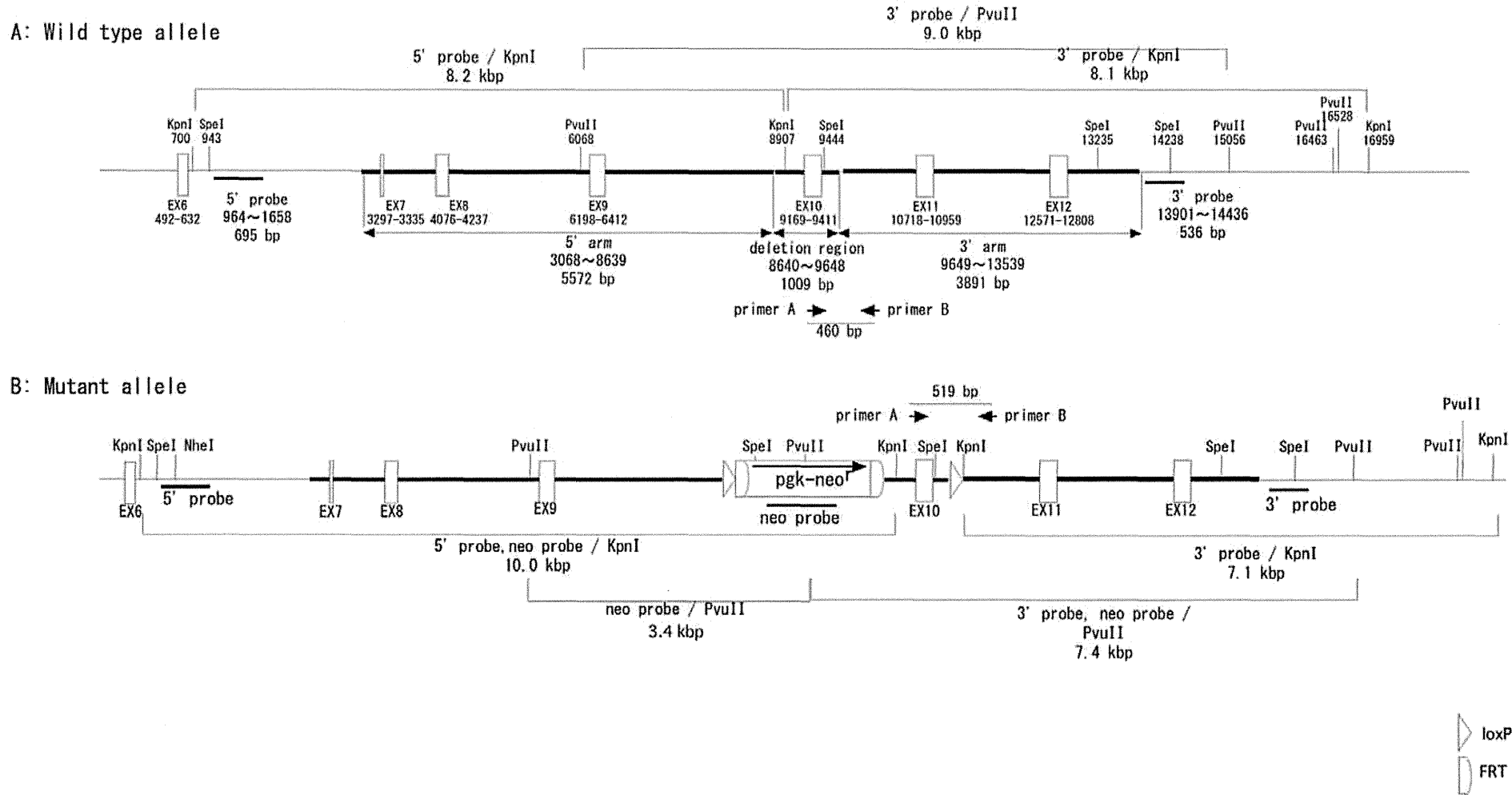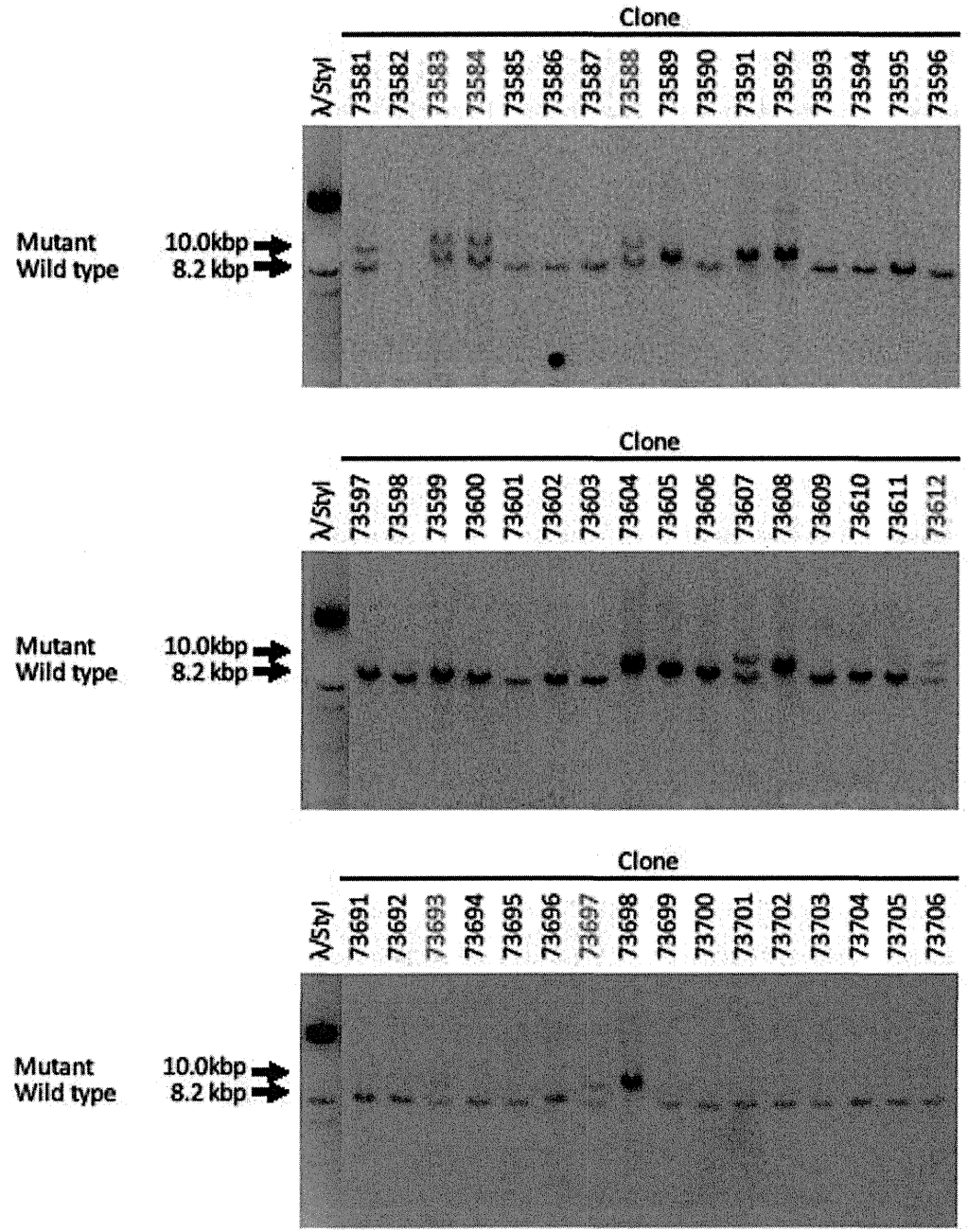Figure S9B).

