総　　説

SIGNIFICANCE OF TRANS FATTY ACIDS IN CANCER

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Abstract : Trans fatty acids (TFAs) are a recent focus of health problems. TFA is a definitive risk factor for cardiovascular diseases and the death. TFA is also possible risk factor for Alzheimer's disease, obesity, diabetes, fatty liver, and ovulation infertility. The relationship between TFA and carcinogenic risk is controversial; however, TFA is reported to increase the risk of breast cancer and prostate cancer. Elaidic acid (EA), a trans form of oleic acid, enhances cancer cell growth, invasion, and anti-apoptotic survival. In animal models, EA promotes tumor growth and metastasis to the lung, liver, and peritoneum. EA induces stemness in cancer cells through transactivation of EFGR via SRC from GPR40/120 as receptors in EA-integrated cholesterol rafts. Activated EGFR relays the signals to activate canonical and non-canonical wnt pathways and to inactivate notch1 pathways. EA also increases miR-494, which inhibits cell differentiation through decrease of target genes. Continuous EA feeding with dosage alteration increased cancer cell stemness. EA diminishes the efficiency of 5-fluorouracil by increasing residual cancer stem cells. These findings suggest that TFA is a relevant cancer promoting factor. The decision to remove TFA from foods made by the FDA might have an impact on cancer clinics.

Key words : trans fatty acid, elaidic acid, conjugated linoleic acid, linoleic acid, colorectal cancer

Trans fatty acid

Unsaturated fatty acids generally have *cis* configurations, which are opposed to *trans* configurations [1]. Trans fatty acids (TFAs) are uncommon in nature and usually generated in industrial processes. TFAs are provided with trans configurations by hydrogenation, which depends mainly on oil temperature, hydrogen pressure, stirring speed, reaction time, and the catalyst type and concentration [1]. TFAs are widely used in margarine and snack foods, and to shorten the frying of fast foods.

The health problem evoked by TFAs are a focus of public interest as well as basic or clinical research. An increase of 2% in the daily energy through TFA is associated with a 23% increase in cardiovascular risk of myocardial infarction and chronic heart disease death [2,3]. The totality

of analyses supports the proportionality of changes in TFA intake and changes in blood lipids (and therefore, CHD risk) and supports the use of a linear regression model to describe this relationship [4].

The American Heart Association recommends limiting trans fats to <1% energy, and the American Dietetic Association, the Institute of Medicine, US Dietary Guidelines, and the National Cholesterol Education Project all recommend limiting dietary trans-fat intake from industrial sources as much as possible [2]. Regulations grounded on maximum limits and mandated labeling can lead to reductions in actual and reported TFAs in food and appear to encourage food producers to reformulate products [5]. Food and Drug Administration (FDA) labeling rules had allowed products containing <0.5 g trans fat per serving to claim 0 g trans fat [2]. June 17, 2015, the FDA issued a final determination that there is no consensus that industrially-produced TFAs are generally recognized as safe for any use in human food. Trans fat must be removed from prepared foods within three years by June 2018 [4].

Cardiovascular disorder and TFA

Monounsaturated fatty acids, on the other hand, have a positive impact on the serum lipid profile, lead to decreased LDL-oxidation and favorably influence the metabolism of diabetics [6]. Concerning polyunsaturated fatty acids, it is important to increase the supply of n-3 fatty acids (ratio of n-6:n-3: about 5:1) as there is substantial evidence for their protective effects [6].

As mentioned above, trans fatty acids have been identified as an important cause of cardiovascular disease and the resulting clinical end points such as strokes and heart attacks [7]. Similar to saturated fatty acids, trans fatty acids increase plasma low density lipoprotein (LDL)-cholesterol concentrations. In contrast to saturated fatty acids, trans fatty acids do not increase high density lipoprotein (HDL)-cholesterol concentrations [8; 9].

TFA may also worsen insulin sensitivity, particularly among individuals predisposed to insulin resistance; possible effects on weight gain and diabetes incidence require further confirmation [3]. TFA upregulated the mRNA levels of resistin and downregulated PPARγ and lipoprotein lipase (LPL) [10]. The alteration in the mRNA levels of PPARγ and resistin could be associated with insulin resistance in TFA-fed rats.

Carcinogenesis and TFA

Literature has proved a direct connection between trans fatty acids and not only cardiovascular diseases but also breast cancer, nonaggressive prostate cancer, ovulatory infertility, Alzheimer's disease, diabetes, fatty liver, obesity and allergy [3; 11-17]. However, in numerous experimental models and human studies, there is insufficient and inconsistent evidence linking specific TFA isomers to cancers of the prostate, colon and breast [18]. The significant changes in TFA intakes between then and the end of follow-up in 1994 limit the reliability of this observation. There is insufficient evidence to allow any differentiation between the effects of TFAs of animal or vegetable origin on cancer risk [19]. In an animal model, 25% EA diet does not enhance colon carcinogenesis in F344 female rats induced by azoxymethan [20].

