Review

MOLECULAR MARKERS OF PERITONEAL DISSEMINATION IN GASTRIC CANCER

KIYOMU FUJII, HIROKI KUNIYASU
Department of Molecular Medicine, Nara Medical University
Received November 8, 2011

Abstract : Peritoneal dissemination is a special condition of gastric cancer metastasis, which worsens the quality of patients' lives and is often difficult to control. In this study, we describe the basic features of the peritoneal dissemination of gastric cancer. Some molecular events are associated with the peritoneal dissemination, including c-met gene amplification, chromosome 7q deletion, and Reg IV overexpression. Especially, Reg IV overexpression enhances the antiapoptotic property of gastric cancer cells. In contrast, activation of PPARg inhibits the peritoneal dissemination by the pro-apoptotic effect.

Key words : gastric cancer, peritoneal metastasis, 7q LOH, Reg IV

INTRODUCTION

Gastric cancer is a leading cause of cancer death in the world, and the second most common cause of cancer death in Japan[1, 2]. One fifth of gastric cancer patients showed peritoneal metastasis and/or liver metastasis at the operation[3] and 30% of patients who died from gastric cancer suffered from peritoneal metastasis[4]. Peritoneal metastasis causes terminal stage of advanced gastric cancer, and diminishes quality of patients' life by intestinal obstruction, ascites, and malnutrition. Control of peritoneal metastasis is expected to make the quality of patients' life better[5, 6]. To understand the mechanism of the peritoneal metastasis of gastric cancer is essential for treatment of the condition.

The molecular mechanism of peritoneal metastasis is an ongoing assignment of cancer research. We have identified loss of heterozygosity of chromosome 7q involving 7q35 locus as a peritoneal metastasis-associated event in gastric cancer[7]. Overexpression of angiogenic factors, such as vascular endothelial growth factor (VEGF) and interleukin (IL)-8, is associated with peritoneal metastasis formation and the ascites production in ovarian cancer[8]. Truncated form of fibroblast growth factor/keratinocyte growth factor receptor 2 IIIb (K-sam) and c-met show gene amplification and/or overexpression in scirrhous type gastric cancer, which frequently produces peritoneal metastasis[9-11]. A gene expression profiling shows alteration of several gene expressions in peritoneal metastasis of scirrhous type gastric cancer, such as up-regulation of trefoil factor 1, α-1-antitrypsin, and galectin 4, and down-regulation of cytidine deaminase[12]. Cell-to-cell adhesion between cancer cells and peritoneal mesothelial cells is thought to be an initial step of peritoneal metastasis. Expressions of CD44, β1 integrin, intercellular adhesion molecule-1 play a role in cancer cell adhesion to mesothelial cells[13-14]. The importance of survival factors in peritoneal metastasis

formation is emphasized in many reports; RUNX3, survivin, nuclear factor κB, Bcl-2/Bag are associated with peritoneal metastasis[15-18].

c-met

The scirrhous type gastric cancer, which frequently produces peritoneal metastasis, shows the gene amplification and overexpression of *c-met* oncogene[9, 10].

A variety of human cancers express multi-autocrine loops of growth factor/receptor system such as EGF, TGF-α and TGF-β, which evidently play a crucial role in tumor progression[19, 20]. In gastric carcinomas, many growth factors are frequently overexpressed without gene amplification, while growth factor receptor type genes such as ERBB (EGF receptor), ERBB2[21, 23] and K-sam (FGF receptor)[22] are often amplified. The amplification of the receptor genes closely correlates with tumor metastasis[23].

An oncogene, met, was initially identified in NIH3T3 cells transfected with DNA from human osteosarcoma cell line (HOS) transformed with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)[24, 25]. Subsequent analyses on the proto-form of this gene revealed that 4.2kb of *c-met* cDNA encoded a receptor type tyrosine kinase with a molecular mass of 145KDa[26, 27]. *c-met* product is revealed to be a hepatocyte growth factor (HGF) receptor consisting of α- and β-subunits[28,29]. These subunits are linked by two disulfide bonds to make an insulin receptor-like structure[30,31]. Moreover, HGF has been found to be identical with scatter factor or lung fibroblast-derived mitogen[32-34].

We examined the amplification of the c-met gene in human esophageal, gastric and colorectal cancers[9]. Six (55%) of the 11 gastric cancer cell lines and 15 (23%) of the 64 advanced gastric cancers show the *c-met* gene amplification. Among them, c-met amplification is detected in 5 gastric cancer cell lines, derived from scirrhous gastric cancer and in 5 (38%) of 13 scirrhous gastric cancer. Scirrhous type stomach cancer corresponds to diffusely infiltrating carcinoma or Borrmann's type IV carcinoma of the stomach showing vast fibrous stroma with rapid and extensive growth. Previous studies have shown the specific genetic features in scirrhous carcinoma. *K-sam* gene amplification preferentially occurs in this type of stomach cancer[22]. Simultaneous overexpression of PDGF and PDGFR mRNA is also detected in stomach cancers associated with plentiful fibrous stroma[35]. TGF-β and basic FGF are also overexpressed in scirrhous type stomach carcinomas[36, 37]. Since *c-met* product is the receptor protein for HGF [28, 29], gastric cancer cells with c-met amplification should be responsible for mitogenic[34, 38, 39] and motogenic[32, 33] properties of HGF. In view of recent evidence that HGF is mainly produced by fibroblasts[32, 33], HGF from fibroblast may bind to *c-met* protein on carcinoma cells, leading to DNA synthesis and migration of tumor cells in scirrhous cancer. In fact, we have confirmed that fibroblast cell line ST-fib obtained from the stomach expresses high levels of HGF mRNA and then secretes a large amount of HGF into the culture media.

The *c-met* gene rearrangement accompanied with gene amplification is not found. In structural study on chromosome 7q using 5 RFLP markers, gene amplification is detected only on *p-met*H, 3'-end probe and *p-met*D[9], upstream probe of *c-met* gene[40]. No amplification is found in three other probes located at telomeric side of c-met gene on the long arm of chromosome 7. Therefore, *c-met* gene amplification occurs on a relatively small range of

chromosome 7 without major rearrangement. Amplification of the *c-met* gene is found in one of paired alleles in all the cases by southern blot analysis[9]. Another allele was not amplified or deleted. It had been reported that GTL-16 gastric carcinoma cell line with the c-met gene amplification[31] has an amplicon including the *c-met* gene region without rearrangement nor mutation[41].

