原　　著

SIGNIFICANCE OF ANGIOTENSIN SYSTEM IN PROGRESSION OF COLORECTAL CANCER

Yi Luo, Takasumi Shimomoto, Hiroki Kuniyasu
Department of Molecular Pathology, Nara Medical University

Correspondence: Hiroki Kuniyasu,
Department of Molecular Pathology, Nara Medical University School of Medicine, 840 Shijo-cho, Kashihara, Japan 634-8521, E-mail: cooninh@zb4.so-net.ne.jp.

Angiotensin in colorectal cancer

Abstract : Colorectal cancer (CRC) cells possess an angiotensin activation mechanism provided by the expression of renin and chymase. Renin expression is induced by hyperglycemic condition. Since angiotensinogen is produced in the liver, CRC cells with angiotensin-activating machinery possess an advantage to metastasize to the liver. In human CRC cases, the diabetes complicated patients show higher concentrations of renin, angiotensin-II in the primary tumors, and more progressed disease stage, especially, liver metastasis in an association with HbA1c levels than those in the patients without diabetes. Concurrent treatment with anti-angiotensin and hypoglycemic agents shows a synergic effect of decrease of liver metastasis and improvement of the survival of diabetic mice of CRC liver metastasis model. The effect of anti-angiotensin treatment and blood sugar control as a baseline management of the colon cancer patients with the diabetic condition is needed to be examined in clinical situation for prevention of liver metastasis.

Key words : angiotensin, liver metastasis, renin, ARB, diabetes

Angiotensin promotes cancer progression

Colorectal cancer (CRC) is the third leading cause of cancer death in Japan, and the cancer mortality is still increasing a s the Western lifestyle gains popularity among the Japanese population [1]. Approximately 24% of CRC cases involving invasion beyond the submucosal layer showed liver metastasis during and/or after the operation [2]. One-third of CRC patients died of liver metastasis [3]. Only one-third or fewer CRC patients with liver metastasis respond to systemic chemotherapy, however, and long-term survival is rare [3]. The 5-year survival rate of CRC patients with liver metastasis is less than 20% [1]. Early detection and control of liver metastasis is therefore an important issue for the treatment of CRCs.

Angiotensin-II (A-II) has multiple physiologic effects; activation of A-II type 1 receptor (ATR1) by A-II eventually leads to vasoconstriction, inflammation, and proliferation in cardiovascular

and neoplastic tissues [4].ATR1 intracellular signaling pathways produce diverse effects: ATR1 induces activation of protein kinase C, angiopoietin 2, vascular endothelial growth factor (VEGF), VEGF receptors, fibroblast growth factor, platelet-derived growth factor, transforming growth factor beta, epidermal growth factor, nitric oxide synthase, and metalloproteinase [4,5]. These properties enhance colon carcinogenesis and disease progression.

We have confirmed the protumoral effect of angiotensin by experiment. Effects of A-II on cell growth, invasion, and apoptosis are examined in the CRC cell lines [6]. A-II enhances cell growth and in vitro invasion into type IV collagen in a dose-dependent manner. In contrast, apoptosis is decreased by A-II in a dose-dependent manner. Reduction of hepatic angiotensinogen (ATG) production by knockdown with cholesterol-conjugated antisense S-oligodeoxynucleotide (S-ODN) suppressed liver metastasis of HT29 cells in nude mice liver metastasis model. ATG-knockdown mice show less pronounced size, number, Ki-67 labeling index, and microvessel density in the metastatic foci than those in the control mice. Knockdown of renin or chymase in HT29 cells show smaller and less numbered metastatic foci in the liver than those in control. Furthermore, the examination of 121 CRC patients shows that the serum A-II concentration is significantly associated with advanced disease stage, especially, the liver metastasis.

Angiotensin activation in CRC cells

ATG is an inactive precursor of A-II (Fig. 1). ATG possesses no effect on cancer cells without conversion to A-II. Effects of ATG on cell growth, invasion, and apoptosis are examined in HT29 cells. Interestingly, ATG enhanced cell growth, in vitro invasion, and anti-apoptotic survival in HT29 cells in a dose-dependent manner as shown as treatment with A-II. This finding suggests that the HT29 cells have angiotensin-activating machinery by themselves.

