Food Science and Human Wellness 8 (2019) 337–343

Contents lists available at ScienceDirect

Food Science and Human Wellness

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

Cytochrome P450 monooxygenase-mediated eicosanoid pathway: A potential mechanistic linkage between dietary fatty acid consumption and colon cancer risk

Weicang Wang[a], Jianan Zhang[a], Guodong Zhang[a,b,*]

[a] Department of Food Science, University of Massachusetts-Amherst, Amherst, MA, 01003, USA
[b] Molecular and Cellular Biology Program, University of Massachusetts-Amherst, Amherst, MA, 01003, USA

ARTICLE INFO

Article history:
Received 12 September 2019
Received in revised form 1 November 2019
Accepted 7 November 2019
Available online 14 November 2019

Keywords:
Linoleic acid
Polyunsaturated fatty acids
ω-3 Fatty acids
Colon cancer
Colonic inflammation
Cytochrome P450
Eicosanoids

ABSTRACT

Human consumption of linoleic acid (LA, 18:2ω-6, abundant in vegetable oils) is very high. Animal experiments showed that excessive LA intake increased azoxymethane-induced colon tumorigenesis, however, the impact of excessive LA on colon cancer in human is not conclusive, making it difficult to make dietary recommendations for optimal intake of LA. Understanding the molecular mechanisms of LA on colon tumorigenesis could help to clarify its health effect, and facilitate development of mechanism-based strategies for preventing colon cancer. Recent studies show that the previously unappreciated cytochrome P450 monooxygenase-mediated eicosanoid pathway is upregulated in colon cancer and plays critical roles in its pathogenesis, and could contribute to the effects of dietary LA, as well as ω-3 fatty acids, on colon tumorigenesis. In this review, we will discuss recent studies about the roles of cytochrome P450 monooxygenases in fatty acid metabolism and its roles in colonic inflammation and colon cancer, and how this information could help us to clarify the health impacts of dietary fatty acids.

1. Introduction

The consumption of linoleic acid (LA, 18:2ω-6), which is abundant in vegetable oil products (such as corn, soybean, and canola oils, as well as fried food, salad dressing, and mayonnaise), is very high in the United States: the average intake of LA is as high as ~12-17 g/person/day [1]. The potential issue with excessive intake of LA is exacerbated because the consumption of LA-rich vegetable oil has significantly increased in the United States in the last century [1], and could be even more prevalent in the future as dietary recommendation continues to encourage greater consumption of LA-rich vegetable oil products [2]. Indeed, the consumption of LA-rich soybean oil has risen more than 50% since 1980 and more than 1000-fold since 1909 [1]. The impact of high dietary intake of LA on human health is an intensively debated topic [3]. Multiple animal experiments have shown that excessive intake of LA exacerbated azoxymethane (AOM)-induced colon tumorigenesis, suggesting its potential adverse effect on colon cancer [4–7]. Epidemiological studies also support that high dietary intake of LA is linked with increased risks of colitis and colon cancer [8–14]. Notably, the European Prospective Investigation into Cancer and Nutrition (EPIC) study has shown that high intake of LA doubled the risks of colitis, and could be responsible for ~30% of ulcerative colitis cases [8]. However, the impact of LA on colon cancer and colonic inflammation in humans remains inconclusive, making it difficult to make dietary recommendations for optimal intake of LA [3]. Exploring the molecular mechanisms of LA on colon tumorigenesis could contribute to clarify its health effect, identify the individuals who are susceptible to excessive LA intake, and facilitate the development of mechanism-based strategies for preventing colon cancer.

A widely recognized explanation for the health effects of dietary polyunsaturated fatty acids (PUFAs), including LA, is that PUFAs could be enzymatically metabolized to generate lipid mediators (LMs), which are lipid signaling molecules with potent biological actions and play critical roles in the pathogenesis of

* Corresponding author at: Department of Food Science, University of Massachusetts-Amherst, Amherst, MA, 01003, USA.
E-mail address: guodongzhang@umass.edu (G. Zhang).
Peer review under responsibility of KeAi Communications Co., Ltd.

