機能情報解析分野　　Division of Biomedical informatics

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◇研究目的

未病とは古代中国の医学書「黄帝内径」に由来する言葉で，健康ではないが，といってはっきりした病気にかかっているわけではない半健康状態を示す。未病には肥満，軽症高血圧，軽症糖尿病などの生活習慣病から，老化による神経・免疫・臓器機能の低下，ストレスによる病的変化，環境要因が引き起こすシックハウス症候群や不妊症等が含まれる。
本部門は，現代科学的手法を駆使し，漢方薬をはじめ，伝統薬物が治療対象とする未病状態における生体機能および生体反応調節物質として伝統医薬品の薬物機能（薬能・薬効）を明らかにするとともに，それらの情報を統合医学的に解析することにより，未病を治療する薬理学的方法論，すなわち未病対策としての未病薬学を確立することをめざしている。

◇研究概要

Ⅰ）　気道液分泌における水チャネル（アクアポリン）の機能調節機構と伝統薬物作用に関する研究

近年，細胞膜の水透過性を調節するアクアポリン(aquaporin, AQP)と呼ばれる水チャンネルが見いだされ 13 種類のアイソフォームが同定されている。我々は麦門冬湯など数種の漢方薬および生薬成分が，気道上皮に発現する AQP5 の発現の誘導や機能の調節により特有の滋潤効果や分泌制御効果を表すことを見いだし，その機序や臨床効果との関連を追求している。

Ⅱ）　ウイルス性疾患に対する感染防御薬の基礎および開発研究

ヘルペスウイルスやロタウイルスなどのウイルス感染に有効な薬を開発するために，プラーク減少法により，生薬成分，微生物代謝産物等の抗ウイルス活性を調べている．今までに，豆科植物の新規トリテルペンサポニン，微生物由来の新規糖脂肪酸や合成ビスフェノール誘導化合物の抗ウイルス活性を明らかにしてきた。漢方薬についても新しい視点からの検討を加えている。

Ⅲ）　細胞賦活化薬食物のトランスレーショナルリサーチ

養命，抗老化につながる生体反応調節薬を開発するために，in vitro と in vivo において，それらの細胞増殖活性，細胞保護活性，抗癌活性，抗酸化活性，抗微生物活性および抗疲労効果を分子薬理学的手法を用いて総合的に解析している。特に熱ショック蛋白 (heat shock protein, HSP) を誘導することにより種々の生体防御効果を示す生薬成分や健康食品に注目している。

◇原著論文

1)　Mibu N., Yokomizo K., Saisho M., Oishi M., Aki H., Miyata T., and Sumoto K.: Synthesis and antiviral activities of some N-acyl-2,6-diaminopyridines and related linker mode identical twin drugs. Heterocycles 83(2), 385-393; 2011.

Abstract: In connection with our studies on biologically active compounds in the class of N-acyl-2,6-diaminopyridines, some molecular modifications were attempted. All of the synthesized compounds were evaluated for biological activity with herpes virus type 1 (HSV-1) by a plaque reduction assay. We observed that most of the synthesized derivatives showed no significant anti-HSV-1 activity, but

we found that compound 5 and 6 with a branched long alkyl chain showed high cytotoxicity to Vero cells.

2) **Miyata T.: Novel approach to curatives of Mibyou (presymptomatic diseases). Yakugaku Zasshi 131(9), 1289-1298; 2011.**

