病態生化学分野　Division of Pathogenic Biochemistry

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◇研究目的　Aims of the research projects

病態生化学部門は病態の生化学的研究を行うとともに，和漢薬を含む種々の薬物の病態に及ばす効果を生化学的，免疫学的，あるいは遺伝学的に研究することを目的としている。和漢薬を中心に，構造の明かにされた成分あるいは化合物を用いて，種々の病態（癌，アレルギーなどの疾患）に有効な薬物の探索とその作用機序を分子レベルで解明する。

また，「証」といわれる病態変化を遺伝子工学的，免疫学的手法等を駆使してその遺伝的背景を解析し，薬物の効果発現との関連性からその科学的基盤を解明する。

◇研究概要　Research projects

Ⅰ）がん転移機構の解明とその制御

1）癌転移病態モデルの作製とその形成に関与する標的分子の探索

2）癌転移成立に対するケモカインの作用機序解明と癌転移治療への応用

3）伝統薬物を中心とした癌および癌転移の抑制物質の探索

Ⅱ）シグナル伝達分子による病態制御機構の解析

1）TAK1 活性化の分子機構

2）NF-κB のリン酸化の解析

3）シグナル伝達系に影響を及ぼす漢方薬の探索

Ⅲ）免疫・アレルギー疾患の病態解析

1）IgE 介在性三相性皮膚反応モデル

2）サイトカイン産生に対する漢方方剤の影響

Ⅳ）漢方方剤テーラーメイド治療法の開発

1）漢方医学の証の解明を目指した血漿プロテオミック・パターン解析，本プロジェクトは，「富山・高岡地域」知的クラスター創成事業「とやま医薬バイオクラスター」及び21世紀 COE プログラムの一環として実施している。

◇著　書　Books

1）小泉桂一，土岐善紀，済木育夫：肺癌の転移機構，先端医療シリーズ20・肺癌　「肺癌の最新医療」，末舛恵一／監修，先端医療技術研究所，pp32-39，2003

2）済木育夫：癌転移成立と Liotta 仮説，日本臨床61巻　増刊号「癌転移」－基礎と臨床アップデート－，日本臨床社，pp17-23，2003

3）櫻井宏明，済木育夫：癌転移阻止に関する研究　癌転移を抑制する薬剤　Curcumin，日本臨床61巻　増刊号「癌転移」－基礎と臨床アップデート－，日本臨床社，pp184-188，2003

4）Hasegawa H. and Saiki I.: Cancer Prevention by Ginseng via its Intestinal Bacterial Metabolites, pp1-149, published by Art Village, Tokyo, 2003.

5）小泉桂一，済木育夫：I-8　がんの浸潤と転移，「臨床腫瘍学　第Ⅲ版」，日本臨床腫瘍研究会／編，癌と化学療法社，pp.66-86，2003

◇原　著　original papers

1）**Okazaki T., Sakon S., Sasazuki T., Sakurai H., Doi T., Yagita H., Okumura K. and Nakano H.: Phosphorylation of serine 276 is critical for p65 NF-κB subunit activation.** ***Biochem. Biophys. Res. Commun.,*** **300: 807-812, 2003.**

Abstract: Phosphorylation of several serine residues especially in the transactivation (TA) domain of p65 NF-κB subunit has been suggested to be important for its transcriptional activity. However, the responsible phosphorylation site of p65 remains controversial. To investigate the biological significance of phosphorylation and to determine the critical phosphorylation sites of p65, we reconstituted murine embryonic fibroblasts (MEFs) from p65(-/-) mice with various serine to alanine (SA)-substituted mutants of p65. Unexpectedly, mutants in the TA domain, including S529A, S536A, and S529A/S536A, completely rescued the defect of p65(-/-) MEFs as assessed by tumor necrosis factor (TNF)- or interleukin-1 (IL-1)-induced IL-6 production and protection from TNF-induced cell death. On the other hand, S276A mutant had an impaired ability to rescue these responses. Moreover, TNF-induced phosphorylation of p65 was severely impaired in S276A mutant, indicating that S276 is the major phosphorylation site of p65 and its phosphorylation is essential for p65-dependent cellular responses.

2）**Lee S. J., Saiki I., Hayakawa Y., Nunome S., Yamada H. and Kim S-H.: Antimetastatic and immunomodulating properties of a new herbal prescription, Bojung-bangam-tang.** ***Int. Immunopharmacol.,*** **3: 147-157, 2003.**

Abstract: We investigated the antimetastatic effect of Bojung-bangam-tang, a new herbal prescription, on liver metastasis by the inoculation of colon 26-L5 carcinoma cells into the portal vein. Oral administration of Bojung-bangam-tang for 15 days from day 7 before tumor inoculation significantly inhibited liver metastasis in a dose-dependent manner. Bojung-bangam-tang enhanced the mitogenic activity of BALB/c whole splenocytes induced by various mitogenic stimuli. Oral administration of Bojung-bangam-tang, by itself, could not induce the production of interleukin (IL)-12 or IFN-γ by macrophages, but enhanced the potential of macrophages to produce cytokines in response to lipopolysaccharide (LPS). Experiments using macrophage- or natural killer (NK) cell-deficient mice revealed that the antimetastatic effect of Bojung-bangam-tang is mediated by macrophages rather than NK cells. Bojung-bangam-tang caused a marked increase of production of Th1 cytokine (IFN-γ) and decrease of production of Th2 cytokine (IL-4) by splenocytes stimulated with concanavalin A (Con A). These results indicated that oral administration of Bojung-bangam-tang inhibited the liver metastasis of colon 26-L5 cells through a mechanism leading to a Th1 dominant immune state and activation of macrophages. Thus, Bojung-bangam-tang may be useful for the prevention of cancer metastasis.

