Supplementary Material

Progress on the biological characteristics and physiological activities of fucoxanthin produced by marine microalgae

Bing Gong1,2, Silu Ma2, Yajun Yan2, Zhaokai Wang2,\*

**\* Correspondence:** Zhaokai Wang: wang@tio.org.cn

# Supplementary Figures and Tables

## Supplementary Tables

Table 2 Pharmacological activities of fucoxanthin

| Physiological and Pharmacological activity |  | Experimental Model | Reference |
| --- | --- | --- | --- |
| Aanti-inflammatory activity | Skin inflammation | UVB-irradiated mice | ([Wang et al., 2018](#R6)) |
| Reconstructed human skin | ([Spagolla Napoleão Tavares et al., 2020](#R8)) |
| Ulcerative colitis | Sodium dextran sulfate-stimulated BALB/c mice | ([Yang et al., 2020](#R9)) |
| Contact hypersensitivity | Dinitrofluorobenzene-stimulated BALB/c mice | ([Sakai et al., 2011](#R10)) |
| Macrophage inflammation | LPS-stimulated RAW264.7 cells | ([Heo et al., 2010](#R61); [Kim et al., 2010](#R62)) |
|  | Palmitic acid-stimulated RAW264.7 cells | ([Li et al., 2020](#R63)) |
| Uveitis | LPS stimulated-Lewis rats | ([Shiratori et al., 2005](#R65)) |
| Allergic | Immunoglobulin E antigen complex-stimulated RBL-2H3 cells | ([Namkoong et al., 2012](#R66)) |
| Antioxidant activity |  | Reduces the rate of vitamin D2 oxidation | ([Li et al., 2000](#R68)) |
| H2O2-stimulated Vero cells | ([Heo et al., 2008](#R69)) |
| UV and visible light-induced mouse fibroblast cell 3T3, reconstructed human skin, HaCaT cells | ([Tavares et al., 2020](#R72)) |
| UVB-induced human fibroblast cells | ([Heo et al., 2009](#R73)) |
| Mouse liver BNL CL.2 cells | ([Liu et al., 2011](#R74)) |
| High-fat diet Sprague-Dawley rats | ([Ha et al., 2013](#R75)) |
| Retinol-deficient rats | ([Ravi Kumar et al., 2008](#R76)) |
| Reduction of potassium ferricyanide by fucoxanthin, scavenging ability of O2- | ([Qin et al., 2013](#R77)) |
| Spontaneous oxidation of mouse liver homogenates and  H2O2-Fe2+-induced oxidation | ([Wu et al., 2011](#R78)) |
| Antitumor activity | Gastric cancer | Gastric adenocarcinoma cells SGC-7901 and BGC-823 | ([Yu et al., 2018](#R79)) |
| Liver cancer | Hepatocellular carcinoma cell HepG2 | ([Liu et al., 2013](#R80); [Das et al., 2008](#R81)) |
| Nasopharyngeal Carcinoma | Nasopharyngeal carcinoma cell C666-1 | ([Long et al., 2020](#R82)) |
| Cervical cancer | Epithelial cervical cancer HeLa, SiHa, CaSki | ([Hou et al., 2013](#R83); [Jin et al., 2018](#R84)) |
| Lung cancer | Lung cancer cells A549, H1299, MRC-5 | ([Moreau et al., 2006](#R85)) |
| Breast cancer | Cancer cell MDA-MB-231, nude mouse model | ([Wang et al., 2019](#R86)) |
| Leukaemia | Human leukemia cell HL-60 | ([Kim et al., 2010](#R87)) |
| Antimetabolic disorder activity | Anti-obesity | Mouse embryonic fibroblasts 3T3-L1 | ([Koo et al., 2019](#R14)) |
| High-fat diet C57BL/6J mice | ([Gille et al., 2019](#R15)) |
| High-fat diet SD rats | ([Hu et al., 2012](#R16)) |
| Clinical double-blind trials | ([Hitoe et al., 2017](#R17)) |
| Clinical trials | ([Abidov et al., 2010](#R18)) |
| Anti-nonalcoholic steatohepatitis | High-fat diet-induced mouse | ([Takatani et al., 2020](#R20)) |
| Oleic and palmitic acids-stimulated human Zhang's hepatocytes | ([Ye et al., 2022](#R21)) |
| High-fat diet C57BL/6 nude mice , human HepaRGTM cells, clinical trials | ([Shih et al., 2021](#R22)) |
| Antidiabetic | Streptozotocin nicotinamide-stimulated Sprague-Dawley rats | ([Sakai et al., 2011](#R10)) |
| High glucose or 4-HNE-stimulated human retinal pigment epithelial cells | ([Kong et al., 2019](#R11)) |
| KK-Ay mice | ([Maeda et al., 2007](#R13)) |
| High-fat diet mice | ([Maeda et al., 2009](#R98)) |
| KK-Ay mice | ([Hosokawa et al., 2010](#R99)) |
| 3T3-L1 adipocytes | ([Kang et al., 2011](#R90)) |
| KK-Ay mice | ([Nishikawa et al., 2012](#R101)) |
| Anti-Alzheimer | Computer simulation | ([Shih et al., 2021](#R22)) |
| Pharmacokinetic simulation, Aβ oligomers-induced mice | ([Xiang et al., 2017](#R24)) |
| PLGA-PEG-Fx nanoparticles | ([Yang et al., 2021](#R25)) |
| Aβ oligomers-induced SH-SY5Y cells | ([Lin et al., 2017](#R26)) |
| D-gal-induced SH-SY5Y cells | ([Zhu et al., 2021](#R27)) |
| Aβ1-42 , H2O2-induced SH-SY5Y cells | ([Zhu et al., 2021](#R27)) |
| Other active | Antiviral | Raji cells | ([Tsushima et al., 1995](#R29)) |
| Antimicrobial | Disc-diffusion and micro-dilution methods | ([Karpiński et al., 2019](#R102)) |
| Disc-diffusion methods | ([Liu et al., 2019](#R103)) |
| \ | ([Peraman et al., 2019](#R104)) |
| Disc-diffusion methods, transmission electron microscope | ([El Shafay et al., 2016](#R105)) |
| Anti-angiogenic | HUVEC | | ([Ganesan et al., 2013](#R108)) |
| HUVEC, male Wistar rats | | ([Sugawara et al., 2006](#R109)) |
| Osteoprotective | TNF-α-stimulated RAW264.7, MC3T3-E1 | | ([Das et al., 2010](#R110)) |
| TNF-α or IL-6-stimulated RAW264. 7 | | ([Ha et al., 2011](#R111)) |

