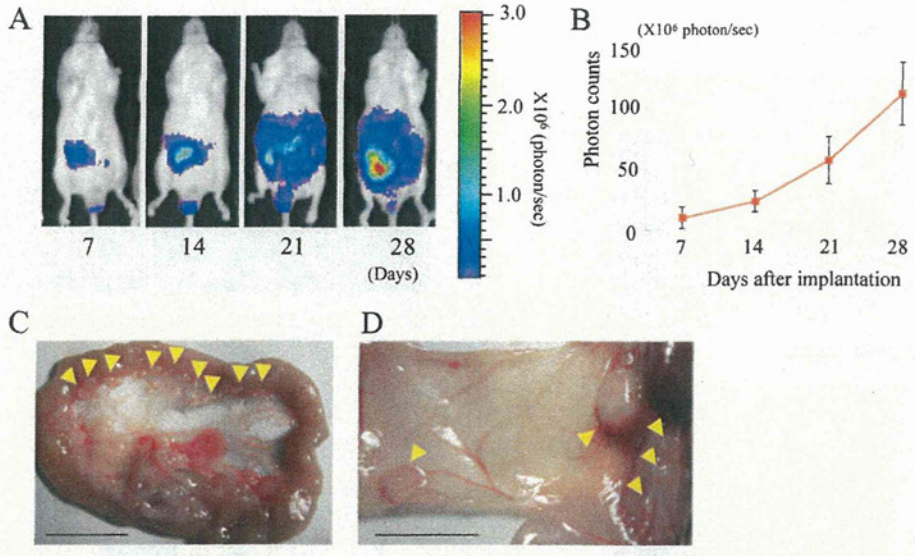


Fig. 1. Characterization of the peritoneal metastasis model. Photon-counting analysis of peritoneal dissemination after the intraperitoneal inoculation of 60As6 Luc cells. Scid mice bearing 60As6Luc tumors in the abdominal cavity were anesthetized and subjected to observations after intraperitoneal administration of luciferin (*A*). Quantitative analysis of the progression of peritoneal disseminated metastasis of tumor cells (n = 5). This experiment was repeated three times, and similar results were observed each time (*B*). Macroscopic evidence of peritoneal dissemination in scid mice bearing tumor cells. There were metastatic lesions in the intestine (*C*, *arrowheads*) and the parietal peritoneum (*D*, *arrowheads*). Scale bar: 10 mm.

semination at intervals of 7 days. Figure 1A shows a typical example. This method made it possible to observe the same animals over time. A tumor growth curve that reflected the progression of peritoneal dissemination was obtained by plotting the number of photons *versus* time (fig. 1B, n = 5). Dissemination to the mesenterium and parietal peritoneum was noted based on the macroscopic appearance of peritoneal dissemination in mice 28 days after inoculation (fig. 1, C and D, *arrowheads*).

Hypersensitivity to Mechanical Stimulation and Visceral Pain-related Behavior Induced by Peritoneal Carcinomatosis in Mice

The hypersensitivity to mechanical stimulation in the abdomen was quantified by counting the number of withdrawal behaviors in response to stimulation with von Frey filaments 14 and 28 days after the inoculation of tumor cells (fig. 2A). Twenty-eight days after inoculation, but not 14 days after inoculation, mice with peritoneal dissemination showed a significant increase in the nociceptive score in response to mechanical stimulation ($P < 0.05$ *vs.* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test, control group: n = 5, tumor group: n = 7). We next examined visceral pain-related behavior, which was assessed in terms of the degree of hunching and the time spent hunching (over 180 s). Hunching behavior has been described previously as a measure of abdominal pain caused by pancreatic cancer in mice.[14] Visceral pain-related behavior was examined at 14 and 28 days after the inoculation of tumor cells (fig. 2B). Spontaneous visceral pain-related behavior became evident at 28 days after inoculation ($P < 0.05$ *vs.* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test, each group: n = 5).

Changes in c-Fos-positive Cells in the Dorsal Horn of the Spinal Cord Induced by Peritoneal Carcinomatosis

Spinal cord tissue samples were obtained from animals 28 days after tumor inoculation and from control mice. In control mice, few cFos-positive cells were found in the superficial layer (L1–2 laminae) or deep layer (L3–5 laminae) of the dorsal horn (fig. 3A). In tumor-bearing mice, the number of c-Fos-positive cells was significantly increased in both the superficial and deep layers of the dorsal horn (fig. 3, B and C, $P < 0.05$ *vs.* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test, each group: n = 5).

Changes in the Expression of Substance P, CGRP, and MOR Induced by Peritoneal Carcinomatosis in Mice

Twenty-eight days after inoculation, the mRNA concentration level of substance P was significantly increased in the DRG of tumor-bearing mice compared with that in control mice, whereas there was no significant difference at 14 days after inoculation (fig. 4A, $P < 0.05$ *vs.* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test, each group: n = 7). In addition, the mRNA concentrations of CGRP in the DRG of tumor-bearing mice were not different from those in the control mice at 14 and 28 days after inoculation (fig. 4B). Under these conditions, 28 days after inoculation, but not 14 days after inoculation, the mRNA concentration of MOR was significantly decreased in the DRG of tumor-bearing mice compared with

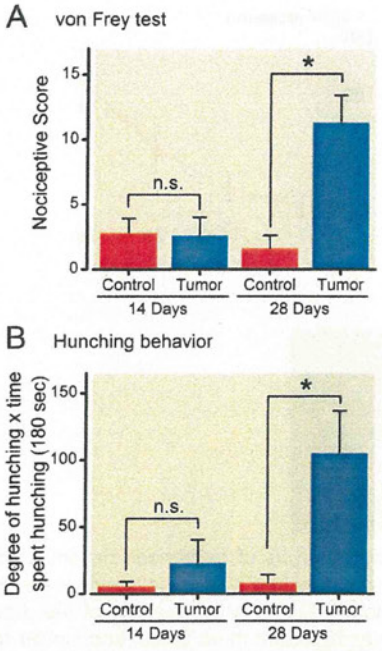


Fig. 2. Abdominal hypersensitivity to mechanical stimulation and visceral pain-related behavior induced by peritoneal carcinomatosis in mice. Hypersensitivity was quantified by counting the number of withdrawal behaviors in response to mechanical stimulation at 14 and 28 days after the inoculation of tumor cells (*A*). Visceral pain-related behavior was assessed in terms of the degree of hunching and time spent hunching (over 180 s) at 14 and 28 days after the inoculation of tumor cells (*B*). Each column represents the mean ± SD. * $P <$ than 0.05 *versus* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test.

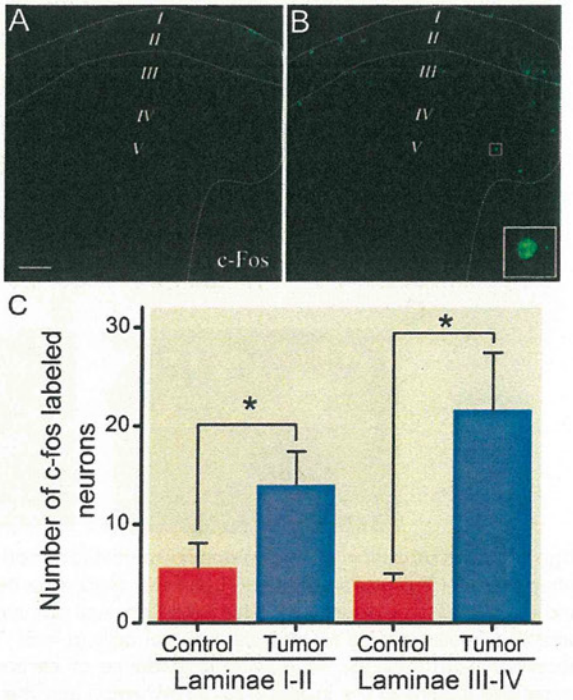


Fig. 3. Changes in c-Fos-positive cells in the dorsal horn of the spinal cord induced by peritoneal carcinomatosis. c-Fos expression in a control mouse (*A*) and in the dorsal horn of a tumor-bearing mouse on day 28. *Inset* is a high magnification image of the squared area (*B*). Increases in c-Fos-positive cells were observed in the superficial layer (L1–2 laminae) and the deep layer (L3–5 laminae) of the dorsal horn in tumor-bearing mice (*C*). The results represent the means ± SD. * $P < 0.05$ *versus* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test. Scale bar: 50 μm.

that in control mice (fig. 4C, $P < 0.05$ *vs.* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test, each group: n = 7).

Changes in MOR in Substance P-positive DRG Neurons Induced by Peritoneal Carcinomatosis in Mice

The expression patterns of MOR in DRG neurons were determined at 28 days after inoculation in control and tumor-bearing mice. In the T10–12 DRGs of control mice, MOR-positive profiles were seen in 39.0 ± 1.6% of all neurons (fig. 5A), which was significantly greater than the value in tumor-bearing mice (26.7 ± 2.9%, fig. 5, B and C, $P <$ 0.05 *vs.* control group, unpaired Student *t* test (two-tailed), each group: n = 3). In addition, the percentage of substance P-positive profiles in tumor-bearing mice (24.2 ± 3.6%) was significantly greater than that in control mice (15.4 ± 1.1%, fig. 5, D, E ,and F, $P < 0.05$ *vs.* control group, unpaired Student *t* test (two-tailed), each group: n = 3). We next investigated the change in the colocalization of MOR with substance P (*arrowhead*). Although 69.6 ± 4.9% of substance P-positive profiles in control mice were also MOR-positive, tumor inoculation significantly decreased this percentage (38.7 ± 0.9%, fig. 5G, $P < 0.05$ *vs.* control group, unpaired Student *t* test [two-tailed], each group: n = 3).

