## 2.7.3 Laboratory : Laboratory of Organic Chemistry in Life Science

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| Member : | Professor | Irie, Kazuhiro, D. Agric. Sci. (comparative agricultural science; Hirai, Nobuhiro, D. Agr. Sci. (collaborative professor) ) |
| | Assistant Professor | Murakami, Akira, D. Agric. Sci. |
| | Assistant Professor | Murakami, Kazuma, D. Agric. Sci. |
| | Doctor's program | 3 |
| | Master's Program | 9 |
| | Undergraduate | 4 |
| | Post-Doctoral fellow | 2 |

**A. Research Activities (2009.4-2010.3)**

**A-1. Main Subjects**

a) Challenges to the development of bryostatin-type anticancer drugs

Protein kinase C (PKC) isozymes are widely recognized as targets for anticancer therapy. However, concerns exist about their therapeutic uses since most PKC activators are potent tumor-promoters. Bryostatin 1 (bryo-1) is a unique PKC activator with little tumor-promoting activities. Bryo-1 is currently undergoing clinical trials for the treatment of cancer. However, its limited availability from natural sources and difficulty in the synthesis hamper further studies on its mode of action and structural optimization. The authors focused on the bryo-1's unique mechanism of activating PKCδ that plays a tumor suppressor role, and found that a simple and less lipophilic analogue (aplog-1) of the tumor-promoting aplysiatoxin showed PKCδ-activating behavior similar to bryo-1. Aplog-1 was easily synthesized in only 22 steps using standard reactions. Moreover, its tumor-promoting activity in vitro was very weak, and its cell growth inhibitory activities were comparable to those of bryo-1. These data suggest that aplog-1 could become another therapeutic lead for cancer.

b) Chemistry of amyloid β peptides

Aggregation of the 42-mer amyloid β protein (Aβ42) plays a critical role in the pathogenesis of Alzheimer's disease (AD). Although clinical trials of immunization targeting Aβ42

aggregates have been proved useful, some adverse effects were unavoidable because of excessive immunoreactions derived from the unintended removal of non-toxic Aβ42. A toxic conformer with a turn at positions 22 and 23 of Aβ42 in aggregates has recently been identified by us. To develop a monoclonal antibody for toxic Aβ42, E22P-Aβ10-35 was used for the generation of antibodies, following the selection of clones using Aβ42 mutants of E22P (turn-inducing) and E22V (turn-preventing). The obtained clone (11A1) inhibited the neurotoxicity of Aβ42 and E22P-Aβ42 in PC12 cells. Immunohistochemical studies showed that not only extracellular but also intracellular amyloid was stained in AD brains. In Western blot using human brains, the trimer rather than the monomer of Aβ was significantly recognized by 11A1. These results imply that the 11A1 antibody could detect toxic Aβ42 oligomers with the turn at positions 22 and 23.

c) Physiological functions of food factors and their undelying molecular mechanisms

(+/-)-13-Hydroxy-10-oxo-trans-11-octadecenoic acid (HOA) is one of the lipoxygenase metabolites of linoleic acid (LA) from corn germ. We investigated the inhibitory effects of HOA on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in ears and skin, as well as tumor promotion in female ICR mice. Pretreatment with HOA (1600 nmol) inhibited ear edema formation by 95% in an inflammation test and reduced tumor incidence and the number of tumors per mouse by 40 and 64%, respectively, in a two-stage skin carcinogenesis model. HOA (40 mM) inhibited TPA-induced activator protein-1 transactivation but not extracellular signal-regulated kinase1/2 activation. Interestingly, HOA (40 mM and 1600 nmol in JB6 cells and mouse skin, respectively) induced expression of programmed cell death 4 (Pdcd4), a novel tumor suppressor protein. Collectively, our results indicate that HOA may be a novel anti-inflammatory and antitumor chemopreventive agent with a unique mode of action.

d) Molecular technology of a plant hormone, abscisic acid

Abscisic acid (ABA) is an important plant hormone that induces adaptative responses in plants upon water stress and low temperature. However, the effect of ABA does not last since ABA is quickly inactivated by the degradation enzyme, ABA 8'-hydroxylase. Inhibitors of the hydroxylase may delay the inactivation of ABA to increase resistance of plants to environmental stress. We have developed hydroxylase inhibitors with the researchers at Shizuoka University and Chiba University. Phaseic acid is a metabolite of ABA having a moderate activity, and its reductase completely inactivates phaseic acid by conversion to dihydrophaseic acid. Inhibitors of the reductase may also keep moderate activitiy of phaseic acid. We have started purification of the phaseic acid reductase for identification of its gene.