Patients of gastric cancer with c-met amplification show significantly advanced tumor stage and poorer prognosis than those without the amplification. Conversely, no amplification is detected in any of the esophageal and colorectal cancer cell lines as well as carcinoma tissues except one colonic cancer. As to clinical features, c-met amplification was closely related to tumor progression. That is, advanced gastric carcinomas (stage III and IV) shared frequent amplification of *c-met*, whereas stage I gastric carcinomas had no amplification. Furthermore, patients with c-met amplification showed significantly worse prognosis than those without the amplification.

These results overall suggest that amplification of the c-met gene might be implicated in carcinogenesis and progression of stomach cancer, especially scirrhous type stomach carcinoma. An aberrant shorter transcript of c-Met is also closely associated with progression of gastric cancer[10]. The normal transcript of the *c-met* gene is 7.0 kb ; in contrast, most gastric cancer cell lines and advanced cancer cases show a 6.0-kb ; in addition to the 7.0-kb transcript. Expression of this 6.0 kb-transcript is closely correlated with tumor stage, lymph node metastasis, and depth of tumor invasion[4]. Park et al. reported a variant of smaller molecular weight of c-Met, which is not confirmed as the protein product of the 6.0 kb transcript.

Chromosome 7q deletion

The development and progression of human cancer are considered to occur as results of accumulation of genetic alteration in a variety of protooncogenes and tumor suppressor genes[42]. Frequent loss of heterozygosity (LOH) on chromosomes 1q, 5q and 17p is reported in gastric cancer[43]. Chromosomes 5q and 17p are well known as the APC and p53 loci.

On the long arm of chromosome 7, c-met gene and human hepatocyte growth factor (hHGF) gene are located. Sano et al. also reported frequent allele loss (39%) on 7q at D7S22 locus in well differentiated gastric cancer[43]. We examined the LOH on the long arm of chromosome 7 using five polymorphic marker probes (D7S95, *c-met*, D7S63, D7S22, and D7S64) in 98 gastric cancers[7]. Twenty-six cases (32%) of 82 informative cases show LOH on 7q on at least one locus of five loci. Among five loci, LOH at D7S95 locus is most frequent, the incidence being 53% in well differentiated gastric cancers and 33% in poorly differentiated and scirrhous gastric cancers, respectively. At three loci, *c-met*, D7S63 and D7S22, the incidence of LOH is about 30% and 10% in well differentiated and poorly differentiated gastric carcinoma cases, respectively. In contrast, LOH at D7S64 is not detected in any gastric carcinoma cases. Deletion mapping of 7q reveals that D7S95 locus is the essential region of LOH. Two types of gene alteration, amplification and deletion, rarely occur in a single gene locus. Of 98 cases examined, 20 reveal the *c-met* gene amplification. However, all these cases are completely different from 26 cases with LOH on 7q. Furthermore, amplification is detected only by *p-met*H and *p-met*D probes, whereas no

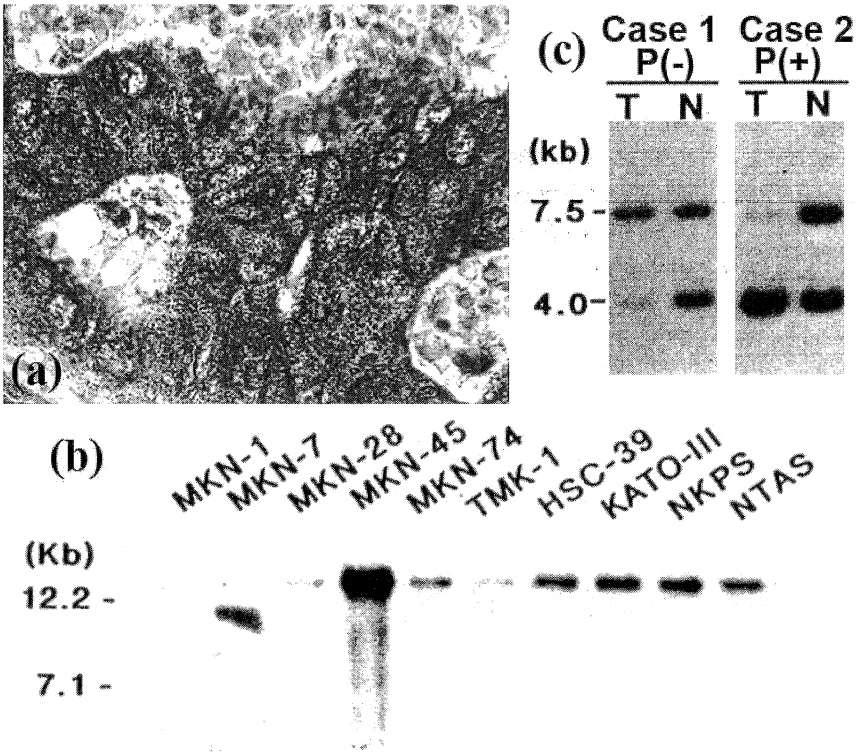

Fig. 1. Expression and gene alteration of c-met in gastric cancer.
(a) Overexpression of c-met protein in peritoneal metastasis of gastric cancer. (b) Gene amplification of c-met in gastric cancer cell lines. MKN-45 cells are derived from poorly differentiated adenocarcinoma. HSC-39, KATO-III, NKPS, and NTAS cells are derived from scirrhous type adenocarcinomas with peritoneal dissemination. (c) Loss of heterozygosity of chromosome 7q in gastric cancer. P(−) case 1 is negative for peritoneal dissemination. P(+) case 2 is positive for peritoneal dissemination.

amplification is observed at loci of D7S22, D7S63, D7S95 and D7S64, respectively. Therefore, the amplification is considered to be a different event from LOH on 7q and the amplicon may be localized in relative short range around *c-met* locus, which is congruent with the literature [41].

Our examination shows that several cases show wide ranges of the LOH at the neighboring 2 to 4 loci[7]. Previous cytogenetic studies have demonstrated the deletion on 7q in gastric cancer[44-45]. The precise mapping of the deletion on 7q has been reported as an interstitial deletion of the distal portion of 7q in leukemia cells. The proximal breakpoint of the interstitial deletion is determined at 7q22[46]. In our study, gastric cancers show no deletion at D7S64 (7q21) in contrast to frequent LOH at the c-met locus (7q31). These results suggest that deletion on 7q in gastric carcinomas might have a similar breakpoint to leukemia. However, since the most telomeric locus, D7S22, is deleted in some cases, an interstitial deletion of 7q is not common in gastric cancer.

In our study, 8 cases (62%) of 13 cases with LOH at D7S95 locus belong to stage IV[4].