Effect of Systemic Administration of Morphine on the Nociceptive Behavior in Response to Mechanical Stimulation in Tumor-bearing Mice, Caerulein-injected Mice, and CFA-injected Mice

Repeated treatment with caerulein (six injections, 50 μg/kg) resulted in abdominal hypersensitivity to mechanical stimulation, indicating that caerulein-injected mice exhibited an acute pancreatitis pain-like state.[19,20] On the other hand, the latency of paw withdrawal induced by mechanical stimulation was reduced dramatically by the intraplantar injection of a CFA solution into the mouse hind paw.[21] In tumor-bearing, caerulein-injected, and CFA-injected mice, subcutaneous injection of saline did not have any effect on the nociceptive score. In caerulein-injected and CFA-injected mice, subcutaneous injection of morphine reduced the nociceptive score in a dose-dependent manner in response to mechanical stimulation compared with that in mice injected with saline ($P < 0.05$, 1 mg/kg: 68.4 ± 17.5% of the basal value, 3 mg/kg: 43.8 ± 21.4% of the basal value, 5 mg/kg: 22.2 ± 10.1% of the basal value, caerulein-saline group [n = 5] *vs.* caerulein-morphine group [n = 6]; $P < 0.05$, 3 mg/kg: 42.9 ± 13.1% of the basal value, 5 mg/kg: 9.5 ± 14.9% of the basal value, CFA-saline group [n = 6] *versus* CFA-mor-

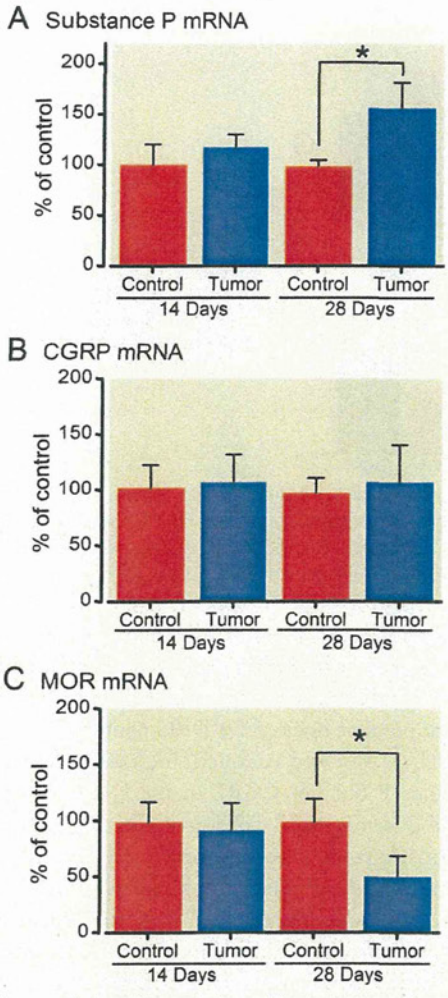


Fig. 4. Transcriptional regulation of substance P, calcitonin gene-related peptide (CGRP), and μ-opioid receptor (MOR) in the dorsal root ganglia (DRG) induced by peritoneal carcinomatosis. Expression of substance P (*A*), CGRP (*B*), and MOR (*C*) in the DRG of tumor-bearing mouse on days 14 and 28. Real-time reverse transcription polymerase chain reaction was carried out on messenger RNA (mRNA) obtained from thoracic 6–13 th and L1–3 DRG. The mRNA concentrations were normalized to those for glyceraldehyde-3-phosphate dehydrogenase (housekeeping gene), and the results are presented as the means ± SD. * $P < 0.05$ *versus* control group, one-way ANOVA followed by the Bonferroni multiple comparisons test.

phine group [n = 7], one-way ANOVA followed by the Bonferroni multiple comparisons test). In tumor-bearing mice, subcutaneous injection of morphine at a dose of 3 mg/kg, which significantly reduced the nociceptive score in both caerulein-injected and CFA-injected mice, did not have any effect on the nociceptive score. In contrast, higher doses of morphine (5 and 10 mg/kg) produced a significant reduction in the nociceptive score ($P < 0.05$, 52.8 ± 15.6% and 39.2 ± 9.3% of the basal value, respectively, tumor-saline group [n = 5] *vs.* tumor-morphine group [n = 5], one-way ANOVA followed by the Bonferroni multiple comparisons test). However, the effect of morphine was less in tumor-bearing mice than in caerulein-injected and CFA-injected mice (fig. 6).

Discussion

Patients with cancer in the advanced stages, especially those with bone metastasis and cancerous peritonitis, endure significant pain. Patients with tumors involving bone destruction and nerve damage are particularly likely to experience severe pain.[27–29] Although published guidelines for pain management are available, the routine use of this treatment does not always alleviate this kind of pain.[3,29,30] Cancer pain often is treated by higher doses of morphine, which can be accompanied by side effects, including sedation, respiratory depression, and interference with gastrointestinal motility, and often provides only incomplete relief. Not surprisingly, novel, more effective analgesics are needed for the treatment of severe opioid-resistant cancer pain, such as abdominal pain caused by cancerous peritonitis.

In this study, we developed a novel mouse model for abdominal pain caused by cancerous peritonitis. Generally, it is difficult to monitor growth and subsequent progression of tumors to cancerous peritonitis, unlike with subcutaneous tumors. In the current study, we observed the progression of dissemination in real-time using luciferase gene-transfected cells and an *in vivo* photon-counting analysis. The level of emitted photon intensity gradually increased over time after tumor cell inoculation, and the intensity level at 28 days after tumor inoculation was approximately 10-fold that at 7 days. Mice with peritoneal dissemination showed dramatic increases in both their nociceptive scores in response to mechanical stimulation and visceral pain-related behavior at 28 days after the inoculation of tumor cells. However, these pain-like behaviors were not observed 14 days after inoculation, suggesting that this abdominal hypersensitivity is observed predominantly in mice at the relatively late stage of cancerous peritonitis.

Nociceptors relay information from the periphery to the spinal cord, where they target secondary neurons in the superficial (L1–2 laminae) and deep layers (L3–5 laminae) of the dorsal horns.[31] Some of these secondary neurons are projection neurons that then pass this nociceptive information to the central nervous system.[31] To visually identify the changes in the activity of spinal neurons related to pain in this model, we observed the changes in the immunoreactivity of c-Fos, an immediate-early gene that is widely used in pain research as a marker for neuronal activation.[32–34] At 28 days after inoculation, there was an increase in c-Fos-positive cells in both the superficial and deep layers of the spinal cord. Second-order neurons in the spinal cord that receive visceral afferent input are located principally in the superficial spinal laminae, deeper in L5 and L10 laminae. Some studies have shown that somatic structures send afferents to L1–3 laminae and to deep L4 and L5 laminae.[35,36] Taken together, the current findings suggest that the progressive insult of the

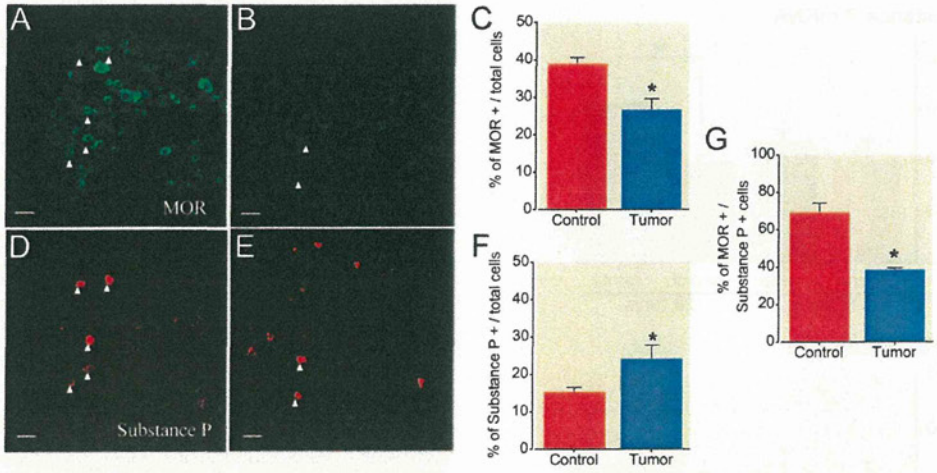


Fig. 5. Expression of μ-opioid receptor (MOR) in the dorsal root ganglia (DRG) of a control mouse (*A*) and a tumor-bearing mouse (*B*). Expression of substance P in the DRG of a control mouse (*D*) and a tumor-bearing mouse (*E*). Percentage of total cells expressing MOR (*C*), substance P (*F*), or MOR/substance P (*G*) in the DRG of control mice and tumor-bearing mice. The results are presented as the means ± SD. * $P < 0.05$ *versus* control group, unpaired Student *t* test (two-tailed). Scale bar: 20 μm.

peripheral nerve on the mesenterium and parietal peritoneum caused by tumor growth may result in the progression of wide-ranging pain accompanied by an increase in c-Fos-positive cells in the spinal cord.

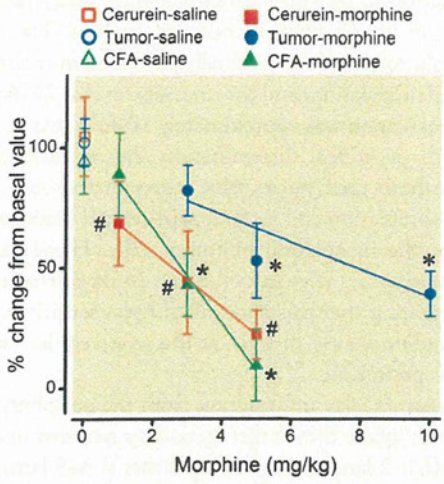


Fig. 6. Effects of systemic morphine treatment on the nociceptive scores in tumor-bearing (*circles*), caerulein-injected (*squares*), or complete Freund's adjuvant (CFA)-injected mice (*triangles*). Six hours after the last injection of caerulein (six injections, 50 μg/kg) or 3 days after CFA-injection, groups of mice were treated with subcutaneous morphine (1, 3, and 5 mg/kg). Twenty-eight days after the inoculation of tumor cells, groups of mice were treated with subcutaneous morphine (3, 5, and 10 mg/kg). The behavioral test was performed 30 min after the injection of morphine. Hypersensitivity to mechanical stimulation was quantified by counting the number of withdrawal behaviors in response to mechanical stimuli. The results are presented as the means ± SD. # $P < 0.05$, caerulein-saline group *versus* caerulein-morphine group. * $P < 0.05$, CFA-saline group *versus* CFA-morphine group. * $P < 0.05$, tumor-saline group *versus* tumor-morphine group, one-way ANOVA followed by the Bonferroni multiple comparisons test.

A variety of neurochemical and other markers have been used to characterize sensory neurons as nociceptive. The neuropeptides substance P and CGRP are considered to be markers of putative nociceptive DRG neurons.[31] In the current model, we observed a dramatic increase in the expression of substance P but not CGRP in the DRG of mice with peritoneal dissemination. Consistent with the data, the percentage of substance P-positive neurons apparently was increased in tumor-bearing mice. Substance P and CGRP have been reported to be up-regulated in both sensory neurons and primary afferent terminals in models of inflammatory pain.[37,38] In contrast, these neurotransmitters have been shown to be down-regulated in the spinal cord of models of nerve injury.[39,40] There was no significant change in the expression of these peptides in a murine model of bone cancer pain.[41] Substance P is abundant in visceral primary afferents (more than 80%),[42] and neurokinin1 receptor knockout mice show a significant deficit in visceral nociceptive perception.[43,44] Furthermore, inflammatory cytokine and neurotropic factors can induce the increased production of substance P and neurokinin1 receptors.[45] These data support the idea that inflammatory cytokine and neurotropic factors released by damaged peripheral nerve and/or tumor cells may promote the novel synthesis of substance P in the DRG of mice with peritoneal carcinomatosis. This phenomenon would correspond to the abdominal pain caused by the peritoneal dissemination of cancer cells in mice.

