病態生化学部門　Department 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ぼす効果を生化学的，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．癌および癌転移の抑止に関する基礎的研究

1）癌および癌転移の抑制物質の探索（伝統薬物を中心に）
2）癌の悪性化・進展モデルの確立とその分子機序の解析
3）癌ワクチンを指向した免疫力増強物質の検索
4）同所移植性転移モデルにおける転移の臓器特異（選択）性とその機序の解析
5）細胞接着の制御に基づく浸潤・転移の抑制
6）基底膜分解酵素の転写・産生・分解レベルでの阻害物質の探索
7）ケモカイン，癌転移治療への応用に関する研究
8）癌転移に及ぼす血管新生およびリンパ管新生の影響に関する研究
9）癌転移におけるストレスシグナル関連分子の役割に関する研究

Ⅱ．免疫抑制に関する基礎的研究

1）アレルギー性／炎症性疾患モデルの確立と有効物質（抑制／増強）の探索
2）免疫応答調節機構解明と和漢薬への応用

Ⅲ．細胞の機能制御とシグナル伝達機構の解析

1）ストレスシグナル伝達系の分子機構解析
2）神経ペプチドによる細胞浸潤の制御と細胞内機能分子の関与

◇著　書　Books

1）小泉桂一，済木育夫：第V編　肺癌の転移機構，1. 血行性転移の分子機構，2）接着分子群，「肺癌の診断と治療－最新の研究動向－」，日本臨床，pp97-102，2002年増刊.

2）済木育夫：「和漢薬の辞典」，富山医科薬科大学/編集，難波恒雄　監修：朝倉書店，2002年.

◇原　著　original papers

1）**Tsuchiya Y., Sawada S., Tsukada K. and Saiki I.: A new pseudo-peptide of Arg-Gly-Asp RGD) inhibits intrahepatic metastasis of orthotopically implanted murine hepatocellular carcinoma. *Int. J, Oncol.*, 20 : 319-324, 2002.**

Abstract: We have previously reported that the expression of matrix metalloproteinase-9 (MMP-9), membrane type-1 matrix metalloproteinase (MT1-MMP) and beta1 integrins in murine hepatocellular carcinoma (HCC) was associated with the occurrence of intrahepatic metastasis, which is considered to be a major modality in recurrence. Here we show that intravenous administration of synthetic RGD pseudo-peptide (FC-336) inhibited intrahepatic metastasis produced by orthotopic implantation of a fragment of murine HCC (CBO140C12) tumor as compared with control administration of vehicle ($p<0.05$), but did not affect the growth of the implanted tumor. To further analyze the anti-metastatic effect of FC-336, we investigated the effects of FC-336 on tumor growth, adhesion and invasion in vitro. FC-336 at non-cytotoxic concentration of less than 5 mg/ml effectively inhibited the adhesion and invasion of CBO140C12 cells ($p<0.05$). We also used zymography to examine the effect of FC-336 on the gelatinolysis of MMPs produced by CBO140C12 cells. FC-336 inhibited the degradation of the gelatin substrate by MMP-9 in a concentration-dependent manner. These results strongly suggest that intrahepatic metastasis of CBO140C12 tumors is partly due to the marked invasive and adhesive abilities of tumor cells mediated by expression of MMP-9 and integrin alpha3beta1 (VLA-3), integrin alpha5beta1 (VLA-5) on the tumor surface, respectively.

2）**Nagakawa O., Murata J., Junicho A., Matsuda T., Fujiuchi Y., Fuse H. and Saiki I.: Vasoactive intestinal peptide (VIP) enhances the cell motility of androgen receptor-transfected DU-145 prostate cancer cells (DU-145/AR). *Cancer Lett.*, 176 : 93-99, 2002.**

Abstract: We established a clonal DU-145 prostate cancer cell line (DU-145/AR) stably transfected with androgen receptor (AR) cDNA and investigated the expression of type 1 vasoactive intestinal peptide (VIP) receptor (VIP1R) and type 2 VIP receptor (VIP2R) mRNA in these cells by reverse transcriptase-polymerase chain reaction analysis and the effect of VIP on the invasion and the haptotactic migration of these cells. DU-145/AR cells constitutively expressed both VIP1R and VIP2R mRNA, but the parent DU-145 cells did not. VIP increased the invasive capacity of DU-145/AR cells. VIP also enhanced the haptotactic migration of these cells to fibronectin. However, the growth of these tumor cells was not affected by VIP at any concentrations used in this study. These results indicate that VIP may play a role in the regulation of the invasion of prostate cancer.

