総　　説

PROTUMORAL EFFECT OF ANGIOTENSIN SYSTEM

RINA FUJIWARA-TANI, SAYAKO MATSUSHIMA-OTSUKA, YUKIKO NISHIGUCHI, SHIORI MORI, HIROKI KUNIYASU
Department of Molecular Pathology, Nara Medical University

Received November 7, 2018

Abstract: Colorectal cancer (CRC) cells possess an angiotensin activation mechanism provided by the expression of renin and chymase. Renin expression is induced by a 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 and angiotensin-II in the primary tumors, and a more progressed disease stage, especially, liver metastasis in association with HbA1c levels than those in the patients without diabetes. Concurrent treatment with anti-angiotensin and hypoglycemic agents shows a synergic effect of decreasing liver metastasis and improving the survival of diabetic mice in the CRC liver metastasis model. MAS1-angiotensin1-7 is a negative regulator of the AGTR1-angiotensin II axis in breast cancer. Notably, MAS1 is overexpressed in triple negative breast cancer, which might be a novel molecular target for the treatment-refractory entity of breast cancer. Nuclear AGTR2 and intracellular angiotensin-II play a role in anti-apoptotic and anti-oxidative stress properties. These functions of nuclear AGTR2 might mitigate "anti-tumoral side effects" of AGTR1 and angiotensin-II system, which enhance mainly tumor progression. The effect of anti-angiotensin treatment, such as ARB and blood sugar control as a baseline management of many cancer patients needs to be examined in a clinical situation for prevention of RAS-induced tumor progression.

Key words: angiotensin, hyperglycemia, MAS1, CD10, AGTR2

Protumoral effect of angiotensin

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 of cardiovascular and neoplastic tissues[1]. 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[1,2]. These properties enhance colon carcinogenesis and disease progression.

We have confirmed the protumoral effect of angiotensin by experiment. The effects of A-II

on cell growth, invasion, and apoptosis are examined in the CRC cell lines[3]. 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 a nude mouse liver metastasis model. ATG-knockdown mice show smaller size, number, and Ki-67 labeling index, and less pronounced microvessel density in the metastatic foci than the control mice. Knockdown of renin or chymase in HT29 cells shows smaller and fewer metastatic foci in the liver than those in the control. Furthermore, the examination of 121 CRC patients shows that the serum A-II concentration is significantly associated with an advanced disease stage, especially liver metastasis.

Angiotensin activation in CRC cells

ATG is an inactive precursor of A-II. ATG has no effect on cancer cells without conversion to A-II. The 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 in 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 have an ACE-like activity, which allow them to be used as a substitute for ACE[4]. Cathepsin D is responsible for producing A-I from ATG in cardiac myocytes, fibroblasts, and vascular smooth muscle cells in place of renin[5]. 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[6]; however, it is not associated with hyperglycemia in CRC cells[3].

MAS1 in breast cancer

MAS1 is a receptor of an angiotensin II (A-II) degenerative product generated by angiotensin converting enzyme 2, angiotensin 1-7 (A1-7), which provides anti-A-II phenotypes, such as vessel dilation and depression of blood pressure[7]. We examine the role of MAS1 in CRC and invasive ductal carcinoma (IDC) of the breast[8]. 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. MAS1 expression is particularly strongly decreased in scirrhous type IDCs compared to other types. The decrease in MAS1 expression is associated with lymph node metastasis but not T factor,

grade, or the status of the estrogen receptor or progesterone receptor. The decrease in MAS1 expression is inversely 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[9]. These findings suggest that MAS1 might act as an inhibitory regulator of breast tissues and the cancer.