Another key finding in the current study was that peritoneal carcinomatosis decreased the mRNA expression of MOR in the DRG. In addition to MOR mRNA, the results of an immunohistochemical analysis revealed that the percentage of substance P-positive neurons that also were MOR-positive was decreased remarkably in the DRG of tumor-bearing mice. In relation to the down-regulation of MOR in the DRG, the dose–response curve for the antihy-

peralgesic effect of morphine against tumor-dependent pain was shifted to the right compared with that found in mice with either caerulein-induced visceral pain or CFA-induced inflammatory pain. Changes in MOR expression in the DRG have been shown in several pain models. Models of peripheral nerve injury have shown a dramatic decrease in MOR expression in the DRG, and such injuries reduce the effects of MOR agonists.[46–49] On the other hand, peripheral inflammation increases MOR expression in the dorsal horn of the spinal cord and the DRG, which are responsible for enhancing the effects of MOR agonists.[50,51] Luger *et al.*[52] and Yamamoto *et al.*[25] showed that higher doses of morphine were required to treat bone cancer pain than to treat inflammatory pain. It has been proposed that an increase in MOR mRNA production increases an opioid's efficacy at individual nociceptors.[53] Taken together, the current findings strongly support the idea that the deceased MOR expression in substance P-positive DRG may correspond to the reduction in the morphine-induced antinociception or analgesia in mice with peritoneal carcinomatosis.

In conclusion, mice with peritoneal carcinomatosis exhibit hypersensitivity to mechanical stimulation and visceral pain-like behavior, which is accompanied by the up-regulation of substance P and the down-regulation of MOR. This newly developed model may be important for studying the pathogenesis of abdominal pain caused by cancerous peritonitis.

The authors thank Masahiko Watanabe, M.D., Ph.D., Professor, Department of Anatomy and Embryology, Hokkaido University Graduate School of Medicine, Sapporo, Japan, for the specific anti-MOR antibody.

References

1. van den Beuken-van Everdingen MH, de Rijke JM, Kessels AG, Schouten HC, van Kleef M, Patijn J: Prevalence of pain in patients with cancer: A systematic review of the past 40 years. Ann Oncol 2007; 18:1437–49
2. Hall EJ, Sykes NP: Analgesia for patients with advanced disease: I. Postgrad Med J 2004; 80:148–54
3. Portenoy RK: Treatment of cancer pain. Lancet 2011; 377: 2236–47
4. WHO: Cancer Pain Relief, 2nd edition. Geneva, World Health Organisation, 1996
5. Jayne D: Peritoneal Carcinomatosis: A Multidisciplinary Approach. New York, Springer Science Business Media, 2006, pp 21–33
6. Cervero F: Sensory innervation of the viscera: Peripheral basis of visceral pain. Physiol Rev 1994; 74:95–138
7. Pacharinsak C, Beitz A: Animal models of cancer pain. Comp Med 2008; 58:220–33
8. Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisy DR, Mantyh PW: Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. J Neurosci 1999; 19:10886–97
9. Lindsay TH, Jonas BM, Sevcik MA, Kubota K, Halvorson KG, Ghilardi JR, Kuskowski MA, Stelow EB, Mukherjee P, Gendler SJ, Wong GY, Mantyh PW: Pancreatic cancer pain and its correlation with changes in tumor vasculature, macrophage infiltration, neuronal innervation, body weight and disease progression. Pain 2005; 119:233–46
10. Nagamine K, Ozaki N, Shinoda M, Asai H, Nishiguchi H, Mitsudo K, Tohnai I, Ueda M, Sugiura Y: Mechanical allodynia and thermal hyperalgesia induced by experimental squamous cell carcinoma of the lower gingiva in rats. J Pain 2006; 7:659–70
11. Honore P, Luger NM, Sabino MA, Schwei MJ, Rogers SD, Mach DB, O'keefe PF, Ramnaraine ML, Clohisy DR, Mantyh PW: Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. Nat Med 2000; 6:521–8
12. Niiyama Y, Kawamata T, Yamamoto J, Furuse S, Namiki A: SB366791, a TRPV1 antagonist, potentiates analgesic effects of systemic morphine in a murine model of bone cancer pain. Br J Anaesth 2009; 102:251–8
13. Furuse S, Kawamata T, Yamamoto J, Niiyama Y, Omote K, Watanabe M, Namiki A: Reduction of bone cancer pain by activation of spinal cannabinoid receptor 1 and its expression in the superficial dorsal horn of the spinal cord in a murine model of bone cancer pain. ANESTHESIOLOGY 2009; 111:173–86
14. Sevcik MA, Jonas BM, Lindsay TH, Halvorson KG, Ghilardi JR, Kuskowski MA, Mukherjee P, Maggio JE, Mantyh PW: Endogenous opioids inhibit early-stage pancreatic pain in a mouse model of pancreatic cancer. Gastroenterology 2006; 131: 900–10
15. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109–10
16. Yanagihara K, Tanaka H, Takigahira M, Ino Y, Yamaguchi Y, Toge T, Sugano K, Hirohashi S: Establishment of two cell lines from human gastric scirrhous carcinoma that possess the potential to metastasize spontaneously in nude mice. Cancer Sci 2004; 95:575–82
17. Yanagihara K, Takigahira M, Tanaka H, Komatsu T, Fukumoto H, Koizumi F, Nishio K, Ochiya T, Ino Y, Hirohashi S: Development and biological analysis of peritoneal metastasis mouse models for human scirrhous stomach cancer. Cancer Sci 2005; 96:323–32
18. Yanagihara K, Takigahira M, Takeshita F, Komatsu T, Nishio K, Hasegawa F, Ochiya T: A photon counting technique for quantitatively evaluating progression of peritoneal tumor dissemination. Cancer Res 2006; 66:7532–9
19. Kawabata A, Matsunami M, Tsutsumi M, Ishiki T, Fukushima O, Sekiguchi F, Kawao N, Minami T, Kanke T, Saito N: Suppression of pancreatitis-related allodynia/hyperalgesia by proteinase-activated receptor-2 in mice. Br J Pharmacol 2006; 148:54–60
20. Takemura Y, Furuta S, Hirayama S, Miyashita K, Imai S, Narita M, Kuzumaki N, Tsukiyama Y, Yamazaki M, Suzuki T, Narita M: Upregulation of bradykinin receptors is implicated in the pain associated with caerulein-induced acute pancreatitis. Synapse 2011; 65:608–16
21. Narita M, Shimamura M, Imai S, Kubota C, Yajima Y, Takagi T, Shiokawa M, Inoue T, Suzuki M, Suzuki T: Role of interleukin-1β and tumor necrosis factor-α-dependent expression of cyclooxygenase-2 mRNA in thermal hyperalgesia induced by chronic inflammation in mice. Neuroscience 2008; 152: 477–86
22. Narita M, Suzuki M, Narita M, Niikura K, Nakamura A, Miyatake M, Aoki T, Yajima Y, Suzuki T: Involvement of spinal metabotropic glutamate receptor 5 in the development of tolerance to morphine-induced antinociception. J Neurochem 2005; 94:1297–305
23. Kasai S, Yamamoto H, Kamegaya E, Uhl GR, Sora I, Watanabe M, Ikeda K: Quantitative detection of μ opioid receptor: Western blot analyses using μ opioid receptor knockout mice. Curr Neuropharmacol 2011; 9:219–22
24. Shimoyama M, Tatsuoka H, Ohtori S, Tanaka K, Shimoyama N: Change of dorsal horn neurochemistry in a mouse model of neuropathic cancer pain. Pain 2005; 114:221–30
25. Yamamoto J, Kawamata T, Niiyama Y, Omote K, Namiki A: Down-regulation of μ opioid receptor expression within

distinct subpopulations of dorsal root ganglion neurons in a murine model of bone cancer pain. Neuroscience 2008; 151:843–53