3) **Kang I-C., Kim S-A., Song G.Y., Baek N-I., Park Y.-D., Ryu S. Y., Saiki I. and Kim S-H.: Effect of ethyl acetate fraction of *Spatholobi Caulis* on tumor cell aggregation and migration. *Phytother. Res.*, 17: 163-167, 2003.**

Abstract: The ethyl acetate (EA) fraction obtained from a methanol extract of Spatholobi caulis (Leguminosae) has been investigated for anti-metastatic activities in vitro. The EA fraction of Spatholobi caulis inhibited platelet aggregation induced by B16BL6 melanoma cells with an IC(50) of 50 microgram/mL. The EA fraction significantly inhibited HT1080 cancer cell invasion through a matrigel-coated filter with an IC(50) of 25 microgram/mL. Messenger RNA expression of uPA was effectively decreased in HT1080 cells by the EA fraction of Spatholobi caulis with an IC(50) of 30 microgram/mL, but the expressions of MMP-2 (matrix metalloproteinase) and TIMPs (tissue inhibitors of metalloproteinases) were not changed. These findings indicated that the EA fraction suppressed tumour cell invasion by downregulation of uPA (urokinase-type plasminogen activator). Taken together, these results suggest that the EA fraction of Spatholobi caulis may have anti-metastatic activities by blocking tumour cell-induced platelet aggregation (TCIPA) and tumour cell invasion.

4) **Zhang H.W., Iida Y., Andoh T., Nojima H., Murata J., Saiki I. and Kuraishi Y.: *Rapid Communication*: Mechanical hypersensitivity and alterations in cutaneous nerve fibers in a mouse model of skin cancer pain. *J. Pharmacol. Sci.*, 91: 167-170, 2003.**

Abstract: Melanoma inoculation induced marked mechanical allodynia and hyperalgesia in the periphery of the melanoma mass in mice from about day 10 post-inoculation. In the middle of the tumor, there were slight hyperalgesia and response disappearance in the early and late phases, respectively. PGP9.5-like immunoreactivities increased in the epidermis of the periphery of the tumor and disappeared from the dermis of the middle on day 18 post-inoculation, without apparent alterations on day 10. When using this pain model, one should consider the tumor site-dependent responses.

5) **Nakamura E. S., Koizumi K., Yamaura T. and Saiki I.: Anti-tumor angiogenic effect of a matrix metalloproteinase inhibitor MMI-270. *Anticancer Res.*, 23: 411-418, 2003.**

Abstract: We investigated the anti-angiogenic effects of a matrix metalloproteinase inhibitor, (MMI), so called MMI270, against B16-BL6 melanoma through the inhibition of the migrating and invasive abilities of hepatic sinusoidal endothelial (HSE) cells, as well as the formation of tube-like structures by HSE cells. MMI270, at the concentration of 12.5 micrograms/ml, significantly inhibited the migration and invasion of HSE cells, in addition to tube formation by approximately 40%. Furthermore, the enzymatic degradation of metalloproteinases MMP-9 and MMP-2 produced by HSE cells was inhibited by treatment with 1 microgram/ml of MMI270, showing 30% and 100% of inhibition in comparison to the control, respectively. The intraperitoneal administration of MMI270 (200 mg/kg, twice daily for 8 days) after the implantation of B16-BL6 melanoma cells into mice reduced the number of vessels towards the established primary tumor on the dorsal side of mice. These results suggest that MMI270 might be useful as an anti-tumor angiogenic drug.

6) **Terasaki K., Kanzaki T., Aoki T., Iwata K. and Saiki I: Effect of recombinant human tissue inhibitor of metalloproteinase-2 (rh-TIMP-2) on migration of epidermal keratinocytes in vitro and wound healing in vivo. *J. Dermatol.*, 30: 165-172, 2003.**

Abstract: Tissue inhibitors of metalloproteinases (TIMP), common inhibitors of matrix proteinases, have cell-promoting activity. We studied the effects of recombinant human tissue inhibitor of metalloproteinases-2 (rh-TIMP-2) on the migration of normal human epidermal keratinocytes (NHEK). An in vitro migration assay revealed that rh-TIMP-2 enhanced random migration (up to 170%, $p<0.05$) in a dose-dependent manner. When we applied rh-TIMP-2 solution (20 microg/20 microl/wound) daily to full-thickness wounds made with an 8-mm punch on the backs of

healthy (n=8), aged (n=9), and diabetic (n=15) rodents, we observed faster wound closure ($p<0.05$) than in vehicle-treated controls. Accelerated wound closure was dose-dependent (0-20 microg/wound) in diabetic mice (n=6), and the optimal concentration was 10-20 microg of rh-TIMP-2/wound. Histological examinations performed on days 0, 5, 10, 15, and 20 in diabetic mice revealed faster migration of epidermal keratinocytes from wound edges. These results suggest that rh-TIMP-2 plays an important role in wound healing.

7) **Kuraishi Y., Iida Y., Zhang H-W., Uehara S., Nojima H., Murata J., Saiki I., Takahata H. and Ouchi H.: Suppression by Gabapentin of pain-related mechano-responses in mice given orthotopic tumor inoculation. *Biol. Pharm. Bull.*, 26 (4): 550-552, 2003.**

Abstract: In this study, we examined whether several types of non-opioid agents would inhibit the pain-related responses of melanoma-bearing mice. Orthotopic inoculation with melanoma into the hind paw induced marked tactile allodynia and mechanical hyperalgesia. A peroral injection (p.o.) of gabapentin (100-300 mg/kg) inhibited the allodynia and hyperalgesia, without effects on gross behaviors. An intraperitoneal injection (i.p.) of ketamine hydrochloride (30 mg/kg) produced partial inhibition in allodynia and hyperalgesia and prostate posture at 15 min after injection. Diclofenac sodium (10 and 30 mg/kg, i.p), mexiletine hydrochloride (20 mg/kg, i.p.), clonidine hydrochloride (0.1 mg/kg, i.p.) and suramin (100 mg/kg, i.p.) were without effects on allodynia and hyperalgesia. Subcutaneous injections of baclofen (3 mg/kg) and N(G)-nitro-L-arginine methyl ester (100 mg/kg) were also without effects. Repeated administration of gabapentin (150 mg/kg, p.o.) produced constant inhibitions, suggesting no analgesic tolerance. Gabapentin may be useful for the management of cancer pain.