Furthermore, 6 cases (75%) of 8 cases with abdominal dissemination show LOH at D7S95. Therefore, cases with LOH at D7S95 show significantly worse prognosis than the cases without LOH in stage III and IV groups. These findings suggest that the deletion at D7S95 locus is closely correlated with tumor progression, especially with peritoneal dissemination of gastric cancer. In some previous literature, LOH on 7q is also associated with rapid progression of leukemia[47] or aggressive tumor status and worse prognosis in breast cancer[48]. D7S95 locus may contain candidate suppressor gene for tumor progression. Detection of LOH at D7S95 may bring clinical prediction for indicator of high malignancy and may make it possible to treat it preventively in advance.

Reg IV

Reg (regenerating) gene family belongs to the calcium-dependent lectin superfamily[49, 50]. *Reg IV* is a new member of the family, which is identified as a gene expressed in the gastrointestinal tracts and pancreas[51, 52]. Human *Reg IV* gene is located on chromosome 1, unlike other *Reg* family genes, which are located on 2p12[53]. *Reg IV* is expressed in Crohn's disease and ulcerative colitis[51, 54]. *Reg IV* is revealed to be associated with malignant potential of colorectal adenocarcinomas, and malignant transformation of colorectal adenomas[55-57]. We revealed that *Reg IV* also associated with malignant potentials of salivary gland cancer[58] and prostate cancer[59]. Recently, *Reg IV* is reported to activate epidermal growth factor receptor (EGFR), protein kinase B/Akt, and activator protein (AP)-1 to accelerate colorectal cancer cell survival by increasing Bcl-2, Bcl-XL, and survivin[60]. Anti-apoptotic property of *Reg IV* is associated with colorectal cancer development[61] and drug resistance in gastric cancer[62]. *Reg IV* expression is expected to be a marker for high malignant potential of cancer[63-65].

We have identified *Reg IV* as a cancer-affiliated expressed gene by serial analysis of gene expression (SAGE) technique, which results were deposited to NCBI SAGE Library in the web (http://www.ncbi.nlm.nih.gov/SAGE/). *Reg IV* protein is immunohistochemically detected in 36% of colorectal adenocarcinomas, which is associated with tumor stage, whereas *Reg IV* production is detected in 29% of gastric cancers, which is associated with both the intestinal mucin phenotype and neuroendocrine differentiation but not with tumor stage or patient prognosis[66]. Thus, the role of *Reg IV* in gastric cancer is still unclear.

In our examination, increase of expression and secretion of *Reg IV*, and levels of BCL-2, BCL-XL, survivin, phosphorylated AKT, and phosphorylated EGFR, and decrease of nitric oxide-induced apoptosis are found in *Reg IV*-transfected gastric cancer cells, whereas those are abrogated in the knockdown cells[67]. Although nitric oxide (NO) is a strong apoptotic inducer[68], the increment of anti-apoptotic factors reduced NO-induced cytotoxicity in these cells. The anti-apoptotic property of *Reg IV* is reported in several studies, which endows cancer cells with advantages for survival, progression, and metastasis[54, 60, 62, 69].

In mice models, increased number and size of peritoneal tumors and decreased apoptosis are found in *Reg IV*-transfectants, whereas those are abrogated by the knockdown cells. Proliferative activity of the transfectant tumors is not different from that of the control cell tumor, whereas the transfectant tumors show reduced necrosis and apoptosis in comparison with control cell tumors (Fig. 2). These findings suggest that anti-apoptotic property of *Reg IV* renders more pronounced potential for peritoneal metastasis to gastric cancer cells. The

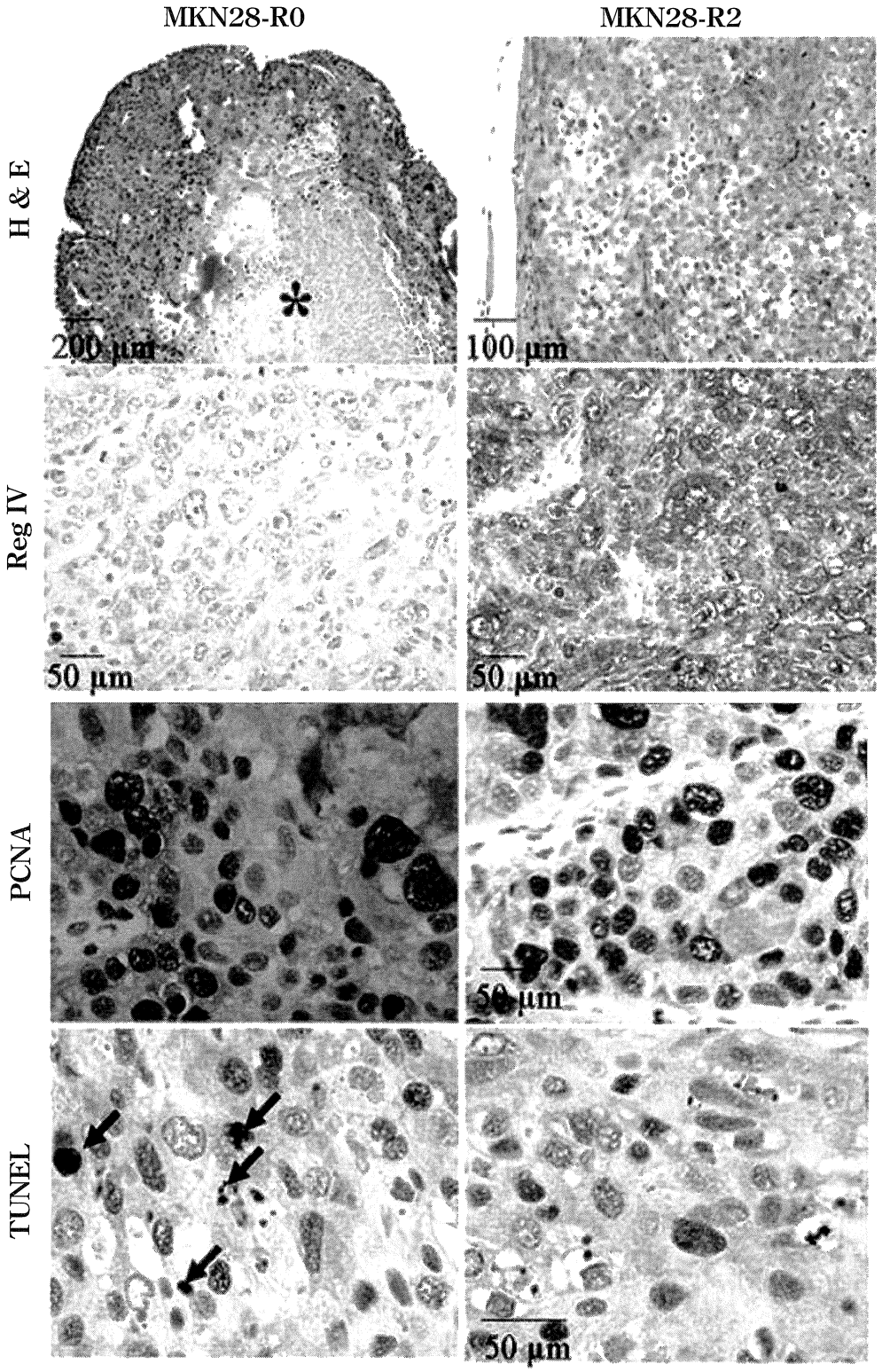
MKN28-R0
MKN28-R2
H & E
Reg IV
PCNA
TUNEL
200 μm
100 μm
50 μm
50 μm
50 μm
50 μm