CD10 in colorectal cancer

CD10, known as common acute lymphoblastic leukemia antigen (CALLA), is a characteristic marker of various subgroups of B-cell type-acute lymphocytic leukemias[10,11]. It is a zinc-dependent membrane metalloendopeptidase, also called neutral endopeptidase (EC 3.4.24.11), enkephalinase, or neprilysin[11]. CD10 is expressed in CRC and is associated with CRC metastases, especially liver metastasis. [12-14]. Met-enkephalin (MENK) is a high affinity substrate of CD10[15,16]. MENK is produced by hepatocytes under the condition of cellular stress, such as hepatitis, bile stasis, and liver metastasis[17-19]. MENK inhibits tumor growth and the establishment of metastatic foci[20]. CD10-positive CRC cells degrade MENK and evade MENK-induced tumor suppression[20]. CD10 has a weak affinity for A-I[21]; however, CD10 shows A-I degrading activity but not A-I conversion activity. Degrading 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[22]. The risk of malignancies is increased at earlier stages in cases of abnormalities in glucose metabolism, and there is a linear relationship between cancer risk and plasma insulin levels[22]. With regard to CRCs, a meta-analysis 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)[23]. High glycated hemoglobin (HbA1c) levels are also associated with an increased risk of CRC (odds ratio, 1.57; 95% CI, 0.94 ± 2.60)[24]. 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[25]. Increased blood concentrations of insulin and insulin-like growth factor are particularly important in enhancing the risk of CRC[26]. 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 only in HT29 cells and 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, similarly to HT29 cells. Then these CRC cells activate angiotensin in a high glucose condition.

In hyperglycemic mice fed a high glucose diet, the size, number, Ki-67 labeling index, and microvessel density in the liver metastatic foci were more pronounced than in the normoglycemic mice fed with the control diet. In 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 [27].

A-Ⅱ and liver metastasis

A-Ⅱ precursor, AGT is mainly produced in the hepatocytes[28]. We confirm that CRC cells that have angiotensin-activating ability establish liver metastasis because these cells can produce abundant A-Ⅱ from AGT in the liver[3]. 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 have advantages for liver metastasis. In CRC cases, A-Ⅱ is associated with renin concentration in the primary tumors[3]. Thus, the presence of a large amount of A-Ⅱ in primary CRC tissues, which suggests the potential angiotensin-activating ability of CRC cells, is 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.

Nuclear angiotensin type Ⅱ receptor in oral cancer

On the other hand, A-Ⅱ binds to angiotensin type 2 receptor (AGTR2) to cause effects that are the opposite of those of AGTR1: vasodilatation and a fall in blood pressure[29]. Recent investigations have reported a role of AGTR2 in cardiovascular system, brain and renal function and also the modulation of various processes in organ development, cell differentiation, and tissue repair[30]. In addition to the function of RAS as a classic hormonal system, intracellular or intranuclear RAS has become the focus of recent attention[31]. In the nucleus, three RAS receptors, AGTR1, AGTR2, and MAS1, have been confirmed. These receptors, which are G protein coupled-protein receptors, are found in the nuclear membrane of human myocardial cells[32]. Subunits of AGTR2, including $G\alpha q/11$, $G\alpha i/3$, and $G\beta$, have been observed in the nuclei of canine atrial fibroblasts[33]. Furthermore, we have observed nuclear immunoreactivity to AGTR2 in CRCs[34]. It is thought that intracellular RAS interacts with extracellular (canonical) RAS[31,35].

In 23 OSCCs, we found that the AGTR1/AGTR2 mRNA ratio is inversely associated with disease progression, while nuclear AGTR2 positivity was associated with disease progression[36].

In the human OSCC cell lines HSC3 and HSC4, AGTR1 is associated with proliferation and invasion, while AGTR2 is associated with anti-apoptosis and anti-oxidative stress[36].

We have previously reported that hyperglycemia promotes A-Ⅱ secretion in CRC cells, which enhances the progression of cancer through AGTR1 activation[3]. Here, we found that AGTR2 levels in the nucleus increase in hypoxic and hyperglycemic conditions and affect cell proliferation, invasion, and survival through the suppression of phosphorylated ERK1/2, and increase in phosphorylation of p38 and Bcl-2. Additionally, the increase in nuclear AGTR2 inhibits apoptosis through the decrease of oxidative stress levels and reduced state. Inhibition of mitogen-activated protein kinase (MAPK) by nuclear AGTR2 activation has been reported in human myocardial cells[37,38]. In contrast, apoptosis is induced by a decrease in Bcl-2, increase in Bax and activation of caspase-3 upon activation of AGTR2 in the cytoplasmic membrane by extrinsic A-Ⅱ[39]. Specifically, extracellular A-Ⅱ activation stimulates the oxidative stress through an increase in p22phox and Nox-1, and a decrease in Nox-4[40]. On the other hand, oxidative stress and nitric oxide increase mitochondrial AGTR2 levels, which suppress oxidative phosphorylation[41].