8) **Nagaoka T., Banskota A.H., Tezuka Y., Harimaya Y., Koizumi K., Saiki I. and Kadota S.: Inhibitory effects of caffeic acid phenethyl ester analogues on experimental lung metastasis of murine colon26-L5 carcinoma cells. *Biol. Pharm. Bull.*, 26: 638-641, 2003.**

Abstract: We have previously examined the antiproliferative activity of caffeic acid phenethyl ester (CAPE) and its 20 analogues against six tumor cell lines, and found that CAPE analogues possess selective antiproliferative activity toward the murine colon 26-L5 carcinoma cell line. To extend our study, the effects of CAPE analogues on the metastatic development of murine colon 26-L5 carcinoma cells in the lung were examined. The oral administration of CAPE (5 mg/mice/d) for 7 d after tumor inoculation decreased the tumor weight and the number of tumor nodules in the lung by 50% and 50%, respectively, compared to the control, while CAPE (5 mg/mice/d) administered for 7 d before tumor inoculation showed no significant effect. Besides CAPE, 4-phenylbutyl caffeate, 8-phenyl-7-octenyl caffeate, 2-cyclohexylethyl caffeate and n-octyl caffeate at an oral dose of 2 mg/mice/d caused a 55%, 43%, 55% and 35% reduction of the tumor nodules in their lung metastasis formation, respectively. These results further elaborate the possibility of CAPE and its analogues to become a new class of chemopreventive agents for the treatment of colon cancer metastasis.

9) **Kanzaki Y., Onoue F., Sakurai H. and Ide T: Telomerase upregulates expression levels of interleukin (IL)-1α, IL-1β,IL-6, IL-8.,and granulocyte-macrophage colony-stimulating factor in normal human fibroblasts. *Biochem. Biophys. Res. Commun.*, 305: 150-154, 2003**

Abstract: Expression of human telomerase reverse transcriptase (hTERT) in normal human fibroblast cell strain, TIG-3, extends their replicative life span. We found that expression levels of interleukin (IL)-1α, IL-1β, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA were up-regulated in hTERT-induced fibroblasts irrespective of population doubling level (PDL). Expression levels of these cytokines were low in growing young TIG-3 cells and in control vector-transfected TIG-3 cells but were up-regulated in growth-arrested young cells maintained at high cell density. In senescent TIG-3 cells, expression of IL-1β, IL-6, and GM-CSF was moderately increased. These results indicate that the introduction of hTERT into normal fibroblasts up-regulates the

expression of some inflammatory cytokines, and caution should be paid when introducing the hTERT gene to establish cell lines with normal phenotype.

10) **Tsuchiya Y., Sawada S., Yoshioka I., Ohashi Y., Matsuo M., Harimaya Y., Tsukada K. and Saiki I.: Increased surgical stress promotes tumor metastasis.** ***Surgery,*** **133: 547-555, 2003.**

Abstract: BACKGROUND: Although it is well-known that excessive surgical stress augments the growth of residual cancer and metastasis, whether surgical stress is increased according to the degree of surgical manipulation and can consequently lead to the enhancement of cancer metastasis has not been thoroughly examined. Moreover, the molecules associated with response for stress-enhanced metastasis have not been well-analyzed. The aim of this study was to examine whether cancer metastasis is enhanced with an increase of surgical stress with an experimental lung metastasis model and to analyze the related molecules responsible for stress-enhanced metastasis. METHODS: Colon 26-L5 carcinoma cells (1.5 x 10^4/mouse) were injected intravenously into 6-week-old female BALB/c mice (Japan SLC, Hamamatsu, Japan). Two hours later, the mice were divided into 5 groups: untreated controls (the C group); mice given anesthesia only (the A group); mice given anesthesia and laparotomy (the AL group); mice given anesthesia, laparotomy, and appendectomy (the ALAp group); and mice given anesthesia, laparotomy, appendectomy, and left hepatic lobectomy (the ALApH group). The anesthesia procedures were the same in all groups (intraperitoneal administration of 0.8 mg/mouse sodium pentobarbital). In the AL, ALAp, and ALApH groups, a 3-cm long laparotomy was performed, and the time of the whole operation was just 5 minutes. All mice were killed 14 days after the procedures, and the number of lung metastases on the lung surface was counted manually. At the same time, BALB/c mice without tumor burden were given the same 5 kinds of surgical stress, and the messenger RNA expression of various metastasis-related molecules in the lung was measured with reverse transcriptase-polymerase chain reaction at 6, 24, and 48 hours after surgical stress. We also examined the effect of ONO-4817 (an inhibitor of matrix metalloproteinases ([MPs]) on lung metastasis in the mice with the 5 kinds of surgical stress. RESULTS: The numbers of lung metastases on the lung surface and the messenger RNA expression of MMP-9, membrane type IBMMP, and urokinase-type plasminogen activator at 24 hours after surgery were enhanced in proportion to the degree of surgical stress. Moreover, ONO-4817 significantly inhibited lung metastasis. CONCLUSION: These results strongly suggest that increased surgical stress augments cancer metastasis via surgical stress-induced expression of proteinases in the target organ of metastasis.

11) **Koizumi K., Tsutsumi Y., Kamada H., Yoshioka Y., Watanabe M., Yamamoto Y., Okamoto T., Mukai. Y, Nakagawa S., Tani Y. and Mayumi T.: Incorporation of adult organ-derived endothelial cells into tumor blood vessel.** ***Biochem. Biophys. Res. Commun.,*** **306: 219-224, 2003.**

Abstract: In this study, we attempted to assess the incorporable potential of vascular endothelial cells derived from adult organ blood vessels into tumor blood vessels. Two kinds of adult organ-derived vascular endothelial cells, human aorta endothelial cells (HAEC) and umbilical vein endothelial cells (HUVEC), were administered into murine tumors inoculated to SCID mice. Many human blood vessel networks were visualized in the murine tumors. These cells in solid tumor not only survived and proliferated, but also incorporated into tumor endothelium. These results suggest that adult organ-derived vascular endothelial cells possess the potential to form the neovascular network in various tissues such as vascular endothelial progenitor-like cells in vivo. We propose that these cells can be regarded as a congenic (autologous) vector for vascular regeneration cell therapy and tumor vascular targeting gene therapy.