**Reference**

Abidov, M., Ramazanov, Z., Seifulla, R., Grachev, S. (2010). The effects of Xanthigen™ in the weight management of obese premenopausal women with non‐alcoholic fatty liver disease and normal liver fat. Diabetes, obesity and metabolism, 12(1), 72-81. <https://doi.org/10.1111/j.1463-1326.2009.01132.x>

Das, S. K., Hashimoto, T., Kanazawa, K. (2008). Growth inhibition of human hepatic carcinoma HepG2 cells by fucoxanthin is associated with down-regulation of cyclin D. Biochimica et biophysica acta, 1780(4), 743–749. https://doi.org/10.1016/j.bbagen.2008.01.003

Das, S. K., Ren, R., Hashimoto, T., Kanazawa, K. (2010). Fucoxanthin induces apoptosis in osteoclast-like cells differentiated from RAW264.7 cells. Journal of agricultural and food chemistry, 58(10), 6090–6095. <https://doi.org/10.1021/jf100303k>

El Shafay, S. M., Ali, S. S., El-Sheekh, M. M. (2016). Antimicrobial activity of some seaweeds species from Red sea, against multidrug resistant bacteria. The Egyptian Journal of Aquatic Research, 42(1), 65-74. <https://doi.org/10.1016/j.ejar.2015.11.006>

Ganesan, P., Matsubara, K., Sugawara, T., Hirata, T. (2013). Marine algal carotenoids inhibit angiogenesis by down-regulating FGF-2-mediated intracellular signals in vascular endothelial cells. Molecular and cellular biochemistry, 380(1-2), 1–9. https://doi.org/10.1007/s11010-013-1651-5