The difference in the roles of AGTR1 and AGTR2 is an important issue in our study. We examine the effect of knockdown of AGTR1 or AGTR2 with or without extrinsic A-Ⅱ[36]. AGTR1-associated (AGTR2 knockdown) phenotypes are significantly enhanced by extrinsic A-Ⅱ, whereas AGTR2-associated (AGTR1 knockdown) phenotypes are not affected by extrinsic A-Ⅱ. A-Ⅱ enhances markedly AGTR1-associated phenotypes, which are pro-tumoral (proliferation and invasion) and anti-survival (apoptosis and oxidative stress). In contrast, AGTR2-associated phenotypes are anti-tumoral and pro-survival regardless of extrinsic A-Ⅱ. These AGTR2-associated phenotypes are resulted by nuclear AGTR2. To examine the phenotypes of nuclear AGTR2, we performed nuclear transportation inhibition assays[36]. Nuclear accumulation of AGTR2 protein decreases 5-FU-induced apoptosis. Notably, serum A-Ⅱ levels in OSCC are not associated with disease progression, while intra-tumoral A-Ⅱ levels positively correlat with nuclear AGTR2 levels and are inversely correlated with the AGTR1-AGTR2 ratio[36]. Our data might show that extrinsic A-Ⅱ-dependent AGTR1 activation and intrinsic A-Ⅱ activated nuclear AGTR2 play complementary roles in OSCC progression. The coexpression of AGTR1 and nuclear AGTR2 provides an advantage for OSCC, which is supported by the data of the survival analyses.

AGTR2 level is not affected by extracellular A-Ⅱ but it is inhibited by knockdown of AGT. Therefore, nuclear AGTR2 is not activated by extracellular A-Ⅱ but by intracellular A-Ⅱ, produced by AGT, renin, and cathepsin G in cancer cells. In this connection, it has been reported that AGTR2 nuclear transport, promoted by hyperglycemia, also increases intracellular A-Ⅱ[32]. Furthermore, hyperglycemia promotes proliferation and invasion in cancer cells upon activation of AGTR1 by extracellular A-Ⅱ, and suppresses apoptosis, decreases the levels of reactive oxygen species, and maintains a reduced redox state through the increase in intracellular A-Ⅱ and activation of nuclear AGTR2[3,34]. Consequently, it is thought that AGTR2 has a cell-protective role in OSCC cells and reduces cell damages caused by extracellular A-Ⅱ[35,42].

Nuclear localization of AGTR2 is confirmed in our data; however, the mechanism of nuclear transportation was not clear. Nuclear transportation of AGTR2 or A-Ⅱ is reported[3,32,34];

however, AGTR2 and A-Ⅱ proteins are found not to have common nuclear transportation domains in a search in the NCBI data base (data not shown). Our data suggest that AGTR2 might be transported by the active transport system with importin/exportin. In contrast, since A-Ⅱ transportation is seemed not to be affected by treatment with LMB or IPZ, nuclear transportation of A-Ⅱ might be independent of the importin/exportin system. Further examination is needed on this issue.

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[1,2,43-46]. Inhibitors of the renin-angiotensin system are widely used to treat hypertension. We have examined some antiangiotensin agents, inhibitors of renin and chymase, suppressed liver metastasis of CRCs[3,47]. ACE inhibitors and/or A-Ⅱ receptor blocker (ARB) have been reported to improve disease prognosis or progression in pancreatic and urogenital cancer[48,49].