12) **Fujiuchi Y., Nagakawa O., Murakami K., Fuse H. and Saiki I.: Effect of hepatocyte growth factor (HGF) on invasion of prostate cancer cell lines.** ***Oncol. Rep.,*** **10: 1001-1006, 2003.**

Abstract: Hepatocyte growth factor (HGF) was suggested to play an important role in the regulation of mitogenesis,

motogenesis, angiogenesis, migration and invasion for various types of cells, and acts through a specific membrane receptor encoded by c-met proto-oncogene. However, the mechanism of the effect of HGF on tumor invasion of prostate cancer cells remains unclear. We investigated the effect of HGF on the invasion of PC-3 and DU-145 prostate cancer cells through a reconstituted basement membrane (Matrigel), the haptotactic migration to fibronectin substrate, the expression of protein and mRNA for matrix metalloproteinases (MMP)-1 and -9, membrane-type 1-MMP (MT1-MMP), urokinase-type plasminogen activator (u-PA) and its receptor (uPAR). HGF increased both Matrigel invasion and haptotactic migration of prostate cancer cells. Furthermore, HGF also increased the production of MMP-1 and -9, MT1-MMP, u-PA and uPAR of these cells. These results suggested that HGF increased the invasive potential of prostate cancer cells probably through enhancement of cell motility and the production of MMPs and u-PA.

13) **Nakamura N., Ochi T., Sawada M., Tanaka H., Inagaki N., Saiki I and Nagai H.: Role of cells in IgE-dependent triphasic cutaneous reaction caused by dinitrofluorobenzene in the mouse ear: Participation of CD8+T cells.** ***Allergology International.,*** **52:31-36,2003.**

Abstract: Background: previous studies have suggested that mice passively sensitized with anti-dinitrophenol (DNP) monoclonal IgE antibody exhibit a triphasic cutaneous reaction with an immediate-pase response (IPR) at 1 h, a late-phase response (LPR) at 24 h and a very late-phase reaction (vLPR) at 8 days after challenge with 2, 4-dinitrofluorobenzene. The present study was conducted to evaluate the role of T cells in this triphasic cutaneous reaction. Methods: Mice were passively sensitized by an intravenous injection of anti-DNP monoclonal antibody. Allergic cutaneous reaction was caused by painting an antigen on the ears of mice and measured an enlargement of the ears. Results: whereas the magnitudes of IPR and LPR in BALB/c nu/nu T cell-deficient mice were similar to those in BALB/c +/+ mice, vLPR was not observed in nu/nu mice. In addition, FK 506 (3 and 5 mg/kg) and cyclosporin A (30 and 50 mg/kg) clearly suppressed the onset of vLPR without affecting IPR and LPR. Because these findings suggest the participation of T cells in the on set of vLPR, the character of the T cells was investigated by using anti-CD4 or anti-CD8 monoclonal antibody (mAb) and interleukin (IL)-4 and IL-5 receptor a chain gene-deficient mice. When mice were treated with anti-CD4 or anti-CD8 mAb, the magnitude of the vLPR was augmented by anti-CD4 mAb and suppressed by anti-CD8 mAb, without affecting IPR and LPR. Disruption of the IL-4 gene slightly suppressed IPR, LPR and vLPR, but the lack of the IL-5Ra chain gene did not affect these triphasic responses. Conclusion: The present findings suggest that vLPR is mainly caused by CD8-positive T cells, probably Tc1 cells, and regulated by CD4-positive t cells.

14) **Matsuo M., Sakurai H. and Saiki I.: ZD1839, a selective epidermal growth factor receptor tyrosine kinase inhibitor, shows antimetastatic activity using a hepatocellular carcinoma model.** ***Mol. Cancer Ther.,*** **2: 557-561, 2003.**

Abstract: The epidermal growth factor receptor (EGFR) is highly expressed in many human tumors and provides a new target for anticancer drug development. EGFR-targeted agents have shown promising antitumor activity in preclinical and clinical trials. However, little is yet known about the effect of these new agents on tumor metastasis. Here, we investigate the effects of ZD1839 (Iressa), a selective EGFR tyrosine kinase inhibitor, on the metastatic properties of murine hepatocellular carcinoma CBO140C12. ZD1839 inhibited not only cell growth but also epidermal growth factor-induced chemotactic migration and production of active matrix metalloproteinase-9 in vitro. In mice, orthotopic implantation of a fragment of CBO140C12 tumor into the liver resulted in the formation of a solitary tumor nodule and intrahepatic metastasis. ZD1839, given p.o., inhibited growth of the implanted tumor and intrahepatic metastasis by approximately 50%. These results indicate that EGFR signaling plays an important role in tumor metastasis and that ZD1839 is effective at inhibiting intrahepatic metastasis.

15) **Lee S.J., Sakurai H.,Oshima K.,Kim S.H.,and Saiki I.: Anti-metastatic and anti-angiogenic activities of a new matrix metalloproteinase inhibitor, TN-6b.** ***Eur.J.Cancer,*** **39: 1632-1641,2003.**

Abstract: We investigated the anti-metastatic and anti-angiogenic effects of TN-6b, a new broad-spectrum inhibitor of matrix metalloproteinases (MMPs), against Lewis lung carcinoma (LLC) and hepatic sinusoidal endothelial (HSE) cells. TN-6b potently inhibited the activities of MMP-2 and -9 secreted by LLC and HSE cells in a zymogram assay. TN-6b, at non-cytotoxic concentrations, caused a marked inhibition of invasion and migration of LLC, and tube-like formation of HSE cells. In contrast, TN-6d, an inactive enantiomer of TN-6b, did not inhibit the invasion and tube-like formation. Daily subcutaneous (s.c.) administration of TN-6b at doses of 30 and 60 mg/kg in mice resulted in a potent inhibition of tumour-induced angiogenesis of B16 melanomas and lymph node metastasis of LLC cells. In conclusion, TN-6b effectively inhibited lymph node metastasis of LLC cells through its anti-invasive and anti-angiogenic properties. These findings suggest that the MMP inhibition correlates well with its anti-angiogenic and anti-metastatic efficacy and TN-6b has the therapeutic potential to inhibit angiogenesis and metastasis in vivo and in vitro.