e) Chemical ecology of ectomycorrhiza

Some ectomycorrhiza form a fairy ring which shows the circular formation of fruit body. The inside of the fairy ring of Tricholoma is a whitish mycelium-soil aggregated zone, and called “shiro”. The density of bacteria and fungi in the shiro is significantly lower than that of the outside of the shiro. The researchers of our University demonstrated in 1967 that the shiro had antimicrobial activity against bacteria. However, the antimicrobial compound(s) has been remained unclear. We have isolated several antimicrobial compounds from the extract roots of Pinus densiflora growing at the Kamigamo research field of Kyoto University, and identified as diterpenes including totarol. We have found another antimicrobial compound from P. densiflora roots growing at the Sakai research field of Kyoto Prefecture. This compound is not diterpene, and water-soluble. Purification of this compoud is under progress.

**A-2.Publications and presentations**

a) Publications

Books

- Murakami, A. and Ohigashi, H.: Anti-inflammatory and Anti-carcinogenesis Potentials of Citrus Coumarins and Polymethoxyflavonoids. In Nutrigenomics and Proteomics in Health and Disease, Mine, Y., Miyashita, K. and Shahidi, F. (Eds.), pp. 311-324, Wiley-Blackwell, New York, 2009

Original Papers

- Nakagawa, Y., Yanagita, R. C., Hamada, N., Takahashi, H., Saito, N., Nagai, H. and Irie, K.: The simple analogue of tumor-promoting aplysiatoxin is an antineoplastic agent rather than a tumor promoter: Development of a bryostatin-like PKCδ activator having the aplysiatoxin skeleton. J. Am. Chem. Soc. 131; 7573–7579, 2009
- Huynh, M. U., Elston, M. C., Hernandez, N. M., Ball, D. B., Kajiyama, S.-i., Irie, K., Gerwick, W. H. and Edwards, D. J.: Enzymatic production of (-)-indolactamV by LtxB, a cytochrome P450 monooxygenase. J. Nat. Prod. 73; 71–74, 2010
- Kondo, T., Kajita, R., Miyazaki, A., Hokoyama, M., Nakamura-Miura, T., Mizuno, S., Masuda, Y., Irie, K., Tanaka, Y., Takada, S., Kakimoto, T. and Sakagami, Y.: Stomatal density is controlled by a mesophyll-derived signaling molecule. Plant Cell Physiol. 51; 1–8, 2010
- Yasuda, M., Nishizawa, T., Ohigashi, H., Tanaka, T., Hou, D. X., Colburn, N. H. and Murakami, A.: Linoleic acid metabolite suppresses skin inflammation and tumor promotion in mice: Possible roles of programmed cell death 4 induction. Carcinogenesis 30; 1209-1216, 2009

- Ohnishi, K., Irie, K. and Murakami, A.: In vitro covalent binding proteins of zerumbone, a chemopreventive food factor. Biosci. Biotechnol. Biochem. 73; 1905-1907, 2009

- Kim, M., Miyamoto, S., Ohigashi, H., Tanaka, T. and Murakami A.: The modifying effects of green tea polyphenols on acute colitis and inflammation-associated colon carcinogenesis in male ICR mice. Biofactors. 36; 43-5, 2010

- Miyamoto, S., Yasui, Y., Ohigashi, H., Tanaka, T. and Murakami, A.: Dietary flavonoids suppress azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJ-db/db mice. Chem. Biol. Int., 2010, 183; 276-283, 2010

- Miyamoto, S., Tanaka, T. and Murakami, A.: Increased visceral fat mass and insulin signaling in colitis-related colon carcinogenesis model mice. Chem. Biol. Int. 183; 271-275, 2010

- Rahimi, R., Murakami, K., Summers, J. L., Chen, C-H. B. and Bitan, G.: RNA aptamrs generated against oligomeric Aβ40 recognize common amyloid aptatopes with low specificity but high sensitivity. PLoS ONE 4: e7694, 2009

- Kajikawa, M., Hirai, H. and Hashimoto, T: A PIP-family reductase is required for biosynthesis of tobacco alkaloids. Plant Mol. Biol. 69; 287-298, 2009

