病態生化学部門

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◇研究目的

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

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遺伝療法の開発と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

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）自己分泌型運動抑制因子の単離・精製とその構造解析
2）細胞運動と細胞内調節分子の関連性の解析
3）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

1）大西康晴，塚田一博，済木育夫：第７章　肝転移と接着分子，「肝転移 −メカニズムと臨床−」，磨伊正義／編集，医学書院，pp86−111，2000.

◇原　著

1）**Banskota A.H., Tezuka Y., Uchiyama M., Tran K.Q., Tanaka K., Saiki I. and Kadota S.: Thirteen Novel cycloartane-type triterpenes from Combretum quadrangulare. J. Nat. Prod., 63: 57-64, 2000.**

2）**Satoh T., Tahara E., Yamada T., Watanabe C., Itoh T., Terasawa K., Nagai H. and Saiki I.: Differential effect of anti-allergic drugs on IgE-mediated cutaneous reaction in passively sensitized mice. Pharmacology, 60: 97-104, 2000.**

Abstract : We investigated the effect of several anti-allergic agents on murine IgE-mediated biphasic cutaneous reaction. Mice were passively sensitized by an intravenous injection of monoclonal anti-dinitrophenol (anti-DNP) IgE antibody. Skin reaction was elicited by an epicutaneous challenge of dinitrofluoro-benzene (DNFB) and occurred biphasically with immediate phase response(IPR) and late phase response(LPR) at 1 h and 24 h, respectively. Classical histamine H1 receptor antagonists and some chemical mediator-release inhibitors, such as diphenhydramine and terfenadine inhibited IPR, but not LPR. In contrast, Leukotriene B4 (LTB4) receptor antagonist (ONO-4057) inhibited LPR only. Antagonists for LTC4, D4, E4 receptor (ONO-1078) and platelet-activating factor (PAF) receptor (Y-24180) significantly inhibited both IPR and LPR, similarly to prednisolone. We recently found that a third phase inflammatory reaction with marked infiltration of eosinophils (named very late phase response; vLPR) , which is supposed to be a more important reaction in allergic diseases, was induced peaking at day 8 following IPR and LPR in this model. The effect of these drugs on the triphasic skin reaction can be scored based on efficacy against IPR / LPR / vLPR; +/+/+ (prednisolone, a PAF receptor antagonist Y-24180, cyclosporin A and FK-506), +/-/- (diphenhydramine), +/+/- (azelastine and LT receptor antagonist ONO-1078), and -/+/+ (a LTB4 receptor antagonist ONO-4057). Thus, the inhibitory effect on vLPR as well as LPR may relate to the inhibition of eosinophil function mediated by LTB4 and PAF and/or T cells. These findings may provide the basis for a treatment modality using various anti-allergic agents in allergic disease.

3）**Hasegawa H., Lee K-S., Nagaoka T., Tezuka Y., Uchiyama M., Kadota S. and Saiki I.: Pharmacokinetics of ginsenoside deglycosylated by intestinal bacteria and its transformation to biologically active fatty acid esters. Biol. Pharm. Bull., 23: 298-304, 2000.**

Abstract : Ginsenosides are deglycosylated by intestinal bacteria to active forms after oral administration. The present study demonstrated the pharmacodynamics of 20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol (M1), an intestinal bacterial metabolite of ginsenosides, and the in vitro and in vivo antitumor activities of M1-metabolites in comparison with M1 using C57BL/6 mice and Wistar rats. M1 was selectively accumulated into the liver soon after its intravenous administration to mice, and mostly excreted as bile; however, some M1 was transformed to fatty acid ester (EM1) in the liver. EM1 was isolated from rats in a recovery dose of approximately 24 mol%. Structural analysis indicated that EM1 comprised a family of fatty acid mono-esters of M1. Because EM1 was not excreted as bile as M1 was, it was accumulated in the liver longer than M1. Although the cytotoxicity of M1 against B16-F10 melanoma cells was attenuated by fatty acid esterification, EM1 inhibited tumor growth more than M1 in vivo. These results suggest that the fatty acid M1 esters may be the real active principles of ginsenosides in the body.

4）**Yamada T., Tahara E., Nagai H., Terasawa K., Tani T., Nunome S. and Saiki I.: Effect of some Kampo medicine, including Tokaku-joki-to (Tao-He-Cheng-Qi-Tang), on IgE-mediated**

triphasic skin reaction in passively sensitized mice. J. Trad. Med., 17: 17-25, 2000.