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A: Wild type allele

3' probe / PvuII
9.0 kbp

5' probe / KpnI
8.2 kbp

3' probe / KpnI
8.1 kbp

KpnI SpeI
700   943

PvuII
6068

KpnI   SpeI
8907   9444

SpeI
13235

SpeI
14238

PvuII
15056

PvuII
16528

PvuII
16463

KpnI
16959

EX6
492~632

5' probe
964~1658
695 bp

EX7
3297-3335

EX8
4076-4237

EX9
6198-6412

EX10
9169-9411

EX11
10718-10959

EX12
12571-12808

3' probe
13901~14436
536 bp

5' arm
3068~8639
5572 bp

deletion region
8640~9648
1009 bp

3' arm
9649~13539
3891 bp

primer A ▶   ◀ primer B
460 bp

B: Mutant allele

519 bp
primer A ▶   ◀ primer B

PvuII

KpnI SpeI NheI

PvuII

SpeI   PvuII

KpnI SpeI KpnI

SpeI

SpeI   PvuII

PvuII KpnI

EX6

5' probe

EX7

EX8

EX9

pgk-neo^r

neo probe

EX10

EX11

EX12

3' probe

5' probe, neo probe / KpnI
10.0 kbp

3' probe / KpnI
7.1 kbp

neo probe / PvuII
3.4 kbp

3' probe, neo probe /
PvuII
7.4 kbp

▷ loxP

⬠ FRT

Figure S1 Vector design of ATP2B1 exon 10 conditional KO mouse

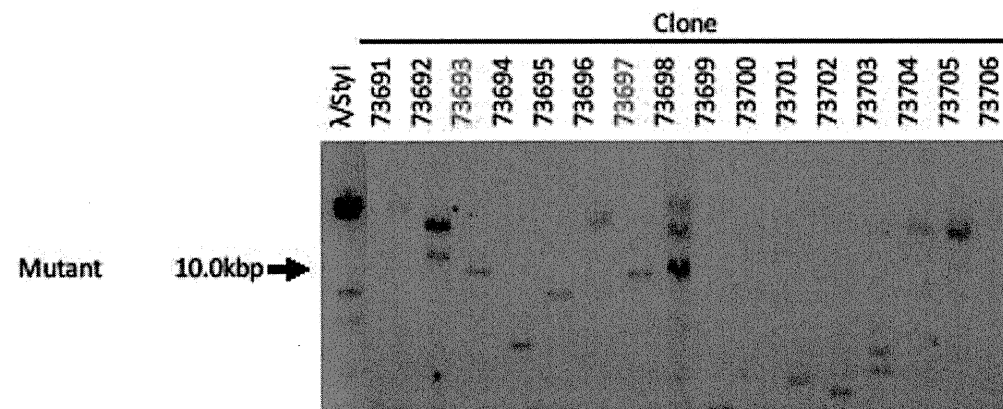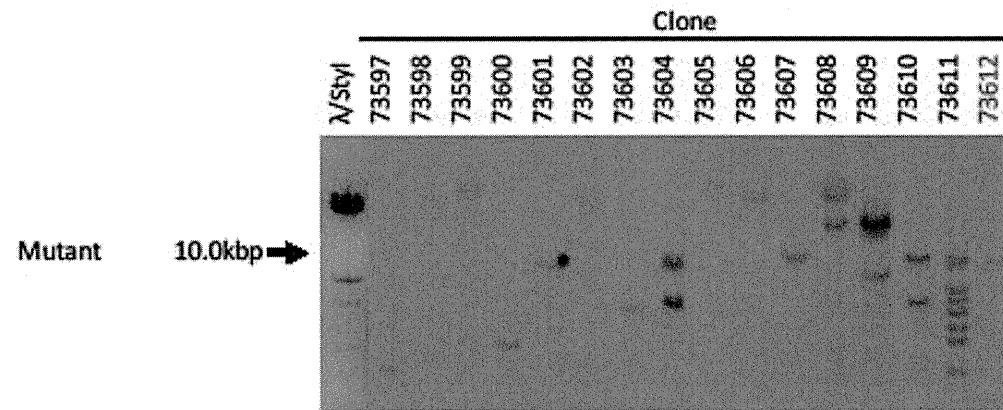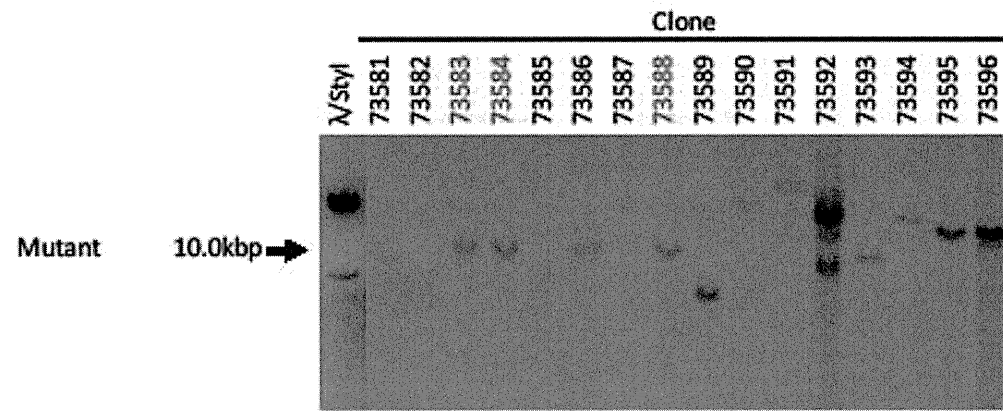Figure S2 Kpn I-digested Southern blot analysis using 5' probe

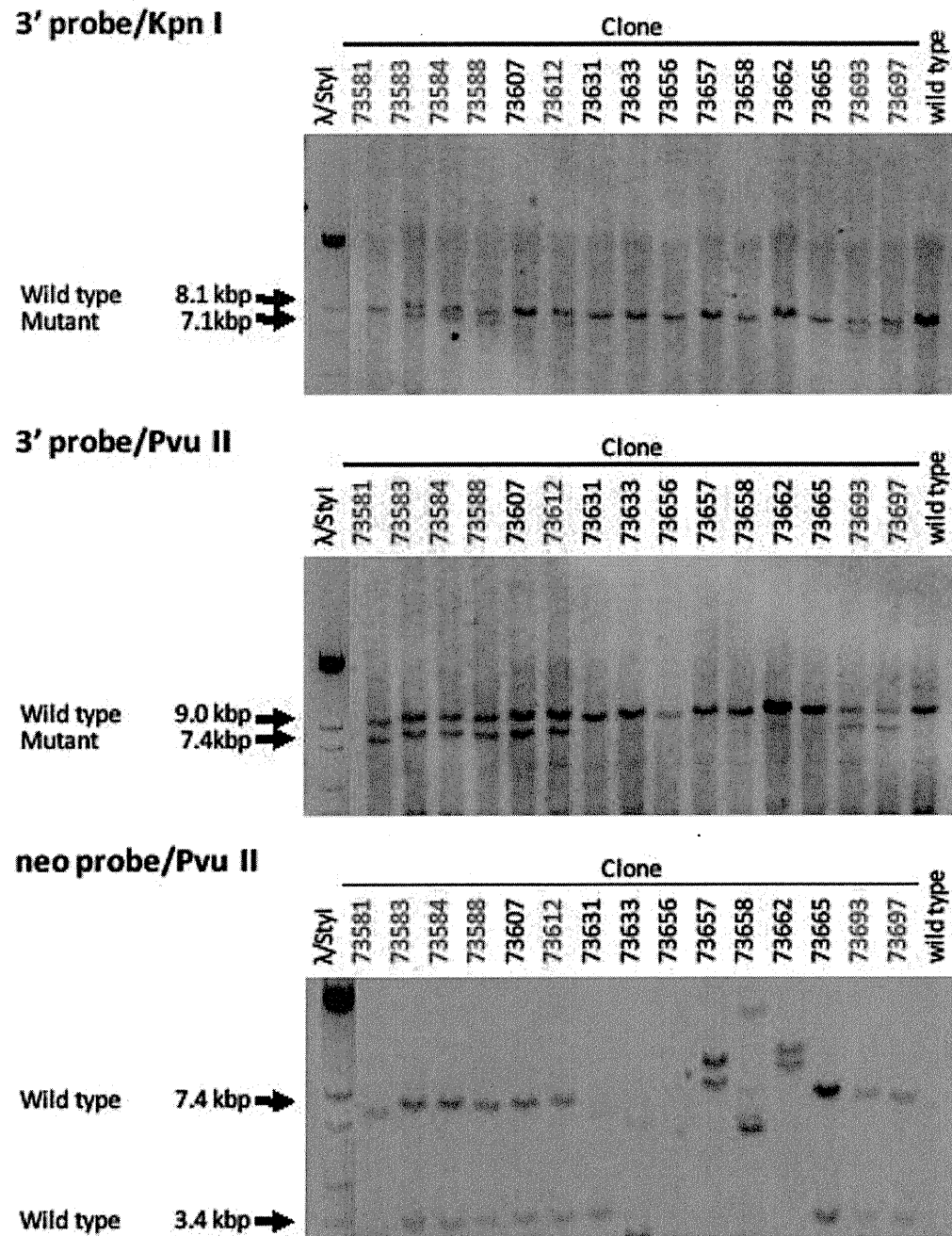**Figure S3 Kpn I-digested Southern blot analysis using neo probe**