26. Livak KJ, Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-ΔΔ C(T)) method. Methods 2001; 25:402–8
27. Mercadante S: Malignant bone pain: Pathophysiology and treatment. Pain 1997; 69:1–18
28. Vecht CJ: Cancer pain: A neurological perspective. Curr Opin Neurol 2000; 13:649–53
29. Forman AD: Peripheral neuropathy and cancer. Curr Oncol Rep 2004; 6:20–5
30. Cherny NI: How to deal with difficult pain problems. Ann Oncol 2005; 16(Suppl 2):ii79–87
31. Todd AJ, Koerber R: Neuroanatomical substrates of spinal nociception, Wall and Melzack's Textbook of Pain, 5th edition. London, Elsevier, 2006, pp 73–90
32. Ma QP, Woolf CJ: Basal and touch-evoked fos-like immunoreactivity during experimental inflammation in the rat. Pain 1996; 67:307–16
33. Coggeshall RE: Fos, nociception and the dorsal horn. Prog Neurobiol 2005; 77:299–352
34. Castellanos DA, Daniels LA, Morales MP, Hama AT, Sagen J: Expansion of formalin-evoked Fos-immunoreactivity in rats with a spinal cord injury. Neurosci Res 2007; 58:386–93
35. Craig AD, Kniffki KD: Spinothalamic lumbosacral lamina I cells responsive to skin and muscle stimulation in the cat. J Physiol 1985; 365:197–221
36. Mense S: Nociception from skeletal muscle in relation to clinical muscle pain. Pain 1993; 54:241–89
37. Ahmed M, Bjurholm A, Schultzberg M, Theodorsson E, Kreicbergs A: Increased levels of substance P and calcitonin gene-related peptide in rat adjuvant arthritis: A combined immunohistochemical and radioimmunoassay analysis. Arthritis Rheum 1995; 38:699–709
38. Donnerer J, Schuligoi R, Stein C: Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: Evidence for a regulatory function of nerve growth factor *in vivo*. Neuroscience 1992; 49:693–8
39. Nahin RL, Ren K, De León M, Ruda M: Primary sensory neurons exhibit altered gene expression in a rat model of neuropathic pain. Pain 1994; 58:95–108
40. Sommer C, Myers RR: Neurotransmitters in the spinal cord dorsal horn in a model of painful neuropathy and in nerve crush. Acta Neuropathol 1995; 90:478–85
41. Honore P, Rogers SD, Schwei MJ, Salak-Johnson JL, Luger NM, Sabino MC, Clohisy DR, Mantyh PW: Murine models of inflammatory, neuropathic and cancer pain each generates a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience 2000; 98:585–98
42. Perry MJ, Lawson SN: Differences in expression of oligosaccharides, neuropeptides, carbonic anhydrase and neurofilament in rat primary afferent neurons retrogradely labelled *via* skin, muscle or visceral nerves. Neuroscience 1998; 85:293–310
43. Muñoz M, Coveñas R: NK-1 receptor antagonists: A new paradigm in pharmacological therapy. Curr Med Chem 2011; 18:1820–31
44. Laird JM, Olivar T, Roza C, De Felipe C, Hunt SP, Cervero F: Deficits in visceral pain and hyperalgesia of mice with a disruption of the tachykinin NK1 receptor gene. Neuroscience 2000; 98:345–52
45. Tuluc F, Lai JP, Kilpatrick LE, Evans DL, Douglas SD: Neurokinin 1 receptor isoforms and the control of innate immunity. Trends Immunol 2009; 30:271–6
46. Lee YW, Chaplan SR, Yaksh TL: Systemic and supraspinal, but not spinal, opiates suppress allodynia in a rat neuropathic pain model. Neurosci Lett 1995; 199:111–4
47. Ossipov MH, Lopez Y, Nichols ML, Bian D, Porreca F: Inhibition by spinal morphine of the tail-flick response is attenuated in rats with nerve ligation injury. Neurosci Lett 1995; 199:83–6
48. Kohno T, Ji RR, Ito N, Allchorne AJ, Befort K, Karchewski LA, Woolf CJ: Peripheral axonal injury results in reduced μ opioid receptor pre- and post-synaptic action in the spinal cord. Pain 2005; 117:77–87
49. Narita M, Imai S, Nakamura A, Ozeki A, Asato M, Rahmadi M, Sudo Y, Hojo M, Uezono Y, Devi LA, Kuzumaki N, Suzuki T: Possible involvement of prolonging spinal μ-opioid receptor desensitization in the development of antihyperalgesic tolerance to μ-opioids under a neuropathic pain-like state. Addict Biol 2011 [Epub ahead of print]
50. Ji RR, Zhang Q, Law PY, Low HH, Elde R, Hökfelt T: Expression of μ-, δ-, and κ-opioid receptor-like immunoreactivities in rat dorsal root ganglia after carrageenan-induced inflammation. J Neurosci 1995; 15:8156–66
51. Maekawa K, Minami M, Masuda T, Satoh M: Expression of μ- and κ-, but not δ-, opioid receptor mRNAs is enhanced in the spinal dorsal horn of the arthritic rats. Pain 1996; 64:365–71
52. Luger NM, Sabino MA, Schwei MJ, Mach DB, Pomonis JD, Keyser CP, Rathbun M, Clohisy DR, Honore P, Yaksh TL, Mantyh PW: Efficacy of systemic morphine suggests a fundamental difference in the mechanisms that generate bone cancer *versus* inflammatory pain. Pain 2002; 99:397–406
53. Silbert SC, Beacham DW, McCleskey EW: Quantitative single-cell differences in μ-opioid receptor mRNA distinguish myelinated and unmyelinted nociceptors. J Neurosci 2003; 23: 34–42

基礎医学セミナー　連載 第7回

ここまでわかってきた漢方薬の「なぜ効くの？」と「本当に効くの？」
―科学的エビデンスに基づいた，がん患者のQOLを高める漢方薬の効果―

独立行政法人国立がん研究センター研究所がん患者病態生理研究分野分野長
上園　保仁

はじめに

2011年12月発行の本誌22巻２号におきまして，基礎医学セミナー「がん患者の症状緩和に役立つ漢方薬―漢方薬の有効性を示す，臨床につながる基礎研究―」を紹介いたしました[1]。そこから１年が経過し，漢方薬ががん患者の生活の質（quality of life；QOL）を改善させる可能性のあることが基礎研究ならびに臨床研究を通してさらに進展いたしました。われわれもこの１年，漢方薬の「なぜ効くの？」について基礎研究を行い，またそれを臨床研究につなげ，「本当に効くの？」を明らかにするための臨床試験を開始しました。本稿では，漢方薬の「なぜ効くの？」と「本当に効くの？」を明らかにするための「漢方薬の基礎研究と臨床研究の最前線」を紹介いたします。

がん患者は，がん自体に加えて手術療法，化学療法，放射線療法などの副作用などにより倦怠感，痛み，しびれ，嘔気・嘔吐など，多くのQOLを低下させる症状に悩まされています。個々の症状について症状緩和を行い，結果として患者の全体症状が和らげばよいのですが，なかなかそういかないのが現実です。日本には，病を個別に診るのでなく人を全体的に診て症状を和らげる「漢方薬」があります。近年，科学的なアプローチにより漢方薬の作用メカニズムの一端が次々と解明されてきました。それらの研究の進展により，今後がん患者のもつさまざまな症状緩和に「漢方薬」が積極的に役立つ可能性が生まれつつあると考えています。世界中で，「西洋薬」に加えて保険でカバーされた「漢方薬」を使える国は日本しかありません。

漢方薬とは？　漢方薬の位置づけは？

漢方薬「Kampo Medicine」は，中国で生まれ発展した中医学（生薬（中薬）をベースに治療を組み立てたもの）を，日本の気候風土や日本人の体質にあわせて17世紀江戸時代に独自に発展させて生まれた薬剤です。1967年に漢方薬は公的医療保険の適用となり，その適用数も徐々に増え，現在では148種の漢方薬が保険収載薬として医療現場で使えるようになっています。これまで漢方薬を使用されてきた先生方の経験に基づいた治療成績により，多くの病人の体質ならびに症状にあわせた漢方処方が可能となっています。くわえて，漢方薬の成分がいかにして一つひとつの症状を抑えるのか？　どのようにして効くのか？　の科学的根拠が明らかとなってきました。特に一部の漢方薬（大建中湯）については，米国食品医薬品局（Food and Drug Administration；FDA）により臨床治験薬として承認され，これまでに終了した分も含めて５本の臨床試験が米国内で行われています（表１）。欧米諸国ではいわゆる科学的根拠のない薬剤についてはその使用が認められないのが通常です。しかし，日本の漢方薬に限っていえば，これまで日本で積み重ねられてきた安全性，ならびに科学的アプローチによる基礎研究の結果をふまえ，さらに臨床研究においても

表1．米国における大建中湯(TU-100)を用いた臨床研究(2012年9月現在)

米国FDAに臨床試験を認可された漢方薬
術後イレウス患者におけるTU-100の忍容性試験—試験終了
術後イレウス患者における腸管機能ならびに腸内食物通過時間におけるTU-100の効果試験—試験終了
クローン病患者におけるTU-100の安全性ならびに効果試験—患者登録中
機能性便秘症患者におけるTU-100の効果確認試験—患者登録中
便秘女性における大腸機能，小腸機能に関する効果試験—患者登録中

(「Clinical Trials. gov」より和訳・引用)

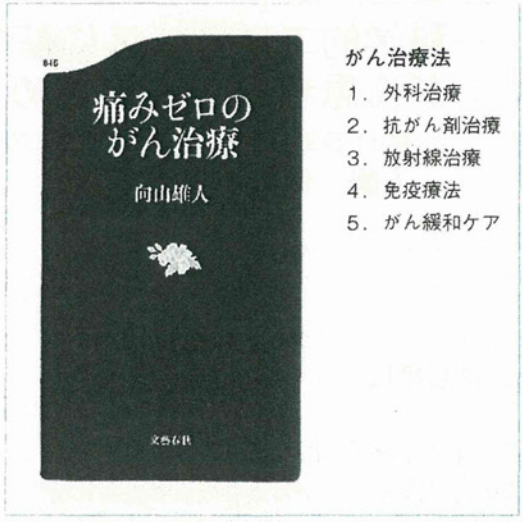


図1．「痛みゼロのがん治療」向山雄人 著(公益財団法人がん研究会有明病院緩和治療科部長)
(文献6)より)

質の高い試験が行われていると米国FDAは判断し，米国での臨床試験にゴーサインを出しました。試験の結果，科学的根拠をもとに有効であると判断されれば，漢方薬は日本発のオリジナル薬として，また米国初の合剤(漢方薬は少なくとも2種類以上の生薬から成ります)として米国ではじめて認可され用いられることになります。大建中湯の米国での臨床試験への道筋を丹念に築いてこられた旭川医科大学消化器病態外科学客員准教授の河野透先生(札幌東徳洲会病院先端外科センター長)の，漢方薬を用いたこの数年の実験結果が一流雑誌に掲載されており，このことも米国FDAの判断に大きく寄与したと思われます[2)-4)]。以前にも述べさせていただきましたが，漢方薬が欧米で注目されるに至ったのは，世界的にも有名な外科の雑誌「Surgery」に，漢方薬はエビデンス(科学的に検証された確かな効果)があるということで「Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines ; is it time yet? (日本の伝統的薬物，「漢方薬」の補完代替医療からの脱出：今その時期か？)」という刺激的なタイトルの漢方総説が2009年に掲載されたことによります[5)]。「Surgery」編集委員長のメイヨー・クリニック外科教授，Michael G. Sarr博士も，「Kampo Medicineは，もはや補完代替医療(CAM)の1つではなく，科学的なエビデンスに基づいた薬物である」という立場に変わられました。

さらに明らかになってきた漢方薬の作用メカニズム

漢方薬のなかには，がん患者のもつさまざまな症状のそれぞれに対応するようなものが現れはじめています。

がんの治療には外科手術，放射線療法，抗がん剤による治療，最近では免疫療法などの新しい方法があります。さらに一歩進んで，がん患者のQOLを向上させることが余命を延ばす1つと考えられ，「緩和ケア療法」が第5の治療法と位置づけられるようになってきました(図1)[6)]。がん治療は患者の全身状態に大きな影響を与えます。手術後は身体の回復が大変ですし，放射線療法や抗がん剤治療には多くの場合，さまざまな副作用

が伴います。漢方薬はそれらのがん患者のQOLを低下させる諸症状を和らげることがこれまでの臨床医の経験を通して知られており，そして現在，その経験が科学的アプローチにより裏打ちされつつあります。

たとえば，外科手術による術後の腸管の癒着，腸管運動不全，イレウスなどに対しての症状緩和に大建中湯が奏効することがわかってきており，外科手術の現場では今や普通に用いられています。大建中湯は腸管の上皮細胞にあるtransient receptor potential(TRP)チャネル，特にTRPV1ならびにTRPA1というチャネルを活性化することにより細胞からアドレノメデュリンというペプチドを放出させ，その結果腸管の血流が増加し，腸管運動が活発化することで腸管癒着を防止することがわかってきました[3)-5)]。