We then examine the expression of angiotensin-associated genes in HT29 cells. They express ATR1, but do not express ATG or angiotensin I (A-I) converting enzyme (ACE). However, they express chymase, which possesses an ACE-like activity. Renin is also expressed in HT29 cells. Moreover, a renin inhibitor abrogates the production of both A-I and A-II. A chymase inhibitor suppresses the production of A-II but not that of A-I. In contrast, an ACE inhibitor does not affect the production of A-I or A-II in HT29 cells. Thus, A-II is produced from ATG by renin and chymase in HT29 cells. Chymase, tonin, and cathepsin G all possess an ACE-like activity, which can be used as a substitute for ACE (Fig. 1) [7]. Cathepsin D is responsible for producing A-I from ATG in cardiac myocytes, fibroblasts, and vascular smooth muscle cells in place of renin [8]. However, the CRC cells in this study do not express cathepsin D. Chymase expression is associated with hypoxia or ischemia in the human left cardiac ventricle [9]; however, it is not associated with hyperglycemia in CRC cells [6].

MAS1

MAS1 is a receptor of an angiotensin II (A-II) degenerative product by angiotensin converting enzyme 2, angiotensin 1-7 (A1-7), which provide anti-A-II phenotypes, such as

vessel dilation, depression of blood pressure (Fig. 1) [10]. We examine a role of MAS1 in CRC and invasive ductal carcinoma (IDC) of the breast [11]. By immunohistochemistry, MAS1 is not detected in CRCs and the normal colon mucosa. The normal mammary lobules and ducts express MAS1 at high levels, whereas MAS1 expression is attenuated in all IDCs. Especially, MAS1 expression is highly decreased in scirrhous type IDCs than that in other types. The decrease of MAS1 expression is associated with lymph node metastasis but not T factor, grade, status of estrogen receptor or progesterone receptor. The decrease of MAS1 expression is reversely associated with HER2 expression. Using a mouse breast cancer cell line, BALB-MC, which expressed MAS1, cell growth and in vitro invasion are examined. A1-7 treatment inhibits growth and invasion of BALB-MC cells, which are abrogated by MAS1 knockdown. MAS1 intracellular signaling involves Akt phosphorylation, protein kinase C activation and mitogen-activated protein (MAP) kinase inhibition [12]. These findings suggest that MAS1 might act as an inhibitory regulator of breast tissues and the cancer.

CD10

CD10, known as common acute lymphoblastic leukemia antigen (CALLA), is a characteristic marker of various subgroups of B-cell type-acute lymphocytic leukemias [13,14]. It is a zinc-dependent membrane metalloendopeptidase, also called as neutral endopeptidase (EC 3.4.24.11), enkephalinase, or neprilysin [14]. CD10 is expressed in CRC and is associated with CRC metastases, especially liver metastasis. [2,15,16]. Met-enkephalin (MENK) is a high affinity substrate of CD10 [17,18]. MENK is produced by hepatocytes under the cellular stress condition, such as hepatitis, bile stasis, and liver metastasis [19-21]. MENK inhibits tumor growth and establish of metastatic foci [22]. CD10-positive CRC cells degrade MENK and escape from MENK-induced tumor suppression [22]. CD10 possesses a weak affinity to A-I [23]; however, CD10 shows A-I degrading activity but not the A-I convertizing activity. Degrading of A-I produces A1-7, a MAS1 ligand. As mentioned above, MAS1 is not expressed in CRCs. CD10-induced A1-7 does not affect CRC progression.

Diabetes and renin/angiotensin system

Diabetes mellitus is a common problem in countries adopting the Western lifestyle. The results of several epidemiological studies show an association between type 2 diabetes and the risk of colorectal, pancreatic, breast, liver, gastric, and endometrial cancer [24]. The risk of malignancies is increased at earlier stages in cases of abnormalities in glucose me tabolism, and there is a linear relationship between cancer risk and plasma insulin levels [24]. With regard to CRCs, a metaanalysis of 15 studies, including 2,593,935 participants, showed that diabetes is associated with an increased risk of CRC (relative risk, 1.30; 95% CI, 1.20 ± 1.40). Diabetes is also associated with CRC mortality (relative risk, 1.26; 95% CI, 1.05 ± 1.50) [25]. High glycated hemoglobin (HbA1c) levels are also associated with an increased risk of CRC (odds ratio, 1.57; 95% CI, 0.94 ± 2.60) [26]. In several studies, it has been demonstrated that hyperinsulinemia, elevated levels of C-peptide,