Figure S4 Kpn I/Pvu II-digested Southern blot analysis using 3'/neo probe
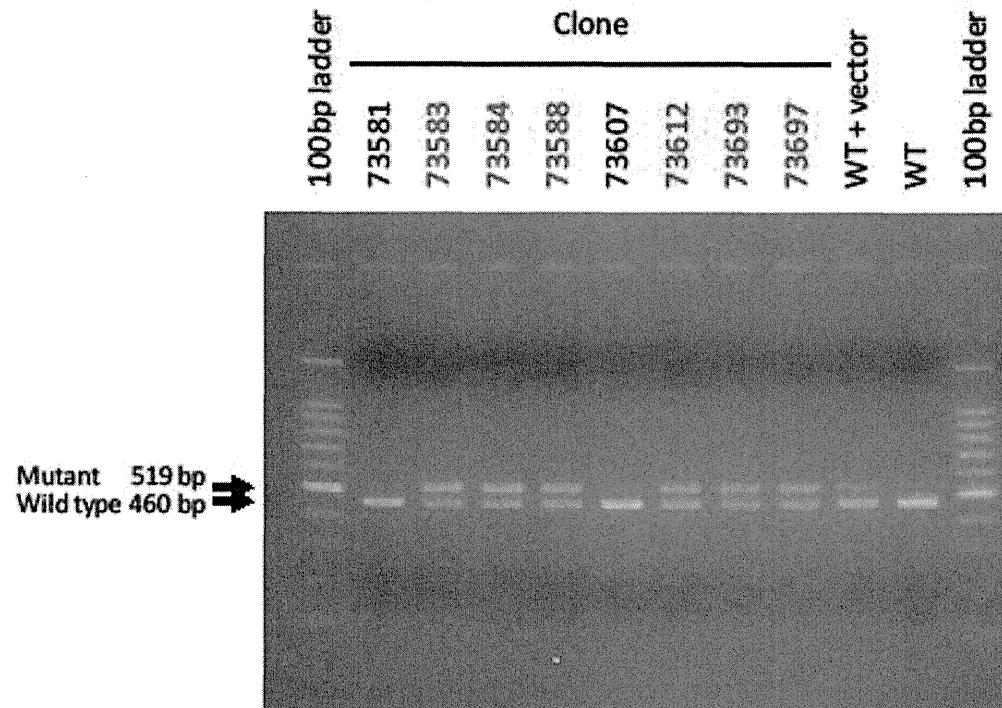
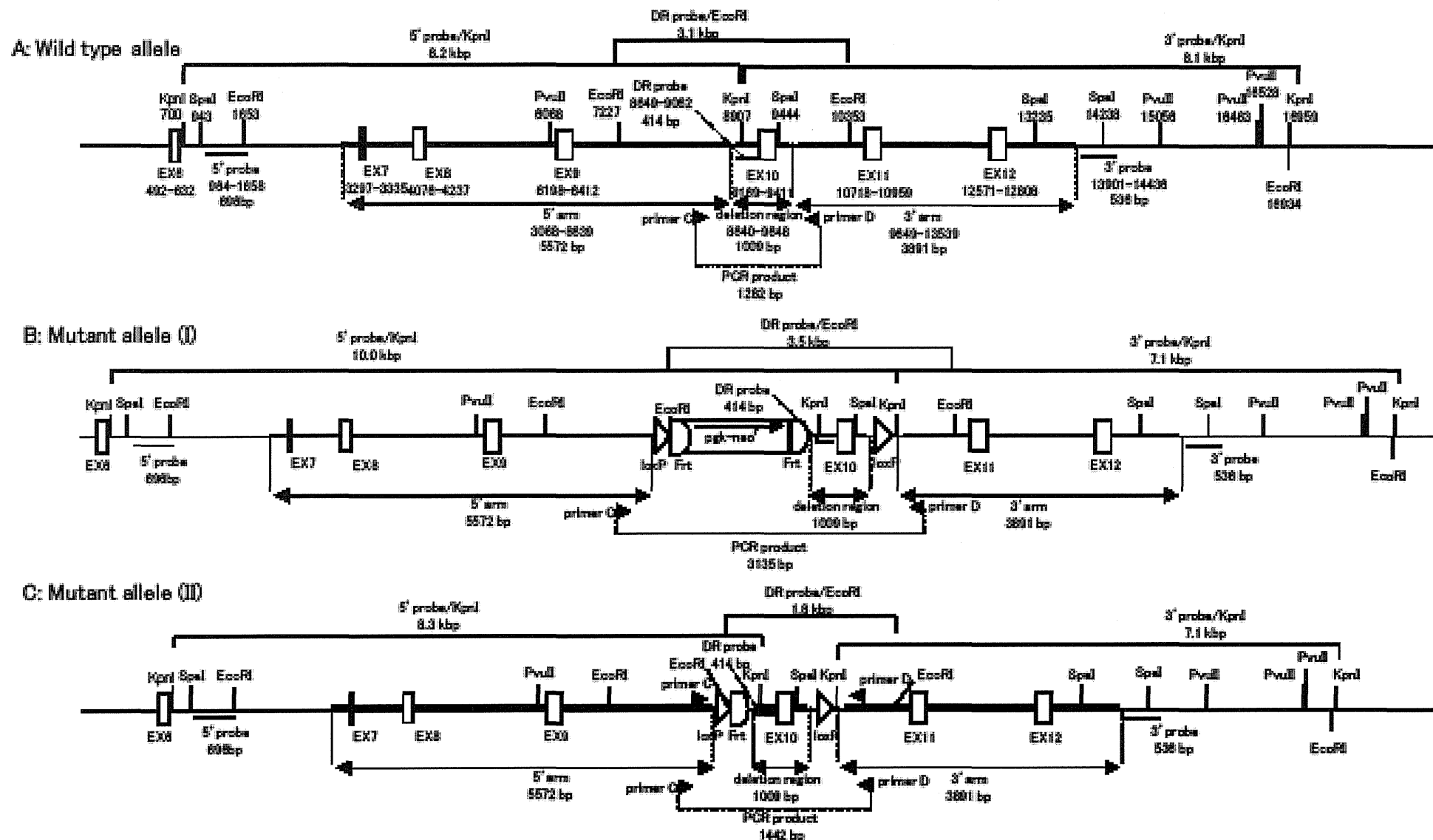**Figure S5 Agarose-gel electrophoresis of PCR products amplified with primer A and primer B**