16) **Yamamoto Y., Majima T., He J-X., Saiki I. and Tani T.: Chemical and pharmaceutical evaluation of Daitou-Gancao in comparison with Chinese Glycyrrhizae Radix.** ***J. Tad. Med.,*** **20: 102-110, 2003.**

17) **Tran Q.L., Tran Q.K., Kouda K., Nguyen N.T., Maruyama Y., Saiki I. and Kadota S.: A. Survey on agarwood in Vietnam.** ***J. Tad. Med.,*** **20: 124-131, 2003.**

18) **Yamamoto Y., Majima T., Saiki I. and Tani T.: Pharmaceutical evaluation of** ***Glycyrrhiza uralensis*** **roots cultivated in eastern Nei-Meng-Gu of China.** ***Biol. Pharm. Bull.,*** **26 (8): 1144-1149,2003.**

Abstract: To clarify the feasibility of medicinal use of the cultivated Glycyrrhiza resources, the equivalency between the G. uralensis roots cultivated in eastern Nei-Meng-Gu of China and medicinal licorice (Glycyrrhizae Radix, Gancao in Chinese and Kanzo in Japanese) was examined. The HPLC fingerprint including glycyrrhizin (GL) of the cultivated roots was similar to that of medicinal Gancao, but different from that of non-medicinal Xinjiang-Gancao (Shinkyo Kanzo in Japanese). Similarity between the cultivated roots and two medicinal Gancao was confirmed quantitatively by hierarchical cluster analysis on the basis of HPLC-7-peak-area data. Moreover, the 4-year-old adventitious roots conformed to the five standards described in the Japanese Pharmacopoeia XIV (JP XIV). The 4-year-old adventitious roots had similar pharmaceutical properties to those of medicinal Dongbei-Gancao (Tohoku Kanzo in Japanese) as determined by examining IgE-mediated triphasic skin reaction in mice and pharmacokinetic profile of glycyrrhetic acid, an anti-allergic metabolite of GL. The present pharmaceutical study suggests that the 4-year-old adventitious roots of G. uralensis cultivated in eastern Nei-Meng-Gu of China are comparable to medicinal Gancao conforming to the JP XIV, and may be a potential medicinal source to compensate for the insufficiency of wild Glycyrrhiza plants caused by collection restriction in China.

19) **Lee S-J., Ohashi Y., Sakurai H. Saiki I.: TAC-101 inhibits intrahepatic metastasis of orthotopically implanted murine hepatocellular carcinoma.** ***Cancer Lett.,*** **198: 169-177, 2003.**

Abstract: The anti-metastatic effect of 4-[3,5-bis(trimethylsilyl)benzamido]benzoic acid (TAC-101) was investigated using our established intrahepatic metastasis model. Orthotopic implantation of a fragment of CBO140C12 hepatoma into the liver resulted in the formation of a solitary tumor nodule and its intrahepatic metastasis. Daily oral administration of TAC-101 at a dose of 8 mg/kg resulted in a significant inhibition of intrahepatic metastasis, but

did not affect the growth of the tumor at the implanted site. The down-regulation of transcriptional anti-activator protein-1 (AP-1) activity by TAC-101 paralleled the inhibition of cell invasion and migration through the repression of expression of the mRNAs for urokinase-type plasminogen activator (u-PA) and its receptor (u-PAR). These findings suggest that TAC-101 may improve therapeutic efficacy for liver cancer patients to prevent intrahepatic metastasis.

20) **Nagakawa O., Fujiuchi Y., Fuse H. and Saiki I.: Differential effect of chromogranin A fragments on invasion and growth of prostate cancer in vitro. *Urology*, 62: 553-558, 2003.**

Abstract: OBJECTIVES: To investigate the effect of various chromogranin A (CgA) fragments on the invasion, haptotactic migration, and growth of prostate cancer cells. METHODS: We investigated the effect of five kinds of CgA fragments (79-115, 286-301, 324-341, 344-374, and 356-374) on the invasion of PC-3 and DU-145 prostate cancer cells through a reconstituted basement membrane (matrigel) and the haptotactic migration of these cells using a Transwell cell culture chamber assay. Cell growth was assessed by the WST-1 Cell Counting Kit. RESULTS: CgA (79-115) inhibited the invasive ability of PC-3 and DU-145 cells ($P = 0.035$ and $P = 0.037$, respectively). CgA (79-115) also inhibited the haptotactic migration of these cells ($P = 0.031$ and $P = 0.021$). On the other hand, other CgA fragments had no significant effect. CgA (79-115) also inhibited the cell growth of PC-3 cells ($P = 0.012$) and DU-145 cells ($P < 0.001$). CgA (324-341), CgA (344-374), and CgA (356-374) inhibited the cell growth of DU-145 cells ($P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively). CONCLUSIONS: These results indicate that some CgA fragments may affect the invasion and growth of prostate cancer cells.

21) **Huh J-E., Kang K-S., Ahn K-S., Kim D-H., Saiki I. and Kim S-H.: Mylabris phalerlata induces apoptosis by caspase activation following cytochrome c release and Bid cleavage. *Life Sci.* 73: 2249-2262, 2003.**

Abstract: Mylabris phalerata (MP) is an insect that has been used for the treatment of cancer in oriental medicine. In the present study, the butanol (BuOH) fraction of MP (BFMP) was examined to determine whether it can exert anti-cancer activity through an apoptotic pathway with little toxicity. BFMP was found to have a specific cytotoxic effect on human monocytic leukemic U937 cells (IC(50) = 140 microg/ml) rather than on peripheral blood mononuclear lymphocytes (PBML, IC(50) = over 500 microg/ml). BFMP also induced the morphological changes of apoptosis, such as chromatin condensation, cell shrinking and DNA fragmentation at a concentration of 31.25 microg/ml. In addition, BFMP significantly increased the portion of apoptotic annexin-V positive cells in a dose-dependent manner, and effectively activated caspases (cysteine aspartase) cascade involving caspases 8, 9 and 3. BFMP also effectively cleaved Bid, a death agonist member of the Bcl-2 family and (poly(ADP-ribose)polymerase) (PARP) and induced the subsequent release of cytochrome c from mitochondria into the cytosol. However, it did not affect Bcl-2 and Bax expression. Taken together, these data suggest that the BuOH extract of Mylabris phalerata can induce apoptosis in U937 cells by caspase cascade activation in conjunction with cytochrome c release, induced by a product of Bid. Therefore, we conclude that BFMP has anti-cancer activity, which is achieved through apoptosis and is associated with little toxicity.