elevated body mass index, high levels of insulin growth factor-1, low levels of insulin growth factor binding protein-3, high leptin levels, and low adiponectin levels are all involved in carcinogenesis [27]. Increased blood concentrations of insulin and insulin-like growth factor are particularly important in enhancing the risk of CRC [28]. However, a detailed understanding of how diabetes might increase the risk of CRC is still lacking.

We examined the expression of renin in HT29 and CT26 cells in association with changing glucose concentration. When the medium contained 100 mg/dl glucose, renin protein was detected in only HT29 cells but not in CT26 cells. When the medium contained glucose at 200 mg/dl or more, the expression of renin increased with increasing glucose concentration in a dose-dependent manner in the two cell lines. CT26 cells also express chymase but not ACE in similar to HT29 cells. Then these CRC cells activate angiotensin in a high glucose condition.

In the hyperglycemic mice fed with high glucose diet, the size, number, Ki-67 labeling index, and microvessel density were pronounced in the liver metastatic foci than those in the normoglycemic mice fed with the control diet. In the clinical situations, this scheme is confirmed. In the examination of 121 CRC patients, the tumoral renin concentration correlated with HbA1c levels and the tumoral A-II concentration correlated with tumoral renin concentration. Moreover, the high blood HbA1c is associated with the higher incidence of liver metastasis in diabetic cases than in nondiabetic cases. In cardiac fibroblasts, a high concentration of glucose significantly increases intracellular A-II levels by increasing the intracellular levels of renin [29].

A-II and liver metastasis

A-II precursor, AGT is mainly produced in the hepatocytes [30]. We confirm that CRC cells possessing angiotensin-activating ability establish liver metastasis because these cells can produce abundant A-II from AGT in the liver [6]. We suppress AGT production in the mouse liver by using pro-AGT antisense S-ODN, which significantly suppresses the liver metastasis of CRC cells. Thus CRC cells with angiotensin-activating ability possess advantages for liver metastasis. In CRC cases, A-II is associated with renin concentration in the primary tumors [6]. Thus the presence of a large amount of A-II in primary CRC tissues, which suggests the potential angiotensin-activating ability of CRC cells, was associated with a high frequency of liver metastasis. Hence, A-II concentration in primary CRC tissues is suggested as a good marker for liver metastasis.

Angiotensin targeting therapy

The renin/angiotensin-activating system is recognized as an important molecular target for CRC prevention and treatment. Several inhibitors of the renin/angiotensin-activating system suppress cancer development, cancer cell growth, angiogenesis, and metastasis [4,5,31-34]. Inhibitors of the renin–angiotensin system are widely used to treat hypertension. We have observed some antiangiotensin agents, inhibitors of renin and chymase, suppressed liver metastasis of CRCs [6,35]. ACE inhibitors and/or A-II receptor blocker (ARB) have been reported to improve disease

prognosis or progression in pancreatic and urogenital cancer [36,37].

Further, we examine the combined effect of anti-angiotensin treatment and hypoglycemic treatment [35]. In a streptozotocin-induced BALB/c mouse diabetes model that is fed a high-calorie diet, the blood sugar level increases and is associated with increasing size and number of CT26 cell liver metastases. In this diabetic mouse model, effect of the concurrent hypoglycemic and anti-angiotensin treatments is examined [35]. Insulin and gliclazide (sulfonylurea) are administered with or without a renin inhibitor, aliskiren in the liver metastasis model using mice fed with high calory diet and treated with streptozotocin injection. Treatment with insulin and gliclazide results in lower blood sugar levels compared to that in the untreated mice. The mice treated with insulin or gliclazide show a decrease in the number of metastatic foci and improved survival than those in the untreated mice. Concurrent treatment with anti-angiotensin using aliskiren or captopril (ARB) and hypoglycemic agents (insulin or gliclazide) results in lower serum A-II concentration, smaller number of metastatic foci, and longer survival than those in the untreated mice or the mice treated with hypoglycemic agents alone. Combined treatment with anti-angiotensin and hypoglycemic agents showed a synergistic inhibitory effect on liver metastasis. The mice treated with the combination show suppression of liver metastasis and improved survival, which is indistinguishable from that of the control mice.