Abstract : Previous studies have reported that the mice passively sensitized with anti-DNP IgE antibody exhibited IgE-mediated biphasic cutaneous reaction with an immediate phase response (IPR) at 1 h and a late phase response (LPR) at 24 h after the challenge of DNFB (dinitrofluorobenzene). We recently found that the third phase inflammatory response with intense and persisting infiltration of eosinophils, named very late phase response (vLPR), was induced following IPR and LPR in response to DNFB in passively sensitized mice, and that the peak response of vLPR was on the 8th day after the challenge. The inhibitory effect of Kampo medicines on the triphasic cutaneous inflammatory reaction was divided into several groups in terms of their inhibition rate of ear swelling. Among the formulations, Tokaku-joki-to (Tao-He-Cheng-Qi-Tang) was effective at inhibiting IPR, LPR and vLPR (+/+/+ group) and scratching behavior in IPR. The Inhibitory effect of Tokaku-joki-to on triphasic cutaneous reaction primarily depends on its composed crude drugs, Glycyrrhizae Radix and Cinnamomi Cortex. These findings indicate that Tokaku-joki-to formulation is usefull for the inhibition of cutaneous inflammatory diseases.

5) **Wu W., Yamaura T., Murakami K., Murata J., Matsumoto K., Watanabe H. and Saiki I.: Social isolation stress enhanced liver metastasis of murine colon 26-L5 carcinoma cells by suppressing immune responses in mice. Life Sci, 66: 1827-1838, 2000.**

Abstract : We investigated the effect of social isolation stress on the formation of experimental liver metastasis resulted from intraportal vein (i.p.v.) injection of colon 26-L5 carcinoma cells in male Balb/c mice, and elucidated some of the underlying mechanism involving the effects of this stress on cellular immunity. Increases in the colony number and tumor burden were observed in the mice socially isolated before and/or after tumor cell challenge, as compared with the group-housed mice. In addition, exposure of mice to 2 weeks of preisolation resulted in decreases in the thymus weight and number of thymocytes by 35.8% and 40.2%, respectively, in comparison with the controls. Reduced proliferative response of splenocytes to various stimuli and suppressed splenic NK activity, as well as decreased macrophage-mediated cytotoxicity, were also found in the mice exposed to social isolation. Thus, these results suggest that social isolation stress enhances tumor metastasis in part via its suppressive effect on the immune system of the host.

6) **Banskota A.H., Tezuka Y., Adnyana K.I., Xiong Q., Hase K., Tran K.Q., Tanaka K., Saiki and Kadota S.: Hepatoprotective effect of Combretum quadrangulare and its constituents. Biol. Pharm. Bull., 23: 456-460, 2000.**

7) **Banskota A.H., Tezuka Y., Tran K.Q., Tanaka K., Saiki I. and Kadota S.: Methyl quadrangularates A-D and related triterpenes from Combretum quadrangulare. Chem. Pharm. Bull., 48: 496-504, 2000.**

8) **Murakami K., Matsuura T., Hasumura S., Nagamori S., Yamada Y. and Saiki I.:Involvement of insulin-like growth factor binding protein-3 in the retinoic receptor-a-mediated inhibition of hepatocellular carcinoma cell proliferation. Cancer Lett, 151: 63-70, 2000.**

Abstract : We examined the relationship between the expression of retinoic acid receptor-alpha (RAR-alpha) and upregulation of insulin-like growth factor binding protein-3 (IGFBP-3) in the retinoid-induced inhibition of hepatocellular carcinoma (HCC) cell proliferation. HCC cell lines showed a marked expression of RAR-alpha, whereas the expression levels of RAR-beta and RAR-gamma were relatively lower. An RAR-alpha agonist significantly inhibited the HCC cell proliferation both in vitro and in vivo. The RAR-alpha expression closely related to the upregulation of IGFBP-3 as compared with RAR-beta or RAR-alpha expressions. RAR-alpha agonist would be beneficial to inhibit the growth of HCC.