Production and hosting by Elsevier

https://doi.org/10.1016/j.fshw.2019.11.002

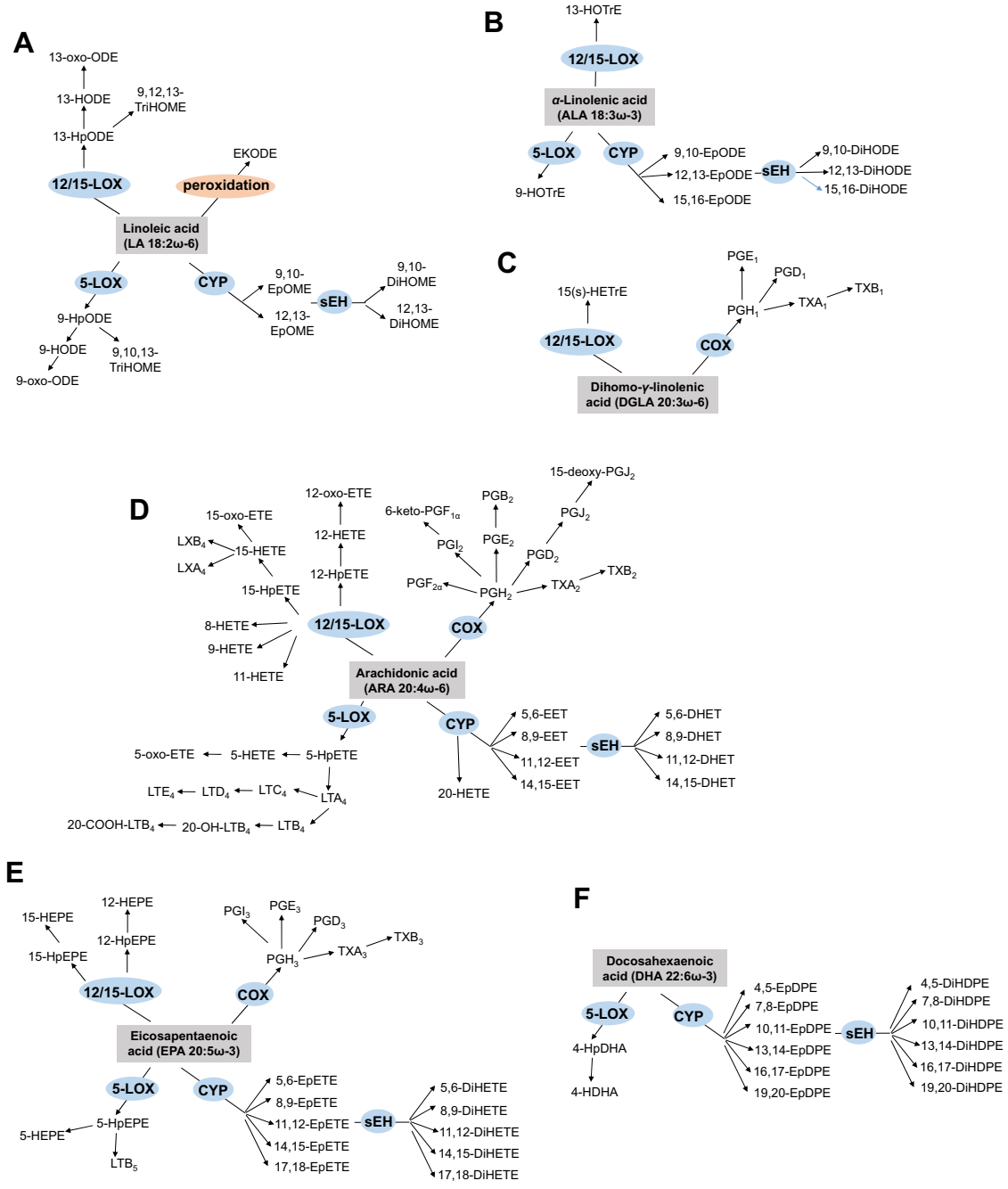

Fig. 1. Multiple dietary PUFAs, including linoleic acid (LA, 18:2ω-6), α-linolenic acid (ALA, 18:3ω-3), dihomo-γ-linolenic acid (DGLA, 20:3ω-6), ARA (ARA, 20:4ω-6), eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acid (DHA, 22:6ω-3), are substrates of COX, LOX, and CYP enzymes. This leads to formation of a large array of metabolites with diverse chemical structures and distinct biological actions.

many human diseases [15–17]. After dietary consumption, the dietary PUFAs are partly incorporated into membrane phospholipids [15–17]. Upon cellular stimulation such as inflammation, the membrane-incorporated PUFAs are released mainly by phospholipase A_2 (PLA_2) to generate free-form intracellular PUFAs, which are then converted by a series of enzymes to generate LMs [15–17]. There are three major enzymatic pathways participated in PUFA metabolism: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450. The COX pathway converts PUFA into prostaglandins, which are well-known mediators of inflammation and pain [16]. The LOX enzymes produce hydroxyl fatty acids and leukotrienes, which are predominately pro-inflammatory mediators playing critical roles in inflammatory disorders such as asthma [16]. The cytochrome P450 monooxygenases convert PUFAs to the corresponding epoxygenated fatty acids (EpFAs) [17,18]. For example, the metabolism of LA by cytochrome P450 monooxygenases generates two regioisomers of 9,10- and 12,13-epoxyoctadecenoic acids (EpOMEs), and the metabolism of arachidonic acid (ARA, 20:4ω-6, a downstream metabolite of LA) by cytochrome P450 monooxygenases generates epoxyeicosatrienoic acids (EETs) that include four regioisomers of 5,6-, 8,9-, 11,12- and 14,15-EET [17]. These EpFAs are then metabolism by soluble epoxide hydrolase (sEH, encoded by *Ephx2*) to generate the corresponding fatty acid diols [19]. Multiple dietary PUFAs, including LA, ARA, α-linolenic

acid (ALA, 18:3ω-3), γ-linolenic acid (γ-LA, 18:3ω-6), dihomo-γ-linolenic acid (DGLA, 20:3ω-6), eicosapentaenoic acid (EPA, 20:5ω-3), and docosahexaenoic acid (DHA, 22:6ω-3), are substrates of these PUFA-metabolizing enzymes (Fig. 1). This leads to formation of a large array of LMs with diverse chemical and biological properties.

Many of these LMs act via directly binding to specific G-protein coupled receptors (GPCRs), and have extremely potent actions to regulate many important biological processes, including inflammation and blood flow [15,16]. For example, the metabolism of DHA by cytochrome P450 monooxygenases (mainly CYP2C and CYP2 J subfamilies) generates epoxydocosapentaenoic acids (EDPs), which have much more potent biological activities compared with the parent fatty acid DHA. Indeed, EDPs dilated porcine coronary arterioles with EC_{50} values ranging from 0.5 to 24 pmol/L, in contrast, DHA only dilated coronary arterioles at range $\geq$ 1 μmol/L [20]. Due to the potent biological effects and modes of actions of the LMs, the tissue profiles of LMs, rather than the parental PUFAs, are more likely to be the biomarkers to predict the health outcomes of dietary PUFA intake. Elucidation of the specific PUFA-metabolizing pathways and metabolites required for the health effects of dietary PUFAs will greatly facilitate the development of PUFA biomarkers, leading to optimized use of dietary PUFAs for disease prevention. In addition, the polymorphisms in the genes encoding PUFA-metabolizing enzymes could affect PUFA metabolism, leading to varying levels of LMs in tissues and altered biological responses to PUFA supplementation. This information will help us to design better human trials to verify the biological effects of dietary PUFAs.

While previous mechanistic research of PUFA metabolism has focused on the COX and LOX pathways [21,22], the roles of cytochrome P450 pathway (the other major pathway besides COX and LOX branches) are not well understood. Recent research supports that the previously unappreciated cytochrome P450 monooxygenase pathway is upregulated in colon cancer, and play vital roles in its pathogenesis. Furthermore, recent research suggests that the cytochrome P450-mediated conversion of dietary LA to EpOMEs could contribute to the colon tumorigenesis-promoting effects of cytochrome P450 monooxygenases [23]. These results suggest a potential novel mechanistic explanation about dietary LA consumption and risks of developing colon cancer. In this essay, we will focus on recent advances in the roles of cytochrome P450 monooxygenases in fatty acid metabolism and its roles in colonic inflammation and colon cancer, and how this information could help us to better clarify the health impact of dietary PUFAs.

