses tumor development at the earliest stages, but its over-expression has been found in later stages of malignancy, during which the already transformed cancer cells need to maintain intracellular antioxidant status by inducing cellular resistance against anticancer therapies [31]. Principal events implicated in the induction of mucosal barrier damage during inflammatory reactions that can lead to tumor growth are shown in Fig. 1.

Dietary lipids, in particular ω6-PUFAs, provide the main substrates for cyclooxygenases (COXs) and lipooxygenases (LOXs) enzymes, and for lipid peroxidation. Phagocytes in the colonic lamina propria generate high levels of eicosanoids produced through COX-2 induction in presence of ω6-arachidonic acid as substrate. Eicosanoids prostaglandins (PGs) and leukotrienes (LTs) may in turn activate lymphocytes to synthesize either pro- or anti-inflammatory mediators in response to dietary antigens [32].

Different studies suggest COX-PG pathway as an emerging signal axis in the regulation of tumorigenesis [33,34]. The well known inflammatory mediator PGE2 has been demonstrated to be responsible for the activation of phosphoinositide 3-kinase (PI3K) p85α, extracellular signal-regulated kinase 1 (ERK1), and NF-κB pathways in epithe-