9) **Takeda K., Hayakawa Y., Van Kaer L., Matsuda H., Yagita H. and Okumura K.: Critical contribution ofliver natural killer T cells to a murine model of hepatitis. Proc. Natl. Acad. Sci. USA, 97: 5498-5503, 2000.**

Abstract : Natural killer T (NKT) cells constitute a distinct subpopulation of T cells with a unique antigen specificity, prompt effector functions, and an unusual tissue distribution. NKT cells are especially abundant in the liver, but their physiological function in this organ remains unclear. In the present study, we examined the possible contribution of NKT cells to a murine model of hepatitis induced by i.v. injection of Con A. CD1-deficient mice lacking NKT cells were highly resistant to Con A-induced hepatitis. Adoptive transfer of hepatic NKT cells isolated from wild-type mice, but not from FasL-deficient gld mice, sensitized CD1-deficient mice to Con A-induced hepatitis. Furthermore, adoptive transfer of hepatic mononuclear cells from wild-type mice, but not from CD1-deficient mice, sensitized gld mice to Con A-induced hepatitis. Upon Con A administration, hepatic NKT cells rapidly up-regulated cell surface FasL expression and FasL-mediated cytotoxicity. At the same time, NKT cells underwent apoptosis leading to their rapid disappearance in the liver. These results implicated FasL expression on liver NKT cells in the pathogenesis of Con A-induced hepatitis, suggesting a similar pathogenic role in human liver diseases such as autoimmune hepatitis.

10) **Takeda K., Oshima H., Hayakawa H., Akiba H., Kobata T., Yagita H. and Okumura K.: CD27-mediated activation of murine NK cells. J. Immunol., 164: 1741-1745, 2000.**

Abstract : CD27, a member of the TNF receptor superfamily, has been implicated in T cell activation, T cell development, and T cell-dependent Ab production by B cells. In the present study we examined the expression and function of CD27 on murine NK cells. Murine NK cells constitutively expressed CD27 on their surface. Stimulation with immobilized anti-CD27 mAb or murine CD27 ligand (CD70) transfectans solely could induce proliferation and IFN-gamma production of freshly isolated NK cells and enhanced the proliferation and IFN-gamma production of anti-NK1.1-sutimulated NK cells. Although NK cell cytotoxicity was not triggered by anti-CD27 mAb or against CD70 transfectants, prestimulation via CD27 enhanced the cytotoxic activity of NK cells in an IFN-gamma-dependent manner. These results suggest that CD27-mediated activation may be involved in the NK cell-mediated innate immunity against virus-infected or transformed cells expressing CD70.

11) **Saiki I.: Review: A Kampo medicine "Juzen-taiho-to" -Prevention of malignant progression and metastasis of tumor cells and the mechanisms of action-. Biol. Pharm. Bull., 23: 677-688, 2000.**

Abstract : Juzen-taiho-to is a Kampo (Japanese and Chinese traditional) medicine, and is a nourishing agent, a so-called "Hozai" (in Japanese), that is used for improving disturbances and imbalances in the homeostatic condition of the body. This drug is administered to patients in various weakened conditions, including post-surgery patients and patients with chronic illnesses, where it can alleviate general symptoms such as extreme fatigue, pale complexion, loss of appetite, dry or scaly skin, night sweating, and dryness of the mouth. Currently, Juzen-taiho-to is often administered to cancer patients, and has been shown to possess various biological activities, such as enhancement of phagocytosis, cytokine induction, antibody production, induction of the mitogenic activity of spleen cells, anti-tumor effects when combined with surgical excision, anti-tumor effects with or without other drugs, and protection against the deleterious effects of anti-cancer drugs as well as radiation-induced immunosuppression and bone marrow toxicity. This article focuses on the antitumor and antimetastatic properties of Kampo formulations and describes the effect of Juzen-taiho-to and related formulations on tumor development, progression and metastasis in vivo. We also discuss the mechanism of the inhibitory action and the importance of the formulation and the constituent drugs in determining the efficacy.

12) **Kurosaki I., Satoh T., Murakami K., Tatsumi T., Mitani N. and Saiki I.: Eotaxin-induced mRNA expression of membrane-type 1 matrix metalloproteinase in human eosinophils. Allergology International, 49: 111-116, 2000.**

Abstract : Eosinophil penetration across the basement membrane (BM) is thought to be dependent on the degradation of membrane components. In this process, matrix metalloproteinases (MMPs) appear to be primarily responsible for degradation of the BM. MMP-2 and MMP-9 degrade type IV collagen, which is a major component of the BM. In the present study, we examined the effects of eotaxin, a selective chemoattractant for eosinophils, on the expressions of mRNA for MMPs. Incubation with chemotactically active concentrations of eotaxin for 24 h enhanced the expression of mRNA for membrane-type 1 MMP (MT1-MMP), but not that for MMP-2 and MMP-9. Increase of protein level of MT1-MMP was also detected in the cell lysate of eotaxin-treated eosinophils. These results suggest that up-regulation of MT1-MMP expression may be involved in the eotaxin-induced penetration of eosinophils.