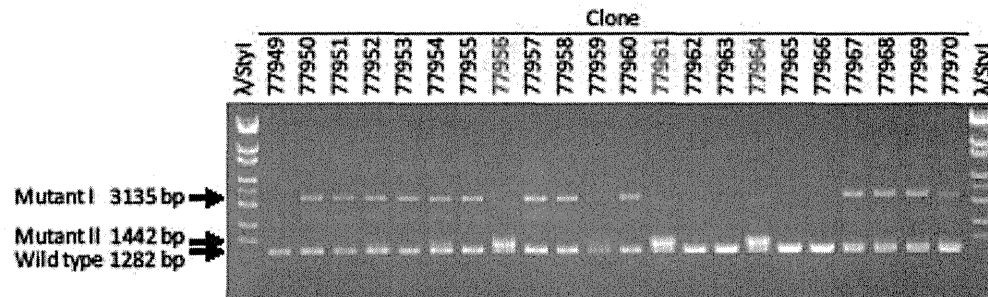**Figure S6 Eliminated the PGK-neo region by Flp recombination system**
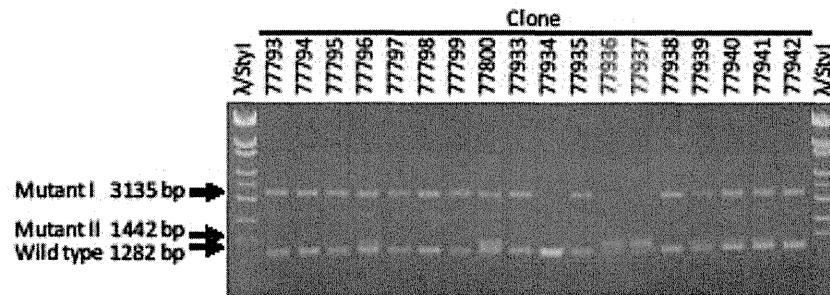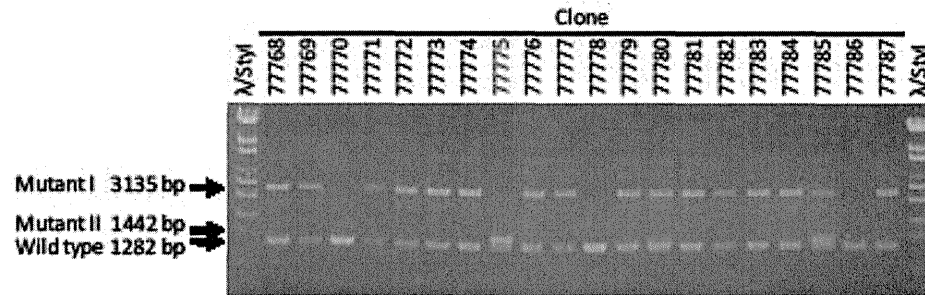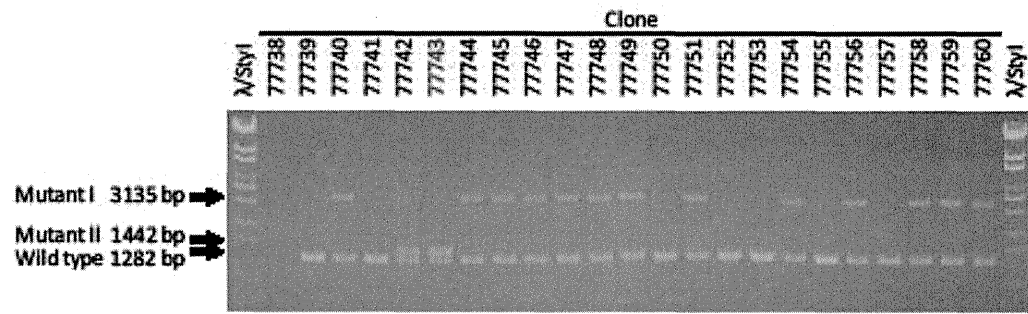
Figure S7 Agarose-gel electrophoresis of PCR products amplified with primer C and primer D
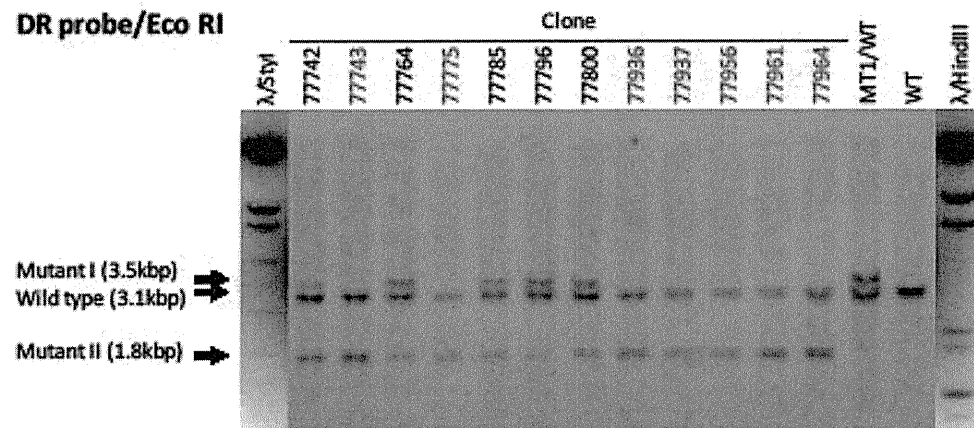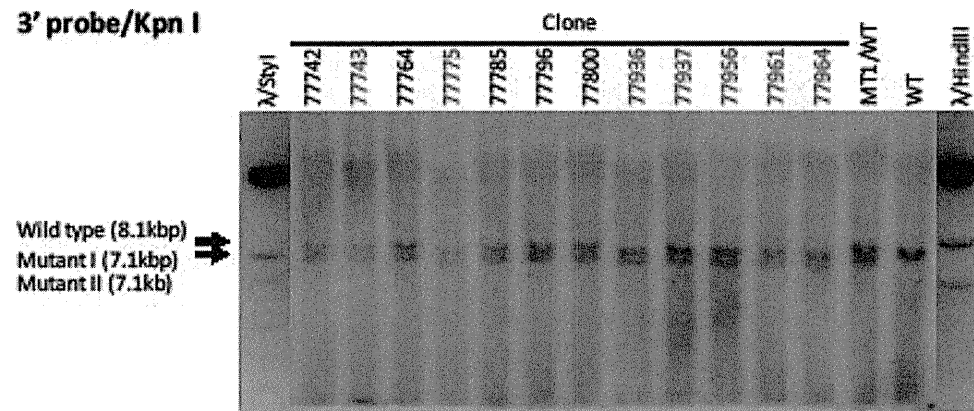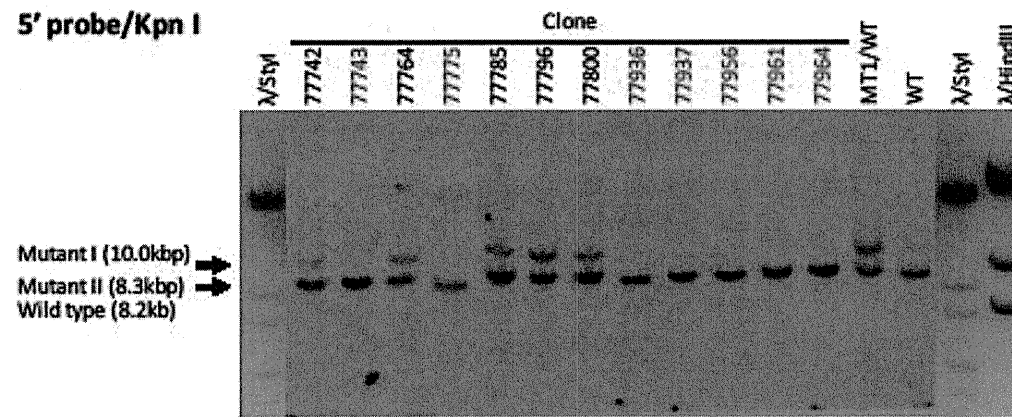
Figure S8 Kpn I/EcoRI-digested Southern blot analysis
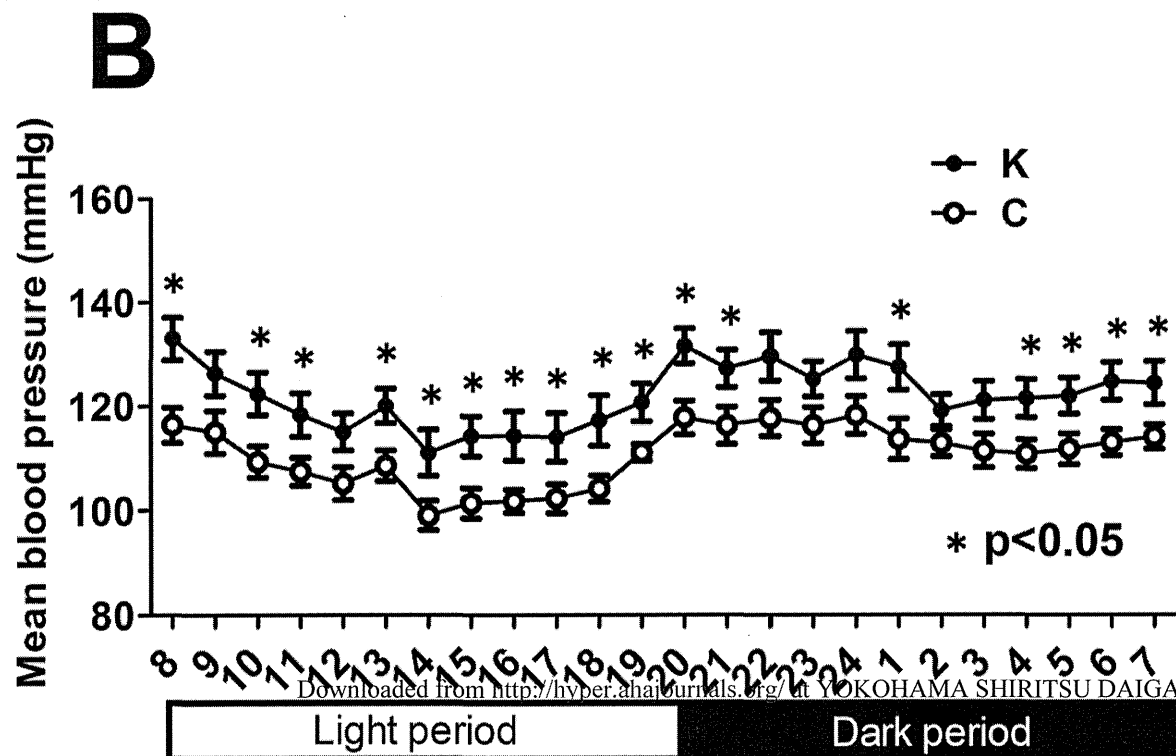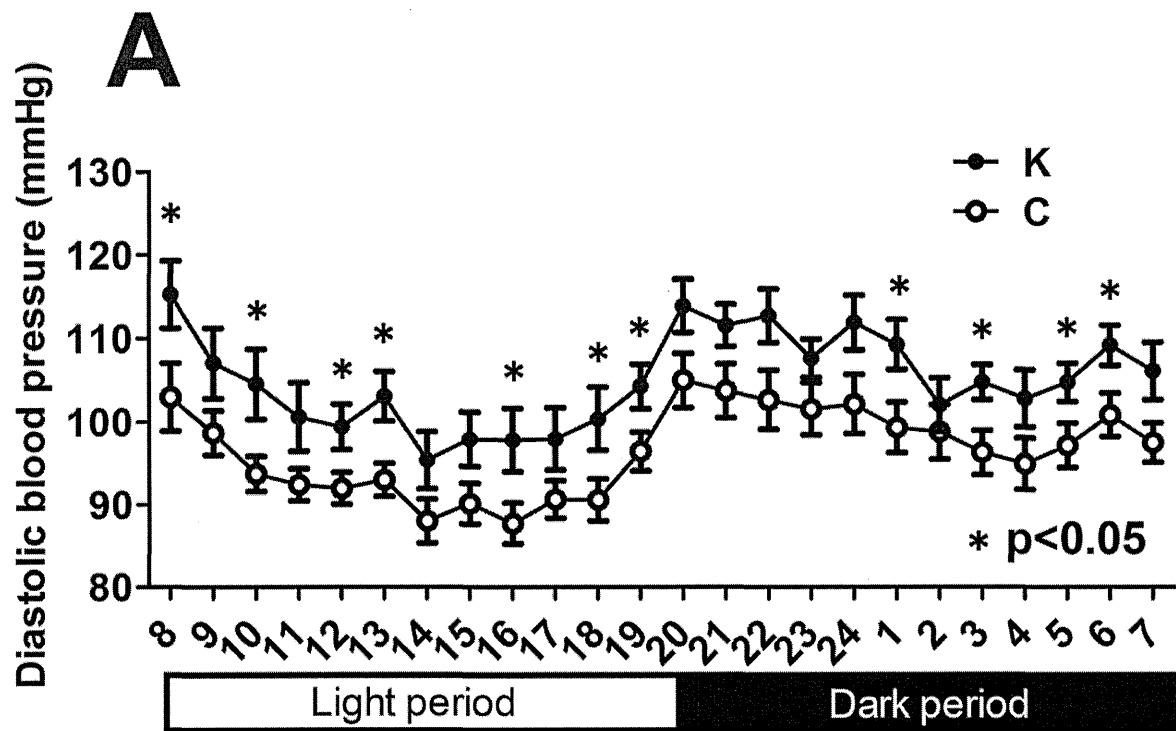
Figure S9 Diastolic and mean blood pressure measured by radiotelemetry

(A) Circadian patterns of diastolic blood pressure in VSMC ATP2B1 KO mice (n=9) and control mice (n=9) on a 12 hour light (8 AM to 8 PM) / dark (8 PM to 8 AM) cycle are shown. Mice were studied on a normal-salt diet. Values plotted are hourly means and standard errors measured over 60 hours (C: control mice, K: VSMC ATP2B1 KO mice). (B) Circadian patterns of mean blood pressure in VSMC ATP2B1 KO mice (n=9) and control mice (n=9) on a 12 hour light (8 AM to 8 PM) / dark (8 PM to 8 AM) cycle are shown. Mice were studied on a normal-salt diet. Values plotted are hourly means and standard errors measured over 60 hours.

ORIGINAL PAPER

Effect of ascorbic acid on reactive oxygen species production in chemotherapy and hyperthermia in prostate cancer cells

Hidenobu Fukumura · Motohiko Sato · Kyouhei Kezuka · Itaru Sato ·
Xianfeng Feng · Satoshi Okumura · Takayuki Fujita · Utako Yokoyama ·
Haruki Eguchi · Yoshihiro Ishikawa · Tomoyuki Saito

Abstract Cellular reactive oxygen species (ROS) production is increased by both temperature and anticancer drugs. Antioxidants are known to suppress ROS production while cancer patients may take them as dietary supplement during chemotherapy and hyperthermic therapy. We examined changes in ROS production in prostate cancer cells in the presence of various anticancer drugs and antioxidants at different temperatures. ROS production was increased with temperature in cancer cells, but not in normal cells; this increase was potently inhibited by ascorbic acid. ROS production was also increased in the presence of some anticancer drugs, such as vinblastine, but not by others. Dietary antioxidant supplements, such as β-carotene, showed variable effects. Ascorbic acid potently inhibited ROS production, even in the presence of anticancer drugs, while β-carotene showed no inhibition. Accordingly, our results suggest that cancer patients should carefully choose antioxidants during their cancer chemotherapy and/or hyperthermic therapy.