2. Expression of cytochrome P450 monooxygenases in colon cancer

Recent studies support that cytochrome P450 monooxygenases are overexpressed in colon cancer in human. More than 70% of tested colon tumor samples have been found to have positive staining of CYP2C9, while it was not found in matched human benign samples [24]. Additionally, CYP2J2 was reported to increase in human colon tumor samples [25]. In addition to CYP2C/2 J enzymes, other cytochrome P450 enzymes, including CYP2W1, CYP1B1, CYP2S1, CYP2U1, CYP3A5, and CYP51, have been found to highly expressed in human colorectal cancer samples compared to normal colorectal samples [26,27]. Further studies are needed to verify and study each individual cytochrome P450 enzyme expression regulation during colon tumorigenesis.

Consistent with the human studies, cytochrome P450 monooxygenases were found to overexpress in colon tumor tissues *in vivo* and colon cancer cells *in vitro*, and the concentrations of cytochrome P450-derived LMs were increased in the circulation and colon of colon cancer mice [28]. Indeed, increased expression of human cytochrome P450 genes, such as *CYP2C8, CYP2C9, CYP2C19, and CYP2 J2*, was been found in human colon cancer cell lines, compared with the normal human colon cell line. In the well-established AOM/dextran sodium sulfate (DSS)-induced colon cancer model, the gene expression of a series of mouse *Cyp* genes, including *Cyp2c38, Cyp2c39, Cyp2c55, Cyp2c65, Cyp2c70, Cyp2j6, Cyp2j9,* and *Cyp2j13*, was increased in colon of AOM/DSS-induced colon cancer mice [28].

There are many possible explanations by which cytochrome P450 monooxygenases are increased in colon tumors. Hypoxia, which is commonly found in the tumor microenvironment, has been shown to increase the expression of cytochrome P450 monooxygenases [18,29]. It is feasible that the hypoxic microenvironment could contribute to the enhanced expression of cytochrome P450 monooxygenases in colon tumors. To support this notion, a previous study showed that CYP2 J2 was overexpressed in the majority (77%) of human samples from many tumor types, including breast, stomach, liver, colon adenocarcinoma, and pulmonary squamous cell carcinoma, adenocarcinoma, small cell carcinoma [25].

3. Roles of cytochrome P450 monooxygenases in colon cancer

A recent study showed that genetic deletion or pharmacological inhibition of cytochrome P450 monooxygenases attenuated AOM/DSS-induced colon cancer *in vivo* [28], supporting a functional role of cytochrome P450 monooxygenase pathway in colon tumorigenesis. Compared with AOM/DSS-induced *Cyp2c*$^{+/+}$ mice, there was lower tumor number and total tumor burden, as well as reduced expression of proliferation marker such as PCNA, in the AOM/DSS-induced of *Cyp2c*$^{+/-}$ mice [28]. Consistently, dietary administration of cytochrome P450 inhibitor, SKF-525A or clotrimazole, suppressed colon cancer development in mice [28]. Treatment of SKF-525A or clotrimazole reduced tumor burden, attenuated colonic expression of proliferation marker PCNA and increased expression of apoptosis marker cleaved caspase-3, further supporting the roles of cytochrome P450 monooxygenases in the development of colon cancer in mice [28]. Together, these results demonstrate that the cytochrome P450 monooxygenases play important roles in colon tumorigenesis. To verify and explore the biological significance of cytochrome P450 monooxygenases in colon cancer development, further studies are needed to study the role of cytochrome P450 monooxygenases in other colon cancer models.

Colonic inflammation has been recognized as the major driving force in promoting colonic tumorigenesis. Therefore, targeting inflammation would be a good strategy to inhibit colon carcinogenesis. Recent studies support the contribution of cytochrome P450 monooxygenases in colon tumorigenesis-related inflammation [23]. In the AOM/DSS-induced colon cancer model, there was decreased expression of pro-inflammatory cytokines *Tnf-α* and *Il-1β* in colon tissue of *Cyp2c*$^{+/-}$ mice, compared to *Cyp2c*$^{+/+}$ mice [23]. Consistently, treatment of the cytochrome P450 inhibitors led to reduced AOM/DSS-induced expression of pro-inflammatory cytokines *Tnf-α, Mcp-1, Il-6, Il-1β,* and *Ifn-γ* in colon tissue [23]. These results demonstrate that inhibition of cytochrome P450 monooxygenases reduces colon cancer-associated inflammatory responses. However, it remains to determine whether cytochrome P450 inhibition or reduced colon tumorigenesis is the direct reason to cause attenuated inflammation.

4. Roles of cytochrome P450 monooxygenases in other types of cancer

Besides colon cancer, previous studies support that cytochrome P450 monooxygenases are involved in the tumorigenesis of other types of cancer. CYP2C8, CYP2C9 and CYP2 J2 have been found to be up-regulated in stomach, liver, ovarian cancer, renal carcinoma and testicular seminoma, compared to adjacent normal tissue [24,25]. In a xenograft tumor model, overexpression of human CYP2C8 in endothelial cell (using Tie2-CYP2C8 Tr mice) led to increased tumor growth of B16F10 melanoma and T241 fibrosarcoma, and Lewis lung carcinoma (LLC) in C57BL/6 mice [30]. Endothelial overexpression of CYP2 J2 (using Tie2-CYP2 J2 Tr mice) also led to increased xenograft growth of B16F10 melanoma and T241 fibrosarcoma in mice [30]. CYP2C11 was found to be increased brain tumor tissue compared to the normal tissue, and treatment of cytochrome P450 monooxygenase inhibitor, 17-octadecynoic acid or miconazole, significantly inhibited glial tumor formation in Fischer rat RG2 cell-induced glioblastoma model, suggesting a potential role of cytochrome P450 monooxygenase in brain tumorigenesis [31]. Human studies support that genetic polymorphisms of CYP2C8 and CYP2C9 are correlated with decreased survival in breast cancer patients [32]. Together, these results demonstrate a critical role of cytochrome P450 monooxygenases in many types of cancer.