13) **Nagakawa O., Murakami K., Fujiuchi Y., Murata J., Fuse H. and Saiki I.: Expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) in prostate cancer cell lines. Cancer Lett., 155: 173-179, 2000.**

Abstract : Membrane-type metalloproteinase-1 (MT1-MMP) is a transmembrane metalloproteinase, which activates proMMP-2 and expressed on the cell surface in many invasive cancer cells. We investigated the expression of MT1-MMP in prostate cancer cell lines. MT1-MMP protein and mRNA were expressed in PC-3, DU-145 and TSU-pr1 cells (androgen-independent prostate cancer cell lines), but in LNCaP cells (androgen-dependent prostate cancer cell line). MT1-MMP protein was negative and mRNA was low to detect by RT-PCR. Cell lysate of PC-3 cleaved proMMP-2 to the active form. In addition, both hepatocyte growth factor (HGF) and gastrin-releasing peptide (GRP) increased Matrigel invasion and induced the expression of MT1-MMP protein in DU-145 prostate cancer cells. These results suggest that MT1-MMP is indeed the tumor-specific activator of proMMP-2 in androgen-independent prostate cancer cells and plays an important role in the invasive properties of prostate cancer cells.

14) **Wu W., Yamaura T., Murakami K., Ogasawara M., Hayashi K., Murata J. and Saiki I.: Involvement of TNF-a in the enhancement of the invasion and metastasis of colon26-L5 carcinoma cells by social isolation. Oncol. Res., 11: 461-469, 2000.**

Abstract : Psychosocial stress has been implicated in tumor metastasis. We have previously reported that social isolation stress exacerbated liver metastasis of colon 26-L5 by partially suppressing the cellular immunity in male Balb/c mice. To further understand the mechanism underlying the influence of isolation stress on liver metastasis, we investigated the effect of social isolation stress on tumor invasion, which is considered to be a pivotal step of tumor metastasis. The invasion and migration of tumor cells obtained from tumor nodules in the isolated mice were more markedly enhanced than that in the group-housed mice. The mRNA expression of proteolytic proteases, including matrix metalloproteinase (MMP)-2, MMP-9, membrane type 1 (MTI)-MMP, and urokinase-type plasminogen activator (u-PA), were increased in the tumor and liver tissues of the isolated mice compared with the control mice. On the other hand, production of plasma TNF-alpha and expression of hepatic TNF-alpha mRNA were elevated in the isolated mice with or without tumor burden. Increased TNF-alpha level was particularly discernible in the liver of tumor-bearing mice. Elevated positive staining for TNF-alpha was immunohistochemically observed within and around tumor mass in the liver from isolated tumor-bearing mice, compared with group-housed mice. In addition, the invasiveness of tumor cells and the expression of proteolytic enzymes, including MMP-9 and u-PA in tumor cells, were enhanced by the treatment of TNF-alpha in vitro. Thus, the data suggested that isolation stress-augmented TNF-alpha may be involved in the enhancement of tumor invasion and metastasis in part by upregulating the proteolytic enzymes such as MMPs and u-PA in tumor and liver tissues.

15) **Kusano Y., Oguri K., Nagayasu Y., Munesue S., Ishihara M., Saiki I., Yonekura H., Yamamoto H. and Okayama M.: Participation of syndecan 2 in the induction of stress fiber formation in cooperation with integrin a5b1: Structural characteristics of heparan sulfate chains with avidity to COOH-terminal heparin-binding domain of fibronectin. Exp. Cell Res., 256: 434-444, 2000.**