Introduction

Physiology of cancer cells has been extensively studied, and the understanding of mechanisms for their rapid growth and proliferation has been advanced in the past decade [1–3]. Accordingly, various therapeutic strategies in cancer treatment have been developed [1, 4]. Although surgical removal of the cancer tissue is still the golden standard for complete cure, it is not always feasible in cases with advanced or metastatic cancer. Surgical stress may be too large for geriatric and/or exhausted patients. In such cases, combination of various therapeutic strategies has been recommended. Among such strategies, hyperthermic therapy may be applied on the top of the conventional cancer chemotherapy or radiation therapy [5, 6]. Although it may not achieve complete remission of cancer by itself, clinical studies have demonstrated that the survival and quality of life may be significantly improved [3, 7].

Molecular mechanism of hyperthermic therapy includes the overstimulation metabolism of rapidly proliferating cancer cells, leading to the induction of apoptosis [8]. Increased production of reactive oxygen species (ROS) from mitochondria may also be involved [9]. Because ROS production may be increased in the presence of anticancer drugs on their own, the combination of chemotherapy and hyperthermic therapy will synergistically increase ROS production, leading to effective cancer cell death [6]. However, ROS production is inhibited in the presence of various antioxidants [10]. In this regard, various antioxidants, which are also used as dietary supplements, may interfere with the

H. Fukumura · T. Saito
Department of Orthopaedic Surgery, Yokohama City University
School of Medicine, 3-9 Fukuura, Kanazawaku,
Yokohama 236-0004, Japan

M. Sato · K. Kezuka · I. Sato · X. Feng · S. Okumura ·
T. Fujita · U. Yokoyama · Y. Ishikawa (✉)
Cardiovascular Research Institute, Yokohama City University
School of Medicine, 3-9 Fukuura, Kanazawaku,
Yokohama 236-0004, Japan
e-mail: yishikaw@med.yokohama-cu.ac.jp

H. Eguchi
IHI Corporation, 1, Shin-nakahara, Isogoku,
Yokohama 235-8501, Japan

efficacy of such chemotherapy and/or hyperthermic therapy. Unfortunately, however, evaluation of the effect of such antioxidants in the combination of cancer chemotherapy has not been well performed [11, 12]. Ascorbic acid, for example, is often used as a dietary supplement. Because ascorbic acid may improve immunity or peripheral circulation [13], people, including cancer patients, take this antioxidant. However, the use of ascorbic acid in cancer patients remains controversial; ascorbic acid may enhance [10] or suppress [13] the efficacy of chemotherapy.

In this study, we examined the effect of temperature, anticancer drugs, and antioxidants on ROS production. We used MAT-Lu prostate cancer cells since hyperthermia therapy has often been applied to prostatic cancer patients [14, 15], and thus it is necessary to evaluate the effect of hyperthermia on this cancer cell type. We demonstrated their effect on ROS production, and make potential suggestions for future use of antioxidants in cancer patients.

Materials and methods

Materials

We used the following anticancer drugs; vinblastine (VBL) (Nihon Kayaku, Japan), cisplatin (CIS), (Pfizer, Japan), adriamycin (ADR), (Wako, Japan), docetaxel (DTX), (Sanofi Aventis, Japan). Similarly, as antioxidants, we used *N*-acetyl-cysteine (NAC), (Sigma, Japan), retinoic acid (Sigma), quercetin (Sigma), catechin (Wako), lutein (Sigma), β-carotene (Sigma), and ascorbic acid (Wako).

Cell culture

Rat prostatic adenocarcinoma cells (R3327-MAT-Lu) were cultured in RPMI-1640 medium supplemented with 10% FBS and 250 nM dexamethasone, which were kindly provided by Dr. J. T. Isaacs (Johns Hopkins University, MD, USA). Cells were incubated at 37°C in 5% CO_2. In some experiments, cells were incubated at 42°C as hyperthermic treatment (see below). Rat cardiac fibroblasts were isolated from adult rats (250–300 g, male) by using a modification of published methods [16]. Fibroblasts were separated from cardiac myocytes by gravity separation and grown to confluence on 10-cm cell culture dishes at 37°C with 90% air with 10% CO_2 in growth media (DMEM with 10% FBS, 1% penicillin, and 1% streptomycin).

Hyperthermic stress and measurement of reactive oxygen species

Cells were plated in 24-well culture plates (5.0×10^4 cells/well) overnight. Cells were then treated with various agents, including anticancer drugs, at 37°C for 3 h. For hyperthermic treatment, cells were further incubated in the presence or absence of various reagents at 42°C for 1 h. The intracellular ROS level was then measured using a fluorescent dye 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (Life technologies, Japan) as previously described [17]. In the presence of oxidant, DCFH is converted into the highly fluorescent 2′,7′-dichlorofluorescein. Cells were first washed with PBS, and serum-free DMEM containing 10 μM DCFH-DA was added to each well. Cells were then incubated at 37°C for 45 min. ROS production was measured using a microplate reader equipped with a spectrofluorometer (PerkinElmer ARVO MX, Japan) at an emission wavelength of 538 nm and extinction wavelength of 485 nm.

Statistical analysis

Data are expressed as means ± SEM. Data was analyzed by one-way ANOVA followed by Tukey post hoc using Graph-pad Prism software. Statistical significance was set at $p < 0.05$.

Results

Effect of temperature on ROS generation

It is known that cancer cells exhibit higher metabolism than normal cells. High metabolic rate may be reflected by increased ROS generation, in particular, upon hyperthermia. Accordingly, we compared the effect of temperature on ROS production between MAT-Lu prostate cancer cells and normal fibroblasts obtained from the cardiac tissue. It is known that fibroblasts grow rapidly and thus possess high metabolic rate in comparison to other normal cell types.

As shown in Fig. 1a, ROS production was lower at 32°C than at 37°C while it was higher at 42°C. Thus, ROS production was increased in a temperature-dependent manner, at least in prostate cancer cells. In contrast, ROS production in cardiac fibroblasts was not increased at 42°C in comparison to that at 37°C (Fig. 1b). Thus, ROS production by hyperthermia was increased only in cancer cells.

Effect of ascorbic acid on ROS production

We then examined the effect of ascorbic acid, which has been used in cancer treatment as part of chemotherapy, but is also known as a major antioxidant. In the presence of an increasing concentration of ascorbic acid (10 μM–100 mM), ROS production was decreased in a concentration-dependent manner at 37°C (Fig. 1c). Similar inhibition was observed at 42°C. Thus, ascorbic acid potently inhibited the production of ROS.
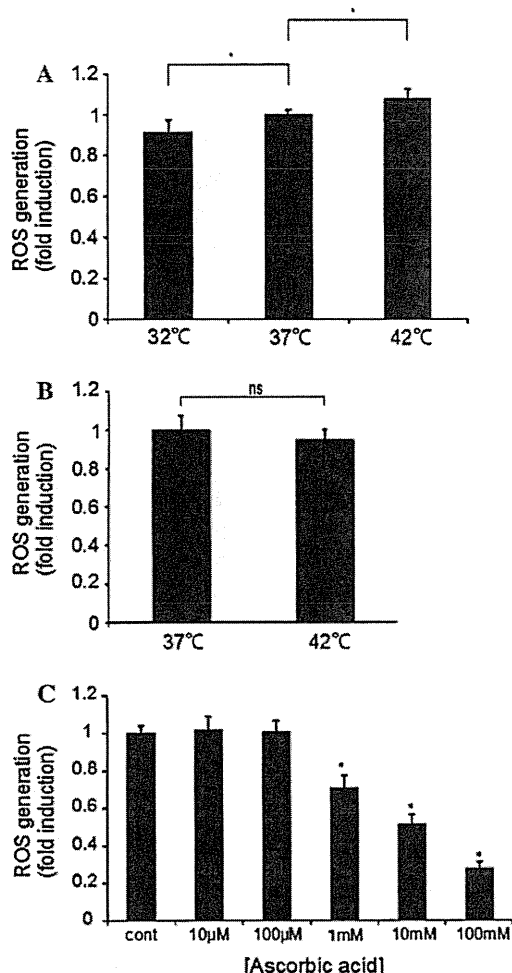
Fig. 1 ROS production in cancer cells and normal cells at different temperatures. a ROS production in cancer cells at 32, 37, and 42°C. Prostate cancer cells were incubated at different temperatures, followed by determination of ROS production (mean ± SEM; $n = 4$, $*p < 0.05$). b ROS production in cardiac fibroblasts at 37 and 42°C. Cardiac fibroblasts were incubated at different temperatures similarly, followed by determination of ROS production (mean ± SEM; $n = 4$, $*p < 0.05$). c ROS production was determined with cancer cells in the presence of an increasing concentration of ascorbic acid (10 µM–100 mM). Prostate cancer cells were incubated at 37°C, followed by determination of ROS production (mean ± SEM; $n = 4$, $*p < 0.05$)

Effect of anticancer drugs on ROS production

Anticancer drugs may induce cytotoxicity through various mechanisms. We examined the effect of these anticancer drugs, which have been widely used in many cancer cell types, including prostate cancer, on ROS production. We first determined the EC_{50} values of these drugs in prostate cancer cells, which were 200 nM for VBL, 15 µM for CIS, 7.5 µM for ADR, and 1 mM for DTX. When prostate cancer cells were incubated with these drugs at the EC_{50}

value concentration, ROS production was slightly, but significantly, increased with VBL and CIS, but not with DTX and ADR at 37°C (Fig. 2a). When hyperthermic treatment at 42°C was added, ROS production by VBL and CIS became even greater (Fig. 2a). Thus, hyperthermia by itself can increase ROS production, which is further enhanced in the presence of certain anticancer drugs.

We then examined the effect of ascorbic acid in the presence of anticancer drugs. ROS production was potently inhibited by 1 mM ascorbic acid in the presence of any anticancer drugs (Fig. 2b). ROS production at 37°C was similar among these anticancer drugs. However, when hyperthermic treatment at 42°C was added, ROS production was significantly greater with VBL (Fig. 2b). Thus, ascorbic acid may negate ROS production induced by certain anticancer drugs at 37°C; however, it cannot negate ROS production of VBL at 42°C. Accordingly, anticancer drug-induced ROS enhancement may be retained in hyperthermia for VBL, but not others.