Abstract The traditional Oriental medicine and health supplement have been empirically practiced but most of them have not come through objective examination to prove their efficacy. From pharmacological aspect, we have been investigating the medical benefits of traditional Oriental medicines and health supplements as curatives and their varied actions and mechanisms. The study on airway inflammation has shown that even a Kampo preparation, Bakumondo-to, has anti-inflammatory, anti-allergic, immunomodulatory, secretory-modulating and metabolic regulatory actions. The base of all its actions is founded on the restoration of normal molecular and cellular functions through DNA transcriptional regulation. In other previous studies, we showed that a health supplement, royal jelly (RJ) has weak estrogenic activity. RJ competes with 17β-estradiol for binding to the human estrogen receptors α and β, though it is much weaker than diethylstilbestrol in binding affinity. Treatment of MCF-7 cells with RJ enhances proliferation, and concomitant treatment with tamoxifen blocked this effect. A reporter gene assay showed that RJ enhanced transcription of the luciferase gene through the estrogen-responsive element in MCF-7 cells. Furthermore, subcutaneous injection of RJ restored the expression of vascular endothelial growth factor gene in the uteri of ovariectomized rats. We suggest that the diverse pharmacological functions of RJ can be ascribed, in part, to its estrogenic effects. We hypothesize that polyherbal medicines and health supplements, which have multiple actions, may be better than Western medicine of single component to treat various diseases including 'Mibyou'

3) **Hisatsune A., Nakayama H., Kawasaki M., Horie I., Miyata T., Isohama Y., Kim K.C., Katsuki H.: Anti-MUC1 antibody inhibits EGF receptor signaling in cancer cells. Biochem Biophys Res Commun 405(3), 377-381; 2011.**

Abstract: MUC1 is a type I transmembrane glycoprotein aberrantly overexpressed in various cancer cells. High expression of MUC1 is closely associated with cancer progression and metastasis, leading to poor prognosis. We previously reported that MUC1 is internalized by the binding of the anti-MUC1 antibody, from the cell surface to the intracellular region via the macropinocytotic pathway. Since MUC1 is closely associated with ErbBs, such as EGF receptor (EGFR) in cancer cells, we examined the effect of the anti-MUC1 antibody on EGFR trafficking. Our results show that: (1) anti-MUC1 antibody GP1.4, but not another anti-MUC1 antibody C595, triggered the internalization of EGFR in pancreatic cancer cells; (2) internalization of EGFR by GP1.4 resulted in the inhibition of ERK phosphorylation by EGF stimulation, in a MUC1 dependent manner; (3) inhibition of ERK phosphorylation by GP1.4 resulted in the suppression of proliferation and migration of pancreatic cancer cells. We conclude that the internalization of EGFR by anti-MUC1 antibody GP1.4 inhibits the progression of cancer cells via the inhibition of EGFR signaling.

4) **Yamada M., Hayashida M., Zhao Q., Shibahara N., Tanaka K., Miyata T., and Matsumoto K.: Ameliorative effects of yokukansan on learning and memory deficits in olfactory bulbectomized mice. J. Ethnopharmacol. 135, 737-746; 2011.**

Abstract: AIM OF THE STUDY: Yokukansan (YKS) is a Japanese traditional herbal medicine and has been used for the treatment of the behavioral and psychological symptoms of dementia (BPSD). The present study aimed to clarify the effects of YKS on learning and memory impairments, and its mechanisms of action in olfactory bulbectomized (OBX) mice, one of the animal models of Alzheimer's disease (AD). **MATERIALS AND METHODS:** OBX or sham-operated ddY mice were treated with YKS or donepezil (DPZ), a reference drug, and their cognitive performances were tested by the modified Y-maze test, novel object recognition test, and fear conditioning test to elucidate the spatial working memory, non-spatial short-term memory, and long-term memory, respectively. After completing the behavioral experiments, the expression level of cholinergic marker proteins and the activity of acetylcholinesterase (AChE) in the brain were analyzed by western blotting and Ellman's method,

respectively. **RESULTS:** OBX caused spatial working memory and non-spatial working memory impairments that were reversed by YKS and also by DPZ; however, YKS failed to affect the long-term memory deficits. Amelioration of the spatial working memory by YKS was reversible by scopolamine, a muscarinic receptor antagonist. YKS treatment reversed OBX-induced down-regulation of choline acetyltransferase and muscarinic muscarinic M_1 receptor expression without affecting muscarinic M_3 receptor expression or AChE activity. **CONCLUSION:** These results demonstrate that YKS improves short-term memory deficit caused by OBX and that the effect is at least partly mediated by muscarinic receptor stimulation and the normalization of central cholinergic systems. The present findings also suggest that YKS has a therapeutic effect not only on BPSD, but also on memory impairment of AD.