Given that the association between hyperglycemia and liver metastasis in colon cancer is a result of renin upregulation, diabetes status is likely to be a risk factor for liver metastasis. Control of blood sugar could, therefore, be important in preventing liver metastasis in colon cancer patients. The use of anti-angiotensin treatment and blood sugar control as a baseline management for colon cancer patients with diabetes deserves to be examined in clinical trials in order to establish whether it helps in the prevention of liver metastasis (Fig. 1).

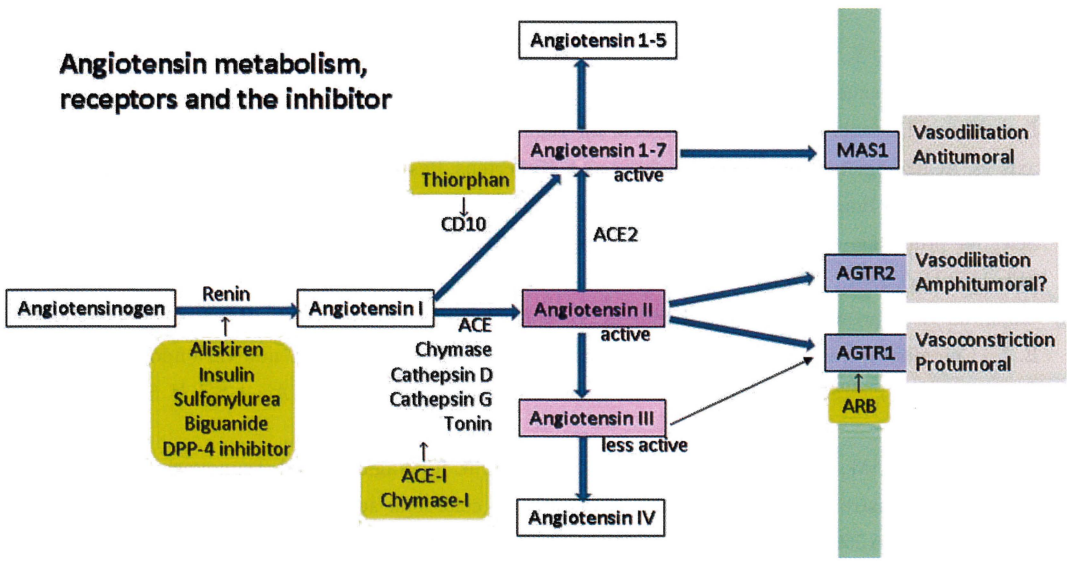

Figure 1. Processing and signaling pathways of angiotensin and potential points at which they can be inhibited.
The signaling and processing pathways of angiotensin are displayed with the enzymes responsible for each process and their inhibitor. Angiotensin II, angiotensin III, and angiotensin 1-7 all have physiological activities.