Effect of ascorbic acid on ROS production by Resovist

Resovist is super-paramagnetic iron oxide nanoparticle that has been used as MRI contrast agent. Because of its magnetic property, similar compounds have been used as source of heat production in hyperthermic therapy. We found that the ROS production was increased in the presence of 10 µM Resovist at 37°C, suggesting that Resovist can produce ROS with cancer cells. When ascorbic acid was added, ROS production was negated or instead decreased (Fig. 3). Thus, ascorbic acid could potently inhibit ROS production induced by Resovist.

Effect of various antioxidants on ROS production

Patients may take various dietary supplements during cancer chemotherapy. In some cases, patients may take supplementary antioxidants on the top of anticancer drugs. We thus examined the effect of these antioxidants and related drugs, namely, N-acetyl cysteine (NAC), retinoic acid, quercetin, catechin, lutein, and β-carotene, on ROS production. We used these antioxidants at concentrations as previously demonstrated to be effective in various assays [11, 18, 19]. We examined their effect on VBL and CIS, which increased ROS production in the above assays.

As shown in Fig. 4a–f, these antioxidative compounds exhibited various degrees of antioxidative effects. NAC showed the most potent inhibition on ROS production; ROS production was decreased by a quarter in prostate cancer cells. VBL or CIS did not further increase ROS production in the presence of NAC at either 37 or 42°C, suggesting the ROS production by these anticancer drugs was completely suppressed by NAC. Thus, NAC showed

Fig. 2 Effect of anticancer drugs and ascorbic acid on ROS production. a ROS production was determined at 37 or 42°C in the presence of 200 nM VBL, 15 μM CIS, 7.5 μM DTX or 1 μM ADR (mean ± SEM; $n = 4$, *$p < 0.05$). b ROS production was similarly determined in the presence of 1 mM ascorbic acid at 37 or 42°C (mean ± SEM; $n = 4$, *$p < 0.05$)
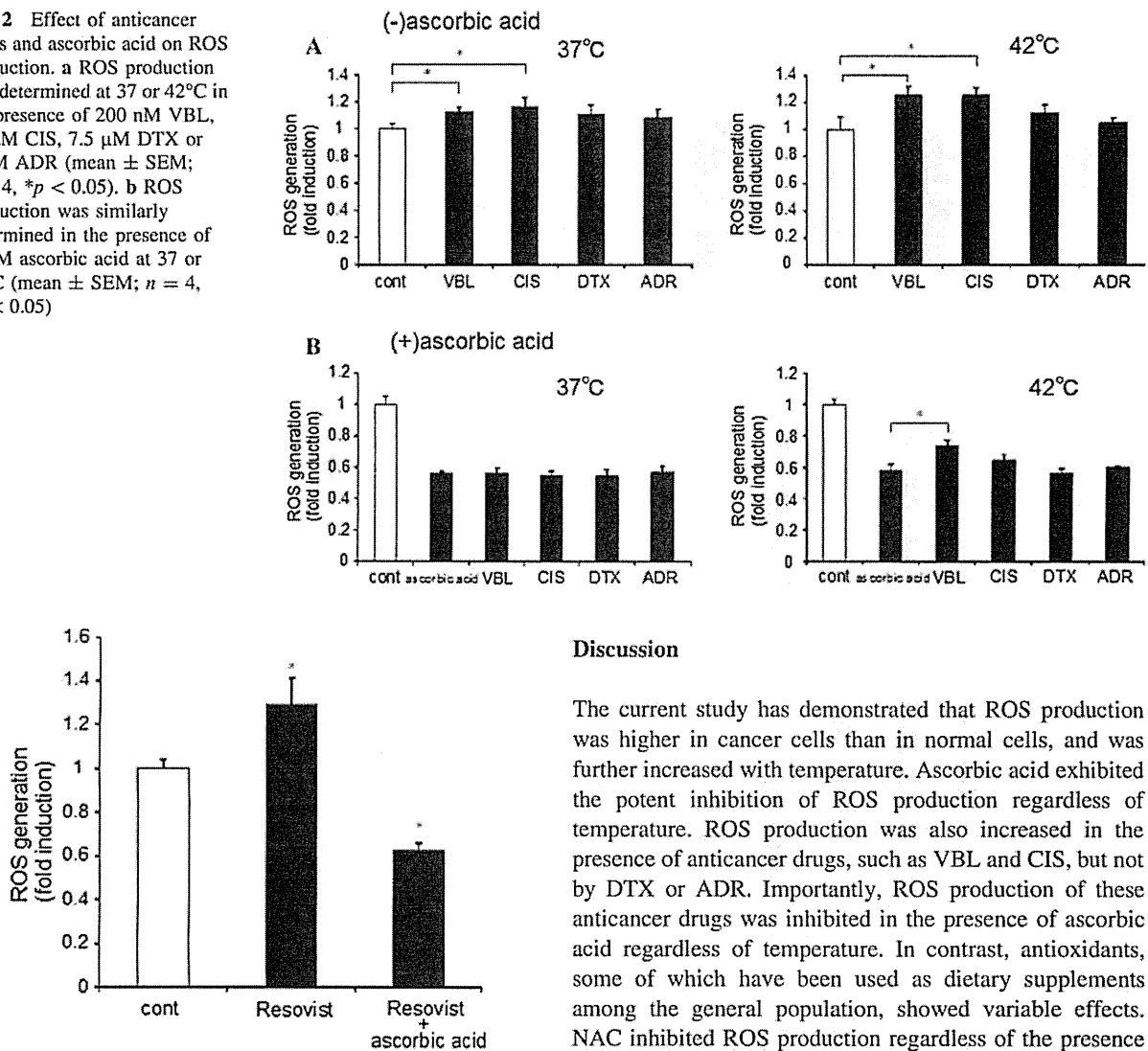

Fig. 3 Effect of Resovist on ROS production. ROS production was determined in the presence of 10 μM Resovist and/or 1 mM ascorbic acid at 37°C. Prostate cancer cells were incubated for 45 min, followed by ROS production assays (mean ± SEM; $n = 4$, *$p < 0.05$)

similar, but perhaps greater, antioxidative effect compared to ascorbic acid. Retinoic acid, quercetin, and lutein showed comparable results to each other. They inhibited ROS production at both 37 and 42°C. However, both VBL and CIS could increase ROS production in the presence of these antioxidants, suggesting that these antioxidants could not inhibit anticancer drug-mediated ROS production. Catechin and β-carotene are best known as general anti-oxidants. However, they did not inhibit ROS production, at either 37 or 42°C, in the absence or presence of anticancer drugs. Thus, the effects of many antioxidants are not always the same.

Discussion

The current study has demonstrated that ROS production was higher in cancer cells than in normal cells, and was further increased with temperature. Ascorbic acid exhibited the potent inhibition of ROS production regardless of temperature. ROS production was also increased in the presence of anticancer drugs, such as VBL and CIS, but not by DTX or ADR. Importantly, ROS production of these anticancer drugs was inhibited in the presence of ascorbic acid regardless of temperature. In contrast, antioxidants, some of which have been used as dietary supplements among the general population, showed variable effects. NAC inhibited ROS production regardless of the presence of anticancer drugs, while catechin or β-carotene did not inhibit ROS production. Lutein, quercetin, and retinoic acid inhibited ROS production in the absence of anticancer drugs, while they did not inhibit the ROS production as induced by anticancer drug. Thus, these antioxidants should be taken carefully by patients since they may variably affect the effect of anticancer drugs, at least in their ROS production.

ROS as a cause of cytotoxicity of anticancer drugs has been extensively studied in the past [20, 21]. CIS may interfere with mitochondrial membrane function and thus increases ROS production. Paclitaxel, which is comparable to DTX, may regulate membrane NOX release, and increases ROS production [22–25]. We found that both CIS and VBL increased ROS production in prostate cancer cells. Hyperthermic therapy potentiates ROS production, leading to enhanced cytotoxicity [26]. We also found that increased temperature enhanced ROS production by CIS

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Fig. 4 Effect of various antioxidants on ROS production. ROS production was determined in the presence of 200 nM VBL or 15 μM CIS at 37 or 42°C. Various antioxidants, i.e., 10 mM NAC (N-acetyl-cysteine), 50 nM retinoic acid, 100 nM quercetin, 50 μM catechin, 100 nM lutein, and 20 μM, β-carotene, were added. Cells were incubated for 45 min, followed by determination of ROS production (mean ± SEM; n = 4, *p < 0.05)
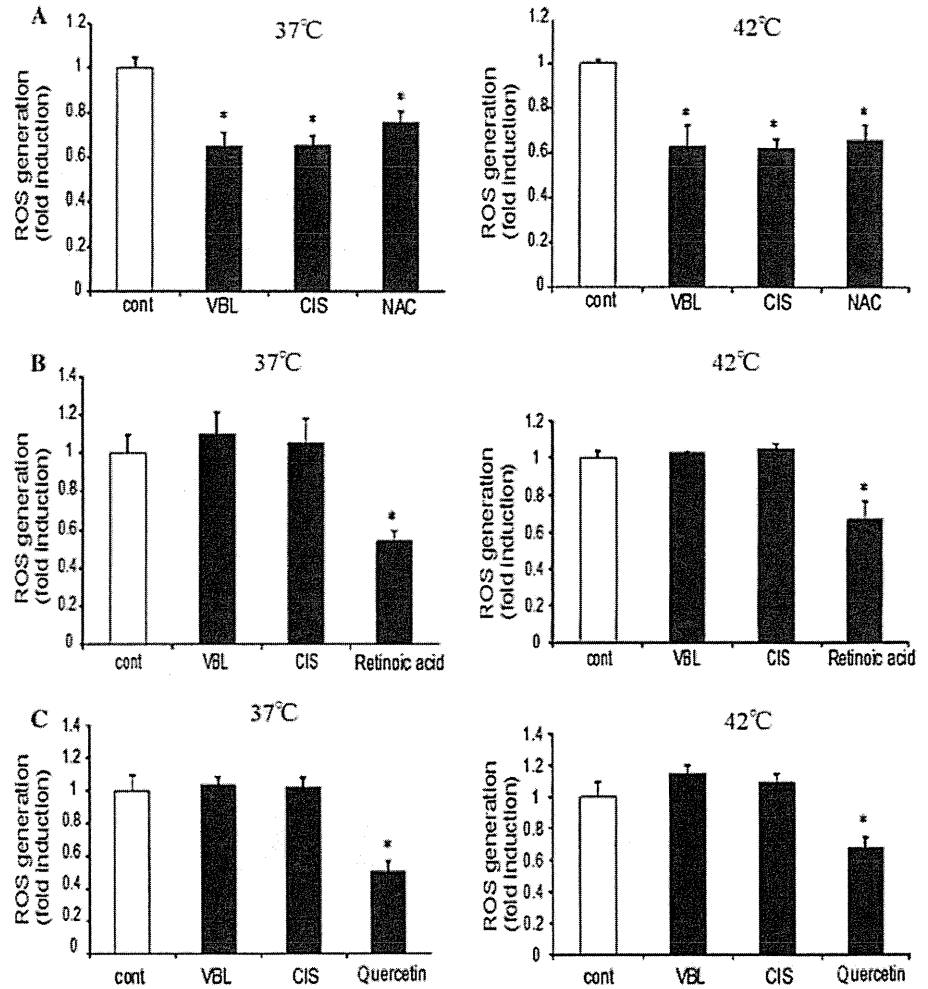