また，放射線療法やがん化学療法で起こる口内炎についても漢方薬が奏効することがわかってきました[7)8)]。2012年の米国消化器病週間(Digestive Disease Week；DDW)においては，実に26題もの漢方薬に関する発表が行われました。この学会は米国の学術集会ですが，消化器病関連では世界で一番権威のある学会とされています。演題採択もかなり難しく，採択されるだけでも素晴らしいと評価される学会です。その内容については，「Nikkei Medical」別冊漢方特集に詳しく紹介されています[9)]。がんに関連した漢方薬の効果についても，食思改善に奏効する六君子湯，口内炎改善に奏効する半夏瀉心湯の演題についての紹介がありますが，化学療法，放射線療法において高い頻度で起こる口内炎に半夏瀉心湯が奏効すること，そしてそのメカニズムを詳しく紹介した河野透先生の発表は多くの諸外国の医師の興味を引いていました[9)]。半夏瀉心湯による口内炎の症状緩和には，構成する7種の生薬成分のそれぞれが鎮痛ならびに組織修復作用を有することが基礎研究により明らかになりつつあります。私たちの研究室でもこの生薬ごとの作用メカニズム解明について鋭意研究を進めているところです。

口内炎に加え，放射線療法，化学療法を受けているがん患者は，食思不振，嘔気・嘔吐，髪が抜ける，下痢，手足のしびれなどさまざまな副作用が起きます。さらに，終末期がん患者は悪液質と呼ばれる症状を呈し，食思不振，全身倦怠感，嘔気・嘔吐，便秘，気持ちの低下が起こります。そのような患者に，たとえば食思不振，嘔気・嘔吐に六君子湯，便秘，イレウスに大建中湯，全身倦怠感に補中益気湯，十全大補湯などがエビデンスをもって奏効することがわかってきました[10)]。

シスプラチンなどの化学療法を受けておられる患者は，食事ができない，できても吐いてしまうといった副作用に悩まされ，このことは患者のQOLを大きく低下させます。消化器症状の改善には六君子湯が江戸時代より用いられてきていますが，北海道大学臨床病態解析学研究室の武田宏司先生は，シスプラチンを投与して食思不振を起こさせたラットを用いて，六君子湯が明らかに食思を改善すること，そのメカニズムに食思増進ペプチドとして知られている内在性ホルモン，グレリンの増加が起こること，その作用はセロトニン2c受容体を抑制すること，さらに六君子湯の8種の生薬の1つ，陳皮(温州ミカンの皮を乾燥させたもの)に含まれるフラボノイド類にその効果があることを証明しました[11)]。一方，鹿児島大学心身内科学の乾明夫先生のグループは，六君子湯はグレリンを分泌させるだけでなくグレリン受容体の活性を増強することを見出しました。さらにその作用が8生薬中の蒼朮(そうじゅつ)に含まれるアトラクチロジンによって起こっていることを証明しました。六君子湯に含まれる異なる生薬がグレリンの分泌ならびにグレリン受容体の機能を高めることで，結果的にグレリンシグナルを強めていることがわかったのです[12)]。このようなターゲットに有効な成分を明らかにしようとする研究は，ほかに抑肝散，牛車腎気丸などでも行われており，最も進んでいるのが人参，山椒，乾姜

という3種の生薬でできている大建中湯です。大建中湯の乾姜に含まれる6-ショーガオールが腸管腔より上皮細胞に働きTRPA1活性化→アドレノメデュリン放出→血流増加となること，山椒に含まれるハイドロキシ-α-サンショールが血中に吸収された後，上皮細胞に働き活動性の閾値を下げることによりTRPV1，TRPA1に6-ショーガオールが反応しやすい環境をつくり出し，また人参に含まれるシンセノサイドなどの成分が6-ショーガオールやハイドロキシ-α-サンショールをターゲット細胞にアクセスしやすくしているのではという仮説が基礎研究により次々と証明されつつあります。結果としての血流の増加，腸管運動亢進作用が大建中湯の効能であり“腹が冷えて痛み，腹部膨満感がある”状況を改善すると考えられます。実際，大建中湯を飲むとお腹が温まると話す患者は多いことからも納得できます。研究が進展すれば，なぜこの組み合わせで漢方薬が構成されているのか，なぜこの組み合わせでないといけないのかという，漢方薬の「組み合わせの妙」が解き明かされる日も近いかもしれません。前述した乾先生のグループは，六君子湯はがん細胞を接種したラットの生存を延ばすことを明らかにしました[12]。同じことがヒトでもいえるのかについて，われわれは北海道大学を中心とした北海道の14病院で，手術のできない膵がんⅣ期患者を対象に「ゲムシタビン投与膵がん患者における軽度悪液質または前悪液質状態に対する六君子湯の悪液質進行抑制効果—無作為化第Ⅱ相比較試験」を計画し，2012年4月14日にキックオフミーティングを行い，現在臨床試験のための患者登録を行っています。その方法とは，患者を2群に分け，六君子湯の薬効を確かめる比較試験です。この結果をもとに2013年にはどちらかに漢方薬，もう一方にプラセボと呼ばれる形・におい・味・食感は同じで有効成分のないものを飲んでもらうランダム化比較試験を行っていく予定です。現在では，漢方薬メーカーの努力によりそのようなプラセボ薬が作られています。

漢方薬市民公開セミナー

2011年11月26日と2012年6月24日に，厚生労働科学研究費補助金 第3次対がん総合戦略研究事業研究班とNPO法人キャンサーネットジャパンとの共催で，市民公開セミナー『もっと知ってほしい「がんと漢方薬」のこと—漢方薬の現状とこれから』を開催いたしました(図2，3)[13]。

同班は，「がん治療の副作用軽減ならびにがん患者のQOL向上のための漢方薬の臨床応用とその作用機構の解明」という研究を2010～2013年の4年をかけて行っています。一方キャンサーネットジャパンは，がん患者を支援している組織・団体の垣根を越えて，抗がん剤の作用などについて科学的エビデンスに基づいたがん情報を発信しているNPO法人です[13]。特に2012年，Health On the Net Foundation(HON)の適性認証(HON code)を得ており，国内におけるがんに関する科学的エビデンスを公平に全国の皆さんに紹介している優れた組織です。同セミナーは，1回目160名，2回目170名の市民の皆様の参加を得て開催されました。内容については動画サイト『YouTube』に公開されており，誰でもその内容を視聴することが可能です。YouTubeで，「上園保仁」と検索するとその内容にアクセスすることができます(http://www.youtube.com/watch?v=WlTn2QVfAAI)。

市民公開セミナーでのQ&Aおよびアンケート調査から

このように2回，「がんと漢方薬のこと—漢方薬の現状とこれから」について公開セミナーを行いましたが，Q&Aならびにアンケート調査から浮き彫りになったことがありました。それは，がん患者とその家族の方が漢方薬に興味をもっており，症状によっては処方してもらいたいと思って

図2．第2回市民公開セミナーポスター

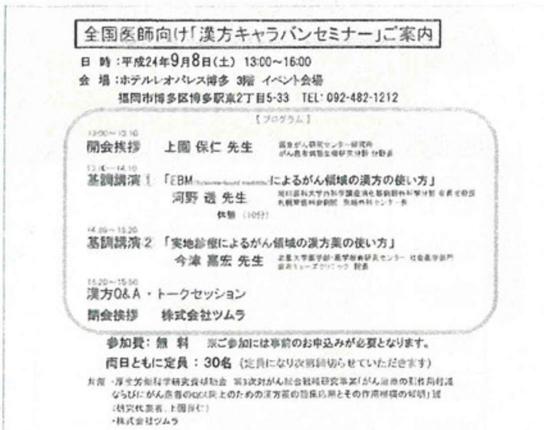
図3．第2回市民公開セミナープログラム

いても，誰に相談していいのかわからない，主治医に尋ねても取り合ってくれない，そもそも科学的根拠があるということを知らなかった，など，漢方薬を自ら使うという段階に行き着くには大きなギャップがあることがわかったのです。

そこでわれわれはこのような現況に鑑み，全国でがん治療，緩和ケアに取り組んでいらっしゃる医師の先生方に，漢方薬の作用メカニズムについての科学的エビデンス蓄積の現状ならびに漢方薬を用いた臨床研究における最新情報について報告できればと考え，全国医師向け「漢方キャラバンセミナー」を計画いたしました。2012年7月7日～9月23日の全7回，北は北海道から南は九州福岡まで全国5ヵ所で開催いたしました。今回のキャラバンでは，河野透先生より漢方薬の基礎，臨床研究のエビデンスを，北里大学薬学部の今津嘉宏先生よりがんの諸症状に応じた漢方薬の使い方を紹介いただきました（図4，5）。またその後Q&Aの時間を設けました。参加された医師の皆様からは「参加してよかった」「ためになった」「早速漢方薬の処方を考えます」という前向きな意見をいただいています。

おわりに

がん患者は，がん自体により，また抗がん剤治療や放射線療法により全身倦怠感，食思不振，痛みや吐き気などQOLを低下させるさまざまな症状に悩まされています。われわれ基礎医学研究者はがん患者のQOLを向上させる薬物療法などについて，その根拠となるデータを科学的に証明し，治療についてのエビデンスのある基盤データを臨床サイドに提供しなければならないと思っています。漢方薬がなぜがん患者の症状を和らげる



全国医師向け「漢方キャラバンセミナー」ご案内

日　時：平成24年9月8日(土)　13:00～16:00

会　場：ホテルレオパレス博多　3階　イベント会場

福岡市博多区博多駅東2丁目5-33　TEL：092-482-1212

【プログラム】

開会挨拶　上園 保仁 先生

基調講演1　「EBMによるがん領域の漢方の使い方」　河野 透 先生

休憩（10分）

基調講演2　「実地診療によるがん領域の漢方薬の使い方」　今津 嘉宏 先生

漢方Q&A・トークセッション

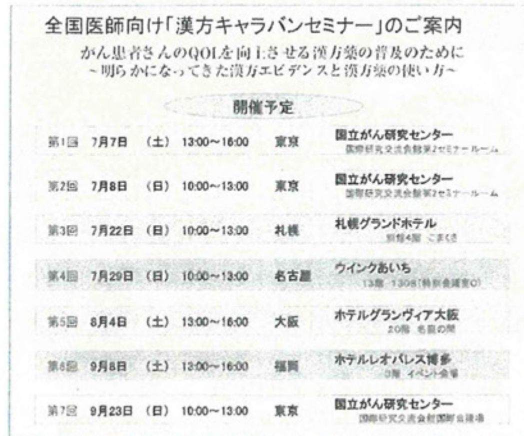
閉会挨拶　株式会社ツムラ

参加費：無　料　※ご参加には事前のお申込みが必要となります。

両日ともに定員：30名

・株式会社ツムラ

図4．全国医師向け「漢方キャラバンセミナー」福岡会場プログラム



全国医師向け「漢方キャラバンセミナー」のご案内

がん患者さんのQOLを向上させる漢方薬の普及のために

～明らかになってきた漢方エビデンスと漢方薬の使い方～

開催予定

第1回	7月7日	(土)	13:00～16:00	東京	国立がん研究センター
第2回	7月8日	(日)	10:00～13:00	東京	国立がん研究センター
第3回	7月22日	(日)	10:00～13:00	札幌	札幌グランドホテル
第4回	7月29日	(日)	10:00～13:00	名古屋	ウインクあいち
第5回	8月4日	(土)	13:00～16:00	大阪	ホテルグランヴィア大阪　20階
第6回	9月8日	(土)	13:00～16:00	福岡	ホテルレオパレス博多　3階　イベント会場
第7回	9月23日	(日)	10:00～13:00	東京	国立がん研究センター