Fig. 2

anti-apoptotic property of cancer cells is emphasized for formation of peritoneal metastasis[15-18]. Moreover, remarkable progressions of metastatic tumors worsen survival rates in mice inoculated with the *Reg IV*-transfected gastric cancer cells than those in mice inoculated with the control cells. In contrast, *Reg IV* knockdown significantly suppresses peritoneal metastasis of gastric cells.

Because *Reg IV* is a secretory small protein[70], detection of *Reg IV* protein in ascites might be expected as a marker for peritoneal metastasis. In our study, the levels of *Reg IV* protein in peritoneal lavage fluids increase in *Reg IV*-transfectants inoculated mice, but decrease in *Reg IV*-knockdown cell inoculated mice[67]. *Reg IV* protein was detected in all cases with macroscopical and cytological peritoneal metastasis. Moreover, all cases with keratin mRNA-positive ascites showed *Reg IV* protein in ascites[67]. Ascites keratin detected by RT-PCR is a sensitive marker for scanty cancer cells in ascites in cytologically metastasis-negative cases [71]. These findings suggest that ascites *Reg IV* might be a sensitive marker for peritoneal metastasis of gastric cancer. *Reg IV* is expected to be a marker for early detection of peritoneal metastasis and a prognostic marker for gastric cancer. In colon cancer, serum *Reg IV* levels are also associated with liver metastasis at the operation and even with recurrence with liver metastasis[57].

PPARγ

Recently, we reported the importance of activation of peroxisome proliferator-activated receptor (PPAR) g in suppression of peritoneal metastasis, which provides tumor growth inhibition and apoptosis induction in gastric and colon cancer cells[72, 73]. As described above, anti-apoptotic property is important in establishing peritoneal dissemination. PPARγ activation induces apoptosis in cancer cells, which is relevant to suppress peritoneal dissemination.

PPARγ is originally identified to induce adipocyte differentiation[74]. PPARg is a nuclear hormone receptor superfamily of ligand-activated transcription factors[75]. PPARγ is dimerized with retinoic X receptor, and binds specific responsive element within promoter DNA sequence to regulate gene expression[76, 77]. PPARγ initiates transcription of genes associated with energy homeostasis, cell growth, and anti-/pro-inflammatory effect[75, 77-80]. PPARγ is activated by endogenous secreted prostaglandins and fatty acids. 15-deoxy-d(12, 14)-prostaglandin J2 is a strong endogenous ligand of PPARγ. PPARγ possesses an anti-carcinogenic effect in colon cancer. Synthesized PPARγ agonists including troglitazon have been shown to be effective chemopreventive agents in a rat model of carcinogenesis and in AOM-induced colon cancer in mice[81]. Moreover, a decrease in PPARγ expression is associated with cancer metastasis[82, 83]. PPARγ play a role in transcriptional regulation of cancer-related

Fig. 2. Effects of Reg IV transfection on peritoneal dissemination of MKN28 human gastric cancer cells. MKN28 cells transfected with control vector (MKN28-R0) or Reg IV expression vector (MKN28-R2) were inoculated into the peritoneal cavity of nude mice. H&E staining showed the large central necrosis in MKN28-R0 tumor but not in MKN28-R2 tumor. Reg IV protein production was found in only MKN28-R2 tumor. PCNA labeling indices of MKN28-R0 and MKN28-R2 tumors were not significantly different. In contrast, apoptotic cells were significantly increased in MKN28-R0 tumor (arrow; apoptotic cells) in comparison with MKN28-R2 tumor.

genes. PPARγ downregulates epithelial growth factor receptor (EGFR), and upregulates Bax, p21Waf-1, and E-cadherin, which are associated with anti-proliferative, pro-apoptotic, and pro-differentiation effects[84-87]. We previously reported that PPARγ induces EGFR and TGF-a expression by CLA and LA treatment in cancer cells[72, 73]. These alterations collectively provide an anti-metastatic effect on cancer cells. Inhibitory effect of PPARγ to cancer metastasis is reported in several cancers, such as non-small cell lung cancer, colon cancer, thyroid cancer, and breast cancer[76, 87-89].

We used CLA as a PPARγ ligand to inhibit peritoneal metastasis[72, 90]. CLA treatment significantly decreases metastatic foci of both cells in the peritoneal cavity[72]. Survival rate in mice inoculated with gastric cancer cells is significantly recovered by CLA treatment. Protein production in gastric cancer cells treated with CLA shows a decrease in EGFR and TGF-a and an increase in Bax. Our results show that CLA inhibits cell growth and invasion, and induces apoptosis in cancer cells. Moreover, CLA inhibited cancer cell colonization in the peritoneal cavity and decreased death rates of cancer-burdened mice. We also reported the tumor suppressive effect of CLA on established peritoneal tumors using a syngeneic mouse peritoneal metastasis model[90]. In CLA-treated tumors, proliferating cells are decreased, whereas apoptotic cells are increased.

Control of peritoneal metastasis is important in improving disease outcome and quality of the patient's life. We hope that the recent advances in basic research on the mechanism of peritoneal dissemination described above will contribute to establishing tactics to control peritoneal dissemination.

REFERENCES

1) **Jamal, A., Murray, T., Samuels, A., Ghafoor, A., Ward, E.** and **Thun, M., J.** : Cancer statistics, 2003. CA Cancer J Clin **53** : 5–26, 2003.

2) National Cancer Center Cancer Information Service (editor) : Cancer Statistics. On line (www.ncc.go.jp/jp/statistics/2001/index-j.html), 2001.