and VBL. Thus, both cancer chemotherapy and hyperthermic treatment enhanced ROS production, at least in prostate cancer cells.

With increasing public interest in antioxidant therapy, many nutritional supplements have been taken by the general public including cancer patients. There have been multiple studies that have examined the interaction between anticancer drugs and antioxidants. However, the results of these studies are not in agreement with each other. Anticancer drugs may produce ROS, which may damage cancer cells [27, 28]. Thereby, some studies demonstrated that antioxidants reduced the effect of these anticancer drugs [29]. In contrast, others demonstrated that ROS production was enhanced by antioxidants [30]. More specifically, ascorbic acid can quench ROS within the cell, and thus stabilize mitochondrial membrane, leading to protection of the cell [13, 26]. Although previous studies have demonstrated that ascorbic acid increased the effect of anticancer drugs, more recently attenuation of anticancer drug effect has also been reported [26].

We found that antioxidants indeed exhibited various effects on ROS production. NAC, which by itself scavenges ROS [18], potently decreased ROS production, and ROS production by anticancer drugs was also negated. Thus, the use of NAC may hamper the effect of anticancer drugs. In contrast, lutein, quercetin, and retinoic acid, which are also known as ROS scavengers, decreased ROS production. However, they were not potent enough to inhibit the ROS-producing effect of anticancer compounds. Thus, these antioxidants may be taken safely by cancer patients during chemotherapy and hyperthermic therapy. Catechin and β-carotene are known as antioxidants and are contained in various kinds of foods, such as green tea or carrot [11, 12]. However, they did not exhibit inhibitory effect on ROS production regardless of the presence of anticancer drugs, suggesting that they do not interfere with such drug effects. Thus, cancer patients may take these antioxidants as well as foods containing these antioxidants.

Putting it together, administration of NAC and ascorbic acid may need caution while other antioxidants may not

Fig. 4 continued
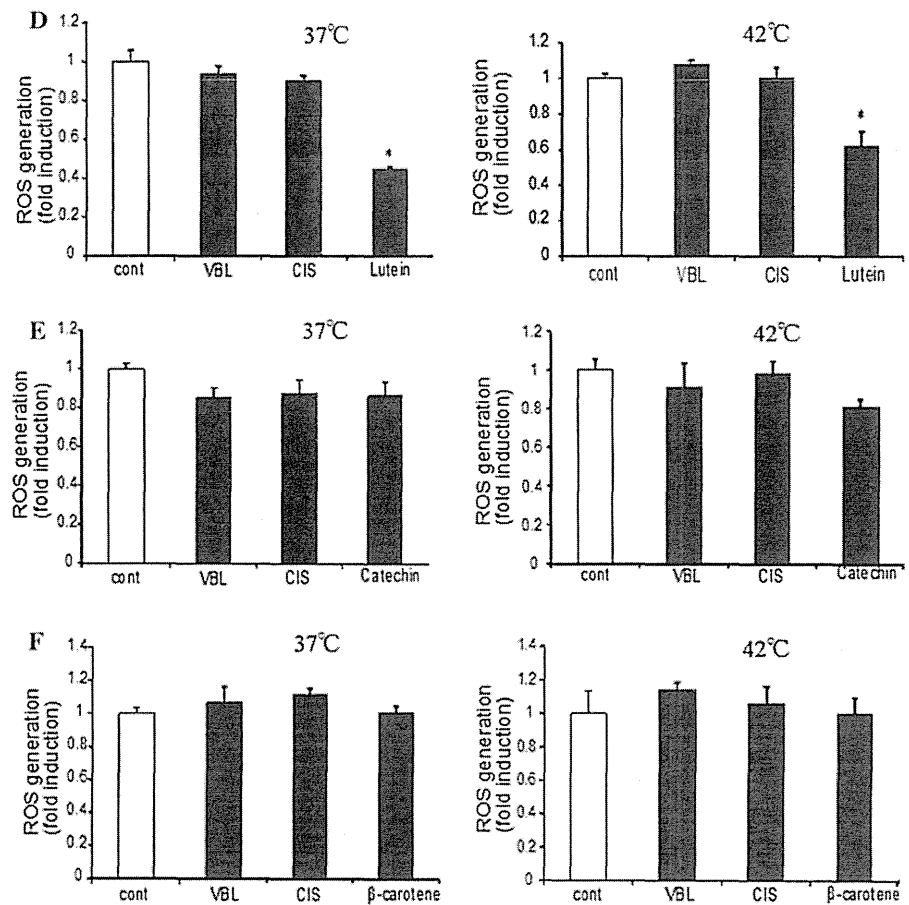
require major attention, at least in terms of ROS production in cancer patients. In particular, ascorbic acid is widely used for multiple purposes, including for viral infection. Accordingly, the current study has suggested that the use of ascorbic acid may be considered carefully by both cancer patients and oncologists. Further, with our findings, the effects of ascorbic acid and its related antioxidants need to be clinically examined in future in cancer patients who are to be treated with chemotherapy and/or hyperthermic therapy.

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Prevention of heart failure in mice by an antiviral agent that inhibits type 5 cardiac adenylyl cyclase

Kosaku Iwatsubo,[1,3] Claudio Bravo,[1] Masami Uechi,[4] Erdene Baljinnyam,[1] Takashi Nakamura,[4] Masanari Umemura,[1] Lo Lai,[1] Shumin Gao,[1] Lin Yan,[1] Xin Zhao,[1] Misun Park,[1] Hongyu Qiu,[1] Satoshi Okumura,[3] Mizuka Iwatsubo,[1] Dorothy E. Vatner,[1,2] Stephen F. Vatner,[1] and Yoshihiro Ishikawa[1,2,3]

[1]*Department of Cell Biology and Molecular Medicine, Cardiovascular Research Institute, New Jersey Medical School-University of Medicine and Dentistry of New Jersey, Newark, NJ;* [2]*Department of Medicine (Cardiology), New Jersey Medical School-University of Medicine and Dentistry of New Jersey, Newark, NJ;* [3]*Cardiovascular Research Institute, Yokohama City University Graduate School of Medicine, Yokohama, Japan; and* [4]*Department of Veterinary Medicine, College of Bio-resource Sciences, Nihon University, Fujisawa, Kanagawa, Japan*

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Iwatsubo K, Bravo C, Uechi M, Baljinnyam E, Nakamura T, Umemura M, Lai L, Gao S, Yan L, Zhao X, Park M, Qiu H, Okumura S, Iwatsubo M, Vatner DE, Vatner SF, Ishikawa Y. Prevention of heart failure in mice by an antiviral agent that inhibits type 5 cardiac adenylyl cyclase. *Am J Physiol Heart Circ Physiol* 302: H2622–H2628, 2012. First published April 13, 2012; doi:10.1152/ajpheart.00190.2012.—Despite numerous discoveries from genetically engineered mice, relatively few have been translated to the bedside, mainly because it is difficult to translate from genes to drugs. This investigation examines an antiviral drug, which also has an action to selectively inhibit type 5 adenylyl cyclase (AC5), a pharmaceutical correlate of the AC5 knockout (KO) model, which exhibits longevity and stress resistance. Our objective was to examine the extent to which pretreatment with this drug, adenine 9-β-D-arabinofuranoside (Ara-A), favorably ameliorates the development of heart failure (HF). Ara-A exhibited selective inhibition for AC5 compared with the other major cardiac AC isoform, AC6, i.e., it reduced AC activity significantly in AC5 transgenic (Tg) mice, but not in AC5KO mice and had little effect in either wild-type or AC6Tg mice. Permanent coronary artery occlusion for 3 wk in C57Bl/6 mice increased mortality and induced HF in survivors, as reflected by reduced cardiac function, while increasing cardiac fibrosis. The AC5 inhibitor Ara-A significantly improved all of these end points and also ameliorated chronic isoproterenol-induced cardiomyopathy. As with the AC5KO mice, Ara-A increased mitogen/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) phosphorylation. A MEK inhibitor abolished the beneficial effects of the AC5 inhibitor in the HF model, indicating the involvement of the downstream MEK-ERK pathway of AC5. Our data suggest that pharmacological AC5 inhibition may serve as a new therapeutic approach for HF.

heart failure; type 5 adenylyl cyclase inhibition; adenine 9-β-D-arabinofuranoside

DESPITE GAINS IN THE TREATMENT of heart failure (HF) with both angiotensin and β-adrenergic receptor (β-AR) blockers, HF still remains a major cause of death and disability. In addition, some patients do not tolerate β-AR blocking therapy (2). It is conceivable that inhibiting mechanisms distal to the β-AR signaling pathway, identified from genetically engineered mouse models, might be a novel approach. While there have been numerous potential therapeutic approaches discovered from studies in genetically engineered mice in the past two decades, there are relatively few of these discoveries that have been translated to the bedside, mainly because it is difficult to translate the effects of disrupting a gene in a mouse to therapy in patients with HF.