REFERENCES

1) Cancer mortality (1958-2011), Center for Cancer Control and Information Services, National Cancer Center, Japan, Tokyo, 2011.
2) Fujimoto, Y., Nakanishi, Y., Sekine, S., Yoshimura, K., Akasu, T., Moriya, Y. and Shimoda, T.: CD10 expression in colorectal carcinoma correlates with liver metastasis. Dis. Colon. Rectum. **48**: 1883-1889, 2005.
3) Fong, Y., Kemeny, N., Paty, P., Blumgart, L. H. and Cohen, A. M.: Treatment of colorectal cancer: hepatic metastasis. Semin. Surg. Oncol. **12**: 219-252, 1996.
4) Escobar, E., Rodriguez-Reyna, T. S., Arrieta, O. and Sotelo, J.: Angiotensin II, cell proliferation and angiogenesis regulator: biologic and therapeutic implications in cancer. Curr Vasc Pharmacol **2**: 385-399, 2004.
5) Wu, X. Z.: New strategy of antiangiogenic therapy for hepatocellular carcinoma. Neoplasma **55**: 472-481, 2008.
6) Shimomoto, T., Ohmori, H., Luo, Y., Chihara, Y., Denda, A., Sasahira, T., Tatsumoto, N., Fujii, K. and Kuniyasu, H.: Diabetes-associated angiotensin activation enhances liver metastasis of colon cancer. Clin. Exp. Metastasis, in press.
7) Belova, L. A.: Angiotensin II-generating enzymes. Biochemistry (Mosc) **65**: 1337-1345, 2000.
8) Kumar, R., Singh, V. P. and Baker, K. M.: The intracellular renin-angiotensin system: implications in cardiovascular remodeling. Curr. Opin. Nephrol. Hypertens. **17**: 168-173, 2008.
9) Nishimura, H., Hoffmann, S., Baltatu, O., Sugimura, K., Ganten, D. and Urata, H.: Angiotensin I converting enzyme and chymase in cardiovascular tissues. Kidney. Int. **Suppl 55**: S18-23, 1996.
10) Ferreira, A. J., Bader, M. and Santos, R. A.: Therapeutic targeting of the angiotensin-converting enzyme 2/Angiotensin-(1-7)/Mas cascade in the renin-angiotensin system: a patent review. Expert Opin. Ther. Pat. **22**: 567-574, 2012.
11) Luo, Y., Chihara, Y., Fujii, K., Matsushima, S., Ohmori, H., Hashizume, A. and Kuniyasu, H.: Expression of MAS1 in breast cancer in submission,
12) Iwai, M. and Horiuchi, M.: Devil and angel in the renin-angiotensin system: ACE-angiotensin II-AT1 receptor axis vs. ACE2-angiotensin-(1-7)-Mas receptor axis. Hypertens. Res. **32**: 533-536, 2009.
13) Patey, G., De La Baume, S., Schwartz, J. C., Gros, C., Roques, B., Fournie-Zaluski, M. C. and Soroca-Lucas, E.: Selective protection of methionine enkephalin released from brain slices by enkephalinase inhibition. Science **212**: 1153-1155, 1981.
14) Shipp, M. A., Vijayaraghavan, J., Schmidt, E. V., Masteller, E. L., D'Adamio, L., Hersh, L. B. and Reinherz, E. L.: Common acute lymphoblastic leukemia antigen (CALLA) is active neutral endopeptidase 24.11 ("enkephalinase"): direct evidence by cDNA transfection analysis. Proc. Natl. Acad. Sci. U. S. A. **86**: 297-301, 1989.
15) Yao, T., Takata, M., Tsutsumi, S., Nishiyama, K., Taguchi, K., Nagai, E. and Tsuneyoshi, M.: Phenotypic expression of gastrointestinal differentiation markers in colorectal adenocarcinomas with liver metastasis. Pathology **34**: 556-560, 2002.
16) Ohji, Y., Yao, T., Eguchi, T., Yamada, T., Hirahashi, M., Iida, M. and Tsuneyoshi, M.: Evaluation of risk of liver metastasis in colorectal adenocarcinoma based on the combination of risk factors including CD10 expression: multivariate analysis of clinicopathological and immunohistochemical factors. Oncol. Rep. **17**: 525-530, 2007.

17) Sumitomo, M., Shen, R. and Nanus, D. M.: Involvement of neutral endopeptidase in neoplastic progression. Biochem. Biophys. Acta **1751**: 52-59, 2005.

18) Ogasawara, M., Murata, J., Ayukawa, K. and Saimi, I.: Differential effect of intestinal neuropeptides on invasion and migration of colon carcinoma cells in vitro. Cancer Lett. **116**: 111-116, 1997.

19) Boyella, V. D., Nicastri, A. D. and Bergasa, N. V.: Human hepatic met-enkephalin and delta opioid receptor-1 immunoreactivities in viral and autoimmune hepatitis. Ann. Hepatol. **7**: 221-226, 2008.

20) Spivey, J. R., Jorgensen, R. A., Gores, G. J. and Lindor, K. D.: Methionine-enkephalin concentrations correlate with stage of disease but not pruritus in patients with primary biliary cirrhosis. Am. J. Gastroenterol. **89**: 2028-2032, 1994.

21) Nicoll, J., Axiotis, C. A. and Bergasa, N. V.: The delta opioid receptor 1 is expressed by proliferating bile ductules in rats with cholestasis: implications for the study of liver regeneration and malignant transformation of biliary epithelium. Med. Hypotheses **65**: 1099-1105, 2005.