- Todoroki, Y., Kobayashi, K., Shirakura, M., Aoyama, H., Takatori, K., Nimitkeatkai, H., Jin, M.-H., Hiramatsu, S., Ueno, H., Kodo, S., Mizutani M. and Hirai, N.: Abscinazole-F-1, a conformationally restricted analogue for the plant growth retardant uniconazole and an inhibitor of ABA 8'-hydroxylase CYP707A with no growth-retardant effect. Bioorg. Med. Chem. 17; 6620-6630, 2009

- Todoroki, Y., Aoyama, H., Hiramatsu, S., Shirakuram, M., Nimitkeatkai, H., Kondo, S., Ueno, K., Mizutani,M. and Hirai, N.: Enlarged analogues of uniconazole, new azole containing inhibitors of ABA 8'-hydroxylase CYP707A. Bioorg. Med. Chem. Lett. 19; 5782-5786, 2009

Reviews

- Masuda, Y. and Irie, K.: Polyphenols as potential preventive agents for Alzheimer's disease. Foods & Food Ingredients Journal of Japan 215; 53-59, 2009

- Murakami, A.: Chemoprevention with phytochemicals targeting inducible nitric oxide synthase. Forum Nutr. 61; 193-203, 2009

Reports

- Monbu-Kagakusho Research Grant. Scientific Research (A): Development of agents against Alzheimer's disease based on the structural analysis of amyloid beta oligomers (Irie, K., Head)

- Monbu-Kagakusho Research Grant. Exploratory Research: Analysis of the reaction mechanism of the enzyme that catalyzes the introduction of a nitrogen atom on the 4 position of indole (Irie, K., Head)
- Monbu-Kagakusho Research Grant. Scientific Research (C): Role of adipocytokines in colon carcinogenesis and identification of preventive food factors (Murakami, A., Head)
- Grant-in-Aid from the Ministry of Health, Labor, and Welfare Molecular mechanisms underlying inflammation-associated carcinogenesis and its prevention (Murakami, A., Head)
- Monbu-Kagakusho Research Grant. Promotion of Science for Young Scientists: Development of therapeutic agents for Alzheimer's disease based on the toxic conformation of Aβ42 and their estimation using novel disease-model mice (Murakami, K., Head)
- Monbu-Kagakusho Research Grant. Scientific Research (B): Development of drugs protecting plants from environmental stress in semi-arid land (Hirai, N., Head)

Patents

- No. 2009-239542 "The antibody that recognizes the turn structure of amyloid b", Irie, K., Murakami, K., Masuda, Y., Shimizu, T., Shirasawa, T., and Kiyofuji, T., 10/16/09, Kyoto University, Tokyo Metropolitan Institute of Gerontology, and Immuno-Biological Laboratories Co., Ltd.

b) Conference and seminar papers presented

- The 2010 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Tokyo): 11 Presentations
- The 32nd Annual Meeting of the Molecular Biology Society of Japan (Yokohama): 1 Presentation
- The 11th International Kyoto Conference on New Aspects of Organic Chemistry (Kyoto): 1 Presentation
- 39th Annual Meeting of Society for Neuroscience (Chicago, USA): 2 Presentation
- The 25th Naito Conference, Chemical Biology [II] -An Emerging Field Inspired by Natural Product Chemistry-, (Sapporo): 1 Invited presentation
- The 57th Symposium of The Kanto Branch of The Society of Synthetic Organic Chemistry, Japan: 1 Presentation
- The 44th Summer School on Natural Products Chemistry (Osaka): 1 Presentation
- The 16th Conference of Japanese Society for Cancer Prevention (Fukuoka): 1 invited

presentation

- The 13th Annual Meeting of Japanese Society for Food Factors (Tokyo): 1 Presentation
- The 21th Annual Meeting of Spice Research (Osaka) : 1 Presentation
- The 38th Annual Meeting of International Society of Environmental Mutagens (Shizuoka): 1 invited presentation
- The 4th Annual Meeting of The Chemical Biology (Kobe): 1 Presentation
- The 44th Annual Meeting of The Japanse Society for Chemical Regulation of Plants (Sendai): 3 Presentations