22) **Sakurai H., Suzuki S., Kawasaki N., Nakano H., Okazaki T., Chino A., Doi T., Saiki I.: TNF-α-induced IKK phosphorylation of NF-κB p65 on serine 536 is medicated through TRAF2, TRAF5 and TAK1 signaling pathway. *J Biol. Chem.*, 278: 36916-36923, 2003.**

Abstract: The activation of NF-κB has been shown to be regulated by multiple phosphorylations of IkappaBs and the NF-κB p65 subunit. Here, we characterized the intracellular signaling pathway leading to phosphorylation of p65 on Ser-536 using a novel anti-phospho-p65 (Ser-536) antibody. The Ser-536 of endogenous p65 was rapidly phosphorylated in response to a wide variety of NF-κB stimulants including TNF-alpha in the cytoplasm and rapidly

dephosphorylated in the nucleus. The TNF-α-but not IL-1beta-induced Ser-536 phosphorylation was severely impaired in murine embryonic fibroblasts derived from traf2-/-traf5-/- mice. Bay 11-7082, an inhibitor of IκB phosphorylation, inhibited the TNF-α-induced phosphorylation in vivo. In addition, overexpression of TGF-β-activated kinase 1 (TAK1), IKKα and IKKβ stimulated the phosphorylation, and their dominant negative mutants blocked the TNF-α-induced phosphorylation. Moreover, small interfering RNAs (siRNAs) against TAK1, IKKα and IKKβ blocked the phosphorylation of endogenous p65. On the other hand, calyculin-A, a protein phosphatase inhibitor, blocked the dephosphorylation in the nucleus in vivo. These results indicate that similar signaling pathways were utilized for the phosphorylations of IκBalpha and p65, which further support the idea that both IκB and NF-κB are substrates for the IKK complex in the activation of NF-κB.

23) **Koizumi K., Tsutsumi Y., Yoshioka Y., Watanabe M., Okamoto T., Mukai Y., Nagakawa S and Mayumi T.: Anti-angiogenic effects of dimethyl sulfoxide on endothelial cells. *Biol. Pharm. Bull.*, 26: 1295-1298, 2003.**

Abstract: Dimethyl sulfoxide (DMSO) has anti-inflammatory and analgesic properties and is the only intravesical agent approved by the FDA for the treatment of interstitial cystitis. While it is known that DMSO has numerous biological effects on cell differentiation and alteration of cell-surface carbohydrate structures, the anti-inflammatory mechanism of DMSO has been not clear yet. Therefore, further investigation of DMSO in terms of inflammation therapy is needed. This study assessed the in vitro anti-angiogenic effects of DMSO on human aorta endothelial cells to clarify one of the mechanisms of its anti-inflammatory activity. DMSO did not affect expression of E-selectin on endothelial cells in the presence of TNF-α. Furthermore, DMSO effectively inhibited capillary tube formation; this mechanism would be due to suppression of matrix metalloproteinase-2 (MMP-2) production. These results provide useful knowledge about the anti-inflammatory effects of DMSO and the regulatory mechanism of MMP-2.

24) **Sano K, Takayama T, Murakami K, Saiki I, Makuuchi M.: Overexpression of retinoic acid receptor α in hepatocellular carcinoma. *Clin. Cancer Res.* 9: 3679-3683, 2003.**

Abstract: PURPOSE: Retinoid analogues have been reported to inhibit the growth of hepatocellular carcinoma (HCC). However, the expression profile of retinoic acid receptors (RARs) in HCC has not been fully clarified. In this study, we investigated the expression of RAR mRNAs and proteins in resected HCC and nontumor liver tissue. EXPERIMENTAL DESIGN: Reverse transcription-PCR and Western blot analysis were applied to investigate the expression of RAR mRNAs and proteins in 32 resected samples of HCC and 14 samples of nontumor liver tissue. A HCC cell line and primary-cultured hepatocyte were treated with RAR-α-selective retinoids in vitro to estimate their antiproliferative activity. RESULTS: The intensities of mRNA and protein for RAR-α in HCC tissue were significantly higher than those in nontumor liver tissue (P = 0.002 and P = 0.002, respectively). The intensity ratios of HCC versus nontumor liver for RAR-α mRNA and protein were significantly higher than those for RAR-β and RAR-γ (mRNA, P = 0.02 and P = 0.006; protein, P = 0.04 and P = 0.007, respectively). There was only one significant correlation between the higher intensity of RAR-beta protein and tumor stage (stage I/II versus stage III/IVA, P = 0.01) among clinicopathological variables in the HCC patients. However, in vitro experiments showed that the growth of a RAR-α-elevated HCC cell line was potently inhibited by treatment with retinoids at concentrations that did not affect the growth of primary-cultured hepatocytes. CONCLUSIONS: These results imply that RAR-α is the dominant receptor in HCC, which suggests that RAR-α-selective retinoid analogues may be useful for chemotherapy.

25) **Ueda J.Y., Tezuka Y., Banskota A.H., Tran Q.L., Tran Q.K, Saiki I., Kadota S.: Constituents of the Vietnamese medicinal plant Streptocaulon juventas and their antiproliferative activity against the human HT-1080 fibrosarcoma cell line.** ***J. Nat. Prod.*** **66: 1427-33, 2003.**

Abstract: The methanolic extract of roots of Streptocaulon juventas, having shown strong antiproliferative activity against the highly metastatic human HT-1080 fibrosarcoma cell line, was subjected to activity-guided isolation to yield 16 cardenolides including five new ones, acovenosigenin A 3-O-beta-digitoxopyranoside (1), digitoxigenin gentiobioside (2), digitoxigenin 3-O-[O-beta-glucopyranosyl-(1-->6)-O-beta-glucopyranosyl-(1-->4)-3-O-acetyl-beta-digitoxopyranoside] (3), digitoxigenin 3-O-[O-beta-glucopyranosyl-(1-->6)-O-beta-glucopyranosyl-(1-->4)-O-beta-digitalopyranosyl-(1-->4)-beta-cymaropyranoside] (4), and periplogenin 3-O-(4-O-beta-glucopyranosyl-beta-digitalopyranoside) (5), and two new hemiterpenoids, (4R)-4-hydroxy-3-isopropylpentyl beta-rutinoside (6) and (R)-2-ethyl-3-methylbutyl beta-rutinoside (7), together with two known phenylpropanoids and a known phenylethanoid. The isolated cardenolides strongly inhibited the proliferation of the HT-1080 cell line (IC(50) values, 54-1600 nM).