22) Kuniyasu, H., Luo, Y., Fujii, K., Sasahira, T., Moriwaka, Y., Tatsumoto, N., Sasaki, T., Yamashita, Y. and Ohmori, H.: CD10 enhances metastasis of colorectal cancer by abrogating the anti-tumoural effect of methionine-enkephalin in the liver. Gut **59**: 348-356, 2010.

23) Skidgel, R. A. and Erdos, E. G.: Angiotensin converting enzyme (ACE) and neprilysin hydrolyze neuropeptides: a brief history, the beginning and follow-ups to early studies. Peptides **25**: 521-525, 2004.

24) Nicolucci, A.: Epidemiological aspects of neoplasms in diabetes. Acta Diabetol. **47**: 87-95, 2010.

25) Larsson, S. C., Orsini, N. and Wolk, A.: Diabetes mellitus and risk of colorectal cancer: a meta-analysis. J. Natl. Cancer Inst. **97**: 1679-1687, 2005.

26) Saydah, S. H., Platz, E. A., Rifai, N., Pollak, M. N., Brancati, F. L. and Helzlsouer, K. J.: Association of markers of insulin and glucose control with subsequent colorectal cancer risk. Cancer Epidemiol. Biomarkers Prev. **12**: 412-418, 2003.

27) Pais, R., Silaghi, H., Silaghi, A. C., Rusu, M. L. and Dumitrascu, D. L.: Metabolic syndrome and risk of subsequent colorectal cancer. World J. Gastroenterol. **15**: 5141-5148, 2009.

28) Giovannucci, E.: Metabolic syndrome, hyperinsulinemia, and colon cancer: a review. Am. J. Clin. Nutr. **86**: s836-842, 2007.

29) Singh, V. P., Baker, K. M. and Kumar, R.: Activation of the intracellular renin-angiotensin system in cardiac fibroblasts by high glucose: role in extracellular matrix production. Am. J. Physiol. Heart Circ. Physiol. **294**: H1675-1684, 2008.

30) Dzau, V. J.: Implications of local angiotensin production in cardiovascular physiology and pharmacology. Am. J. Cardiol. **59**: 59A-65A, 1987.

31) Grossman, E., Messerli, F. H. and Goldbourt, U.: Carcinogenicity of antihypertensive therapy. Curr. Hypertens. Rep. **4**: 195-201, 2002.

32) Attoub, S., Gaben, A. M., Al-Salam, S., Al Sultan, M. A., John, A., Nicholls, M. G., Mester, J. and Petroianu, G.: Captopril as a potential inhibitor of lung tumor growth and metastasis. Ann. N. Y. Acad. Sci. **1138**: 65-72, 2008.

33) Deshayes, F. and Nahmias, C.: Angiotensin receptors: a new role in cancer? Trends Endocrinol. Metab. **16**: 203-299, 2005.

34) Fyhrquist, F. and Saijonmaa, O.: Renin-angiotensin system revisited. J. Intern. Med. **264**: 224-236, 2008.

35) Luo, Y., Ohmori, H., Shimomoto, T., Fujii, K., Sasahira, T., Chihara, Y. and Kuniyasu, H.: Anti-angiotensin and hypoglycemic treatments suppress liver metastasis of colon cancer cells. Pathobiology **78**: 285-290, 2011.

36) Nakai, Y., Isayama, H., Ijichi, H., Sasaki, T., Sasahira, N., Hirano, K., Kogure, H., Kawakubo, K., Yagioka, H., Yashima, Y., Mizuno, S., Yamamoto, K., Arizumi, T., Togawa, O., Matsubara, S., Tsujino, T., Tateishi, K., Tada, M., Omata, M. and Koike, K.: Inhibition of renin-angiotensin system affects prognosis of advanced pancreatic cancer receiving gemcitabine. Br. J. Cancer **103**: 1644-1648, 2010.

37) Miyajima, A., Kikuchi, E., Kosaka, T. and Oya, M.: Angiotensin II Type 1 Receptor Antagonist as an Angiogenic Inhibitor in Urogenital Cancer. Rev. Recent Clin. Trials **4**: 75-78, 2009.