Abstract : The present study provides direct evidence that syndecan 2 participates selectively in the induction of stress fiber formation in cooperation with integrin alpha5beta1 through specific binding of its heparan sulfate side chains to the fibronectin substrate. Our previous study with Lewis lung carcinoma-derived P29 cells demonstrated that the cell surface heparan sulfate proteoglycan, which binds to fibronectin, is syndecan 2 (N. Itano et al., 1996, Biochem. J. 315, 925-930). We here report that in vitro treatment of the cells by antisense oligonucleotide for syndecan 2 resulted in a failure to form stress fibers on fibronectin substrate in association with specific suppression of its cell surface expression. Instead, localization of actin filaments in the cytoplasmic cortex occurred. A similar response of the cells was observed when the cells were treated to eliminate functions of cell surface heparan sulfates, including exogenous addition of heparin and pretreatment with anti-heparan sulfate antibody, F58-10E4, and with proteinase-free heparitinase I. Size- and structure-defined oligosaccharides prepared from heparin and chemically modified heparins were utilized as competitive inhibitors to examine the structural characteristics of the cell surface heparan sulfates involved in organization of the actin cytoskeleton. Their affinity chromatography on a column linked with a recombinant H-271 peptide containing a C-terminal heparin-binding domain of fibronectin demonstrated that 2-O-sulfated iduronates were essential for the binding. Inhibition studies revealed that a heparin-derived dodecasaccharide sample enriched with an IdoA(2OS)-GlcNS(6OS) disaccharide completely blocked binding of the syndecan 2 ectodomain to immobilized H-271 peptide. Finally, the dodecasaccharide sample was shown to inhibit stress fiber formation, triggered by adhesion of P29 cells to a CH-271 polypeptide consisting of both the RGD cell-binding and the C-terminal heparin-binding domains of fibronectin in a fused form. All these results consistently suggest that syndecan 2 proteoglycan interacts with the C-terminal heparin-binding domain of fibronectin at the highly sulfated cluster(s), such as [IdoA(2OS)-GlcNS(6OS)](6) present in its heparan sulfate chains, to result in the induction of stress fiber formation in cooperation with integrin alpha5beta1.

16) **Ikeda T., Murakami K., Sakukawa R., Hayakawa Y. and Saiki I.: Characterization of a liver metastatic variant of murine K1735M2 melanoma cells. In Vivo, 14: 519-528, 2000.**

Abstract : Intraportal vein injection of highly metastatic K1735M2L5 cells consistently resulted in liver metastases (increases in the number of tumor nodules in the liver), whereas inoculation of K1735M2 cells rarely did so. K1735M2L5 cells invaded the basement membrane Matrigel in greater numbers than did K1735M2 cells, suggesting that the metastatic potential of K1735M2L5 cells is partly related to enhanced invasive properties. The adhesion to Matrigel- and laminin-coated substrates was enhanced in K1735M2L5 cells. The up-regulated expression of VLA-4 and VLA-6 on the surface of K1735M2L5 cells was detected by flow cytometry. The RT-PCR and gelatin zymography study revealed that the secretion of MMP-2 was higher in K1735M2L5 cells than in K1735M2 cells. These results indicate that the invasive ability of K1735M2L5 cells may also be attributed to enhanced gelatinolytic activity as well as adhesiveness. K1735M2L5 cells grew more rapidly than K1735M2 cells and showed fibroblastoid morphology with loose cell-cell contacts as compared with K1735M2 cells. Thus, experimental models using highly metastatic K1735M2L5 cells would be useful for analyzing the molecular mechanism of tumor metastasis and for evaluating the efficacy of treatments for metastases which may have already occured at the time of the diagnosis.

17) **Suda K., Murakami K., Murata J., Hasegawa H. and Saiki I.: An intestinal bacterial metabolite (M1) of ginseng protopanaxadiol saponin inhibits tumor-induced neovascularization. J. Trad. Med., 17: 144-150, 2000.**

Abstract : The present study demonstrated that an intestinal bacterial metabolite (M1) of protopanaxadiol-type ginsenosides significantly inhibited the growth of implanted tumor and the intrahepatic metastasis by the implantation of a small fragment of colon 26-L5 tumor into the liver when it was administered orally. These findings indicates that M1 was effective for the inhibition of the growth and metastasis of colon26-L5 cells in addition to lung metastasis of B16-BL6 melanoma cells as have been reported previously. The conditioned medium of colon 26-L5 cells (CM-L5) induced in vitro tube formation of hepatic sinusoidal endothelial (HSE) cells on Matrigel-coated substrates, which is considered to be an important step in the processes of tumor angiogenesis. This activity of CM-L5 was abrogated by noncytotoxic concentrations of M1 in a concentration-dependent manner. Similarly, M1 eliminated the ability of CM-L5 to promote the migration of HSE cells concentration-dependently. These findings indicate that M1-induced inhibition of tumor growth and intrahepatic metastasis may be partly related to the suppression of tumor angiogenic responses including capillary tube formation and migration of HSE cells.