The goal of this investigation was to examine the extent to which a pharmacological inhibitor of type 5 adenylyl cyclase (AC5), 9-β-D-arabinofuranoside (Ara-A), could mimic the salutary effects observed in the AC5 knockout (KO) mice model, which protects against cardiac stress (10, 11) and increases longevity (15). The first goal was to determine the extent to which Ara-A selectively inhibits AC5. The next goal was to determine whether pretreatment with the pharmacological AC5 inhibitor ameliorates the development of cardiomyopathy and HF following either permanent coronary artery occlusion (CAO) or chronic isoproterenol (ISO) infusion. An additional goal was to determine if the mechanism involved the mitogen/extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, a key protective signaling pathway in the AC5KO (15). The latter was accomplished by repeating the experiments with permanent CAO in the presence of a specific MEK blocker, U-0126.

The AC5 inhibitor Ara-A is a Food and Drug Administration (FDA)-approved drug, also known as vidarabine (adenine 9-β-D arabinofuranoside), and has been used as an anti-herpes virus drug for many decades. The advantage of identifying Ara-A as a potential drug for HF is that the drug has already been FDA approved and could be rapidly moved to clinical trials.

METHODS

Animal models. Three- to five-month-old male AC5KO (9) (on C57Bl/6 background) and cardiac-specific overexpression of AC5 [AC5 transgenic (Tg)] (7) or AC6 (AC6Tg) (7) (on FVB background) mice were used in this study. In the CAO model, mini-osmotic pumps delivering Ara-A (15 mg·kg^{-1}·day^{-1}), the MEK blocker U-0126 (5 mg·kg^{-1}·day^{-1}) (15), or a combination of U-0126 with Ara-A were subcutaneously implanted 1 wk before the CAO of the left anterior descending artery. Chronic infusion of ISO (Sigma-Aldrich, St. Louis, MO) was performed for 7 days at a dose of 60 mg·kg^{-1}·day^{-1} with or without Ara-A delivered with the mini-osmotic pumps. The dose of Ara-A was selected on the basis of that previously used for viral encephalopathy (13). Animals used in this study were maintained in
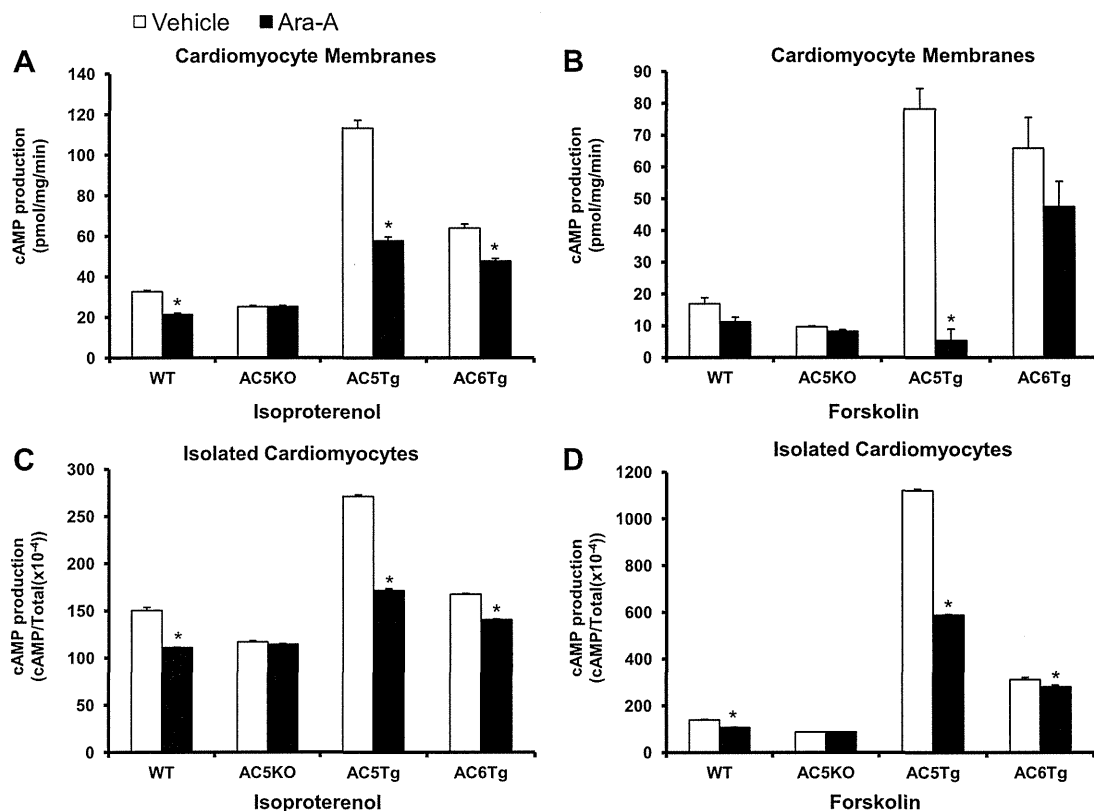
☐ Vehicle ■ Ara-A

Fig. 1. Selective inhibition of type 5 adenylyl cyclase (AC5) by adenine 9-β-D-arabinofuranoside (Ara-A) (in vitro). Mouse cardiac membrane preparations were used for A and B. Adult mouse cardiac myocytes were used for C and D. A and B: cAMP reduction with Ara-A (10 μM) was measured in cardiac membrane preparations from myocardium of wild-type (WT), AC5 knockout (KO), AC5 transgenic (Tg), and AC6Tg mice with isoproterenol (ISO) (5 μM, A) or with forskolin (50 μM, B). C and D: cAMP reduction with Ara-A (10 μM) was measured in adult cardiac myocytes from myocardium of WT, AC5KO, AC5Tg, and AC6Tg mice with ISO (C) or with forskolin (D). cAMP reduction by Ara-A was greater in AC5Tg than in WT, was similar between WT and AC6Tg, and was absent in the AC5KO. These data indicate that Ara-A selectively suppresses AC5 enzymatic activity. t-Test: $*P < 0.01$ vs. vehicle; $n = 4$ experiments for A and B and $n = 5$ for C and D.
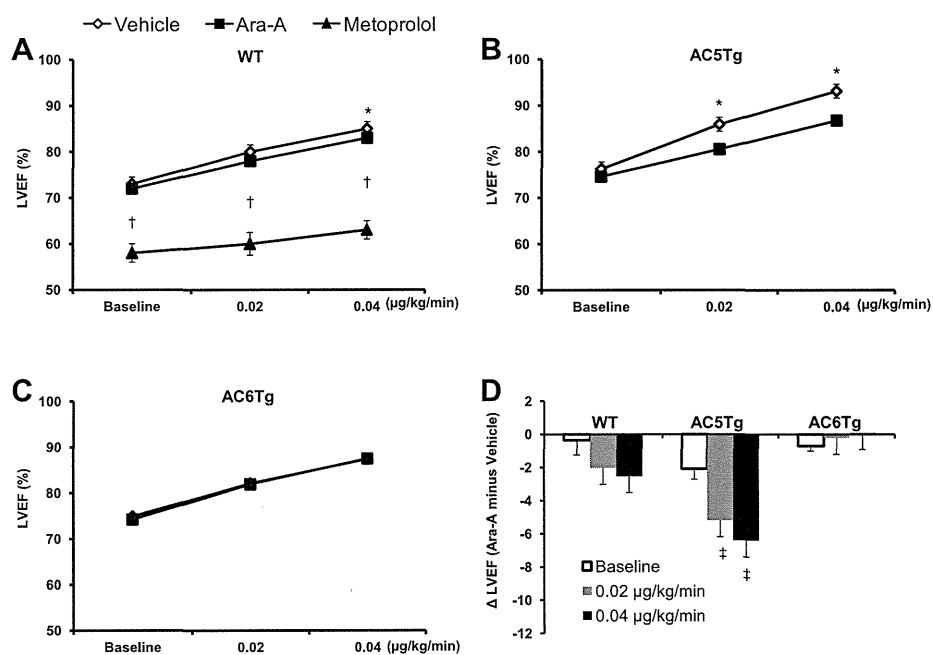

Fig. 2. Selective inhibition of AC5 by Ara-A (in vivo). Left ventricle ejection fraction (LVEF) was measured in response to ISO challenges in WT (A), AC5Tg (B), and AC6Tg (C) in the presence of either vehicle, Ara-A, or metoprolol ($n = 6$ animals/group). D: negative inotropic effect of Ara-A at baseline and at a dose of 0.02 and 0.04 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of ISO. Ara-A decreased ISO-induced increases in LVEF more in AC5Tg mice than WT or AC6Tg mice (A–D). These data show that Ara-A selectively suppresses AC5 in vivo. In contrast to Ara-A, the β-adrenergic receptor (β-AR) blocker metoprolol decreased basal left ventricle (LV) function and also completely blocked the positive inotropic response to ISO (A). t-Test: $*P < 0.05$, Ara-A vs. vehicle; $^{†}P < 0.05$, metoprolol vs. Ara-A or vs. vehicle; and $^{‡}P < 0.05$ vs. same ISO dose in WT or vs. same ISO dose in AC6Tg.