3) **Maehara, Y., Moriguchi, S., Kakeji, Y., Kohnoe, S., Korenaga, D., Haraguchi, M.** and **Sugimachi, K.** : Pertinent risk factors and gastric carcinoma with synchronous peritoneal dissemination or liver metastasis. Surgery **110** : 820–823, 1991.

4) **Moriguchi, S., Maehara, Y., Korenaga, D., Sugimachi, K.** and **Nose, Y.** : Risk factors which predict pattern of recurrence after curative surgery for patients with advanced gastric cancer. Surg Oncol **1** : 341–346, 1992.

5) **Sugarbaker, P., H., Yu, W.** and **Yonemura, Y.** : Gastrectomy, peritonectomy, and perioperative intraperitoneal chemotherapy: the evolution of treatment strategies for advanced gastric cancer. Semin Surg Oncol **21** : 233–248, 2003.

6) **Vogel, I.,** and **Kalthoff, H.** : Disseminated tumour cells. Their detection and significance for prognosis of gastrointestinal and pancreatic carcinomas. Virchows Arch **439** : 109–117, 2001.

7) **Kuniyasu, H., Yasui, W., Yokozaki, H., Akagi, M., Akama, Y., Kitahara, K., Fujii, K., Tahara, E.** : Frequent loss of heterozygosity of the long arm of chromosome 7 is closely associated with progression of human gastric carcinomas. Int J Cancer **59** : 597–600, 1994.

8) **Yoneda, J., Kuniyasu, H., Crispens, M., A., Price, J., E., Bucana, C., D., Fidler, I., J.** : Expression of

angiogenesis-related genes and progression of human ovarian carcinomas in nude mice. J Natl Cancer Inst **90** : 447-454, 1998.

9) **Kuniyasu, H., Yasui, W., Kitadai, Y., Yokozaki, H., Ito, H., Tahara, E.** : Frequent amplification of the c-met gene in scirrhous type stomach cancer. Biochem Biophys Res Commun **189** : 227-232, 1992.

10) **Kuniyasu, H., Yasui, W., Yokozaki, H., Kitadai, Y., Tahara, E.** : Aberrant expression of *c-met* mRNA in human gastric carcinomas. Int J Cancer **55** : 72-75, 1993.

11) **Takada, M., Koizumi, T., Toyama, H., Suzuki, Y., Kuroda, Y.** : Differential expression of RAGE in human pancreatic carcinoma cells. Hepatogastroenterol **48** : 1577-1578, 2001.

12) **Hippo, Y., Yashiro, M., Ishii, M., Taniguchi, H., Tsutsumi, S., Hirakawa, K., Kodama, T., Aburatani, H.** : Differential gene expression profiles of scirrhous gastric cancer cells with high metastatic potential to peritoneum or lymph nodes. Cancer Res **61** : 889-895, 2001.

13) **Alkhamesi, N., A., Ziprin, P., Pfistermuller., K., Peck, D., H., Darzi., A., W.** : ICAM-1 mediated peritoneal carcinomatosis, a target for therapeutic intervention. Clin Exp Metastasis **22** : 449-459, 2005.

14) **Skubitz, A., P.** : Adhesion molecules. Cancer Treat Res **107** : 305-329, 2002.

15) **Sakakura, C., Hasegawa, K., Miyagawa, K., Nakashima, S., Yoshikawa, T., Kin, S., Nakase, Y., Yazumi, S., Yamagishi, H., Okanoue, T, Chiba T, Hagiwara, A.** : Possible involvement of RUNX3 silencing in the peritoneal metastases of gastric cancers. Clin Cancer Res **11** : 6479-6488, 2005.

16) **Tsuburaya A, Noguchi Y, Yoshikawa T, Saito A, Doi C, Okamoto T, Fukuzawa K** : An anti-apoptosis gene, survivin and telomerase expression in gastric cancer. Hepatogastroenterology **49** : 1150-1152, 2002.

17) **Sasaki N, Morisaki T, Hashizume K, Yao T, Tsuneyoshi M, Noshiro H, Nakamura K, Yamanaka T, Uchiyama A, Tanaka M, Katano M** : Nuclear factor-κB p65 (RelA) transcription factor is constitutively activated in human gastric carcinoma tissue. Clin Cancer Res **7** : 4136-4142, 2001.

18) **Yawata A, Adachi M, Okuda H, Naishiro Y, Takamura T, Hareyama M, Takayama S, Reed JC, Imai K** : Prolonged cell survival enhances peritoneal dissemination of gastric cancer cells. Oncogene **16** : 2681-2686, 1998.

19) **Yasui W, Hata J, Yokozaki H, Nakatani H, Ochiai A, Ito H, Tahara E** : Interaction between epidermal growth factor and its receptor in progression of human gastric carcinoma. Int J Cancer **41** : 211-217, 1988.

20) **Tahara, E.** : Growth factors and oncogenes in human gastrointestinal carcinomas. J Cancer Res Clin Oncol **116** : 121-131, 1990.

21) **Yoshida, K., Tsuda, T., Matsumura, T., Tsujino, T., Hattori, T., Ito, H., Tahara, E.** : Amplification of epidermal growth factor receptor (EGFR) gene and oncogenes in human gastric carcinomas. Virchows Arch B **57** : 285-290, 1989.

22) **Hattori, Y., Odagiri, H., Nakatani, H., Miyazawa, K., Naito, K., Sakamoto, H., Katoh, O., Yoshida, T., Sugimura, T., Terada, M.** : K-sam, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. Proc Natl Acad Sci U S A **87** 5938-5987, 1990.

23) **Tsujino, T., Yoshida, K., Nakayama, H., Ito, H., Shimosato, T., Tahara, E.** : Alterations of oncogenes in metastatic tumours of human gastric carcinomas. Br J Cancer **62** : 226-230, 1990.

24) **Cooper, C., S., Park, M., Blair, D., G., Tainsky, M., A., Heubner, K., Croce, C., M., Vande Woude, G., F.** : Molecular cloning of a new transforming gene from a chemically transformed human cell line. Nature **311** : 29-33, 1984.

25) **Dean, M., Park, M., Le Beau, M., M., Robins, T., S., Diaz, M., O., Rowley, D., Blair, D., G.** and **Vande Woude, G., F.** : The human met oncogene is related to the tyrosine kinase oncogenes. Nature **318** : 385-388, 1985.

26) **Park, M., Dean, M, Kaul, K., Braun, M., J., Gonda, M., A, Vande Woude GF** : Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors.

Proc Natl Acad Sci U S A **84** : 6379–6383, 1987.