Further, we examine the combined effect of anti-angiotensin treatment and hypoglycemic treatment[47]. In a streptozotocin-induced BALB/c mouse diabetes model involving feeding 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, the effect of the concurrent hypoglycemic and anti-angiotensin treatments is examined[47]. Insulin and gliclazide (sulfonylurea) are administered with or without a renin inhibitor, aliskiren, in the liver metastasis model using mice fed with high-calorie diet and treated with streptozotocin injection. Treatment with insulin and gliclazide results in lower blood sugar levels than those in untreated mice. The mice treated with insulin or gliclazide show a decrease in the number of metastatic foci and improved survival compared to the untreated mice. Concurrent treatment with anti-angiotensin using aliskiren or captopril (ARB) and hypoglycemic agents (insulin or gliclazide) results in a lower serum A-Ⅱ concentration, a smaller number of metastatic foci, and longer survival compared to 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.

Considering that hyperglycemia is associated with liver metastasis of colon cancer via renin upregulation, diabetic status is thought 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 effect of anti-angiotensin treatment and blood sugar control as a baseline management of the colon cancer patients with the diabetic condition needs to be examined in a clinical situation for prevention of liver metastasis. Antiangiotensin systemic therapy and hypoglycemic therapy should therefore be tested for prevention of liver metastasis in cases of colon cancer.

The effects of losartan (LOS), an ARB used as an RAS antagonist, are examined on nuclear AGTR2[36]. LOS does not affect AGTR2 mRNA expression but suppresses its nuclear transport.

LOS also reduces the intracellular A-Ⅱ levels by repressing AGT, renin, and cathepsin G and importantly, cell survival is limited by the increased oxidative stress and enhanced oxidized redox state. Inhibition of extracellular RAS through AGTR1 blockage suppressed nuclear AGTR2. In the mouse tumor model, treatment with LOS alone is associated with weak suppression of tumor growth. However, cotreatment with LOS and the chemotherapeutic drug 5-FU provides synergistic growth inhibition[36]. Therefore, ARBs, by inhibiting both extracellular and intracellular RAS, might increase the effectiveness of chemotherapy in OSCC.

References

1) 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.
2) Wu, X. Z.: New strategy of antiangiogenic therapy for hepatocellular carcinoma. Neoplasma **55**: 472-481, 2008.
3) 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 **29**: 915-925, 2012.
4) Belova, L. A.: Angiotensin Ⅱ-generating enzymes. Biochemistry (Mosc) **65**: 1337-1345, 2000.
5) 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.
6) Nishimura, S., Chung, Y. S., Yashiro, M., Inoue, T. and Sowa, M.: Role of alpha 2 beta 1- and alpha 3 beta 1-integrin in the peritoneal implantation of scirrhous gastric carcinoma. Br J Cancer **74**: 1406-1412, 1996.
7) 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.
8) Luo, Y., Tanabe, E., Kitayoshi, M., Nishiguchi, Y., Fujiwara, R., Matsushima, S., Sasaki, T., Sasahira, T., Chihara, Y., Nakae, D., Fujii, K., Ohmori, H. and Kuniyasu, H.: Expression of MAS1 in breast cancer. Cancer Sci **106**: 1240-1248, 2015.
9) 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.
10) 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.
11) 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.
12) 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.
13) Yao, T., Takata, M., Tustsumi, 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.

14) 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.

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

16) 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.

17) 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.

18) 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.

19) 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.

20) 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.

21) 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.

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

23) 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.

24) 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.

25) 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.

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

27) 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.

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

29) Kuniyasu, H. : Multiple roles of angiotensin in colorectal cancer. World J Clin Oncol **3** : 150–154, 2012.

30) Kaschina, E. and Unger, T. : Angiotensin AT1/AT2 receptors : regulation, signalling and function. Blood Press **12** : 70–88, 2003.

31) Costa-Besada, M. A., Valenzuela, R., Garrido-Gil, P., Villar-Cheda, B., Parga, J. A., Lanciego, J. L. and Labandeira-Garcia, J. L. : Paracrine and Intracrine Angiotensin 1-7/Mas Receptor Axis in the Substantia

Nigra of Rodents, Monkeys, and Humans. Mol Neurobiol **55**: 5847–5867, 2018.