Previous studies support that EETs, which are cytochrome P450 metabolites derived from ARA, contribute to the pro-cancer effects of cytochrome P450 monooxygenases. Panigrahy et al. found that treatment with 14,15-EET, at a dose as low as 15 μg/kg/day, increases primary tumor growth in several models, including xenograft LLC model, transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model, human liposarcoma in SCID mice, and orthotopic PC3M-LN4 human prostate cancer in SCID mice [30]. These results demonstrate that EETs have extremely potent pro-cancer effects *in vivo*, supporting that these metabolites are critical in mediating the biological activities of cytochrome P450 monooxygenases on tumorigenesis.

Angiogenesis, which is the process of formation of new blood vessels from pre-existing vessels, is a hallmark of tumorigenesis [33]. Previous studies have shown that cytochrome P450 monooxygenases and their derived metabolites enhance tumor angiogenesis, resulting in increased tumorigenesis. In a xenograft mouse model, endothelial overexpression of human CYP2C8 or CYP2 J2 led to increased formation of CD31-positive vessels in B16F10 tumors, illustrating enhanced tumor angiogenesis [30]. Similarly, in a corneal tumor angiogenesis model, overexpression of CYP2C8 in endothelial cells increased neovascularization in the cornea [30]. Systemic treatment with the cytochrome P450 metabolites EETs also increased tumor angiogenesis. Administration of 14,15-EET in the TRAMP mice increased the formation of blood vessels in prostate tumor [30]. These results demonstrate that cytochrome P450 monooxygenases and their metabolites have potent biological activities to promote tumor angiogenesis, which could contribute to their pro-cancer effects.

Cytochrome P450 monooxygenases also contribute to tumor metastasis by increasing tumor cells to spread from its primary location to other organs, which is the reason for ~90% of cancer-related death [34]. Panigrahy et al. showed that cytochrome P450 monooxygenases and metabolites EETs play critical roles in promoting tumor metastasis, and therefore could be important therapeutic targets to treat metastasis [30]. Indeed, using a LLC-resection metastasis model, endothelial overexpression of human CYP2C8 or CYP2 J2 led to increased surface and invasive metastases in lung tissues [30]. In a hematogenic metastasis model, overexpression of CYP2C8 also led to increased lung and liver metastases [30]. Consistent with the pro-metastatic effect of cytochrome P450 monooxygenases, treatment with EETs also promoted tumor metastasis. In the LLC-resection metastasis model, administration of 14,15-EET at a dose of 15 μg/kg/day increased axillary and inguinal lymph node metastasis, and finally lung metastasis in C57BL/6 mice [30]. These results support that the cytochrome P450 monooxygenase eicosanoid pathway plays critical roles in regulating tumor metastasis. Considering the critical roles in metastasis in cancer patient survival, it is of critical importance to study the roles of cytochrome P450 monooxygenases in metastasis and develop targeted therapies.

5. Roles of EpOMEs, the cytochrome P450 monooxygenase metabolites of LA, in colon cancer and other diseases

Recent studies support that EpOMEs, which are the metabolites of LA produced by cytochrome P450 monooxygenases, play critical roles in inducing inflammatory responses and enhancing colon tumorigenesis. In the AOM/DSS-induced colon cancer model, increased concentrations of 9,10- and 12,13-EpOMEs were been found in the circulation and colon tissue of colon cancer mice. These two metabolites are also among the most abundant lipid metabolites detected in the circulation and tissues of mice, which could be at least in part due to the high abundance of LA in tissues [28]. Treatment with low-dose 12,13-EpOME *via* mini-pumps increased tumor number, size, and total burden, and enhanced colonic expression of tumorigenic markers in colon tissue of C57BL/6 mice in colon cancer model. Consistent with the animal data, treatment with 12,13-EpOME at a concentration of 100 nmol/L increased production of pro-inflammatory cytokines and induced JNK phosphorylation in both macrophage and colon cancer cells [28]. Together, these results demonstrate the pro-inflammation and pro-cancer effects of EpOMEs in colon tumorigenesis.

These results are largely in agreement with previous studies which showed that EpOMEs have been associated with a series of detrimental effects, including chemotaxis, inflammation, cardiovascular diseases, and pulmonary injury [35–40]. In human studies, EpOMEs have been found to contribute to respiratory distress syndrome and even multiple organ failure in severe burn patients, and were termed as “leukotoxins” [36,38,41]. In Wistar rats, treatment of EpOME led to alveolar epithelial and endothelium damage, promoted neutrophil infiltration into lung tissues, and induced pulmonary edema and acute lung injury [37]. In cell culture studies, EpOMEs enhanced neutrophil chemotaxis at the dose of 10 nmol/L, demonstrating the potent pro-inflammatory effects of EpOMEs [40].

EpOMEs is biological unstable and could be further converted into corresponding fatty acid diols termed dihydroxyoctadecenoic acids (DiHOMEs) by sEH [18]. DiHOMEs have also been shown to induce chemotaxis, tissue injury, and cause mortality in animal models [41,42]. In both zymosan-induced and Freund’s Adjuvant-induced inflammatory pain models, the concentrations of 12,13-DiHOME were increased in peripheral nervous tissue in mice [43]. Injection of 12,13-DiHOME led to thermal hyperalgesia caused inflammatory pain *via* transient receptor potential vanilloid 1 (TRPV1)-dependent mechanisms [43]. In addition, administration of DiHOMEs could cause massive bronchiolar, peribronchiolar, and alveolar edema in the lungs of Swiss Webster mice[37]. The toxic effect of DiHOMEs might due to cellular mitochondrial dysfunction. DiHOMEs was found to change mitochondrial inner membrane permeability and induce mitochondrial membrane leakage in living cells, resulting in the release of cytochrome c and causing cell toxicity [44]. Together, these results show that not only EpOMEs but also their down-stream metabolites contribute to a series of detrimental biological effects.