図5．全国医師向け「漢方キャラバンセミナー」(全7回)

のか？　これまでの多くの経験，そしてさまざまな臨床試験により，効くことは事実であろうことがわかりはじめています。しかし，しっかりとした客観的で質の高い臨床試験を計画し，また漢方薬のプラセボ薬を用いた二重盲検でランダム化臨床比較試験を行い，事実を明らかにしていくことが必要であると考えています。くわえて，漢方薬がどのようなメカニズムで効くのかについて，生薬のどの成分が作用し，組み合わせではどのようなことが起こるのかなどについても科学的エビデンスに基づいたデータを集積したいと考えています。少しでも早く，漢方薬をはじめとして基礎医学の研究結果ががん患者のQOL向上に役立てばと願い，研究を進めているところです。

文　献

1）上園保仁：がん患者の症状緩和に役立つ漢方薬―漢方薬の有効性を示す，臨床につながる基礎研究―．がん患者と対療 **22**：140-146，2011

2）河野　透，上園保仁：腸管血流からみた大建中湯の役割―アメリカ臨床治験薬TU-100になった理由．医のあゆみ **241**：163-169，2012

3）Kono T, Omiya Y, Hira Y, et al : Daikenchuto (TU-100) ameliorates colon microvascular dysfunction via endogenous adrenomedullin in Crohn's disease rat model. J Gastroenterol **46** : 1187-1196, 2011

4）Kono T, Kaneko A, Hira Y, et al : Anti-colitis and -adhesion effects of daikenchuto via endogenous adrenomedullin enhancement in Crohn's disease mouse model. J Crohns Colitis **4** : 161-170, 2010

5）Kono T, Kanematsu T, Kitajima M : Exodus of Kampo, traditional Japanese medicine, from the complementary and alternative medicines ; is it time yet? Surgery **146** : 837-840, 2009

6）向山雄人：痛みゼロのがん治療．東京，文藝春秋，2012

7）Kono T, Mamiya N, Chisato N, et al : Efficacy of goshajinkigan for peripheral neurotoxicity of oxaliplatin in patients with advanced or recurrent colorectal cancer. Evid Based Complement Alternat Med : 2011(Epud ahead of print)

8）Kono T, Mishima H, Shimada M, et al ; GONE Investigators : Preventive effect of goshajinkigan on peripheral neurotoxicity of FOLFOX therapy ; a placebo-controlled double-blind randomized phase Ⅱ study (the GONE Study). Jpn J Clin Oncol **39** : 847-849, 2009

9）米国消化器病週間(DDW)2012. Nikkei Medical 2012年8月号別冊付録：11-23, 2012

10）北島政樹 監，今津嘉宏 編：がん漢方．東京，南山堂，2012

11) Takeda H, Sadakane C, Hattori T, et al : Rikkunshito, an herbal medicine, suppresses cisplatin-induced anorexia in rats via 5-HT2 receptor antagonism. Gastroenterology **134** : 2004-2013, 2008
12) Fujitsuka N, Asakawa A, Uezono Y, et al: Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival. Translational Psychiatry **1** : e23, 2011
13) NPO法人キャンサーネット(http://www.cancernet.jp/):『がんと漢方薬』のこと 漢方薬の現状とこれから．第1回，2011(http://www.cancerchannel.jp/posts/2011-11-26/7499.html)，第2回，2012(http://www.cancerchannel.jp/posts/2012-06-24/14942.html)

A Review of Traditional Japanese Medicines and their Potential Mechanism of Action

Yasuhito Uezono*, Kanako Miyano, Yuka Sudo, Masami Suzuki, Seiji Shiraishi and Kiyoshi Terawaki

Division of Cancer Pathophysiology, National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan

Abstract: Traditional Japanese herbal, or Kampo medicine was developed and modified from Chinese herbal medicine. After the Japanese government approved Kampo for clinical use, much attention has been paid to establishing scientific evidence for the effectiveness of these medicines. Recent progress has been made in elucidating the mechanisms of action of some types of Kampo medicine, including rikkunshito (RKT), daikenchuto, and yokukansan. In this review, we focused on identifying the target molecules and the active ingredients of RKT.

Thus far, many target molecules have been implicated in the mechanism of action of Kampo medicines, such as ion channels, enzymes, and receptors. In particular, G protein-coupled receptors are attractive candidates for explaining herbal medicine activity. This is particularly true of RKT, which is composed of 8 independent, crude drug extracts. Recent reports have shown that RKT elicits its effects through dual action to the G protein-coupled receptors: inhibition of serotonergic 5-HT_{2C} and 5-HT_{2B} receptors and activation of ghrelin receptors via specific ingredients of RKT.

In addition, we suggest that the identification of the effective ingredients from Kampo medicines could contribute to the discovery and development of new drugs by means of modern high-throughput drug screening technology.

Keywords: Rikkunshito, GPCR, Kampo medicine, herbal medicine, ghrelin.

1. KAMPO MEDICINES

Chinese herbal medicine, one of the oldest forms of traditional medicine, has been used in China and other countries for more than 3000 years. Traditional Japanese herbal, or Kampo medicine originated from its Chinese counterpart after the latter was introduced into Japan and subsequently modified and developed to suit Japanese culture and environmental factors [1]. These Kampo medicines, systemically developed in the 16th century into a more specifically Japanese form, have a wide range of indications for maintaining quality of life, rather than curing patients [2]. Kampo medicine is thus intended to boost the body's own healing power (i.e., immune system) and help restore its natural balance.

Since Japan's Ministry of Health, Labour and Welfare approved a series of approximately 130 Kampo medicines for use in clinical practice, they have been increasingly employed to help maintain the quality of life in patients with diseases such as gastrointestinal disorders, cancer, and lifestyle-related diseases [3]. However, there is little scientific evidence supporting the reliable clinical use of these treatments. This is because Kampo medicines are composed of several crude drug products, mainly extracted from plants, and it is difficult to maintain consistent quality and quantity of these ingredients. Consequently, evidence-based medicine has avoided the field of Kampo medicine. Recently, however, Kampo medicines have been developed in Japan through clinical and laboratory studies based on Western-adopted, scientific, experiment-based approaches [1, 4]. Thus, in the past decade, to support the use of Kampo medicine, scientific evidence has been accumulated and is continuously increasing.

In this review, we focused on the top 10 best-selling Kampo medicines in Japan and reviewed their mechanisms of action with laboratory-based, scientific approaches. In particular, we focused on G protein-coupled receptors (GPCRs). These molecules comprise the largest superfamily of cell-surface receptors, mediate many important physiological functions, and are considered some of the most successful therapeutic targets for a broad spectrum of diseases, serving as the target for more than 30 % of the current therapeutic agents on the market [5, 6]. Even though Kampo medicines are composed of many active substances, GPCR assay development and GPCR ligand screening of the substances in Kampo medicines appear quite successful in identifying new bioactive substances, as compared to ready-made, low molecule panels. We therefore examined the cellular signaling pathways of Kampo medicines primarily through GPCRs. In this review, we chose to focus on a widely used Kampo medicine in Japan, namely, "rikkunshito (RKT)," for the analysis of RKT-mediated GPCR signaling.

*Address correspondence to this author at the Division of Cancer Pathophysiology, National Cancer Center Research Institute, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan; Tel: +81-3-3547-5248; Fax: +81-3-3542-1886; E-mail: yuezono@ncc.go.jp

2. PUBLISHED STUDIES REGARDING THE MOST COMMONLY USED KAMPO MEDICINES

The most frequently, clinically used Kampo medicines in Japan, as reflected by the top 10 best-selling products, are made by Tsumura Co. Ltd., and are listed in (Table **1**) [7]. The table also lists the number of relevant scientific reports found via the PubMed database. We found a direct relationship between sales rank and number of peer-reviewed research papers published on the product (Table **1**). As a result, we have chosen the top 10, bestselling Kampo medicines in Japan (and an additional 2 products with a significant number of scientific reports) for our study. We introduced and summarized the scientific reports for these products by elucidating each medicine's signaling mechanism and site of action. (Table **2**) lists the pathological symptoms currently treated by the Kampo medicines listed in (Table **1**).

3. PHARMACOLOGICAL EFFECTS AND THE MECHANISMS OF ACTIONS OF THE KAMPO MEDICINES

(Table **3**) shows the pharmacology and mechanisms of actions of the 12 Kampo medicines listed in (Table **1**), without listing GPCR-mediated signaling (these pathways are explained later, in an independent section). For each listed Kampo medicine, a variety of cellular signaling processes related to the drug's pharmacological action are reported in the literature, e.g., activation of the transient

1873-4286/12 $58.00+.00

Table 1. The Ranking of Sales and Numbers of Scientific Reports of Kampo Medicines

Kampo	Sales in 2010		Numbers of Paper	
	The Ranking [7]	(Million of Yen) [7]	The Ranking	(Searched by PubMed)
Daikenchuto	1	7,960	4	59
Hochuekkito	2	6,218	12	19
Rikkunshito	3	5,288	7	46
Yokukansan	4	3,984	2	64
Kamishoyosan	5	3,653	10	27
Goshajinkigan	6	3,531	8	41
Bakumondouto	7	3,510	11	24
Saireito	8	3,336	1	108
Shakuyakukanzoto	9	3,309	6	51
Shouseiryuto	10	2,757	9	40
Saibokuto	Out of Top 10		3	60
Keishibukuryogan			5	55

Table 2. Medical Claims of Kampo

Sales Ranking (see Table 1)		Medical Claims
1	Daikenchuto	Sense of abdominal distension and pain by feeling cold in abdomen
2	Hochuekkito	Loss of appetite, languor and enhancement of physical strength of the convalescence
3	Rikkunshito	Loss of appetite, gastritis, stomach pain and dyspepsia
4	Yokukansan	Neurosis, insomnia, irritability in children
5	Kamishoyosan	Climacteric disturbance, coldness of hands and legs, nervousness and menoxenia
6	Goshajinkigan	Lumbago, pain of lower extremities and numbness
7	Bakumondouto	Bronchitis, bronchial asthma and cough accompanying severe sputum
8	Saireito	Diarrhea, edema and acute gastroenteritis
9	Shakuyakukanzoto	Muscle cramp, pain with muscle cramp
10	Shouseiryuto	Allergic rhinitis, rhinitis, bronchial asthma and bronchitis
Out of Top 10	Saibokuto	Anxiety neurosis, bronchial asthma, bronchitis and cough
	Keishibukuryogan	Climacteric disturbance, hot flash, menoxenia and endometritis

receptor potential channel vanilloid 1 (TRPV1) in daikenchuto (DKT) [8], enhancement of cytokine production in hochuekkito and induction of nitric oxide (NO) synthesis in RKT [9, 10], inhibition of *N*-methyl-D-asparatic acid (NMDA) receptor [11], or activation of γ-aminobutyric acid A ($GABA_A$) receptor [12] in yokukansan (YKS). These findings suggest that there are numerous potential target molecules in the cells for the pharmacological action of Kampo medicines. This is probably because Kampo medicines are composed of mixtures of more than 2 crude drug extracts, and thus contain multiple substances that could activate many signaling pathways in the cells.

