論　文　内　容　要　旨

Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells

（オメガ３系多価不飽和脂肪酸は血管内皮細胞における DNA 損傷を軽減する）

PLoS ONE, 12(11): e0187934, 2017.

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Background and Objective: Recent findings suggest that DNA damage likely has a pivotal role in the pathogenesis and progression of cardiovascular disease. This is substantiated by atherosclerosis being one of the major symptoms of progeroid syndromes, which are genetic disorders cause premature aging mostly due to mutations in genes associated with genome integrity. We have previously reported the accumulation of DNA damage in human atherosclerotic lesions (Ishida *et al.* PLoS ONE, 9(8): e103993, 2014). Regarding the body of evidence, attenuating DNA damage may be crucial for treatment and prevention of atherosclerosis and related cardiovascular disease.

Considerable evidence supports that fish derived omega-3 polyunsaturated fatty acids (n-3 PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), hinder cardiovascular disease. EPA and DHA are known to decrease plasma triglyceride levels and improve endothelial function. However, whether their cardioprotective effects are mediated through the DNA damage response is currently unclear. In this study, we investigated the effects of EPA and DHA on DNA damage in vascular endothelial cells to clarify the cardioprotective mechanisms of n-3 PUFAs.

Approach and Results: We examined the effect of EPA and DHA on H_2O_2-induced DNA damage response in human aortic endothelial cells (HAECs). DNA damage was detected by immunofluorescence staining as a cytologically visible 'foci' using an antibody against the phosphorylated form of the histone H2AX (γH2AX), a prominent marker of DNA double-strand breaks. H_2O_2-induced γH2AX foci formation after 30 min and 24 h recovery was significantly reduced in HAECs treated with EPA (by 30% and 47%, respectively) and DHA (by 27% and 48%, respectively). H_2O_2-induced activation of ATM, a primary transducer of the DNA damage response signaling, was detected by western blotting. Phosphorylation of ATM was significantly reduced with EPA and DHA treatment (by 31% and 33%, respectively). These findings suggested that EPA and DHA attenuated DNA damage, which was not induced by activating the DNA damage response. We further determined the effects of EPA and DHA on oxidative stress, a cause of DNA damage. Intracellular reactive oxygen species were detected by using a molecular probe, chloromethyl-2',7'-dichlorofluorescein diacetate (CM-H_2DCFDA). Treatment with EPA and DHA significantly reduced intracellular reactive oxygen species under both basal condition (by 9.4% and 17.1%, respectively) and H_2O_2 stimulation (by 14.1% and 16.4%, respectively). In addition, we performed real-time RT-PCR analysis to determine the mRNA expression levels of renowned antioxidant molecules, which were heme oxygenase-1 (HO-1), NADPH dehydrogenase quinone 1, ferritin heavy chain (FTH), ferritin light chain (FTL), thioredoxin reductase 1 (TXNRD1), manganese superoxide dismutase (SOD2), catalase and peroxiredoxin 5. Although EPA and DHA did not influence all of these molecules, they

significantly increased the mRNA levels of HO-1, FTH, FTL, TXNRD1 and SOD2. These antioxidant molecules are known to be regulated by nuclear factor erythroid 2-related factor 2 (NRF2), which is a transcription factor primarily modulates oxidative stress responses. Gene silencing with the small interfering RNA against *NRF2* abrogated the increased mRNA levels of antioxidant molecules mentioned above. SOD2 is known to be regulated by the forkhead box O (FOXO) family transcription factors. Thus we performed double knockdown of *NRF2* and *FOXO1*. Compared to *NRF2* knockdown alone, the n-3 PUFA-induced increase of SOD2 mRNA expression was additively abrogated by double knockdown of *NRF2* and *FOXO1*. In addition, H_2O_2-induced increase of intracellular reactive oxygen species was decreased with EPA treatment, which was also abrogated by *NRF2* silencing. Furthermore, we determined the effect of EPA and DHA on cell senescence, which is a consequent manifestation of sustained DNA damage. Senescence-associated beta-galactosidase activity assay, a marker of cell senescence, showed that treatment with EPA and DHA significantly reduced H_2O_2-induced cell senescence in HAECs (by 31% and 22%, respectively). This effect was revoked by *NRF2* silencing.

Conclusions: We demonstrated for the first time that both EPA and DHA attenuated oxidative stress-induced DNA damage and cell senescence in human endothelial cells. These effects were exerted, at least partly, through upregulation of NRF2-mediated antioxidant response. Therefore n-3 PUFAs likely help prevent coronary artery disease in part by their genome protective properties.