6. Potential roles of cytochrome P450 monooxygenases in mediating the colon cancer-promoting effects of excessive LA intake

As discussed above, recent research has shown that: (1) the concentrations of 9,10- and 12,13-EpOME, which are metabolites of LA produced by cytochrome P450 monooxygenases, are significantly increased in the plasma and colon of AOM/DSS-induced colon cancer mice; (2) treatment with 12,13-EpOME, at nM doses, increases production of pro-inflammatory cytokines and induced JNK phosphorylation in both macrophage and colon cancer cells; (3) treatment with low-dose 12,13-EpOME, *via* mini-pumps, exacerbates AOM/DSS-induced colon tumorigenesis in mice; (4) the enzymes that produce EpOMEs, cytochrome P450 monooxygenases, are overexpressed in colon tumor tissues *in vivo* and colon cancer cells *in vitro*; and (5) pharmacological inhibition or genetic ablation of cytochrome P450 monooxygenases attenuates AOM/DSS-induced colon tumorigenesis *in vivo* [28]. Together, these results support that the previously unappreciated cytochrome P450/EpOME axis could play a critical role in the carcinogenesis of colon cancer, and could mediate the colon cancer-enhancing effects of dietary LA.

Based on these findings, overconsumption of LA could increase tissue concentrations of EpOMEs, which could enhance colon inflammation and tumorigenesis, therefore could result in enhanced colonic inflammation and colon cancer. A recent study by Taha et al. [45] showed that the circulating concentration of EpOMEs, but not other metabolites, was dose-dependently increased with increasing levels of LA in the diet: after a 15-week treatment with diet containing 0.4%, 5.2%, and 10% calories LA, the plasma concentrations of EpOMEs in rats were ~27.9, 175.4, and 346.3 pmol/mL, respectively. These results support a critical role of EpOMEs in mediating the biological actions of dietary LA. Based on these studies, the polymorphisms in the genes encoding cytochrome P450 monooxygenases, as well as its downstream fatty acid-metabolizing enzyme sEH, could affect the metabolism of LA to generate EpOMEs [46–52]. This could in part explain the inconsistent results from human studies. Further study of the biological significance of cytochrome P450/EpOME pathway in colon tumorigenesis could establish a new mechanistic linkage between excessive LA intake and increased risks of colon cancer. The obtained results could provide the fundamental knowledge that is critical to perform better designed human studies to clarify the health impact of dietary LA, and broadly enhance our understanding of how diet-gene interactions affect human health.

7. Potential roles of cytochrome P450 monooxygenases in mediating the colon cancer-suppressing effects of ω-3 PUFAs

A well-known theory to explain the effects of ω-3 PUFAs is that they suppress the enzymatic metabolism of ω-6 PUFAs (such as ARA) to generate ω-6-series LMs that are predominately pro-inflammatory and pro-angiogenic, or they act as alternative substrates to form ω-3-series LMs which usually have less detrimental or beneficial effects [53–56]. For example, EPA can effectively compete with ARA for metabolism by COX-2, leading to the reduced level of ARA-derived prostaglandin E_2 (PGE_2) which potently induces inflammation and tumor progression, and increased levels of EPA-derived prostaglandin E_3 (PGE_3) which is less detrimental [56,57]. The 4-hydroxy-docosahexaenoic acid (4-HDHA), the metabolite of DHA from 5-lipoxygenase (5-LOX), has potent anti-angiogenic effect; while the corresponding metabolite from ARA stimulates angiogenesis [55].

Recent research supports that the cytochrome P450 monooxygenase pathway could play critical roles in mediating the health-promoting effects of ω-3 PUFAs. Animal and human studies support that ω-3 PUFAs are mainly metabolized by the cytochrome P450 pathway [58–62], and they are known to be relatively poor substrates of other PUFA-metabolizing enzymes, such as COX and LOX [63–65]. In addition, cytochrome P450-derived ω-3 LMs, notably EDPs derived from DHA, have been shown to potently inhibited tumor growth, angiogenesis and metastasis [18,66,67]. A recent study showed that treatment with EDPs *via* mini-pumps at a dose of 0.5 mg/kg/day inhibited xenograft colon tumor growth in mice [68]. These findings are supported by other studies, which showed that EDPs had potent actions to suppress angiogenesis in the mouse model of laser-induced macular degeneration [69,70]. Treatment with 19,20-EDP inhibited pathological angiogenesis during macular degeneration by reducing the size of choroidal neovascularization lesions and suppressing vascular leakage [69]. Moreover, administration of 19,20-EDP inhibited choroidal neovascularization progression by regulating leukocyte recruitment in local microenvironment of choroidal neovascularization lesions and reducing the expression of adherent molecules on both leukocytes and endothelial cells [70]. These results suggest a potential role of the cytochrome P450 monooxygenase pathway in mediating the beneficial effects of ω-3 PUFAs.

8. Summary

Colon cancer is a major public health issue: it is estimated to cause 140,250 new cases and 50,630 deaths per year in the United States, making colon cancer the third most common type of cancer and the second leading cause of cancer death in the United States [71]. A better understanding of the molecular linkage between dietary PUFA consumption and colon cancer risks could help to develop safe and effective strategies for preventing colon cancer, and therefore leads to significant and positive impact on public health.

Recent research supports that the previously unappreciated cytochrome P450 monooxygenase pathway could play a critical role in mediating the opposite actions of ω-6 PUFAs *versus* ω-3 PUFAs on colon cancer. Indeed, studies show that the ω-6-series cytochrome P450 metabolites, including EETs derived from ARA and EpOMEs derived from LA, promote tumorigenesis of colon cancer and potentially other types of cancer [28,30]; in contrast, the ω-3-series cytochrome P450 metabolites, notably EDPs derived from DHA, potently inhibits angiogenesis, tumor growth, and tumor metastasis [66,68]. Further exploration of the cytochrome P450 monooxygenase pathway could help us to clarify the health impacts of dietary PUFAs.