27) **Chan, A., M., King, H., W., S., Tempest, P., R., Deakin, E., A., Cooper, C., S.** and **Brookes, P.** : Primary structure of the met protein tyrosine kinase domain. Oncogene **1** : 229–233, 1987.

28) **Naldini, L., Vigna, E., Narsimhan, R., P., Gaudino, G., Zarnegar, R., Michalopoulos, G., K.** and **Comoglio, P., M.** : Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET. Oncogene **6** : 501–504, 1991.

29) **Bottaro, D., P., Rubin, J., S., Faletto, D., L., Chan A., L., Kmiecik, TE, Vande Woude, G., F.** and **Aaronson, S., A.** : Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science **251** : 802–804, 1991.

30) **Tempest, P., R., Stratton, M., R.** and **Cooper, C., S.** : Structure of the met protein and variation of met protein kinase activity among human tumour cell lines. Br J Cancer **58** : 3–7, 1988.

31) **Giordano, S., Ponzetto, C., Di Renzo, M., F., Cooper, C., S.** and **Comoglio, P., M.** : Tyrosine kinase receptor indistinguishable from the c-met protein. Nature **339** : 155–156, 1989.

32) **Weidner, K., M., Arakaki, N., Hartmann, G., Vandekerckhove, J., Weingart, S., Rieder, H., Fonatsch, C,. Tsubouchi H, Hishida T, Daikuhara Y** and **Birchmeier W**: Evidence for the identity of human scatter factor and human hepatocyte growth factor. Proc Natl Acad Sci USA **88** : 7001–7005, 1991.

33) **Konishi, T., Takahara, T., Tsuji, T., Ohsato, K., Matsumoto, K.** and **Nakamura, T.** : Scatter factor from human embryonic lung fibroblasts is probably identical to hepatocyte growth factor. Biochem Biophys Res Commun **180** : 765–773, 1991.

34) **Rubin, J., S., Chan, A., M., Bottaro, D,. P., Burgess, W., H., Taylor, W., G., Cech, A., C., Hirschfield, D., W., Wong, J., Miki., T., Finch, P., W.** and **Aaronson, S., A.** : A broad-spectrum human lung fibroblast-derived mitogen is a variant of hepatocyte growth factor. Proc Natl Acad Sci U S A **88** 415–419, 1991.

35) **Tsuda, T., Yoshida, K., Tujino, T., Nakayama, H., Kajiyama, G., Tahara, E.** : Coexpression of platelet-derived growth factor (PDGF) A-chain and PDGF receptor genes in human gastric carcinomas. Jpn J Cancer Res **80** : 813–817, 1989.

36) **Yoshida, K., Yokozaki, H., Niimoto, M., Ito, H., Ito, M.** and **Tahara, E.** : Expression of TGF-– and procollagen type I and type III in human gastric carcinomas. Int J Cancer **44** : 394–398, 1989.

37) **Tanimoto, H., Yoshida, K., Yokozaki, H., Yasui, W., Nakayama, H., Ito, H., Ohama, K,** and **Tahara E** : Expression of basic fibroblast growth factor in human gastric carcinomas. Virchows Arch B **61** : 263–267, 1991.

38) **Nakamura, T., Teramoto, H.** and **Ichihara, A.**: Purification and characterization of a growth factor from rat platelets for mature parenchymal hepatocytes in primary cultures. Proc Natl Acad Sci U S A **83** : 6489–6493, 1986.

39) **Igawa, T., Kanda, S., Kanetake, H., Saitoh, Y., Ichihara, A., Tomita, Y.** and **Nakamura, T.** : Hepatocyte growth factor is a potent mitogen for cultured rabbit renal tubular epithelial cells. Biochem Biophys Res Commun **174** : 831–838, 1991.

40) **Park, M., Dean, M., Cooper, C., S., Schmidt, M., O'Brien, S., J., Blair, D., G.** and **Vande Woude., G., F.** : Mechanism of met oncogene activation. Cell **45** : 895–904, 1986.

41) **Ponzetto, C., Giordano, S., Peverali, F., Della Valle, G., Abate, M.,L.,** and **Vaula, G, Comoglio PM** : c-met is amplified but not mutated in a cell line with an activated met tyrosine kinase. Oncogene **6** : 553–559, 1991.

42) **Tahara, E.** : Molecular mechanism of stomach carcinogenesis. J Cancer Res Clin Oncol **119** : 265–272, 1993.

43) **Sano, T., Tsujino, T., Yoshida, K., Nakayama, H., Haruma, K., Ito, H., Nakamura, Y.** and **Kajiyama, G., Tahara, E** : Frequent loss of heterozygosity on chromosome 1q, 5q, and 17p in human gastric carcinomas. Cancer Res **51** : 2926–2931, 1991.

44) **Xiao, S., Geng, J., S., Feng, X., L., Liu, X., Q., Liu, Q., Z., Li, P.** : Cytogenetic studies of eight primary gastric cancers. Cancer Genet Cytogenet **58** : 79-84, 1992.

45) **Ochi, H., Douglass, Jr. H.O.** and **Sandberg, A., A.**: Cytogentic studies in primary gastric cancer. Cytogenet **22** : 295-307, 1986.

46) **Kere, J., Ruutu, T., Davies, K., A., Roninson, I., B., Watkins, P., C., Winqvist, R.** and **de la Chapelle, A.** : Chromosome 7 long arm deletion in myeloid disorders: a narrow breakpoint region in 7q22 defined by molecular mapping. Blood **73** : 230-234, 1989.

47) **Michalova, K., Musilova, J.** and **Zemanova, Z.** : Consecutive chromosomal studies in patients with myelodysplastic syndrome (MSD). Ann Genet 1991; **34** : 212-218, 1991.

48) **Bieche, I., Champeme, M., H., Matifas, F., Hacene, K., Callahan, R.** and **Lidereau, R.** : Loss of heterozygosity on chromosome 7q and aggressive primary breast cancer. Lancet **369** : 139-143, 1992.

49) **Chakraborty, C., Katsumata, N., Myal, Y., Schroedter, I., C., Brazeau, P., Murphy, L., J., Shiu, R., P.** and **Friesen, H., G.** : Age-related changes in peptide-23/pancreatitis-associated protein and pancreatic stone protein/reg gene expression in the rat and regulation by growth hormone-releasing hormone. Endocrinology **136** : 1843-1849, 1995.

50) **Lasserre, C., Simon, M., T., Ishikawa, H., Diriong, S., Nguyen, V., C., Christa, L., Vernier, P.** and **Brechot, C.** : Structural organization and chromosomal localization of a human gene (HIP/PAP) encoding a C-type lectin overexpressed in primary liver cancer. Eur J Biochem **224** : 29-38, 1994.