Gille, A., Stojnic, B., Derwenskus, F., Trautmann, A., Schmid-Staiger, U., Posten, C., et al. (2019). A Lipophilic Fucoxanthin-Rich Phaeodactylum tricornutum Extract Ameliorates Effects of Diet-Induced Obesity in C57BL/6J Mice. Nutrients, 11(4), 796. https://doi.org/10.3390/nu11040796

Ha, A. W., Na, S. J., Kim, W. K. (2013). Antioxidant effects of fucoxanthin rich powder in rats fed with high fat diet. Nutrition research and practice, 7(6), 475–480. https://doi.org/10.4162/nrp.2013.7.6.475

Ha, Y. J., Choi, Y. S., Oh, Y. R., Kang, E. H., Khang, G., Park, Y. B., et al. (2021). Fucoxanthin Suppresses Osteoclastogenesis via Modulation of MAP Kinase and Nrf2 Signaling. Marine drugs, 19(3), 132. https://doi.org/10.3390/md19030132

Heo, S. J., Jeon, Y. J. (2009). Protective effect of fucoxanthin isolated from Sargassum siliquastrum on UV-B induced cell damage. Journal of photochemistry and photobiology. B, Biology, 95(2), 101–107. https://doi.org/10.1016/j.jphotobiol.2008.11.011

Heo, S. J., Ko, S. C., Kang, S. M., Kang, H. S., Kim, J. P., Kim, S. H., et al. (2008). Cytoprotective effect of fucoxanthin isolated from brown algae Sargassum siliquastrum against H 2 O 2-induced cell damage. European food research and technology, 228, 145-151. https://doi.org/10.1007/s00217-008-0918-7

Heo, S. J., Yoon, W. J., Kim, K. N., Ahn, G. N., Kang, S. M., Kang, D. H., et al. (2010). Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. Food and chemical toxicology, 48(8-9), 2045-2051. https://doi.org/10.1016/j.fct.2010.05.003

Hitoe, S., Shimoda, H. (2017). Seaweed fucoxanthin supplementation improves obesity parameters in mild obese Japanese subjects. Functional Foods in Health and Disease, 7(4), 246-262. doi: [10.31989/ffhd.v7i4.333](https://doi.org/10.31989/ffhd.v7i4.333)

Hosokawa, M., Miyashita, T., Nishikawa, S., Emi, S., Tsukui, T., Beppu, F., et al. (2010). Fucoxanthin regulates adipocytokine mRNA expression in white adipose tissue of diabetic/obese KK-Ay mice. Archives of biochemistry and biophysics, 504(1), 17–25. <https://doi.org/10.1016/j.abb.2010.05.031>

Hou, L. L., Gao, C., Chen, L., Hu, G. Q., Xie, S. Q. (2013). Essential role of autophagy in fucoxanthin-induced cytotoxicity to human epithelial cervical cancer HeLa cells. Acta pharmacologica Sinica, 34(11), 1403–1410. https://doi.org/10.1038/aps.2013.90

Hu, X., Li, Y., Li, C., Fu, Y., Cai, F., Chen, Q., et al. (2012). Combination of fucoxanthin and conjugated linoleic acid attenuates body weight gain and improves lipid metabolism in high-fat diet-induced obese rats. Archives of biochemistry and biophysics, 519(1), 59-65. <https://doi.org/10.1016/j.abb.2012.01.011>

Jin, Y., Qiu, S., Shao, N., Zheng, J. (2018). Fucoxanthin and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Synergistically Promotes Apoptosis of Human Cervical Cancer Cells by Targeting PI3K/Akt/NF-κB Signaling Pathway. Medical science monitor : international medical journal of experimental and clinical research, 24, 11–18. https://doi.org/10.12659/msm.905360

Kang, S. I., Ko, H. C., Shin, H. S., Kim, H. M., Hong, Y. S., Lee, N. H., et al. (2011). Fucoxanthin exerts differing effects on 3T3-L1 cells according to differentiation stage and inhibits glucose uptake in mature adipocytes. Biochemical and biophysical research communications, 409(4), 769–774. https://doi.org/10.1016/j.bbrc.2011.05.086

Karpiński, T. M., Adamczak, A. (2019). Fucoxanthin-An Antibacterial Carotenoid. Antioxidants (Basel, Switzerland), 8(8), 239. https://doi.org/10.3390/antiox8080239