Recent advances in molecular pathological epidemiology (MPE) demonstrate that various exogenous and endogenous factors from dietary, immune response and gut microbial will work together to influence colon tumorigenesis [72–74]. Indeed, ω-3 PUFA intake has been associated with a lower risk of colorectal carcinomas only with increased regulatory T cell in tumor tissue instead of lower regulatory T cell density [75]. Additionally, dysregulation of the gut microbial could also contribute to colon tumorigenesis *via* increasing pro-inflammatory response. For example, intake of Western diets rich in red and processed meat is associated with increased risk of colorectal cancer when there is the presence of the intestinal *Fusobacterium nucleatum* in tumor tissue, while not with a risk in *F. nucleatum*-null cancer [76]. *F. nucleatum* has been found to promote colorectal carcinogenesis through downregulating antitumor T cell-mediated adaptive immunity and activation of *E*-cadherin/β-catenin signaling pathways in colon cancer cells [77,78]. More studies are needed to further elucidate the inter-

action between host cytochrome P450 monooxygenase pathway and gut microbiota, such as *F. nucleatum*, in regulating the immune response and development of colon cancer.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This research is supported by a new faculty start-up from the University of Massachusetts Amherst, USDA NIFA2016-67017-24423, USDA NIFA 2019-67017-29248, USDA/HatchMAS00492, and NIH/NCIR03 CA218520 (to G.Z.).