4. GPCRS

GPCRs comprise the largest superfamily of cell-surface receptors, accounting for 4 % of the human genome [13]. Although the GPCR family members share a structural similarity—namely, a characteristic, 7-transmembrane-spanning architecture linked by an N-terminal extracellular domain, C-terminal intracellular domain, 3 extracellular loops, and 3 intracellular loops—different GPCRs react with different types of ligands, including photons, ions, biogenic amines, peptide and non-peptide neurotransmitters, hormones, growth factors, and lipids. Each ligand transduces its specific signal to the intracellular effectors via a conformational

Table 3. Pharmacological Actions and it's Mechanism of Kampo: Expect for GPCRs

Sales Ranking (see Table 1)		Pharmacological Actions	Mechanism of Actions	References
1	Daikenchuto	Enhancement of gastrointestial motility	Activation of TRPV1	[8]
2	Hochuekkito	Improvement of immunosuppression	Enhancement of cytokine production (IFNα, IFNγ, IL-12)	[97, 98]
3	Rikkunshito	Gastric emptying effects	Induction of NO synthesis	[10]
		Protective effects of gastric mucosal injury	Induction of NO synthesis	[9]
			Inhibition of myeloperoxidase (MPO) activity	[99]
			Increase of heat-shock protein 60 (HSP60) expression	[100]
		Suppression of secretion of gastric acid	Inhibition of activity of H^+, K^+-ATPase	[101]
4	Yokukansan	Suppression of calming effects	Attenuation of abnormal glutamate release	[102]
			Amelioration of the levels of both glutamate uptake and expression of glutamate aspartate transporter	[103]
			Inhibition of NMDA receptor	[11]
		Improvement of insomnia	Activation of $GABA_A$ receptor	[12]
5	Kamishoyosan	Anxiolytic effects in climacteric women	Activation of $GABA_A$ receptor	[104]
6	Goshajinkigan	Antinociceptive effects (numbness)	Induction of NO synthesis	[53]
		Increase of blood flow		[105]
7	Bakumondouto	Antiallergic effects	Suppression of degranulation	[55]
8	Saireito	Hydragogue effects	Antagonistic action on the mineral corticoid receptor	[106]
		Antiinflammation effects	Inhibition of prostaglandin E_2 production and cyclooxygenase-2 expression	[107, 108]
			Production of proopiomelanocortin (POMC)	[109]
9	Shakuyakukanzoto	Antispasmodic effects	Increase of cAMP by inhibition of phosphodiesterase III	[110]

(Table 3) Contd....

Sales Ranking (see Table 1)		Pharmacological Actions	Mechanism of Actions	References
10	Shouseiryuto	Antiallergic and antiinflammatory effects on respiratory	Suppression of cytokine production (IL-3, IL-4)	[111, 112]
			Generation of NO in airway epithelium	[113]
Out of Top 10	Saibokuto	Antiallergic and antiinflammatory effects on respiratory	Suppression of cytokine production (IL-3, IL-4)	[114]
			Generation of NO in airway epithelium	[115]
	Keishibukuryogan	Regulation of female hormone	Regulation of luteinizing hormone (LH) and follicle-stimulating hormone (FSH)	[116]
		Effects on adenomyosis	Suppression of activity of thymidylate synthetase in uteri	[117]

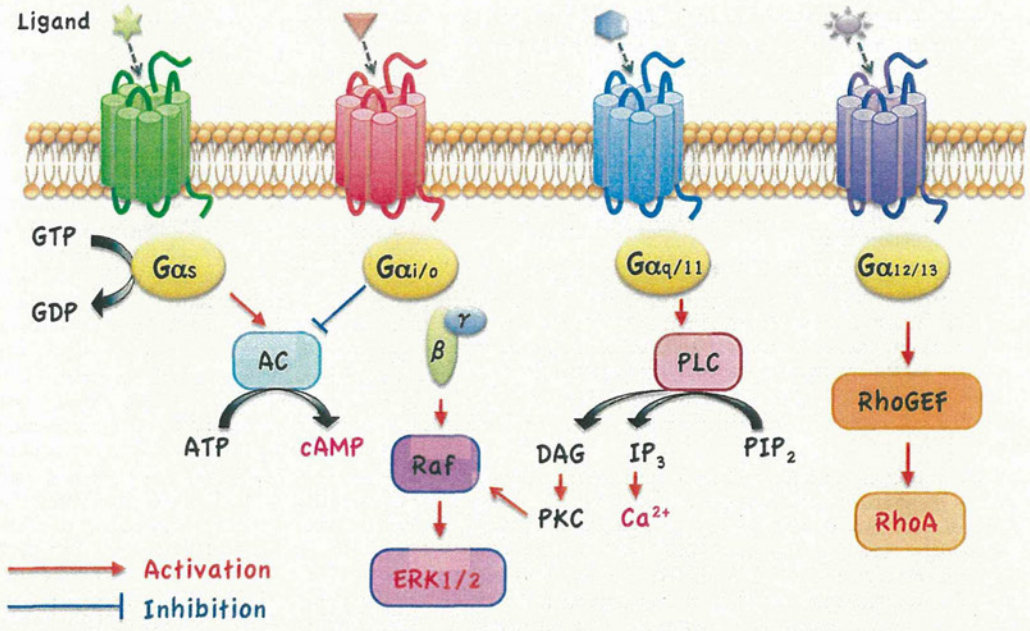


Fig. (1). G protein-coupled receptor (GPCR)-mediated signaling. Schematic representation of major pathways of GPCR signaling by different G_{α} proteins. Red line: activation, blue line: inhibition. AC: adenylyl cyclase, ERK1/2: extracellular signal-regulated kinase 1/2, PLC: phospholipase C, RhoGEF: Rho guanine nucleotide exchange factor.

rearrangement linked to an increasing number of cytosolic interactants [14]. When an agonist binds to its specific receptors, these receptors catalyze the exchange of guanidine diphosphate (GDP) for guanidine triphosphate (GTP) on the α-subunit of heterotrimeric G proteins that are composed of 3 independent proteins (namely α-, β-, and γ-subunits), change their conformation, and cause dissociation of Gα from the dimeric Gβγ subunits [15]. As shown in (Fig. **1**), GPCRs coupled to Gαs activate adenylyl cyclase, whereas those coupled to Gαi/o inhibit this enzyme. Cyclic adenosine 3′, 5′-monophosphate (cAMP), produced by adenylyl cyclase from adenosine 5′-triphosphate (ATP), serves as a second messenger that activates protein kinase A (PKA) and other downstream effectors. In addition, Gβγ subunits released from $G\alpha_{i/o}$-coupled, receptor-bound, trimeric G proteins activate Raf and extracellular signal-regulated kinase (ERK) 1/2 pathways. GPCRs coupled to $G\alpha_{q/11}$ activate phospholipase C (PLC), which catalyzes the formation of diacylglycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3) from phosphatidylinositol 4,5-biphosphate (PIP_2). The IP_3 thus produced, binds to and opens the endoplasmic IP_3-gated calcium channel, releasing calcium into the cytoplasm, eventually increasing intracellular Ca^{2+} concentrations. The DAG thus produced activates protein kinase C (PKC), which also activates Raf-mediated path-

ways. GPCRs coupling to $G\alpha_{12/13}$ activate the Rho guanine nucleotide exchange factor (RhoGEF), which in turn activates the small G protein RhoA. Signals elicited by G proteins stimulate downstream signaling, such as enhancement of transcription and translation, which induce a specific GPCR-mediated cellular response [16, 17].

5. ASSAY OF GPCR-MEDIATED SIGNALING

GPCRs mediate many important physiological functions and are considered some of the most effective therapeutic targets for a broad spectrum of diseases. Hence, the establishment and development of a GPCR assay for GPCR ligand screening is very important. Receptor binding assays are used for the characterization of the interaction between receptors and their ligands, such as the intrinsic affinity of ligands for the receptor, association/dissociation rates, and the density of receptors in tissues or cells [18]. Receptor binding assays have been used to obtain information about agonists and antagonists in previous experiments [18]. The availability of labeled ligands, however, greatly limits the usefulness of this assay. Additionally, upon ligand binding, GPCRs change their conformation and activate coupled G proteins, which subsequently promote second-messenger production via downstream effectors, and such cellular changes are not detected with this assay. As a result, corresponding assays measuring either G protein activation or G protein-mediated events are necessary in addition to the binding assay (Fig. **1**).

GTPγS-binding assays directly measure the guanine nucleotide exchange of G proteins, an early and simple event following GPCR activation, since it is not subjected to amplification or regulation by other cellular effector systems [19]. Typically, the accumulation of a nonhydrolysable GTP analog, such as [^{35}S]-GTPγS, on a plasma membrane prepared from cells expressing GPCRs of interest is measured after agonist stimulation. The merit of the GTPγS-binding assay over the ligand binding assay is that it can discriminate between full or partial agonists, neutral antagonists, inverse agonists, and allosteric regulators [20].

Assays measuring cellular levels of cAMP are dependent on the activity of adenylyl cyclase, which is regulated by GPCRs coupled to $G\alpha_s$ or $G\alpha_{i/o}$ proteins. As shown in (Fig. **1**), $G\alpha_s$ stimulates the activity of adenylyl cyclase to increase cellular cAMP levels. In contrast, $G\alpha_{i/o}$ negatively regulates adenylyl cyclase to decrease cAMP levels. The ERK1/2 assay, which measures levels of phosphorylated ERK1/2, could also be used to measure $G\alpha_{i/o}$-mediated signaling.