Kim, K. N., Heo, S. J., Kang, S. M., Ahn, G., Jeon, Y. J. (2010). Fucoxanthin induces apoptosis in human leukemia HL-60 cells through a ROS-mediated Bcl-xL pathway. Toxicology in vitro : an international journal published in association with BIBRA, 24(6), 1648–1654. https://doi.org/10.1016/j.tiv.2010.05.023

Kim, K. N., Heo, S. J., Yoon, W. J., Kang, S. M., Ahn, G., Yi, T. H., et al. (2010). Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-κB and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. European journal of pharmacology, 649(1-3), 369-375. https://doi.org/10.1016/j.ejphar.2010.09.032

Kong, Z.-L., Sudirman, S., Hsu, Y.-C., Su, C.-Y., Kuo, H.-P. (2019). Fucoxanthin-Rich Brown Algae Extract Improves Male Reproductive Function on Streptozotocin-Nicotinamide-Induced Diabetic Rat Model. International Journal of Molecular Sciences, 20(18), 4485. <https://doi.org/10.3390/ijms20184485>

Koo, S. Y., Hwang, J.-H., Yang, S.-H., Um, J.-I., Hong, K. W., Kang, K. (2019). Anti-Obesity Effect of Standardized Extract of Microalga Phaeodactylum tricornutum Containing Fucoxanthin. Marine Drugs, 17(5), 311. https://doi.org/10.3390/md17050311

Li, S., Ren, X., Wang, Y., Hu, J., Wu, H., Song, S., et al. (2020). Fucoxanthin alleviates palmitate-induced inflammation in RAW 264.7 cells through improving lipid metabolism and attenuating mitochondrial dysfunction. Food & function, 11(4), 3361-3370.

Li, T. L., King, J. M., Min, D. B. (2000). Quenching mechanisms and kinetics of carotenoids in riboflavin photosensitized singlet oxygen oxidation of vitamin D2. Journal of food biochemistry, 24(6), 477-492. https://doi.org/10.1111/j.1745-4514.2000.tb00717.x

Lin, J., Yu, J., Zhao, J., Zhang, K., Zheng, J., Wang, J., et al. (2017). Fucoxanthin, a marine carotenoid, attenuates β-amyloid oligomer-induced neurotoxicity possibly via regulating the PI3K/Akt and the ERK pathways in SH-SY5Y cells. Oxidative Medicine and Cellular Longevity, 2017. https://doi.org/10.1155/2017/6792543

Liu, C. L., Chiu, Y. T., Hu, M. L. (2011). Fucoxanthin enhances HO-1 and NQO1 expression in murine hepatic BNL CL.2 cells through activation of the Nrf2/ARE system partially by its pro-oxidant activity. Journal of agricultural and food chemistry, 59(20), 11344–11351. https://doi.org/10.1021/jf2029785

Liu, C. L., Lim, Y. P., Hu, M. L. (2013). Fucoxanthin enhances cisplatin-induced cytotoxicity via NFκB-mediated pathway and downregulates DNA repair gene expression in human hepatoma HepG2 cells. Marine drugs, 11(1), 50–66. https://doi.org/10.3390/md11010050

Liu, Z., Sun, X., Sun, X., Wang, S., Xu, Y. (2019). Fucoxanthin Isolated from Undaria pinnatifida Can Interact with Escherichia coli and lactobacilli in the Intestine and Inhibit the Growth of Pathogenic Bacteria. Journal of Ocean University of China, 18, 926-932. https://doi.org/10.1007/s11802-019-4019-y

Long, Y., Cao, X., Zhao, R., Gong, S., Jin, L., Feng, C. (2020). Fucoxanthin treatment inhibits nasopharyngeal carcinoma cell proliferation through induction of autophagy mechanism. Environmental toxicology, 35(10), 1082–1090. https://doi.org/10.1002/tox.22944

Maeda, H., Hosokawa, M., Sashima, T., Miyashita, K. (2007). Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. J. of Agricultural and Food Chemistry, 55(19), 7701-7706. <https://doi.org/10.1021/jf071569n>