References

[1] T. Blasbalg, J. Hibbeln, C. Ramsden, et al., Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century, Am. J. Clin. Nutr. 93 (2011) 950–962.
[2] U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015 – 2020 Dietary Guidelines for Americans. 8th Edition. December 2015. Available at: http://health.gov/dietaryguidelines/2015/guidelines/.
[3] R.J. Jandacek, Linoleic acid: a nutritional quandary, Healthcare (Basel) 5 (2) (2017).
[4] R.T. Enos, K.T. Velazquez, J.L. McClellan, et al., High-fat diets rich in saturated fat protect against azoxymethane/dextran sulfate sodium-induced colon cancer, Am. J. Physiol. Gastrointest. Liver Physiol. 310 (11) (2016) G906–19.
[5] B. Wu, R. Iwakiri, A. Ootani, et al., Dietary corn oil promotes colon cancer by inhibiting mitochondria-dependent apoptosis in azoxymethane-treated rats, Exp. Biol. Med. (Maywood) 229 (10) (2004) 1017–1025.
[6] T. Fujise, R. Iwakiri, T. Kakimoto, et al., Long-term feeding of various fat diets modulates azoxymethane-induced colon carcinogenesis through Wnt/beta-catenin signaling in rats, Am. J. Physiol. Gastrointest. Liver Physiol. 292 (4) (2007) G1150–6.
[7] B.S. Reddy, T. Tanaka, B. Simi, Effect of different levels of dietary trans fat or corn oil on azoxymethane-induced colon carcinogenesis in F344 rats, J. Natl. Cancer Inst. 75 (4) (1985) 791–798.
[8] A. Tjonneland, K. Overvad, M.M. Bergmann, et al., Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study, Gut 58 (12) (2009) 1606–1611.
[9] N. Sakamoto, S. Kono, K. Wakai, et al., Dietary risk factors for inflammatory bowel disease: a multicenter case-control study in Japan, Inflamm. Bowel Dis. 11 (2) (2005) 154–163.
[10] A.N. Ananthakrishnan, Environmental risk factors for inflammatory bowel disease, Gastroenterol. Hepatol. 9 (6) (2013) 367–374.
[11] J.K. Hou, B. Abraham, H. El-Serag, Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature, Am. J. Gastroenterol. 106 (4) (2011) 563–573.
[12] S. Rashvand, M.H. Somi, B. Rashidkhani, et al., Dietary fatty acid intakes are related to the risk of ulcerative colitis: a case-control study, Int. J. Colorectal Dis. 30 (9) (2015) 1255–1260.
[13] A.R. Hart, R. Luben, A. Olsen, et al., Diet in the aetiology of ulcerative colitis: a European prospective cohort study, Digestion 77 (1) (2008) 57–64.
[14] G.K. Pot, A. Geelen, E.M. van Heijningen, et al., Opposing associations of serum n-3 and n-6 polyunsaturated fatty acids with colorectal adenoma risk: an endoscopy-based case-control study, Int. J. Cancer 123 (8) (2008) 1974–1977.
[15] M.W. Buczynski, D.S. Dumlao, E.A. Dennis, Thematic Review Series: Proteomics. An integrated omics analysis of eicosanoid biology, J. Lipid Res. 50 (6) (2009) 1015–1038.
[16] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, Science (New York, N.Y.) 294 (5548) (2001) 1871–1875.
[17] D.C. Zeldin, Epoxygenase pathways of arachidonic acid metabolism, J. Biol. Chem. 276 (39) (2001) 36059–36062.
[18] G. Zhang, S. Kodani, B.D. Hammock, Stabilized epoxygenated fatty acids regulate inflammation, pain, angiogenesis and cancer, Prog. Lipid Res. 53 (0) (2014) 108–123.
[19] J.W. Newman, C. Morisseau, B.D. Hammock, Epoxide hydrolases: their roles and interactions with lipid metabolism, Prog. Lipid Res. 44 (1) (2005) 1–51.
[20] D. Ye, D. Zhang, C. Oltman, et al., Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels, J. Pharmacol. Exp. Ther. 303 (2) (2002) 768–776.
[21] D. Wang, R.N. Dubois, The role of COX-2 in intestinal inflammation and colorectal cancer, Oncogene 29 (6) (2010) 781–788.
[22] C.V. Rao, N.B. Janakiram, A. Mohammed, Lipoxygenase and Cyclooxygenase Pathways and Colorectal Cancer Prevention, Curr. Colorectal Cancer Rep. 8 (4) (2012) 316–324.
[23] W. Wang, J. Yang, M.L. Edin, et al., Targeted metabolomics identifies the cytochrome P450 monooxygenase eicosanoid pathway as a novel therapeutic target of colon tumorigenesis, Cancer Res. 79 (8) (2019) 1822–1830.
[24] A.E. Enayetallah, R.A. French, D.F. Grant, Distribution of soluble epoxide hydrolase, cytochrome P450 2C8, 2C9 and 2J2 in human malignant neoplasms, J. Mol. Histol. 37 (3-4) (2006) 133–141.
[25] J.G. Jiang, C.L. Chen, J.W. Card, et al., Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors, Cancer Res. 65 (11) (2005) 4707–4715.
[26] F.F. Chung, C.W. Mai, P.Y. Ng, et al., Cytochrome P450 2W1 (CYP2W1) in colorectal cancers, Curr. Cancer Drug Targets 16 (1) (2016) 71–78.
[27] M. Kumarakulasingham, P.H. Rooney, S.R. Dundas, et al., Cytochrome p450 profile of colorectal cancer: identification of markers of prognosis, Clin. Cancer Res. 11 (10) (2005) 3758–3765.
[28] W. Wang, J. Yang, M.L. Edin, et al., Targeted metabolomics identifies the cytochrome P450 monooxygenase eicosanoid pathway as a novel therapeutic target of colon tumorigenesis, Cancer Res. 79 (8) (2019) 1822–1830.
[29] P. Vaupel, A. Mayer, Hypoxia in cancer: significance and impact on clinical outcome, Cancer Metastasis Rev. 26 (2) (2007) 225–239.
[30] D. Panigrahy, M.L. Edin, C.R. Lee, et al., Epoxyeicosanoids stimulate multiorgan metastasis and tumor dormancy escape in mice, J. Clin. Invest. 122 (1) (2012) 178–191.
[31] D. Zagorac, D. Jakovcevic, D. Gebremedhin, et al., Antiangiogenic effect of inhibitors of cytochrome P450 on rats with glioblastoma multiforme, J. Cereb. Blood Flow Metab. 28 (8) (2008) 1431–1439.
[32] H. Jernstrom, E. Bageman, C. Rose, et al., CYP2C8 and CYP2C9 polymorphisms in relation to tumour characteristics and early breast cancer related events among 652 breast cancer patients, Br. J. Cancer 101 (11) (2009) 1817–1823.
[33] M. De Palma, D. Biziato, T.V. Petrova, Microenvironmental regulation of tumour angiogenesis, Nat. Rev. Cancer 17 (8) (2017) 457–474.
[34] C.L. Chaffer, R.A. Weinberg, A perspective on Cancer cell metastasis, Science (New York, N.Y.) 331 (6024) (2011) 1559–1564.
[35] Y. Hanaki, H. Kamiya, M. Ohno, et al., Leukotoxin, 9, 10-epoxy-12-octadecenoate: a possible responsible factor in circulatory shock and disseminated intravascular coagulation, Jpn. J. Med. 30 (3) (1991) 224–228.
[36] M. Hayakawa, K. Kosaka, S. Sugiyama, et al., Proposal of leukotoxin, 9,10-epoxy-12-octadecenoate, as a burn toxin, Biochem. Int. 21 (3) (1990) 573–579.
[37] J.N. Hu, F. Taki, S. Sugiyama, et al., Neutrophil-derived epoxide, 9,10-epoxy-12-octadecenoate, induces pulmonary edema, Lung 166 (6) (1988) 327–337.
[38] K. Kosaka, K. Suzuki, M. Hayakawa, et al., Leukotoxin, a linoleate epoxide: its implication in the late death of patients with extensive burns, Mol. Cell. Biochem. 139 (2) (1994) 141–148.
[39] T. Ozawa, M. Nishikimi, S. Sugiyama, et al., Cytotoxic activity of leukotoxin, a neutrophil-derived fatty acid epoxide, on cultured human cells, Biochem. Int. 16 (2) (1988) 369–373.
[40] Y. Totani, Y. Saito, T. Ishizaki, et al., Leukotoxin and its diol induce neutrophil chemotaxis through signal transduction different from that of fMLP, Eur. Respir. J. 15 (1) (2000) 75–79.
[41] J. Zheng, C.G. Plopper, J. Lakritz, et al., Leukotoxin-diol: a putative toxic mediator involved in acute respiratory distress syndrome, Am. J. Respir. Cell Mol. Biol. 25 (4) (2001) 434–438.
[42] M.F. Moghaddam, D.F. Grant, J.M. Cheek, et al., Bioactivation of leukotoxins to their toxic diols by epoxide hydrolase, Nat. Med. 3 (5) (1997) 562–566.
[43] B. Zimmer, C. Angioni, T. Osthues, et al., The oxidized linoleic acid metabolite 12,13-DiHOME mediates thermal hyperalgesia during inflammatory pain, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1863 (7) (2018) 669–678.
[44] M.F. Sisemore, J. Zheng, J.C. Yang, et al., Cellular characterization of leukotoxin diol-induced mitochondrial dysfunction, Arch. Biochem. Biophys. 392 (1) (2001) 32–37.
[45] A.Y. Taha, M. Hennebelle, J. Yang, et al., Regulation of rat plasma and cerebral cortex oxylipin concentrations with increasing levels of dietary linoleic acid, Prostaglandins Leukotrienes Essential Fatty Acids 138 (2018) 71–80.
[46] P.K. Srivastava, V.K. Sharma, D.S. Kalonia, et al., Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure, Arch. Biochem. Biophys. 427 (2) (2004) 164–169.
[47] A.W. Dreisbach, S. Japa, A. Sigel, et al., The Prevalence of CYP2C8, 2C9, 2J2, and soluble epoxide hydrolase polymorphisms in African Americans with hypertension, Am. J. Hypertens. 18 (10) (2005) 1276–1281.
[48] M. Spiecker, H. Darius, T. Hankeln, et al., Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2, Circulation 110 (15) (2004) 2132–2136.
[49] C.R. Lee, K.E. North, M.S. Bray, et al., Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study, Hum. Mol. Genet. 15 (10) (2006) 1640–1649.
[50] Q. Wei, P.A. Doris, M.V. Pollizotto, et al., Sequence variation in the soluble epoxide hydrolase gene and subclinical coronary atherosclerosis: interaction with cigarette smoking, Atherosclerosis 190 (1) (2007) 26–34.
[51] M. Fornage, E. Boerwinkle, P.A. Doris, et al., Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in