3）**Sizemore N., Lerner N., Dombrowski N., Sakurai H. and Stark G.R.: Distinct Roles of the IkB kinase a and b subunits in liberating nuclear factor kB (NF-kB) from IkB and in phosphorylating the p65 subunit of NF-kB. *J. Biol. Chem.*, 277 : 3863-3869, 2002.**

Abstract: Phosphatidylinositol 3'-kinase (PI3K) and the serine/threonine kinase AKT have critical roles in phosphorylating and transactivating the p65 subunit of nuclear factor kappaB (NF-kappaB) in response to the pro-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF). Mouse embryo fibroblasts (MEFs) lacking either the alpha or beta subunit of IkappaB kinase (IKK) were deficient in NF-kappaB-dependent transcription following treatment with IL-1 or TNF. However, in contrast to IKKbeta-null MEFs, IKKalpha-null MEFs were not substantially defective in the cytokine-stimulated degradation of Ikappabetaalpha or in the nuclear translocation of NF-kappaB. The IKK complexes from IKKalpha- or IKKbeta-null MEFs were both deficient in PI3K-mediated

phosphorylation of the transactivation domain of the p65 subunit of NF-kappaB in response to IL-1 and TNF, and constitutively activated forms of PI3K or AKT did not potentiate cytokine-stimulated activation of NF-kappaB in either IKKalpha- or IKKbeta-null MEFs. Collectively, these data indicate that, in contrast to IKKbeta, which is required for both NF-kappaB liberation and p65 phosphorylation, IKKalpha is required solely for the cytokine-induced phosphorylation and activation of the p65 subunit of NF-kappaB that are mediated by the PI3K/AKT pathway.

4) **Mizukami J., Takaesu G., Akatsuka H., Sakurai H. Ninomiya-Tsuji J., Matsumoto K.and Sakurai N.: Receptor activator of NF-kB ligand (RANKL) activates TAK1 mitogen-activated protein kinase kinase kinase through a signaling complex containing RANK, TAB2, and TRAF6. *Mol. Cell. Biol.*, 22 : 992-1000, 2002.**

Abstract: The receptor activator of NF-kappaB (RANK) and its ligand RANKL are key molecules for differentiation and activation of osteoclasts. RANKL stimulates transcription factors AP-1 through mitogen-activated protein kinase (MAPK) activation, and NF-kappaB through IkappaB kinase (IKK) activation. Tumor necrosis factor receptor-associated factor 6 (TRAF6) is essential for activation of these kinases. In the interleukin-1 signaling pathway, TAK1 MAPK kinase kinase (MAPKKK) mediates MAPK and IKK activation via interaction with TRAF6, and TAB2 acts as an adapter linking TAK1 and TRAF6. Here, we demonstrate that TAK1 and TAB2 participate in the RANK signaling pathway. Dominant negative forms of TAK1 and TAB2 inhibit NF-kappaB activation induced by overexpression of RANK. In 293 cells stably transfected with full-length RANK, RANKL stimulation facilitates the formation of a complex containing RANK, TRAF6, TAB2, and TAK1, leading to the activation of TAK1. Furthermore, in murine monocyte RAW 264.7 cells, dominant negative forms of TAK1 and TAB2 inhibit NF-kappaB activation induced by RANKL and endogenous TAK1 is activated in response to RANKL stimulation. These results suggest that the formation of the TRAF6-TAB2-TAK1 complex is involved in the RANK signaling pathway and may regulate the development and function of osteoclasts.