5) **Zhao Q., Yokozawa T., Tsuneyama T., Tanaka K., Miyata T., Shibahara N., Matsumoto K.: Chotosan-induced improvement of cognitive deficits in senescence-accelerated mouse (SAMP8) involves normalization of angiogenic/neurotrophic factors and neuroplasticity systems in the brain. Chin. Med. 6, 33 (on line); 2011.**

Abstract: BACKGROUND: Chotosan (CTS, Diaoteng San), a Kampo medicine (ie Chinese medicine) formula, is reportedly effective in the treatment of patients with cerebral ischemic insults. This study aims to evaluate the therapeutic potential of CTS in cognitive deficits and investigates the effects and molecular mechanism(s) of CTS on learning and memory deficits and emotional abnormality in an animal aging model, namely 20-week-old senescence-accelerated prone mice (SAMP8), with and without a transient ischemic insult (T2VO). **METHODS:** Age-matched senescence-resistant inbred strain mice (SAMR1) were used as control. SAMP8 received T2VO (T2VO-SAMP8) or sham operation (sham-SAMP8) at day 0. These SAMP8 groups were administered CTS (750 mg/kg, p.o.) or water daily for three weeks from day 3. **RESULTS:** Compared with the control group, both sham-SAMP8 and T2VO-SAMP8 groups exhibited cognitive deficits in the object discrimination and water maze tests and emotional abnormality in the elevated plus maze test. T2VO significantly exacerbated spatial cognitive deficits of SAMP8 elucidated by the water maze test. CTS administration ameliorated the cognitive deficits and emotional abnormality of sham- and T2VO-SAMP8 groups. Western blotting and immunohistochemical studies revealed a marked decrease in the levels of phosphorylated forms of neuroplasticity-related proteins, N-methyl-D-aspartate receptor 1 (NMDAR1), Ca2+/calmodulin-dependent protein kinase II (CaMKII), cyclic AMP responsive element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) in the frontal cortices of sham-SAMP8 and T2VO-SAMP8. Moreover, these animal groups showed significantly reduced levels of vasculogenesis/angiogenesis factors, vascular endothelial growth factor (VEGF), VEGF receptor type 2 (VEGFR2), platelet-derived growth factor-A (PDGF-A) and PDGF receptor α (PDGFRα). CTS treatment reversed the expression levels of these factors down-regulated in the brains of sham- and T2VO-SAMP8. **CONCLUSION:** Recovery of impaired neuroplasticity system and VEGF/PDGF systems may play a role in the ameliorative effects of CTS on cognitive dysfunction caused by aging and ischemic insult.

6) **Zhao Q., Matsumoto K., Tsuneyama K., Tanaka K., Li F., Shibahara N., Miyata T., and Yokozawa T.: Diabetes-induced central cholinergic neuronal loss and cognitive deficit are attenuated by tacrine and a Chinese herbal prescription, kangen-karyu: elucidation in type 2 diabetes db/db mice. J. Pharmacol. Sci. 117, 230-242; 2011.**