**A-3.Off-campus activities**

Membership in academic societies

- Irie, Kazuhiro, D. Agric. Sci. : Japan Society for Bioscience, Biotechnology, and Agrochemistry in Kansai Branch (councilor), Japan Society for Dementia Research (councilar), Organizing committee of Symposium on The Chemistry of Natural Products (member), Organizing committee of Summer School on Natural Products Chemistry (adviser), The Japanese Association for the Pursuit of New Bioactive Resources (councilor)
- Murakami, Akira, D. Agric. Sci. : Japanese Society for Oxidative Stress Research (councilor), Japanese Society for Food Factors (councilor), Japanese Association for Cancer Prevention (councilor)
- Murakami, Kazuma, D. Agric. Sci. : Organizing committee of Summer School on Natural Products Chemistry (member)

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- Scientific Research (A) : Irie, Kazuhiro, D. Agric.Sci : Development of agents against Alzheimer's disease based on the structural analysis of amyloid beta oligomers
- Challenging Exploratory Research : Irie, Kazuhiro, D. Agric.Sci : Analysis of the reaction mechanism of the enzyme that catalyzes the introduction of a nitrogen atom on the 4 position of indole
- Scientific Research (C) : Murakami, Akira, D. Agric.Sci : Role of adipocytokines in colon carcinogenesis and identification of preventive food factors
- Grant-in Aid for JSPS Fellows : Murakami, Kazuma, D. Agric.Sci : Development of therapeutic agents for Alzheimer’s disease based on the toxic conformation of Aβ42 and their estimation using novel disease-model mice
- Scientific Research (B) : Kondo, Satoru (Hirai, Nobuhiro, D. Agric. Sci., cooperator) :

Development of a method increasing water-stress resistance in fruit trees by regulation of abscisic acid hydroxylase

2.Other Research Grants

- Grant-in-Aid from the Ministry of Health, Labor, and Welfare : Murakami, Akira, D. Agric.Sci : Molecular mechanisms underlying inflammation-associated carcinogenesis and its prevention

**A-4.International cooperation and overseas activities**

Membership in academic societies

- Irie, Kazuhiro, D. Agric.Sci: American Chemical Society (member) , The Society for Neuroscience (member)
- Murakami, Akira, D. Agric. Sci.: American Association for Cancer Research (member)
- Murakami, Kazuma, D. Agric. Sci.: The Society for Neuroscience (member), American Chemical Society (member)

International meetings(country,roles)

- Irie, Kazuhiro, D. Agric. Sci : The 11th International Kyoto Conference on New Aspects of Organic Chemistry (Japan, 1 presentation), 39th Annual Meeting of Society for Neuroscience (USA, 1 presentation), The 25th Naito Conference, Chemical Biology [II] -An Emerging Field Inspired by Natural Product Chemistry-, (Japan, 1 invited presentation)
- Murakami, Akira, D. Agric. Sci : The 2nd Joint Symposium Between Japan and Italy on Natural Products and Functional Foods (Japan, 1 invited presentation), The 3rd International Meeting for Translational Cancer Research (India, 1 invited presentation)
- Murakami, Kazuma, D. Agric. Sci. : 39th Annual Meeting of Society for Neuroscience (USA, 2 presentation)

**B.Educational Activities(2009.4-2010.3)**

**B-1.On-campus teaching**

a) Courses given

- Undergraduate level : Organic Chemistry in Food Science I (Irie, K.), Organic Chemistry in Food Science II (Irie, K.), Organic Chemistry in Food Science III (Irie, K.), Organic Chemistry in Life Science (Irie, K.), Laboratory Course in Organic Chemistry (Irie, K., Murakami, A. and Hirai, N.), Introduction and Practice in the Department of Food Science and

Biotechnology (Irie, K., Murakami, A. and Hirai, N., a partial charge)

- Graduate level : Seminar of Organic Chemistry in Life Science (Irie, K., Murakami, A., Murakami, K. and Hirai, N.), Experimental Course of Organic Chemistry in Life Science (Irie, K., Murakami, A., Murakami, K. and Hirai, N.)

**B-2.Off-campus teaching etc.**

Part-time lecturer

- Irie, Kazuhiro, D. Agric.Sci: Department of Applied Molecular Biosciences, Graduate School of Bioagricultural Sciences, Nagoya University, Closely-packed series of lecture "Organic Chemistry in Life Science"

**B-3.Overseas teaching**

International students

- International students : Master 1 (Thailand)

**C.Other Remarks**

- Irie, Kazuhiro, D. Agric.Sci : Committee of Collaboration among Medicine, Technology, and Life Science in Kyoto City