18) **Yamaura T., Murakami K., Doki Y., Sugiyama S., Misaki T., Yamada Y. and Saiki I.: Solitary lung tumors and their spontaneous metastasis in athymic nude mice orthotopically implanted with human non-small cell lung cancer. Neoplasia., 2: 315-324, 2000.**

Abstract : We examined the tumorigenic and metastatic potentials of three human non-small cell lung cancer (NSCLC) cell lines, PC-14, A549 or Lu-99 cell lines suspended in Matrigel-containing phosphate-buffered saline were orthotopically implanted into the lungs of nude mice. The formation of a solitary tumor nodule in the lung was observed after the implantation of all cell lines. Intrapulmonary implantation of PC-14 or Lu-99 cells resulted in spontaneous distant metastases. In contrast, A549 cells caused multiple intrapulmonary metastases to the right and left lobes of the lung without producing visible lymphatic metastasis. We also investigated the expression of matrix metalloproteinases (MMPs), urokinase-type plasminogen activator (u-PA), u-PA receptor (u-PAR) and c-MET in these cell lines in vitro and in vivo. Reverse transcription polymerase chain reaction (RT-PCR) analysis showed that the expression of MMP-2 and membrane-type 1 MMP (MT1-MMP) was elevated in PC-14 as compared with the other two cell lines. In contrast, stronger expression of c-MET was observed in A549 than in PC-14 or Lu-99. These results indicate that differential patterns of metastasis of lung cancer might be associated with differential expression of metastasis-associated molecules. Our orthotopic implantation models display clinical features resembling those of NSCLC, and may provide a useful basis for lung cancer research.

19) **Takeda K., Hayakawa H., Atsuta M., Hong S., Kaer V.L., Kobayashi K., Ito M., Yagita H. and Okumura K.: Relative contribution of NK and NKT cells to the anti-metastatic activities of IL-12. Int. Immunol., 12: 909-914, 2000.**

Abstract : Conventional T cells, NK cells and NKT cells have been implicated in the anti-tumor activities induced by IL-12. Here we show that IL-12-induced immune responses are partially impaired in T and NKT cell-deficient RAG-2(-/-) mice, and in NKT cell-deficient CD1(-/-) mice. In response to a small dose (<1000 U) of IL-12, RAG-2(-/-) and CD1(-/-) mice demonstrated reduced cytotoxicity, serum IFN-gamma elevation and anti-metastatic activities; in contrast, in response to a high dose (>2000 U) of IL-12, the IL-12-induced immune responses of RAG-2(-/-) and CD1(-/-) mice were indistinguishable from wild-type mice. The defective responses to low-dose IL-12 of RAG-2(-/-) mice were corrected by adoptive transfer of NKT cells but not NK cells. These findings indicate that both NK and NKT cells contribute to the anti-metastatic responses induced by IL-12, and that NKT cells are mostly responsible for the low-dose activities of this cytokine.

20) **Nagao N., Nakayama T., Etoh T., Saiki I. and Miwa N.: Tumor invasion is inhibited by phosphorylated ascorbate via enrichment of intracellular vitamin C and decreasing of oxidative stress. J. Cancer Res. Clin. Oncol., 126: 511-518, 2000.**

Abstract : Tumor metastasis and invasion were shown to be inhibited by the 2-O-phosphorylated form (Asc2P) of L-ascorbic acid (Asc); intact Asc did not inhibit tumor invasion when added once, but appreciably inhibited it upon repeated addition. The anti-metastatic effect is attributable to a marked enrichment of intracellular Asc by Asc2P, subsequently dephosphorylated. Asc2P scavenged most of the intracellular reactive oxygen species (ROSin), and notably inhibited production of matrix metalloproteases and cell motility. ROSin was decreased by Asc2P more markedly than by Asc added once. Thus, involvement of ROSin in tumor invasion and a potent anti-metastatic therapy by ROSin-decreasing agents are suggested.