Maeda, H., Hosokawa, M., Sashima, T., Murakami-Funayama, K., Miyashita, K. (2009). Anti-obesity and anti-diabetic effects of fucoxanthin on diet-induced obesity conditions in a murine model. Molecular medicine reports, 2(6), 897–902. https://doi.org/10.3892/mmr\_00000189

Moreau, D., Tomasoni, C., Jacquot, C., Kaas, R., Le Guedes, R., Cadoret, J. P., et al. (2006). Cultivated microalgae and the carotenoid fucoxanthin from Odontella aurita as potent anti-proliferative agents in bronchopulmonary and epithelial cell lines. Environmental toxicology and pharmacology, 22(1), 97–103. https://doi.org/10.1016/j.etap.2006.01.004

Namkoong, S., Joo, H. M., Jang, S. A., Kim, Y. J., Kim, T. S., Sohn, E. H. (2012). Suppressive Effects of Fucoxanthin on Degranulation in IgE-antigen complex-stimulated RBL-2H3 Cells. Korean Journal of Plant Resources, 25(3), 339-345.

Nishikawa, S., Hosokawa, M., Miyashita, K. (2012). Fucoxanthin promotes translocation and induction of glucose transporter 4 in skeletal muscles of diabetic/obese KK-A(y) mice. Phytomedicine : international journal of phytotherapy and phytopharmacology, 19(5), 389–394. https://doi.org/10.1016/j.phymed.2011.11.001

Peraman, M., Nachimuthu, S. (2019). Identification and quantification of fucoxanthin in selected carotenoid-producing marine microalgae and evaluation for their chemotherapeutic potential. Pharmacognosy Magazine, 15(Suppl 2), S243-S249.

Qin, Y., Meng, L., Wang, F. (2013). Complex enzymatic extraction of kelp fucoxanthin and its antioxidant activity analysis. Food Science (16), 279-283. https://kns.cnki.net/kcms/detail/11.2206.TS.20130107.1632.027.html

Ravi Kumar, S., Narayan, B., Vallikannan, B. (2008). Fucoxanthin restrains oxidative stress induced by retinol deficiency through modulation of Na (+) K (+) - ATPase [corrected] and antioxidant enzyme activities in rats. European journal of nutrition, 47(8), 432–441. https://doi.org/10.1007/s00394-008-0745-4

Sakai, S., Sugawara, T., Hirata, T. (2011). Inhibitory effect of dietary carotenoids on dinitrofluorobenzene-induced contact hypersensitivity in mice. Bioscience, biotechnology, and biochemistry, 75(5), 1013-1015. https://doi.org/10.1271/bbb.110104

Shih, P.-H., Shiue, S.-J., Chen, C.-N., Cheng, S.-W., Lin, H.-Y., Wu, L.-W., et al. (2021). Fucoidan and Fucoxanthin Attenuate Hepatic Steatosis and Inflammation of NAFLD through Modulation of Leptin/Adiponectin Axis. Marine Drugs, 19(3), 148. <https://doi.org/10.3390/md19030148>

Shiratori, K., Ohgami, K., Ilieva, I., Jin, X. H., Koyama, Y., Miyashita, K., et al. (2005). Effects of fucoxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. Experimental eye research, 81(4), 422-428. https://doi.org/10.1016/j.exer.2005.03.002

Spagolla Napoleão Tavares, R., Stuchi Maria-Engler, S., Colepicolo, P., Debonsi, H. M., Schäfer-Korting, M., Marx, U., et al. (2020). Skin Irritation Testing beyond Tissue Viability: Fucoxanthin Effects on Inflammation, Homeostasis, and Metabolism. Pharmaceutics, 12(2), 136. https://doi.org/10.3390/pharmaceutics12020136

Sugawara, T., Matsubara, K., Akagi, R., Mori, M., Hirata, T. (2006). Antiangiogenic activity of brown algae fucoxanthin and its deacetylated product, fucoxanthinol. Journal of agricultural and food chemistry, 54(26), 9805–9810. https://doi.org/10.1021/jf062204q

Takatani, N., Kono, Y., Beppu, F., Okamatsu-Ogura, Y., Yamano, Y., Miyashita, K., et al. (2020). Fucoxanthin inhibits hepatic oxidative stress, inflammation, and fibrosis in diet-induced nonalcoholic steatohepatitis model mice. *Biochemical and Biophysical Research Communications*, *528*(2), 305-310. <https://doi.org/10.1016/j.bbrc.2020.05.050>