Stimulation of $G\alpha_{q/11}$-coupled-GPCRs activates PLC, which in turn produces IP_3 and DAG. While DAG activates PKC, IP_3 results in an efflux of Ca^{2+} from the endoplasmic reticulum into the cytoplasm, eventually elevating the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Thus, measuring $[Ca^{2+}]_i$ is a reliable and easy assay for these receptors. Measurement of $[Ca^{2+}]_i$ is very popular in GPCR screening, employing commercially available, cell-permeable, highly Ca^{2+}-sensitive fluorescent dyes such as Fluo-3 and Fluo-4. Thus Ca^{2+}assays are robust and easily amenable to high throughput assay. One drawback of such an assay is that calcium flux occurs rapidly and transiently and is thus unsuitable for detecting long-lasting cellular events. Assays of $G\alpha_{12/13}$ protein-mediated signaling face the drawback that it remains relatively difficult to measure their downstream second messengers [21].

6. GPCRS AS SITES OF ACTION OF KAMPO MEDICINES

Among the 12 Kampo medicines we studied, a significant number stimulate or inhibit some GPCR-mediated pathways, as shown in (Table **4**). To study these pathways, we next introduce and describe GPCR-mediated signaling using 2 particular examples, DKT and YKS. Others are also introduced (Table **4**).

1) DKT

DKT is widely prescribed for patients with gastrointestinal obstruction, such as postoperative ileus (POI) or postoperative intestinal paralysis [22-24]. Studies based on several animal disease-models have shown that DKT is therapeutically effective. POI is a transient bowel motor dysfunction that occurs after surgery; DKT has been found to ameliorate delayed gastrointestinal transit in a rat POI model, created by inducing laparotomy via intestinal manipulation [25, 26] or talc-induced intestinal adhesion [27]. These results were repeated in a hypoperistalsis model induced by chlorpromazine in mice [28]. These ameliorative effects were partially inhibited by SB204070, a serotonergic 5-HT_4 receptor antagonist, and the effects were completely abolished by atropine, a muscarinic receptor antagonist [26]. These results thus demonstrate that DKT performed its action via muscarinic and 5-HT_4 receptors.

Additional evidence further linked DKT's effects to 5-HT_4 receptors. DKT induced contractions in a dose-related manner in the isolated guinea pig ileum, and this contraction was completely suppressed by atropine and tetrodotoxin, an inhibitor of voltage-gated Na^+ channels—and this contraction was partially suppressed in the presence of a 5-HT_4 receptor antagonist [29]. Intraduodenal and intrajejunal application of DKT induced phasic contractions in the duodenum and proximal jejunum, respectively, which were both inhibited by atropine [30]. These results suggest that the contractile response induced by DKT is partially mediated by acetylcholine released from cholinergic nerve endings and 5-HT_4 receptors.

In addition, tachykinins, such as substance P (SP)—which is also classified in the GPCR family agonist—may also be involved in the atropine-resistant contraction induced by DKT [8]. A single oral administration of DKT caused significant increases in plasma levels of neurotransmitters, such as motilin [31], vasoactive intestinal peptide (VIP), 5-HT [32], calcitonin gene-related peptide (CGRP), and SP [33]—but not of gastrin or somatostatin [31]. It has been shown that DKT induces the release of motilin and VIP into plasma mainly through the activation of muscarinic M_1 receptors [34]. Although intestinal motility of DKT has been suggested as an important mechanism underlying its effective motility, increased intestinal or colonic blood flow caused by DKT is another potentially important mechanism. Administration of DKT to rats increased blood flow in both the small and large intestine via the CGRP/ adrenomedullin (ADM) receptor family [24, 35-37]. The CGRP receptor is composed of the calcitonin receptor-like receptor (CRLR) and single transmembrane domain receptor activity modifying protein 1 (RAMP1), and ADM receptors are composed of CRLR with RAMP2 [38, 39]. Interestingly, mRNA expression for CRLR and RAMP1 were upregulated by DKT in the rat colon [36], demonstrating that increased blood flow by DKT is mainly mediated by CGRP and its receptors. Collectively, DKT's effects are mediated, at least in part, by some GPCRs, such as 5-HT receptors, muscarinic receptors, and CGRP/ADM receptors.

2) YKS

YKS has been reported to improve behavioral and psychological symptoms associated with dementia (BPSD), such as hallucinations, agitation, and aggressiveness in patients with Alzheimer's disease, dementia with Lewy bodies, and other forms of senile dementia [2, 40, 41]. Research supporting these significant clinical effects of YKS has been performed in a series of disease-related animal models. In these studies, YKS ameliorated BPSD-like symptoms, such as aggressive behavior, anxiety-like behavior, and decreases in social behaviors in thiamine-deficient rats [42, 43], zinc-deficient mice [44], amyloid β protein (Aβ)-intracerebroventricular (i.c.v.)–injected mice [45, 46], Aβ precursor protein-transgenic mice [47], and para-chloroamphetamine (PCA)-injected rats [48]. The mechanisms behind these ameliorative effects have been shown to involve serotonergic function via 5-HT receptors.

Table 4. Effects of Several Kampo on GPCRs and it's Pharmacological Actions

Sales Ranking (see Table 1)		Pharmacological actions	Mechanism of actions	References
1	Daikenchuto	Enhancement of gastrointestial motility	Mediation by muscarinic ACh receptor	[8, 30, 118]
			Mediation by neurokinin-1 receptor	[8]
			Activation of 5-HT_4 receptor receptor	[29]
		Increase of blood flow in intestinal	Mediation by CGRP	[35, 36]
			CGRP receptor mRNA up-regulation	[24, 36]
			Mediation by VIP and ACh	[35]
			ADM release	[24, 37, 119]
		Improvement on postoperative ileus	Mediation by 5-HT_4 receptor	[26]
			Mediation by ACh	[26]
3	Rikkunshito	**See Table 6**		
4	Yokukansan	Antiagressive behavior	Mediation by 5-HT_{1A} receptor	[48]
		5-HT_{1A} receptor partial agonistic effect		[49]
		Improvement of memory disturbance	Up-regulation of expression of muscarinic M_1 receptor	[52]
		Suppression of head twitch induced by 5-HT_{2A} agonist	5-HT_{2A} receptor expression down-regulation	[51]
6	Goshajinkigan	Antinociceptive effects (numbness)	Activation of κ-opioid receptor	[53, 54]
7	Bakumondouto	Bronchodilation	Augmentation of responses stimulated by adrenergic β receptor	[55]
		Expectoration	Increase of expression of adrenergic β_1 receptor	[56]
9	Shakuyakukanzoto	Antinociception	Activation of spinal descending pain inhibitory adrenergic α_2 system	[57]

(Table 4) Contd....

Sales Ranking (see Table 1)		Pharmacological actions	Mechanism of actions	References
10	Shouseiryuto	Antiallergic and antiinflammatory effects on respiratory	Suppression of histamine release	[59]
			Attenuation of LT release	[120]
			Suppression of the mRNA level of histamine H_1 receptor	[58]
Out of Top 10	Saibokuto	Antiallergic and antiinflammatory effects on respiratory	Augmentation of responses stimulated by adrenergic β receptor	[56]
			Suppression of both production and release of LT	[61, 121, 122]
			Attenuation of production of PAF	[62]
			Inhibition of histamine release	[60]
	Keishibukuryogan	Alleviation of climacteric symptom	Increase of CGRP in serum	[63]
			Regulation of CGRP receptors in mesenteric artery	[63]

The ameliorative effects of chronic YKS specifically on aggressive behavior were counteracted by co-administration of a 5-HT_{1A} antagonist, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclo-hexanecarboxamide trihydrochloride (WAY-100635) [48], suggesting that one of YKS's mechanisms may be related to an agonistic effect on 5-HT_{1A} receptors. Indeed, Terawaki *et al.* (2010) [49] showed, using competitive binding assays and [^{35}S] GTPγS for 5-HT_{1A} receptors, that YKS had a partial agonistic effect on 5-HT_{1A} receptors. This effect has been mainly attributed to *Uncaria hook*, one of the medicine's constituent herbs. Furthermore, geissoschizine methyl ether (GM), a plant indole alkaloid from *Uncaria hook*, has been shown to behave as a partial agonist at the 5-HT_{1A} receptor [50]. Taken together, these results suggest that GM contained in YKS is the source of the medicine's partial agonist effects on 5-HT_{1A} receptors. A recent report has also shown that GM has antagonistic effects at the serotonin 5-HT_{2A}, 5-HT_{2C} and 5-HT_7 receptors, and partial-agonist/antagonist effects at the dopamine D_{2L} receptor [50]. In addition, YKS was reported to decrease expression of 5-HT_{2A} receptors in the prefrontal cortex, which is a part of the circuitry mediating inhibition of 2,5-dimethoxy-4-iodoamphetamine (DOI)-induced head-twitch responses [51]. In addition, it has been shown that amelioration of the spatial working memory by YKS was reversible by a muscarinic receptor antagonist scopolamine; additionally, YKS treatment reversed olfactory bulbectomized (OBX)-induced downregulation of choline acetyltransferase and muscarinic M_1 receptor expression [52].

Thus, YKS potentially has multiple effects on various 5-HT receptor subtypes. Finally, YKS elicits its actions on receptor other than 5-HT receptors, such as dopamine and muscarinic receptors, suggesting that these integrated actions via several GPCR-mediated signaling pathways contribute to the psychotropic effects of YKS.

3) Other Kampo Medicines

Kampo medicines other than DKT and YKS also have been reported to exhibit their signaling effects via GPCR-mediated pathways (see Table **4**), as follows:

Goshajinkigan, which is prescribed for pain or numbness in the lower extremities, has been shown to stimulate spinal κ-opioid receptors via the release of the endogenous κ-opioid agonist dynorphin [53, 54].

Bakumondouto, which is prescribed for bronchitis or bronchial asthma, has been shown to increase levels of mRNA for adrenergic β_1 receptors in a concentration-dependent manner [55], and potentiate responses stimulated by a β-adrenergic receptor agonist in airway smooth muscle [56].

Shakuyakukanzoto, which is prescribed for muscle cramps and associated pain, has been shown to have an antinociceptive effect in diabetic mice, which is enacted via selective activation of the spinal descending inhibitory adrenergic α_2 systems [57].

Shouseiryuto, which is prescribed for allergic rhinitis or bronchial asthma, has been shown to suppress histamine release from rat peritoneal mast cells and the mRNA levels of histaminergic H_1 receptors in the nasal mucosa of toluene 2, 4-diisocyanate (TDI)-sensitized rats that model nasal allergies [58, 59].

Saibokuto, which is prescribed for anxiety neurosis or bronchial asthma, has been shown to inhibit compound 48/80-induced degranulation and histamine release from mast cells, production of leukotrienes (LTs), and LTB4 and 5-lipoxygenase activities in cultured rat basophilic leukemia-1 cells. It also inhibited platelet-activating-factor (PAF) production by human neutrophils. These results imply that saibokuto inhibited the release or production of endogenous ligands for LT or PAF receptors [60-62]. Saibokuto