5) **Sawada S., Murakami K., Yamaura T., Mitani N., Tsukada K. and Saiki I.: Therepeutic and analysis model of intrahepatic metastasis reflects clinical behavior of hepatocellular carcinoma. *Jpn. J. Cancer Res.*, 93 : 190-197, 2002.**

Abstract: This study was designed to establish an intrahepatic metastasis model to investigate the biology and therapy of hepatocellular carcinoma (HCC) in mice. A fragment of mouse HCC tumor CBO140C12 was orthotopically implanted into the mouse liver. The number of intrahepatic metastatic colonies and the volume of the implanted tumor increased in a time-dependent manner. At 28 days after fragment implantation, all mice showed intrahepatic metastasis. Intravenous administrations of cisplatin and doxorubicin at 7 and 21 days after the implantation significantly suppressed the growth of the primary tumor nodule, but tended to inhibit intrahepatic metastasis. However, a marked decrease of body weight was observed during the experiment. On the other hand, an inhibitor of matrix metalloproteinases (MMPs), ONO-4817, decreased the gelatinase activity of MMP-9 secreted by CBO140C12 cells, and significantly reduced the number of colonies of intrahepatic metastasis when administered orally. Our established model, which is focused on intrahepatic metastasis, is suitable for evaluating the therapeutic effect of HCC and for analyzing intrahepatic metastasis, because this model reflects the clinical features of HCC and all the steps of tumor metastasis.

6) **Tatsumi T., Terasawa M., Tega E., Hayakawa Y., Terasawa K. and Saiki I.: Immunopharmacological properties of Oren-gedoku-to (a Kampo medicine, Huang-Lian-Jie-Du-Tang) on contact hypersensitivity reaction in mice. *J. Trad. Med.*, 19 : 21-27, 2002.**

Abstract: We investigated the effects of Oren-gedoku-to (Huang-Lian-Jie-Du-Tang), a Kampo medicine, on DNFB-induced contact hypersensitivity (CHS) response in mice in order to further clarify the immunopharmacological

properties of this formulation. 1) Administration of Oren-gedoku-to decreased the magnitude of ear swelling in the CHS response and shortened the affected period. The inhibitory effect on ear swelling was observed even when Oren-gedoku-to was given orally with different timing schedules. 2) The expressions of mRNAs for CD8, IFN-γ and TNF-α in the ear of Oren-gedoku-to-treated mice were markedly decreased 24 h after the challenge. 3) The number of skin-draining regional lymph node cells (LNC) was decreased without affecting the ratio of $CD8^+/CD4^+$ T cells. Oren-gedoku-to resulted in a marked impairment of the hapten-specific development of lymphocytes. These results suggest that the suppressive effect of Oren-gedoku-to on ear swelling was partly caused by the suppression of lymphocyte proliferation.

7) **Muraishi Y., Mitani N., Fuse H. and Saiki I.: Effect of a Matrix metalloproteinase inhibitor (ONO-4817) on lung metastasis of murine renal cell carcinoma.** ***Anticancer Res.*****, 21 : 3845-3852, 2002.**

Abstract: We examined the anti-metastatic effect of a newly developed inhibitor of synthetic matrix metalloproteinase (MMP), ONO-4817, on experimental pulmonary metastasis of murine renal cell carcinoma (Renca) cells and on tumor cell invasion, through reconstituted basement membrane (Matrigel) in vitro using the same cells. Oral administration of ONO-4817 (50-200 mg/kg/day) to Renca-bearing mice resulted in a dose-dependent inhibition of lung metastasis without a loss of body weight. ONO-4817 at the high dose of 200 mg/kg showed a tendency to prolong the survival of the mice. We also found that oral administration of ONO-4817 significantly inhibited the angiogenic response (number of vessels oriented towards the tumor mass) and the growth of tumors inoculated i.d. in syngeneic mice. In addition, ONO-4817, at non-cytotoxic concentrations of less than 10 microM, caused a marked inhibition of the invasion of Renca cells as compared to the vehicle control. Gelatin zymography revealed that ONO-4817 inhibited the enzymatic activity of MMP-2 produced by Renca cells in a concentration-dependent manner. In conclusion, ONO-4817 effectively inhibited lung metastasis of Renca cells through its anti-invasive and anti-angiogenic properties. These results suggest that use of the MMP inhibitor (MMPI) ONO-481 7 may provide a therapeutic basis for preventing lung recurrence and metastasis of renal cell carcinoma.