Cancer progression and EA

Elaidic acid (EA; C18:1 Δ 9, *trans*) is a *trans* form of oleic acid (OA; C18:1 Δ 9, *cis*), and constitutes the largest quantity in dietary TFAs. In a previous study, Ehrlich ascites tumors in EA-fed mice showed higher proliferative activity than those in OA-fed mice and this shortened the survival rate of mice by 45% at maximum [21]. EA tends to be more incorporated into Ehrlich ascites tumor cells and less oxidized than OA [22].

EA increases cell growth, invasion and anti-apoptotic survival in colorectal cancer (CRC) cells [23]. In mouse models, EA enhances tumor growth and metastasis of CRC cells to the lung, liver, and peritoneum at more pronounced levels than OA treatment [23]. The same amount of peritoneal metastasis occurred with $1x10^3$ cells unber EA treatment as with $1x10^5$ cells under OA treatment [23]. These results suggest that EA might induce stemness of the CRC cells.

Cancer stem cell and EA

Recent studies have focused on the stemness of the cancer cells in the responsibility for carcinogenis and metastasis [24]. Dysregulation of normal stem cells produces transformed stem cells, which are the tumor-initiating cells or cancer stem cells CSCs [25; 26]. In the CSC model, only CSCs can propagate tumors at carcinogenesis and cancer metastasis [27]. Activation of the signal pathways of wnt, hedgehog, and notch is deeply associated with cancer stem cell dysregulation [27-31]. The diversity of normal stem cells is reflected in CSCs, generating heterogeneity and plasticity of the progeny [32]. Thus, CSCs are an essential therapeutic target, however, the conventional anti-cancer therapies are not effective against CSCs [33; 34]. The study of substances affecting CSCs might provide some novel valuable information on handling CSCs. In the present study, the pro-tumoral effect of EA is studied with a focus on cancer stem cell modification.

EA increases sphere formation of CRCs, which possess high stemness [23; 35]. EA treatment induces greater expression of nucleostemin in a monolayer culture and CD133 in sphere assays in comparison with OA treatment [23]. Spheres of EA-treated CRCs show larger size and higher proliferative activity than OA-treated cell spheres [23]. The increase of stemness caused by EA provides resistance to 5-fluorouracil treatment [36].

Receptor, intracellular signal of EA

G-protein coupled receptors (GPRs) are known as receptors of long chain fatty acids. GPR40 and GPR120 in particular function as receptors for unsaturated long-chain free fatty acids, such as OA or LA [37]. Binding of EA to GPR40 and 120 is confirmed by surface protein internalization assay; however, knockdown of these two receptors does not abrogate completely the increased proliferative activity of spheres [23]. EA might possess another receptor for the function.

EA is involved into triglycerides, cholesterol esters, and phospholipids as well as other long chain fatty acids, such as OA, LA [38]. However, EA shows low affinity to intestinal lymph triglyceride in comparison with OA [39]. EA is incorporated into the plasma membrane as well as

incorporated into triglyceride [40]. EA in the plasma membrane is integrated in the cholesterol raft, which involves EGFR and GPR40/120. The transactivation of EGFR from GPR40/120 occurrs through Src activation [23]. Crosstalk between GPCR and EGFR is reported to activate phospholipases C, D, and A2, which subsequently activates PKC/PKD, Raf/MEK/ERK, and Akt/mTOR/p70S6K [41].

EGFR signaling upregulates nucleostemin and snail, and downregulates notch1 and E-cadherin in CT26 cells carrying wild type APC. In contrast, the signal upregulates nucleostemin, wnt5a and CD44, and downregulates notch1 in APC null HT29 cells [23]. Thus EA provides enhancement of proliferative stem activity and epithelial-mesenchymal transition phenotype. These effects are closely linked to the prominent metastatic potential of EA-treated cancer cells.

microRNA and EA [42]

The expression levels of many micro-RNAs (miRNAs) are altered in EA treated CT26 CRC cells examined by miRNA array [42]. miRNA-494 is one of the increased miRNAs, whose function, especially in cancer, has not been elucidated. The effect of the treatment of miR-494 inhibitor or miR-494 mimic concurrently with EA on stemness is examined by a sphere assay. Sphere density was increased by EA; however, a strong increase is observed with mimic treatment. Meanwhile, sphere density is decreased by inhibitor treatment. CD133 and NS increased with mimic and EA, whereas the inhibitor treatment abrogated the expression of CD133 and NS [42]. The target genes of miR-494 contained differentiation factors for cancer stem cells. The expressions of PPARγ coactivator 1A, PTEN, and activin A receptor 1C are reduced by treatment with EA and the mimic, and increased by the inhibitor [42]. Thus, miR-494 expression is suggested to be promoted by EA, to maintain the stemness of cancer stem cells by suppressing differentiation factors [42].