32) da Silva Novaes, A., Ribeiro, R. S., Pereira, L. G., Borges, F. T. and Boim, M. A.: Intracrine action of angiotensin II in mesangial cells: subcellular distribution of angiotensin II receptor subtypes AT1 and AT2. Mol Cell Biochem, 2018.

33) Tadevosyan, A., Xiao, J., Surinkaew, S., Naud, P., Merlen, C., Harada, M., Qi, X., Chatenet, D., Fournier, A., Allen, B. G. and Nattel, S.: Intracellular Angiotensin-II Interacts With Nuclear Angiotensin Receptors in Cardiac Fibroblasts and Regulates RNA Synthesis, Cell Proliferation, and Collagen Secretion. J Am Heart Assoc **6**, 2017.

34) Zhou, L., Luo, Y., Sato, S., Tanabe, E., Kitayoshi, M., Fujiwara, R., Sasaki, T., Fujii, K., Ohmori, H. and Kuniyasu, H.: Role of two types of angiotensin II receptors in colorectal carcinoma progression. Pathobiology **81**: 169–175, 2014.

35) Villar-Cheda, B., Costa-Besada, M. A., Valenzuela, R., Perez-Costas, E., Melendez-Ferro, M. and Labandeira-Garcia, J. L.: The intracellular angiotensin system buffers deleterious effects of the extracellular paracrine system. Cell Death Dis **8**: e3044, 2017.

36) Matsushima-Otsuka, S., Fujiwara-Tani, R., Sasaki, T., Ohmori, H., Nakashima, C., Kishi, S., Nishiguchi, Y., Fujii, K., Luo, Y. and Kuniyasu, H.: Significance of intranuclear angiotensin-II type 2 receptor in oral squamous cell carcinoma. Oncotarget: in press, 2018.

37) Tadevosyan, A., Maguy, A., Villeneuve, L. R., Babin, J., Bonnefoy, A., Allen, B. G. and Nattel, S.: Nuclear-delimited angiotensin receptor-mediated signaling regulates cardiomyocyte gene expression. J Biol Chem **285**: 22338–22349, 2010.

38) Lehtonen, J. Y., Daviet, L., Nahmias, C., Horiuchi, M. and Dzau, V. J.: Analysis of functional domains of angiotensin II type 2 receptor involved in apoptosis. Mol Endocrinol **13**: 1051–1060, 1999.

39) Luetzen, U., Zhao, Y., Lucht, K., Zuhayra, M., Hedderich, J., Cascorbi, I. and Culman, J.: Activation of the cell membrane angiotensin AT2 receptors in human leiomyosarcoma cells induces differentiation and apoptosis by a PPARgamma - dependent mechanism. Neoplasma **64**: 395–405, 2017.

40) Chabrashvili, T., Kitiyakara, C., Blau, J., Karber, A., Aslam, S., Welch, W. J. and Wilcox, C. S.: Effects of ANG II type 1 and 2 receptors on oxidative stress, renal NADPH oxidase, and SOD expression. Am J Physiol Regul Integr Comp Physiol **285**: R117–124, 2003.

41) Valenzuela, R., Costa-Besada, M. A., Iglesias-Gonzalez, J., Perez-Costas, E., Villar-Cheda, B., Garrido-Gil, P., Melendez-Ferro, M., Soto-Otero, R., Lanciego, J. L., Henrion, D., Franco, R. and Labandeira-Garcia, J. L.: Mitochondrial angiotensin receptors in dopaminergic neurons. Role in cell protection and aging-related vulnerability to neurodegeneration. Cell Death Dis **7**: e2427, 2016.

42) Re, R. N.: Role of intracellular angiotensin II. Am J Physiol Heart Circ Physiol **314**: H766–H771, 2018.

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

44) 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.

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

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

47) 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.

48) 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.

49) 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.