8) **Ueda J., Tezuka Y., Banskota A.J., Tran G.L., Tran Q.K., Harimaya Y., Saiki I. and Kadota S.: Antiproliferative activity of vietnamese medicinal plants.** ***Biol. Pharm. Bull.*****, 25 : 753-760, 2002.**

9) **Sasamura T., Nakamura S., Iida Y., Fujii H., Murata J., Saiki I. Nojima H. and Kuraishi Y.: Morphine analgesia suppresses tumor growth and metastasis in a mouse model of cancer pain produced by orthotopic tumor inoculation.** ***Eur. J. Pharmacol*****, 441 : 185-191, 2002.**

10) **Tran Q.L., Adnyana I.K., Tezuka Y., Harimaya Y., Saiki I., Kurashige Y., TranQ.K. and Kadota S.: Hepatoprotective effect of majonoside R2, the major saponin from Vietnamese ginseng (Panax vietnamensis).** ***Planta Med.*****, 68 : 402-406, 2002.**

11) **Hasegawa H. Suzuki R., Nagaoka T., Tezuka Y., Kadota S. and Saiki I.: Prevention of growth and metastasis of murine melanoma through enhanced natural-killer cytotoxicity by fatty acid-conjugate of protopanaxatriol.** ***Biol. Pharm. Bull.*****, 25 : 861-866, 2002.**

Abstract: Ginsenosides, the glycosides of Panax ginseng, are metabolized (deglycosylated) by intestinal bacteria after oral administration. 20(S)-Protopanaxatriol (M4) is the main bacterial metabolite of protopanaxatriol-type ginsenosides and mediates their antitumor effects. To clarify the mechanism of the M4-mediated antitumor effect, the antitumor activity and metabolism of M4 was examined, using the C57BL/6 mice implanted with B16-BL6 melanoma. The chronic oral administration of M4 inhibited the growth of B16-BL6 melanoma at the implanted site.

Analyses using TLC, HPLC, MS and NMR suggest that orally administered M4 was absorbed from the small intestine into the mesenteric lymphatics followed by the rapid esterification of M4 with fatty acids and its accumulation in the tissues including the liver and lung. The administration of M4 prior to the intravenous injection of B16-BL6 cells abrogated the enhanced lung metastasis in the mice pretreated with 2-chloroadenosine more effectively than in those pretreated with anti-asialo GM1. The esterified M4 (EM4) did not directly affect tumor growth in vitro, whereas it stimulated splenic NK cells to become cytotoxic to tumor cells. These results indicate that the antitumor activity of M4 is based on the NK cell-mediated tumor lysis enhanced by EM4.

12) Nakamura E.S., Kurosaki F., Arisawa M., Mukainada T., Takayasu J., Okuda M., Nishino H. Pastore F. Jr.: Cancer chemopreventive effects of a Brazilian folk medicine, Juca, on in vivo two-stage skin carcinogenesis. *J. Ethnopharmacol.*, 81 : 135-137, 2002.

Abstract: Gallic acid (1) and methyl gallate (2) were isolated from Juca, a Brazilian folk medicine, fruits of Caesalpinia ferrea MART (Leguminosae), decreased significantly the average number of papillomas per mouse in the experiment of the promoting effects of 12-O-tetra- decanoylphorbol-13-acetate (TPA) on skin tumor formation in mice initiated with 7,12-dimethylbenz[a]anthracene (DMBA).

13) Matsuo M., Tani T. and Saiki I.: Organ selectivity of Juzen-taiho-to and Ninjin-yoei-to in the expression of anti-metastatic efficacy. *J. Trad. Med.*, 19 : 93-97, 2002.

Abstract: We investigated the inhibitory effect of oral administration of Juzen-taiho-to and Ninjin-yoei-to on the liver metastasis caused by intraportal vein injection of colon 26-L5 cells and lung metastasis by intravenous injection of same tumor cells. Juzen-taiho-to significantly inhibited liver metastasis but not lung metastasis. In contrast, Ninjin-yoei-to was effective at inhibiting lung metastasis but not liver metastasis. In the experimental liver and lung metastasis model using same tumor in syngeneic mice system, oral administration of both formulations showed differential pattern with organ selectivity for the expression of anti-metastatic effects. These results suggest that the different expression of the anti-metastatic effects of both Kampo medicines on tumor metastasis are partly based on the medicinal guides according to the theory of Jing and Lun (Inkei-hoshi) formed in 13th century.