26) **Ueda J., Tezuka Y., Banskota A.H., Tran Q.L., Tran Q.K., Saiki I. and Kadota S.: Antiproliferative activity of cardenolides isolated from** ***Streptocaulon juventas.*** ***Biol. Pharm. Bull.,*** **26: 1431-1435, 2003.**

Abstract: Sixteen cardenolides, two hemiterpenoids, two phenylpropanoids and a phenylethanoid isolated from the roots of Streptocaulon juventas (LOUR.) MERR. were examined for their antiproliferative activity toward three human-derived (HT-1080 fibrosarcoma, lung A549 adenocarcinoma, cervix HeLa adenocarcinoma) and three murine-derived (colon 26-L5 carcinoma, Lewis lung carcinoma, B16-BL6 melanoma) cell lines. The cardenolides selectively and strongly inhibited proliferation of the HT-1080 (IC(50) values, 0.054-1.6 microM) and A549 (IC(50), 0.016-0.65 microM) cell lines. The characteristic morphological changes and ladder-like DNA fragmentation in those cells treated with the cardenolides indicated the antiproliferative activity was due to the induction of apoptosis.

27) **Fujita F., Delhase M., Taniguchi Y., Narita Y., Kato T., Furuya A., Ogawa T., Sakurai H., Joh T., Itoh M., Karin M. and Nakanishi M.: Identification of NAP1: a regulatory subunit of IKK-related kinases that activates NF-κB signaling.** ***Mol. Cell. Biol.,*** **23: 7780-7793, 2003.**

Abstract: The IκB kinase (IKK)-related kinase NAK (also known as TBK or T2K) contributes to the activation of NF-κB-dependent gene expression. Here we identify NAP1 (for NAK-associated protein 1), a protein that interacts with NAK and its relative IKK varepsilon (also known as IKKi). NAP1 activates NAK and facilitates its oligomerization. Interestingly, the NAK-NAP1 complex itself effectively phosphorylated serine 536 of the p65/RelA subunit of NF-κB, and this activity was stimulated by tumor necrosis factor alpha (TNF-α). Overexpression of NAP1 specifically enhanced cytokine induction of an NF-κB-dependent, but not an AP-1-dependent, reporter. Depletion of NAP1 reduced NF-κB-dependent reporter gene expression and sensitized cells to TNF-α-induced apoptosis. These results define NAP1 as an activator of IKK-related kinases and suggest that the NAK-NAP1 complex may protect cells from TNF-α-induced apoptosis by promoting NF-κB activation.

28) **Ueda Y., Yamagishi T., Samata K., Ikeya H., Hirayama N., Takashima H., Nakaike S., Tanaka M., and Saiki I.: A novel low molecular weight antagonist of vascular endothelial growth factor receptor binding: VGF1155.** ***Mol. Cancer Ther.,*** **2: 1105-1111, 2003.**

Abstract: Vascular endothelial growth factor (VEGF) plays a pivotal role in the processes of angiogenesis, which is essential for the growth of solid tumors and their metastasis. Because VEGF is a critical factor in tumor survival, inhibiting VEGF would provide significant benefits in tumor therapy. To identify a compound that inhibits the binding of VEGF to its receptor, we used a high throughput screening method, finding that small molecular compounds inhibited VEGF binding. Among active compounds, 5-[N-methyl-N-(4-octadecyloxyphenyl)acetyl]amino-2-

methylthiobenzoic acid (VGA1155) was selected for its potent inhibition of binding. VGA1155 inhibited [(125)I] VEGF binding to two cell lines, NIH3T3-fms-like tyrosine kinase-1 (VEGF receptor 1 transfected) cells and NIH3T3-kinase insert domain containing receptor/fetal liver kinase-1 (KDR/Flk-1; VEGF receptor 2 transfected), in a concentration-dependent manner. VGA1155 did not inhibit the binding of several other growth factors or cytokines to their receptors. Based on the results of surface plasmon resonance analysis using Biacore S51 system, it appears that this binding inhibitory property may be based on the association of VGA1155 with VEGF receptor 2 (KDR/Flk-1). Further, the interference in VEGF binding by VGA1155 in turn induces the inhibition of VEGF-induced KDR/Flk-1 autophosphorylation. VGA1155 also reduced intradermal VEGF-induced vascular permeability in guinea pigs. These findings indicate that VGA1155 inhibits not only VEGF binding to its receptors through association with KDR/Flk-1 but also VEGF function in vivo. These VGA1155 activities may provide a useful basis for the development of antiangiogenic and antitumor agents.

29) **Ohashi Y., Tsuchiya Y., Koizumi K., Sakurai H. and Saiki I.: Prevention of intrahepatic metastasis by curcumin in an orthotopic implantation model. *Oncology,* 65: 250-258, 2003.**

Abstract: Curcumin has been shown to have potent anti-metastatic activity, however, its mechanism of action is still unclear. Here, we analyzed the anti-metastatic mechanism using hepatocellular carcinoma, CBO140C12 cells. Daily oral administration of curcumin suppressed intrahepatic metastasis in a dose-dependent manner, whereas the growth of implanted tumors was not affected. We next examined the effect of curcumin on several metastatic properties in vitro. Curcumin inhibited the invasion of tumor cells through Matrigel-coated filters and the production of MMP-9. In addition, curcumin significantly inhibited adhesion and haptotactic migration to fibronectin and laminin without affecting the expression of integrins on the cell surface. Furthermore, the formation of actin stress fibers was affected by treatment with curcumin. These results suggested that curcumin suppressed the intrahepatic metastasis mediated by the inhibiton of several metastatic properties, in which the functional alteration of cytoskeletal organization, at least in part, could play an important role.

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2）済木育夫：基礎医学から呼吸器へのメッセージ，肺癌の縦隔リンパ節転移の病態モデル：分子呼吸器病，7(3)：91-94，2003

3）済木育夫：漢方薬の将来展望：漢方と最新治療，12(3)：141-148，2003.

4）済木育夫：和漢薬研究の最前線-ゲノムからのアプローチ-，漢方薬の使用指針である「証」の解明に向けて，日本東洋医学雑誌，54(3)：490-498，2003.

5）済木育夫：漢方トピックス，証の科学的解明に向けて産学官共同で研究がスタートノーベル化学賞受賞の「TOF-MS」解析技術を応用，日経メディカル，5：35-36，2003.