Abstract: We investigated the effect of kangen-karyu (KK), a Chinese herbal prescription, on cognitive deficits and central cholinergic systems of type 2 diabetic db/db mice. Seven-week-old db/db (Y-db/db) mice received daily administration of test drugs during an experimental period of 12 weeks. At 18 weeks of age (O-db/db), the animals underwent the water maze test. Compared with age-matched control strain mice (O-m/m), vehicle-treated O-db/db mice showed impaired learning and memory performance. KK (100 - 200 mg/kg per day) and the reference drug tacrine (THA: 2.5 mg/kg per day) ameliorated the performance of O-db/db mice without affecting their serum glucose level. O-db/db mice had lower levels of brain-derived neurotrophic factor (BDNF) mRNA and its protein in the brain than O-m/m mice. Expression levels of central cholinergic marker proteins in the hippocampus and the number of cholinergic cells in the medial septum and basal forebrain were also significantly lower in O-db/db than in O-m/m mice, whereas no significant differences in the expression levels of these factors and the cell

number were found between Y-m/m and Y-db/db mice. KK and THA treatment significantly reversed the down-regulated levels of cholinergic markers, choline acetyltransferase-positive cell number, and BDNF expression in db/db mice. These findings suggest that KK as well as THA prevents diabetes-induced cognitive deficits by attenuating dysfunction of central cholinergic systems.

◇その他

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2) 宮田健：清肺湯による湿性咳漱の抑制（去痰）作用，漢方医学 35(6), 54-55; 2011

◇学会報告 （*：特別講演，シンポジウム，ワークショップ等）

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2) Zhao Q., Yokozawa T., Park C.H., Tsuneyama K., Miyata T., and Matsumoto K.：Kangenkaryu, a heerbal Chinese prescription, ameliorates dysfunction of central cholinergic systems in db/db mice. 第 84 回日本薬理学会年会，2011, 3/22-24，横浜.
3) 内田航，壬生伸子，横溝和美，宮田健，須本國弘 : 抗ウイルス活性を指向したトリス（2-アミノエチル）アミン誘導体の探索研究．日本薬学会第 131 年会，2011, .3/29-31，静岡.
4) 竹村知，壬生伸子，横溝和美，宮田健，須本國弘 : 1,3,5-トリアジン誘導体合成と抗ウイルス活性日本薬学会第 131 年会．3/29-31，静岡.
5) 周建融，横溝和美，宮田健 : Polyherb supplement の行動薬理学的と BDNF 産生に及ぼす影響．日本薬学会第 131 年会，2011, 3/29-31，静岡.
6) 横溝和美，周建融，國香清，宮田健 : Polyherb supplement 美露仙寿の抗腫瘍効果と腸内フローラ改善効果．第 18 回日本未病システム学会学術総会 2011, 11/19-20，名古屋.
7) 礒濱洋一郎，坂本唯真，久恒昭哲，宮田健，香月博志 : 肺胞上皮細胞のアクアポリン-5 は炎症応答の亢進機構か？第 10 回肺サーファクタント分子病態研究会，2011, 6/25，札幌.
8) 礒濱洋一郎，久恒昭哲，香月博志，宮田健 : ケラチノサイトのアクアポリン-3 発現に対する生薬の作用とその意義．第 28 回和漢医薬学会学術大会シンポジウム，2011, 8/28-29, 富山.
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* 10) 宮田健: 日中保健・医療・福祉交流，－未病を治す－．日中保健・医療・福祉交流フォーラム, 2011, 10/15，熊本.
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12) 松本欣三，趙琦，常山幸一，田中謙，李峰，宮田健，横澤隆子 : 加齢・糖尿病に起因する認知行動障害と漢方薬による実験的予防・治療．第 11 回日本臨床中医薬学会学術大会シンポジウム，2011, 11/12，東京.
13) 松本欣三，山田麻利名，林田未希，柳雅樹，趙琦，田中謙，宮田健 : 嗅球摘出マウスの学習記憶障害に対する抑肝散の効果 : 中枢コリン神経系を介した改善作用．第 39 回薬物活性シンポジウム，2011, 11/21-22，福岡.

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2) Mohamed A. Morsy: Department of Pharmacology, School of Medicine, Minia University, Egypt ‘ Airway pharmacology of Egyptian traditional medicine’ September, 2002～
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