14) Hasegawa H. and Saiki I.: Psychosocial stress augments tumor development through β-adrenergic activation in mice. *Jpn. J. Cancer Res.*, 93 : 729-735, 2002.

Abstract: Housng conditions affect behavioral and biological responses of animals. We investigated the effect of same-sex-grouped (G), crowded (GC) and isolated (I) conditions on the growth of B16 melanoma or Meth A fibrosarcoma implanted in the footpad of syngeneic male C57BL / 6 or BALB / c mice. Differential housing altered host resistance to tumor growth. The host responses to stress were reflected in thymic atrophy, which was lowest in the G mice, highest in the GC mice and intermediate in the I mice. The GC condition was a more stressful social environment than the I condition in both male C57BL / 6 and BALB / c mice. Reflecting the extent of psychosocial stress, tumor growth was augmented in the order of GC, I and G condition, and a negative mass correlation between tumor and thymus was observed, thus clearly indicating that the host resistance to tumors was attenuated by psychosocial stress. Furthermore, the stress-enhanced tumor growth and thymus atrophy were completely abrogated by the oral administration of the non-selective beta-adrenergic antagonist, propranolol. On the contrary, the chronic administration of corticosterone significantly induced the atrophy of thymus and spleen without affecting tumor growth. These results suggest an interrelationship among psychosocial stress, tumor growth and beta-adrenergic activation.

15) Nagaoka T., Banskota A.H., Tezuka Y., Saiki I. and Kadota S.: Selective antiproliferative activity of caffeic acid phenethyl ester analogues on highly liver-metastatic murine colon 26-L5

carcinoma cell line. Bioorg. Med. Chem., 10 : 3351-3359, 2002.

16) Nakamura E.S., Kurosaki F., Arisawa M., Mukainada T., Okuda M., Takayasu J., Nishino H. Pastore F. Jr.: Cancer chemopreventive effects of constituents of *Caesalpinia ferrea* and related compounds. *Cancer Lett.*, 177 : 119-124, 2002.

Abstract: The anti-tumor promoting effects of fruits of Caesalpinia ferrea MART. (Leguminosae) were tested by the in vitro Epstein-Barr virus early antigen (EBV-EA) activation assay, and its active constituents were identified as gallic acid (1) and methyl gallate (2). A total of 49 related compounds of 1 and 2 were analysed for the effects by this assay, and the structure activity relationships have been proposed. Three acetophenone derivatives, 2,6-dihydroxyacetophenone (48), 2,3,4-trihydroxyacetophenone (50) and 2,4,6-trihydroxy- acetophenone (51) were found to show potent inhibitory

17) Hayakawa Y., Takeda K., Yagita H., Smyth M.J., Kae L.V., Okumura K. and Saiki I.: IFN-γ-mediated inhibition of tumor angiogenesis by the natural killer T cell ligand, α-galactosylceramide. *Blood*, 100 : 1728-1733, 2002.

Abstract : Alpha-galactosylceramide (alpha-GalCer), which is a specific ligand for CD1d-restricted variable-alpha14 chain (V(alpha)14) natural killer T (NKT) cells, exerts a potent antitumor effect. We recently demonstrated that interferon-gamma (IFN-gamma) secreted by both NKT cells and NK cells plays a critical role in mediating the antimetastatic effect of alpha-GalCer; however, the IFN-gamma-dependent antitumor mechanisms remain poorly defined. In the present study, we demonstrate IFN-gamma-dependent inhibition of tumor angiogenesis by alpha-GalCer. In alpha-GalCer-treated mice, subcutaneous tumor growth and tumor-induced angiogenesis were inhibited in an IFN-gamma-dependent manner. The alpha-GalCer-activated splenic or hepatic mononuclear cells inhibited murine endothelial cell proliferation in vitro, and this inhibitory effect was mediated mostly by IFN-gamma produced by NKT cells and NK cells. NK cell depletion resulted in significant but partial inhibition of tumor growth and angiogenesis in vivo. These results suggest that the IFN-gamma-mediated inhibition of tumor angiogenesis is critically involved in the effector mechanisms of antitumor effects evoked by alpha-GalCer.