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1）永川　修，明石拓也，藤内靖喜，十二町　明，古谷雄三，布施秀樹，済木育夫：ヒト前立腺癌細胞株DU-145/AD のインテグリン発現と浸潤について，第 7 回北陸泌尿器科 Basic Research Meeting，2003，02. 08，金沢

2）福家洋子，永田郁子，澤木佐重子，小泉桂一，済木育夫：B16-BL6 メラノーマ肺転移モデルを用いたワサビ 6-(methylsulfinyl) hexyl isothiocyanate の転移抑制効果，日本農芸化学会，2003. 04. 01-03，東京

3）永川　修，明石拓也，早川芳弘，藤内靖喜，十二町　明，古谷雄三，済木育夫，布施秀樹：ヒト前立腺癌細胞株DU-145/ADの細胞外マトリックスへの接着と浸潤について，第91回日本泌尿器学会総会，2003，04. 02-05，徳島

4）小泉桂一，小澤陽子，大橋養賢，中村エリアネ静，青塚保志，櫻井宏明，済木育夫：Secondary Lymphoid-tssue Chemokine（SLC）によるヒト非小細胞肺癌（NSCLC）のリンパ節転移亢進の可能性，第7回癌分子標的治療研究会，2003. 06. 02-03，東京

5）李　秀眞，櫻井宏明，済木育夫：新しいMMP阻害剤TN-6bの抗転移及び血管形成阻害効果，第12回日本がん転移学会，2003. 06. 27-28，金沢

6）吉岡伊作，土屋康紀，青塚保志，塚田一博，済木育夫：外科侵襲による癌転移増強に対するUrinary trypsin inhibitorの抑制効果の検討，第12回日本がん転移学会，2003. 06. 27-28，金沢

7）安本和生，松尾光浩，磨伊正義，済木育夫：ヒト大腸癌細胞株同所性移植自然転移モデルを用いた転移関連因子群の解析，第12回日本がん転移学会，2003. 06. 27-28，金沢

8）Nakamura E.S., Koizumi K. and Saiki I.: Matrix metalloproteinases as mediators of lymphangiogenesis-related properties of mirine lymphatic endothelial cells and lymph node metastasis of lung cancer，第12回日本がん転移学会，2003. 06. 27-28，金沢

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11）地野充時，手賀栄治，櫻井宏明，谿　忠人，寺澤捷年，済木育夫：Th1/Th2バランスに及ぼす新しい和漢薬製剤（富山オリジナルブランド）の効果，第20回日本和漢医薬学会大会，2003.08.30-31，熊本

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14）山本　豊，間嶋孝美，済木育夫，谿　忠人：中国内蒙古自治区東部で栽培したGlycyrrhizae uralensis根の薬材規格と薬財特性評価，第50回生薬学会，2003.09.12-13，東京

15）永川　修，明石拓也，小泉桂一，藤内靖喜，古谷雄三，済木育夫，布施秀樹：ヒト前立腺癌細胞のIL-6産生に及ぼすVIP（vasoactive intestinal peptide）の影響，第62回日本癌学会総会，2003.09.25-27，名古屋

16）青塚保志，小泉桂一，斉藤百合花，植田康嗣，櫻井宏明，済木育夫：APN/CD13による腫瘍血管新生メカニズムの解明と癌転移治療への応用，第62回日本癌学会総会，2003.09.25-27，名古屋

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22) 鈴木俊輔，川崎範隆，中野祐康，地野充時，土井貴裕，済木育夫，櫻井宏明：NF-κB p65サブユニット Ser-536 のリン酸化機構の解析，第26回日本分子生物学会，2003，12. 10-13，神戸

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16) 済木育夫：越後湯沢漢方セミナー，講演：「癌転移抑制と漢方薬」2003. 07. 05-06，越後湯沢

17) 済木育夫：第19回茨城県東洋医学研究会学術講演会，講演：「漢方薬による癌転移の抑制効果とその作用機序」2003. 07. 15-16，つくば

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20) 済木育夫：漢方医学セミナー，講演：「漢方薬理の基礎」2003. 07. 26-27，名古屋

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25）済木育夫：中信漢方医学講座，講演：「漢方薬による癌転移抑制効果」，2003. 11. 05-06，松本
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3）岩崎輝明：玄米酵素（株），「FBRA の経口投与によるマウスリンパ球の増殖及びサイトカイン産生に及ぼす効果」，2002，4〜

◇非常勤講師

1）済木育夫：富山医科薬科大学医学部専門教育　講義「免疫学」，2003，01，07，富山.
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◇研究費取得状況　Acquisition of research funds

1）文部省科学研究費，特定領域研究 C（1）（分担：済木育夫）「制癌剤スクリーニング」，（分担課題）基底膜浸潤阻害物質の検定
2）平成15年度文部科学省科学研究費補助金若手研究 B（代表：櫻井宏明）「ストレスシグナル伝達分子 TAK1 の病態制御分子としての役割」
3）平成15年度文部科学省科学研究費補助金若手研究B（代表：小泉桂一）「患者血清のプロテオミクス解析による漢方医学診断基準（証）の客観的評価法の構築」
4）平成14年度東京生化学研究会研究助成金（代表：櫻井宏明）「TAK1 の炎症制御分子としての役割に関する研究」
5）平成14年度上原記念生命科学財団研究助成金（代表：櫻井宏明）「新しい NF-κB 活性化機構：RelA リン酸化に関する研究」
6）平成15年度富山第一銀行奨学財団研究助成金（代表：櫻井宏明）「転写因子 NF-κBリン酸化の生理機能解析」
7）平成15年度 富山県受託研究：和漢薬・バイオテクノロジー研究，（代表：渡邊裕司）「免疫系・血液血管系に作用する家庭薬や薬食同源食品の開発」
8）平成15年度 知的クラスター創成事業「とやま医薬バイオクラスター」，（代表：済木育夫）漢方方剤テーラーメード治療法の開発について
9）平成15年度21世紀 COE プログラム「東洋の知に立脚した個の医療の創生」（分担：済木育夫）臨床研究（遺伝子多型と血漿プロテオーム解析）

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卒業論文：
鈴木俊輔：NF-κB p65サブユニット Ser-536のリン酸化および脱リン酸化の解析
修士論文：
小澤陽子：ヒト非小細胞肺癌に対する Secondary Lymphoid-tissue Chemokine（SLC）のリンパ節転移亢進因子としての可能性
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◇人事移動

林　和子：本学技官　退官（2003. 03. 31）
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