51) **Hartupee, J., C., Zhang, H., Bonaldo., M., F., Soares, M., B.** and **Dieckgraefe, B., K.** : Isolation and characterization of a cDNA encoding a novel member of the human regenerating protein family: Reg IV. Biochim Biophys Acta **1518** : 287-293, 2001.

52) **Li, A., Crimmins, D., L., Luo, Q., Hartupee, J., Landt, Y., Ladenson, J., H., Wilson, D.** and **Anant, S., Dieckgraefe, B., K.** : Expression of a novel regenerating gene product, Reg IV, by high density fermentation in Pichia pastoris: production, purification, and characterization. Protein Expr Purif **31** : 197-206, 2003.

53) **Nata, K., Liu, Y., Xu, L., Ikeda, T., Akiyama, T., Noguchi, N., Kawaguchi, S., Yamauchi, A., Takahashi, I., Shervani, N., J., Onogawa, T., Takasawa, S., Okamoto, H.**: Molecular cloning, expression and chromosomal localization of a novel human REG family gene, REG III. Gene **340** : 161-170, 2004.

54) **Nanakin, A., Fukui, H., Fujii, S., Sekikawa, A., Kanda, N., Hisatsune, H., Seno, H., Konda, Y., Fujimori, T.** and **Chiba, T.** : Expression of the REG IV gene in ulcerative colitis. Lab Invest **87** : 304-314, 2007.

55) **Violette, S., Festor, E., Pandrea-Vasile, I., Mitchell, V., Adida, C., Dussaulx, E., Lacorte, J., M., Chambaz, J., Lacasa, M.** and **Lesuffleur, T.** : Reg IV, a new member of the regenerating gene family, is overexpressed in colorectal carcinomas. Int J Cancer **103** : 185-193, 2003.

56) **Zhang, Y., Lai, M., Lv, B., Gu, X., Wang, H., Zhu, Y., Shao, L.** and **Wang, G.** : Overexpression of Reg IV in colorectal adenoma. Cancer Lett **200** : 69-76, 2003.

57) **Oue, N., Kuniyasu, H., Noguchi, T., Sentani, K., Ito, M., Tanaka, S., Setoyama, T., Sakakura, C., Natsugoe, S.** and **Yasui, W.** : Serum concentration of Reg IV in patients with colorectal cancer: overexpression and high Reg IV serum level is associated with liver metastasis. Oncology **72** : 371-380, 2008.

58) **Sasahira, T., Oue, N., Kirita, T., Luo, Y., Bhawal, U., K., Yasui, W.** and **Kuniyasu, H.** : Reg IV expression is associated with cell growth and prognosis of adenoid cystic carcinoma in the salivary gland. Histopathol **53** : 667-675, 2008.

59) **Ohara, S., Oue, N., Matsubara, A., Mita, K., Hasegawa, Y., Hayashi, T., Usui, T., Amatya, V., Takeshima, Y., Kuniyasu, H.** and **Yasui, W.** : Reg IV is an independent prognostic factor for relapse in patients with clinically localized prostate cancer. Cancer Sci **99** : 1570-1577, 2008.

60) **Bishnupuri, K., S., Luo, Q., Murmu, N., Houchen, C., W., Anant, S.** and **Dieckgraefe, B., K.** : Reg IV activates the epidermal growth factor receptor/Akt/AP-1 signaling pathway in colon adenocarcinomas.

Gastroenterology **130** : 137-149, 2006.

61) **Bishnupuri, K., S., Luo, Q., Korzenik, J., R., Henderson, J., O., Houchen, C., W., Anant, S.** and **Dieckgraefe, B., K.** : Dysregulation of Reg gene expression occurs early in gastrointestinal tumorigenesis and regulates anti-apoptotic genes. Cancer Biol Ther **5** : 1714-1720, 2006.

62) **Mitani, Y., Oue, N., Matsumura, S., Yoshida, K., Noguchi, T., Ito, M., Tanaka, S., Kuniyasu, H., Kamata, N.** and **Yasui, W.** : Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy. Oncogene **26** : 4383-4393, 2007.

63) **Gu, Z., Rubin, M., A., Yang, Y., Deprimo, S., E., Zhao, H., Horvath, S., Brooks, J., Loda, M.** and **Reiter, R., E.** : Reg IV: a promising marker of hormone refractory metastatic prostate cancer. Clin Cancer Res 2005; **11** : 2237-2243, 2005.

64) **Heiskala, K., Giles-Komar, J., Heiskala, M.** and **Andersson, L., C.** : High expression of RELP (Reg IV) in neoplastic goblet cells of appendiceal mucinous cystadenoma and pseudomyxoma peritonei. Virchows Arch **448** : 295-300, 2006.

65) **Pankova-Kholmyansky, I.,** and **Arber, N.** : Reg IV Can Serve for Early Diagnosis and Therapy. Cancer Biol Ther **6** : 123-124, 2007.

66) **Oue, N., Mitani, Y., Aung, P., P., Sakakura, C., Takeshima, Y., Kaneko, M., Noguchi, T., Nakayama, H.** and **Yasui, W.** : Expression and localization of Reg IV in human neoplastic and non-neoplastic tissues: Reg IV expression is associated with intestinal and neuroendocrine differentiation in gastric adenocarcinoma. J Pathol **207** : 185-198, 2005.

67) **Kuniyasu, H., Oue, N., Sasahira, T., Luo, Y., Moriwaka, Y., Shimomoto, T., Fujii, K.** and **Ohmori H, Yasui W** : Reg IV enhances peritoneal metastasis in gastric carcinomas. Cell Prolif **42** : 701-709, 2009.

68) **Sasaki, T., Yoshida, K., Kondo, H., Ohmori, H.** and **Kuniyasu, H.** : Heme oxygenase-1 accelerates protumoral effects of nitric oxide in cancer cells. Virchows Arch **446** : 525-531, 2005.

69) **Zhang, Y., W., Ding, L., S., Lai, M., D.** : Reg gene family and human diseases. World J Gastroenterol **9** : 2635-2641, 2003.

70) **Li, A., Crimmins, D., L., Luo, Q., Hartupee, J., Landt, Y., Ladenson, J., H., Wilson, D., Anant, S.** and **Dieckgraefe, B., K.**: Expression of a novel regenerating gene product, Reg IV, by high density fermentation in Pichia pastoris: production, purification, and characterization. Protein Expr Purif **31** : 197-206, 2003.