21) **Hasegawa H. and Saiki I.: Oleoyl triterpene glycoside biotransformed from ginseng suppresses growth and metastasis of murine B16-F10 melanoma via immunostimulation. J. Trad. Med., 17: 186-193, 2000.**

Abstract : The effects of 20 (S)-protopanaxadiol 20-O-b-D-glucopyranoside (M1) and 3-O-oleoyl M1 (OM1) on the growth and metastasis of murine B16-F10 melanoma cells were examined in C57BL/6 mice. A single co-injection of M1 (5 mg/kg) with B16-F10 cells into the liver inhibited tumor growth at the inoculation site by 23% (not significant compared to untreated control). In contrast, the same dosage of OM1 caused a 2.6-fold suppression of tumor growth, compared with M1 treatment ($p<0.02$). Concerning the pharmacokinetics, both M1 and OM1 were selectively taken up into the liver soon after i.v. administration (30 mg/kg). Thereafter, M1 was cleared immediately from the liver; however, OM1 was retained in the liver at a level of more than 25% of the administered dose for 24h after administration. Thus, the antitumor activity paralleled the pharmacokinetic behavior. Moreover, three consecutive i.v. administrations of OM1 (30 mg/kg) inhibited the liver metastasis produced by intrasplenic inoculation of B16-F10 cells by 95%. OM1 did not directly affect tumor growth *in vitro*, whereas it promoted tumor cell lysis by lymphocytes, particularly non-adherent splenocytes, in a concentration-dependent manner. These results suggest that fatty acid esterification of M1 potentiates the antitumor activity of the parental M1 through delay of the clearance and through immunostimulation.

22) **Muraishi Y., Mitani N., Yamaura T., Fuse H. and Saiki I.: Effect of interferon-a A/D in combination with the Japanese and Chinese traditional herbal medicine Juzen-taiho-to on lung metastasis of murine renal cell carcinoma. Anticancer Res., 20: 2931-2938, 2000.**

Abstract : Several studies have shown that the Kampo medicine Juzen-taiho-to (Si-Quan-Da-Bu-Tang in Chinese) has various biological activities, including anti-tumor effects when combined with surgical excision or with chemotherapeutic drugs. Here we investigated the effect of combined therapy with interferon (IFN)-alpha A/D and Juzen-taiho-to on experimental lung metastasis of murine renal cell carcinoma (Renca) cells. Five consecutive administrations of IFN-alpha A/D to Renca-bearing mice resulted in dose-dependent inhibition of lung metastasis. IFN-alpha A/D at the dose of 100,000 IU/mouse significantly inhibited the metastasis, but a marked loss of body weight was observed during and after the administration. In contrast, oral administration of Juzen-taiho-to (50 mg/-mouse) alone tended to inhibit the metastasis, but the effect was not statistically significant. The combination treatment of suboptimal doses of IFN-alpha A/D and Juzen-taiho-to markedly augmented the antimetastatic effect without causing any loss of body weight, as compared with either treatment alone. Similar results were also obtained by treatment with IFN-gamma in combination with Juzen-taiho-to. Clinically, immunotherapy with IFNs has been primarily approved for the treatment of patients with metastatic renal cell carcinoma, but sufficient efficacy has not yet been obtained. Therefore, the combination of IFNs with Juzen-taiho-to may provide a means to increase the therapeutic potential of IFNs and to decrease their toxicity for the treatment of metastatic renal cell carcinoma.

23) **Suda K., Murakami K., Hasegawa H. and Saiki I.: Induction of apoptosis in Lewis lung carcinoma cells by an intestinal bacterial metabolite produced from orally administered ginseng protopanaxadiol saponins. J. Trad. Med., 17: 236-244, 2000.**

Abstract : The present study demonstrated that oral administration of an intestinal bacterial metabolite (M1) of protopanaxadiol-type saponin significantly inhibited the tumor growth at the implantation site after intrapulmonary implantation of Lewis lung carcinoma (LLC) cells, and tended to suppress the metastasis to mediastinal lymph nodes. We also investigated the inhibitory mechanism of M1 on the growth of LLC cells. M1 inhibited the proliferation of LLC cells in a concentration-dependent manner, with characteristic morphological changes at the concentration of 30 μM. Treatment of LLC cells with M1 resulted in marked elevation of the caspase-3 activity, peaking at 2 h, and a subsequent time-dependent induction of apoptosis during the period from 3 to 24 h, as evidenced by DNA fragmentation analysis. Since M1-induced growth inhibition of LLC cells was completely abrogated by the pretreatment with a specific inhibitor of caspase-3, Z-DEVD-FMK, M1 functions via the activation of caspase-3 in the process of apoptosis in LLC cells. Thus, the anti-proliferative activity of M1 against LLC cells is primarily due to the induction of apoptosis via promotion of caspase-3 activity, and this induction may lead to the anti-tumor activity *in vivo*.

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