18) Harimaya Y., Koizumi K., Andoh T., Nojima H., Kuraishi Y. and Saiki I.: Potential ability of morphine to inhibit the adhesion, invasion and metastasis of metastatic colon 26-L5 carcinoma cells. *Cancer Lett.*, 187 : 121-127, 2002.

Abstract: Morphine is frequently used for cancer patient's terminal medical care to relieve cancer pain. In the present study, we examined the inhibitory effect of morphine on experimental lung metastasis and invasion of colon 26-L5 cells. Morphine was found to significantly reduce the number of tumor colonies and the weight of the tumor-containing lung. Morphine inhibited the adhesion and migration of colon 26-L5 cells to extracellular matrix components and invasion into reconstituted basement membrane Matrigel, without affecting the cell proliferation in vitro. Notably, naloxone, an antagonist of morphine, abrogated morphine-induced inhibition of tumor cell adhesion, but did not affect the inhibitory effect on the production of matrix metalloproteinases (MMPs) from tumor cells. These results suggest that morphine inhibited the adhesive and invasive properties of tumor cells by different inhibitory mechanisms that involved the mediation of an opioid receptor.

19) Sakurai H., Nishi A., Sato N., Mizukami J., Miyoshi H. and Sugita T.: TAK1-TAB1 fusion protein: a novel constitutively active mitogen-activated protein kinase kinase kinase that stimulates AP-1 and NF-kB signaling pathway. *Biochem. Biophys. Res. Commun.* 297 : 1277-1281, 2002.

Abstract: TAK1 mitogen-activated protein kinase kinase kinase (MAP3K) is activated by its specific activator,

TAK1-binding protein 1 (TAB1). A constitutively active TAK1 mutant has not yet been generated due to the indispensable requirement of TAB1 for TAK1 kinase activity. In this study, we generated a novel constitutively active TAK1 by fusing its kinase domain to the minimal TAK1-activation domain of TAB1. Co-immunoprecipitation assay demonstrated that these domains interacted intra-molecularly. The TAK1-TAB1 fusion protein showed a significant MAP3 activity in vitro and activated c-Jun N-terminal kinase/p38 MAPKs and IkappaB kinase in vivo, which was followed by increased production of interleukin-6. These results indicate that the fusion protein is useful for characterizing the physiological roles of the TAK1-TAB1 complex.

20) Ogasawara M., Matsunaga T., Takahashi S., Saiki I. and Suzuki H.: Anti-invasive and metastatic activities of evodiamine. *Biol. Pharm. Bull.*, 25 : 1491-1493, 2002.

Abstract: We have recently reported that evodiamine can suppress in vitro invasion and lung metastasis by colon 26-L5 carcinoma cells. To extend our study, we examine here the anti-invasive and metastatic effects of evodiamine on Lewis lung carcinoma (LLC) and B16-F10 melanoma in addition to colon 26-L5 carcinoma. Critical structures of evodiamine for the activities were also evaluated by comparison with compounds possessing structures similar to that of evodiamine. Evodiamine concentration-dependently inhibited the invasion of B16-F10, LLC and colon 26-L5 cells with IC(50) values of 2.4 micro M, 4.8 micro M and 3.7 micro M, respectively. Pre-treatment of colon 26-L5 cells with evodiamine before inoculation into mice caused significant suppression of the liver metastasis as well as the lung metastasis. Lung metastasis by LLC is also inhibited significantly by pre-exposure to evodiamine. When the anti-migratory activity of evodiamine was compared with that of evodiamine-like compounds, rutaecarpine lacking a methyl group at N-14 and a hydrogen at C-13 b exhibited much less effect than evodiamine. In addition, reserpine, having beta-configurated hydrogen at C-13 b, inhibited tumor cell migration more potently than yohimbine, having alpha-configurated hydrogen at the same position. These results suggest that evodiamine may be useful as a leading compound for agents in tumor metastasis therapy. Also, the presence of a methyl group at N-14 and the configuration of hydrogen at C-13 b may be responsible for the inhibitory activities of evodiamine.