71) **Mori, K., Aoyagi, K., Ueda, T., Danjoh, I., Tsubosa, Y., Yanagihara, K., Matsuno, Y., Sasako, M., Sakamoto, H., Mafune, K., Kaminishi, M., Yoshida, T., Terada, M.** and **Sasaki, H.** : Highly specific marker genes for detecting minimal gastric cancer cells in cytology negative peritoneal washings. Biochem Biophys Res Commun **313** : 931-937, 2004.

72) **Kuniyasu, H., Yoshida, K., Sasaki, T., Sasahira, T., Fujii, K.** and **Ohmori, H.** : Conjugated linoleic acid inhibits peritoneal metastasis in human gastrointestinal cancer cells. Int J Cancer **118** : 571-576, 2006.

73) **Sasaki, T., Fujii, K., Yoshida, K., Shimura, H., Sasahira, T., Ohmori, H.** and **Kuniyasu, H.** : Peritoneal metastasis inhibition by linoleic acid with activation of PPAR- in human gastrointestinal cancer cells. Virchows Archiv **448** : 422-427, 2005.

74) **McCarty, M., F.** : Activation of PPAR- may mediate a portion of the anticancer activity of conjugated linoleic acid. Med Hypotheses **55** : 187-188, 2000.

75) **Wolf, G.** : The function of the nuclear receptor peroxisome proliferator-activated receptor - in energy homeostasis. Nutr Rev **61** : 387-390, 2003.

76) **Liu, H., Zang, C., Fenner, M., H., Possinger, K.** and **Elstner, E.** : PPAR- ligands and ATRA inhibit the invasion of human breast cancer cells in vitro. Breast Cancer Res Treat **79** : 63-74, 2003.

77) **Berger, J.** and **Wagner, J., A.** : Physiological and therapeutic roles of peroxisome proliferator-activated receptors. Diabetes Technol Ther **4** : 163-174, 2002.

78) **Bassaganya-Riera, J., Hontecillas, R., Beitz, D., C.** : Colonic anti-inflammatory mechanisms of conjugated linoleic acid. Clin Nutr **21** : 451-459, 2002.

79) **Desreumaux, P., Dubuquoy, L., Nutten, S., Peuchmaur, M., Englaro, W., Schoonjans, K., Derijard, B., Desvergne, B., Wahli, W., Chambon, P., Leibowitz, M., D., Colombel, J., F.** and **Auwerx, J.** : Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor γ (PPARγ) heterodimer. A basis for new therapeutic strategies. J Exp Med **193** : 827-838, 2001.

80) **Tanaka, T., Kohno, H., Yoshitani, S., Takashima, S., Okumura, A., Murakami, A.** and **Hosokawa, M.** : Ligands for peroxisome proliferator-activated receptors α and γ inhibit chemically induced colitis and formation of aberrant crypt foci in rats. Cancer Res **61** : 2424-2428, 2001.

81) **Osawa, E., Nakajima, A., Wada, K., Ishimine, S., Fujisawa, N., Kawamori, T., Matsuhashi, N., Kadowaki, T., Ochiai, M., Sekihara, H.** and **Nakagama, H.** : Peroxisome proliferator-activated receptor γ ligands suppress colon carcinogenesis induced by azoxymethane in mice. Gastroenterology **124** : 361-367, 2003.

82) **Badawi, A., F., Eldeen, M., B., Liu, Y., Ross, E., A.** and **Badr, M., Z.** : Inhibition of rat mammary gland carcinogenesis by simultaneous targeting of cyclooxygenase-2 and peroxisome proliferator-activated receptor γ. Cancer Res **64** : 1181-1189, 2004.

83) **Terashita, Y., Sasaki, H., Haruki, N., Nishiwaki, T., Ishiguro, H., Shibata, Y., Kudo, J., Konishi, S., Kato, J., Koyama, H., Kimura, M., Sato, A., Shinoda, N., Kuwabara, Y.** and **Fujii, Y.** : Decreased peroxisome proliferator-activated receptor γ gene expression is correlated with poor prognosis in patients with esophageal cancer. Jpn J Clin Oncol **32** : 238-243, 2002.

84) **Chen, A.,** and **Xu, J.** : Activation of PPARγ by curcumin inhibits Moser cell growth and mediates the suppression of the gene expression of cyclinD1 and EGFR. Am J Physiol Gastrointest Liver Physiol **288** : G447-56, 2004.

85) **Liu, J., J., Huang, R., W., Lin, D., J., Peng, J., Wu, X., Y., Lin, Q., Pan, X., L., Song, Y., Q., Zhang, M., H., Hou, M.** and **Chen., F.** : Expression of survivin and bax/bcl-2 in peroxisome proliferator activated receptor-γ ligands induces apoptosis on human myeloid leukemia cells in vitro. Ann Oncol **16** : 455-9, 2005.

86) **Majumder, B., Wahle, K., W., Moir, S., Schofield, A., Choe, S., N., Farquharson, A., Grant, I., Heys, S., D.** : Conjugated linoleic acids (CLAs) regulate the expression of key apoptotic genes in human breast cancer cells. Faseb J **16** : 1447-1449, 2002.

87) **Yoshizumi, T., Ohta, T., Ninomiya, I., Terada, I., Fushida, S., Fujimura, T., Nishimura, G., Shimizu, K., Yi, S.** and **Miwa, K.** : Thiazolidinedione, a peroxisome proliferator-activated receptor-□ ligand, inhibits growth and metastasis of HT-29 human colon cancer cells through differentiation-promoting effects. Int J Oncol **25** : 631-639, 2004.

88) **Bren-Mattison, Y., Van Putten, V., Chan, D., Winn, R., Geraci, M., W.** and **Nemenoff, R., A.** : Peroxisome proliferator-activated receptor-□ (PPAR□) inhibits tumorigenesis by reversing the undifferentiated phenotype of metastatic non-small-cell lung cancer cells (NSCLC). Oncogene **24** : 1412-22, 2004.

89) **Galusca, B., Dumollard, J., M., Chambonniere, M., L., Germain, N., Prades, J., M., Peoc'h, M.** and **Estour, B.** : Peroxisome proliferator activated receptor □ immunohistochemical expression in human papillary thyroid carcinoma tissues. Possible relationship to lymph node metastasis. Anticancer Res **24** : 1993-1997, 2004.

90) **Sakai, Y., Sasahira, T., Ohmori, H., Yoshida, K.** and **Kuniyasu, H.** : Conjugated linoleic acid reduced metastasized LL2 tumors in mouse peritoneum. Virchows Arch **448** : 422-427, 2006.