Tavares, R. S. N., Kawakami, C. M., Pereira, K. C., do Amaral, G. T., Benevenuto, C. G., Maria-Engler, S. S., et al. (2020). Fucoxanthin for Topical Administration, a Phototoxic vs. Photoprotective Potential in a Tiered Strategy Assessed by In Vitro Methods. Antioxidants (Basel, Switzerland), 9(4), 328. https://doi.org/10.3390/antiox9040328

Tsushima, M., Maoka, T., Katsuyama, M., Kozuka, M., Matsuno, T., Tokuda, H., et al. (1995). Inhibitory effect of natural carotenoids on Epstein-Barr virus activation activity of a tumor promoter in Raji cells. A screening study for anti-tumor promoters. Biological and Pharmaceutical Bulletin, 18(2), 227-233. https://doi.org/10.1248/bpb.18.227

Wang, J., Ma, Y., Yang, J., Jin, L., Gao, Z., Xue, L., et al. (2019). Fucoxanthin inhibits tumour-related lymphangiogenesis and growth of breast cancer. Journal of cellular and molecular medicine, 23(3), 2219–2229. https://doi.org/10.1111/jcmm.14151

Wang, S., Verma, S. K., Hakeem Said, I., Thomsen, L., Ullrich, M. S., Kuhnert, N. (2018). Changes in the fucoxanthin production and protein profiles in Cylindrotheca closterium in response to blue light-emitting diode light. Microbial cell factories, 17, 1-13. https://doi.org/10.1186/s12934-018-0957-0

Wu, C., Ren, D., Chen, Q. (2011). Effects of kelp fucoxanthin on the inhibition of lipid peroxidation in mice. Journal of Dalian Ocean University (05) ,428-431. doi :10.16535/j.cnki.dlhyxb.2011.05.006.

Xiang, S., Liu, F., Lin, J., Chen, H., Huang, C., Chen, L., et al. (2017). Fucoxanthin inhibits β-amyloid assembly and attenuates β-amyloid oligomer-induced cognitive impairments. Journal of agricultural and food chemistry, 65(20), 4092-4102. <https://doi.org/10.1021/acs.jafc.7b00805>

Yang, M., Jin, L., Wu, Z., Xie, Y., Zhang, P., Wang, Q., et al. (2021). PLGA-PEG nanoparticles facilitate in vivo anti-Alzheimer’s effects of fucoxanthin, a marine carotenoid derived from edible brown algae. Journal of Agricultural and Food Chemistry, 69(34), 9764-9777. https://doi.org/10.1021/acs.jafc.1c00569

Yang, Y. P., Tong, Q. Y., Zheng, S. H., Zhou, M. D., Zeng, Y. M., Zhou, T. T. (2020). Anti-inflammatory effect of fucoxanthin on dextran sulfate sodium-induced colitis in mice. Natural product research, 34(12), 1791-1795. https://doi.org/10.1080/14786419.2018.1528593.

Ye, J., Zheng, J., Tian, X., Xu, B., Yuan, F., Wang, B., et al. (2022). Fucoxanthin Attenuates Free Fatty Acid-Induced Nonalcoholic Fatty Liver Disease by Regulating Lipid Metabolism/Oxidative Stress/Inflammation via the AMPK/Nrf2/TLR4 Signaling Pathway. *Marine Drugs*, *20*(4), 225. https://doi.org/10.3390/md20040225

Yu, R. X., Yu, R. T., & Liu, Z. (2018). Inhibition of two gastric cancer cell lines induced by fucoxanthin involves downregulation of Mcl-1 and STAT3. Human cell, 31(1), 50–63. https://doi.org/10.1007/s13577-017-0188-4

Zhu, M., Li, Y., Zhang, M., Li, X., Cheng, M., Zhou, Z., et al. (2021). Anti-senescence effect of fucoxanthin on D-gal-induced SH-SY5Y cells and its mechanism. Guangxi Science(03), 310-320.doi:10.13656/j.cnki.gxkx.20210608.001