21) Siripong P., Nakamura E.S., Kanokmedhakul K., Ruchirawat S. and Saiki I.: Anti-invasive effects of curucuminoid compounds from *Curcuma aromatica* Salisb. on murine colon 26-L5 carcinoma cells. *J. Trad, Med.*, 19 : 209-215, 2002.

Abstract: Bioassay-directed fractionation of the active chloroform extract from the rhizomes of Curcuma aromatica Salisb. (Zingiberaceae) led to the isolation of four main curcuminoid constituents: curcumin (CA-1), demethoxycurcumin (CA-2), 5'-methoxycurcumin (CA-3) and bisdemethoxycurcumin (CA-4). This is the first report to describe the isolation of CA-3 from C. aromatica. The chemical structures of these compounds were determined on the basis of spectral analysis and their inhibitory effects on the proliferation, invasion and migration of murine colon 26-L5 adenocarcinoma cells were evaluated in vitro. Curcumin and its analogues (CA-2, 3 and 4), at the non-cytotoxic concentration of 10 mM, inhibited the invasive ability of colon 26-L5 cells to the ranges of 22.8, 28.9, 10.3 and 62.0%, respectively. A similar effect of these constituents on the migration of colon 26-L5 cells was also observed. Among these curcuminoids, CA-4 showed the strongest activities, inhibiting both tumor cell invasion and migration in a concentration-dependent manner.

◇総　説　Review Paper

1) 済木育夫：特集「EBM にもとづくアレルギー疾患の漢方治療」，講座　アレルギー性皮膚疾患に用いられる漢方方剤，アレルギー・免疫，**9** : 790-799, 2002.
2) 済木育夫：連載「現代西洋医学からみた東洋医学」，漢方薬の抗腫瘍効果とその作用機序　－補剤を用いた癌転移の抑制－，医学のあゆみ，**202** : 205-209, 2002.
3) 済木育夫：スペシャルインタビュー「スピルリナ」，体と栄養のメカニズム，元気生活，**90** : 34-41, 2002.

4）済木育夫：4. 補剤　現代医学の立場から，第１４回伝統医学臨床セミナー「虚弱者に頻用される処方」，日本東洋医学雑誌，**53**：297-309，2002.

5）済木育夫：特別講演「漢方方剤のアレルギー性疾患治療への応用　－基礎からの提言－」，日本小児東洋医学会誌，**18**：21-26，2002.

6）済木育夫：特集　癌免疫治療と漢方の位置づけ，BRMとしての十全大補湯はIFN-αA/Dのマウス腎細胞癌に対する肺転移抑制効果を著明に増強する，T-webs，**6**：10-13，2002.

◇学会報告　Scientific presentation（*: 特別講演，シンポジウム，ワークショップ等）

*1）済木育夫：　シンポジウム「アレルギー疾患における漢方薬の特性と有用性」，アレルギー性疾患に用いられる漢方方剤の基礎的研究，第21回漢方免疫アレルギー研究会学術集会，2002，01. 26，東京.

2）済木育夫：アレルギー疾患に用いられる漢方薬の基礎的研究，皮膚科漢方入門セミナー，2002，03. 17. 富山.

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3）平成13年度上原記念生命科学財団　海外留学助成ポストドクトラルフェローシップ（代表：早川芳弘），NK細胞による腫瘍監視機構の解明

4）平成13年度上原記念生命科学財団　研究助成金（代表：済木育夫），アレルギー性皮膚炎に及ぼす漢方

薬の効果とその作用機序の解析

5）平成13年度東京生化学研究会　研究助成金（代表：小泉桂一），がん組織リンパ管を標的とした新規がん抗体療法の構築

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7）平成14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，

8）平成14年度漢方医薬研究振興財団　研究助成金（代表：櫻井宏明），自然免疫系に及ぼす漢方薬の効果に関する分子生物学的研究

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修士論文：

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播磨谷優子：放線菌二次代謝物TP-1の癌細胞に対するアポトーシス誘導効果とその作用機序の解析

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