Continuous feeding of EA diet [43]

The effect of intermittent feeding restriction (IFR) is simulating human habits of feeding, which are quite variable both temporally and quantitatively. The effect of IFR and food contents on cancer are studied using a mouse model. CT26 CRC cells were inoculated into the back of a syngeneic BALB/c mouse. A 24-hour fast was implemented once a week for 4 weeks. The effect of oral intake of TFAs on tumor stem cells with IFR was examined using the model. Mice fed with 10% EA diet (EA)+IFR, and a control diet (CD)+IFR are compared with EA alone or CD alone. The order from highest to lowest in terms of tumor weight and tumor MIB1 index (cell proliferation)is: EA+IFD, CD+IFR, EA, and CD. In EA+IFR, NS-positive tumor cells are transiently increased on the next day of feeding restriction. After 6 weeks with 12 IFRs, NS-positive CT26 cells are increased in EA+IFR in comparison with EA alone. These findings suggest that alteration of feeding habits might enhance cancer stemness.

Ruminant TFA

Recent research has now identified an important cardioprotective role for a sub-category of trans fats, the ruminant trans fats [7]. TFAs contained in meat of the ruminant animals or vegetable oils have different features from industrial TFAs [44]. However, no relationship is found between ruminant TFA intake levels of up to 4.2% of daily energy intake and changes in cardiovascular risk factors such as total-cholesterol: HDL-cholesterol and LDL-cholesterol: HDL-cholesterol ratios. Moreover, a multivariate regression analysis that included other dietary variables shows that doses of ruminant TFA did not significantly influence the changes in the lipid ratio [45].

Conjugated linoleic acid

Conjugated linoleic acid (CLA) is composed of positional- and stereo-isomers of octadecadienoate (18:2), which is recognized as one of the ruminant TFAs [44]. The chemoprotective properties of CLA are reported in experimental cancer models and *in vitro* examinations [44; 46]. In contrast, LA has carcinogenic properties. LA, an ω-6 polyunsaturated fatty acid, is a stereoisomer of CLA. COX-2 metabolizes LA to PGE2, which plays roles in carcinogenesis due to its properties of pro-inflammation, pro-proliferatation, and immunosuppression [47; 48]. CLA activates PPARγ as a ligand [49; 50]. PPARγ is a nuclear hormone receptor superfamily of ligand-activated transcription factors, which initiate transcription of genes associated with energy homeostasis [51].

CLA affects the risk of cardiovascular disease (CVD) and cancer more than industrial TFAs [52]. CLA supplementation is associated with a significant decrease in LDL cholesterol, and not associated significantly with decrease in HDL cholesterol, increase in total cholesterol and decrease in TAG [53]. Supposed adverse effects such as oxidative stress, insulin resistance, irritation of the intestinal tract and milk fat depression are also examined. It seems that no consistent result was observed even in similar studies conducted at different laboratories, this may be due to variations in age, gender, racial and geographical disparities, coupled with type and dose of CLA supplemented [54].

The effect of CLA was examined using gastrointestinal cancer cell lines [55]. CLA inhibited cell growth and invasion of MKN28 and Colo320 cancer cells with an increment in apoptosis. CLA induced growth inhibition was recovered by knockdown of PPARγ in both cell lines. In a nude mouse peritoneal dissemination model, CLA treatment significantly decreases the metastatic foci of both cells in the peritoneal cavity and the survival rate in mice is significantly recovered by CLA treatment. CLA treatment provides a decrease in EGFR and TGF-α and an increase in Bax [55] in these cells. The anti-tumoral effect of CLA is confirmed with a syngeneic mouse peritoneal dissemination model of C57BL6 mice and LL2 cells [56].

Conclusion

Drawing on the above overview, numerous reports indicate that TFA is a pivotal factor threatening health, evoking risks for almost all of the lifestyle-related diseases. Cardiovascular diseases are already recognized as a disease associated with TFAs. Our recent studies show that TFAs are closely associated with cancer promotion through induction of cancer stemness. Now TFAs are a social problem for regulation, i.e. subject to FDA decision. At the same time, the importance of elucidating the bioactive cancer-related functions of fatty acids is suggested.

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