African-American subjects: the Coronary Artery Risk Development in Young Adults (CARDIA) study, Circulation 109 (3) (2004) 335–339.
[52] B.D. Przybyla-Zawislak, P.K. Srivastava, J. Vazquez-Matias, et al., Polymorphisms in human soluble epoxide hydrolase, Mol. Pharmacol. 64 (2) (2003) 482–490.
[53] D.P. Rose, J.M. Connolly, Omega-3 fatty acids as cancer chemopreventive agents, Pharmacol. Ther. 83 (3) (1999) 217–244.
[54] C.N. Serhan, N.A. Petasis, Resolvins and protectins in inflammation resolution, Chem. Rev. 111 (10) (2011) 5922–5943.
[55] P. Sapieha, A. Stahl, J. Chen, et al., 5-Lipoxygenase metabolite 4-HDHA is a mediator of the antiangiogenic effect of omega-3 polyunsaturated fatty acids, Sci. Transl. Med. 3 (69) (2011), 69ra12.
[56] D. Bagga, L. Wang, R. Farias-Eisner, et al., Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion, Proc. Natl. Acad. Sci. U. S. A. 100 (4) (2003) 1751–1756.
[57] M. Szymczak, M. Murray, N. Petrovic, Modulation of angiogenesis by omega-3 polyunsaturated fatty acids is mediated by cyclooxygenases, Blood 111 (7) (2008) 3514–3521.
[58] C. Arnold, M. Markovic, K. Blossey, et al., Arachidonic acid-metabolizing cytochrome P450 enzymes are targets of omega-3 fatty acids, J. Biol. Chem. 285 (43) (2010) 32720–32733.
[59] R. Fischer, A. Konkel, H. Mehling, et al., Dietary Omega-3 fatty acids modulate the eicosanoid profile in man primarily via the CYP-epoxygenase pathway, J. Lipid Res. 55 (6) (2014) 1150–1164.
[60] A. Zivkovic, J. Yang, K. Georgi, et al., Serum oxylipin profiles in IgA nephropathy patients reflect kidney functional alterations, Metabolomics 8 (6) (2012) 1102–1113.
[61] A.H. Keenan, T.L. Pedersen, K. Fillaus, et al., Basal omega-3 fatty acid status affects fatty acid and oxylipin responses to high-dose n3-HUFA in healthy volunteers, J. Lipid Res. 53 (8) (2012) 1662–1669.
[62] G.C. Shearer, W.S. Harris, T.L. Pedersen, et al., Detection of omega-3 oxylipins in human plasma and response to treatment with omega-3 acid ethyl esters, J. Lipid Res. 51 (8) (2010) 2074–2081.
[63] D.B. Jump, The biochemistry of n-3 polyunsaturated fatty acids, J. Biol. Chem. 277 (11) (2002) 8755–8758.
[64] M.G. Malkowski, E.D. Thuresson, K.M. Lakkides, et al., Structure of eicosapentaenoic and linoleic acids in the cyclooxygenase site of prostaglandin endoperoxide H synthase-1, J. Biol. Chem. 276 (40) (2001) 37547–37555.
[65] O. Laneuville, D.K. Breuer, N. Xu, et al., Fatty acid substrate specificities of human prostaglandin-endoperoxide H synthase-1 and −2: formation of 12-hydroxy-(9Z,13E/Z,15Z)-octadecatrienoic acids from α-linolenic acid, J. Biol. Chem. 270 (33) (1995) 19330–19336.
[66] G. Zhang, D. Panigrahy, L.M. Mahakian, et al., Epoxy metabolites of docosahexaenoic acid (DHA) inhibit angiogenesis, tumor growth, and metastasis, Proc. Natl. Acad. Sci. U. S. A. 110 (16) (2013) 6530–6535.
[67] W. Wang, J. Zhu, F. Lyu, et al., Omega-3 polyunsaturated fatty acids-derived lipid metabolites on angiogenesis, inflammation and cancer, Prostaglandins Other Lipid Mediat. 1 (13-115) (2014) 13–20.
[68] W. Wang, J. Yang, Y. Nimiya, et al., ω-3 polyunsaturated fatty acids and their cytochrome P450-derived metabolites suppress colorectal tumor development in mice, J. Nutr. Biochem. 48 (2017) 29–35.
[69] R. Yanai, L. Mulki, E. Hasegawa, et al., Cytochrome P450-generated metabolites derived from omega-3 fatty acids attenuate neovascularization, Proc. Natl. Acad. Sci. U. S. A. 111 (26) (2014) 9603–9608.
[70] E. Hasegawa, S. Inafuku, L. Mulki, et al., Cytochrome P450 monooxygenase lipid metabolites are significant second messengers in the resolution of choroidal neovascularization, Proc. Natl. Acad. Sci. U. S. A. 114 (36) (2017) E7545–E7553.
[71] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin. 68 (1) (2018) 7–30.
[72] S. Ogino, J.A. Nowak, T. Hamada, et al., Insights into pathogenic interactions among environment, host, and tumor at the crossroads of molecular pathology and epidemiology, Annu. Rev. Pathol. 14 (2019) 83–103.
[73] T. Hamada, J.A. Nowak, D.A. Milner Jr., et al., Integration of microbiology, molecular pathology, and epidemiology: a new paradigm to explore the pathogenesis of microbiome-driven neoplasms, J. Pathol. 247 (5) (2019) 615–628.
[74] S. Ogino, A.T. Chan, C.S. Fuchs, et al., Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field, Gut 60 (3) (2011) 397–411.
[75] M. Song, R. Nishihara, Y. Cao, et al., Marine omega-3 polyunsaturated fatty acid intake and risk of colorectal cancer characterized by tumor-infiltrating T cells, JAMA Oncol. 2 (9) (2016) 1197–1206.
[76] R.S. Mehta, R. Nishihara, Y. Cao, et al., Association of dietary patterns with risk of colorectal cancer subtypes classified by Fusobacterium nucleatum in tumor tissue, JAMA Oncol. 3 (7) (2017) 921–927.
[77] K. Mima, Y. Sukawa, R. Nishihara, et al., Fusobacterium nucleatum and t cells in colorectal carcinoma, JAMA Oncol. 1 (5) (2015) 653–661.
[78] M.R. Rubinstein, X. Wang, W. Liu, et al., Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin, Cell Host Microbe 14 (2) (2013) 195–206.