However, it has been reported that mean arterial blood pressure in mice heterozygous for the APE1/REF-1 allele (APE1/REF-$1^{+/-}$) was higher than that in wild-type mice (APE1/REF-$1^{+/+}$).[10,11]

Hypertension affects 25% of adults in most populations and is a major risk factor for death from cerebral infraction, myocardial infarction, and congestive heart failure.[12] The most prevalent form of hypertension is EH. EH is considered to be a polygenic disease resulting from the inheritance of a number of susceptibility genes. The causal genes identified may account for 30–50% of the variations in blood pressure seen among individuals.[13] These genetic determinants interact with environmental factors, such as dietary salt, obesity, drinking, and smoking to produce the final disease phenotype.[14]

The aim of this study was to investigate the relationship between EH and the human *APE1/REF-1* gene through a haplotype-based case–control study using single-nucleotide polymorphisms (SNPs).

METHODS

Subjects. EH subjects were 265 patients diagnosed with EH according to the following criteria: seated systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥100 mm Hg, on two occasions within 2 months after the first medical examination. None of the EH subjects were using antihypertensive medication or Cox inhibitors (nonsteroidal anti-inflammatory drugs). Patients with secondary forms of hypertension were excluded based on the results of clinical and laboratory examinations: (i) measurement of fasting blood sugar, glycosylated hemoglobin A_{1c}, plasma aldosterone, plasma renin activity, and plasma catecholamine; and (ii) computed tomography and magnetic resonance imaging to assess the condition of adrenal glands and check for pituitary tumors. These tests were performed in all patients. For comparison, we included 266 healthy normotensive (NT) controls. None of the NT subjects had a family history of hypertension, and all NT subjects had a systolic blood pressure of <130 mm Hg and diastolic blood pressure of <85 mm Hg. A family history of hypertension was defined as prior diagnosis of hypertension in a grandparent, uncle, aunt, parent, or sibling. Hypercholesterolemia was defined as plasma total cholesterol >220 mg/dl, or current use of a lipid-lowering drug in addition to a confirmed diagnosis of hypercholesterolemia. Both groups were recruited from the northern area of Tokyo, Japan. Informed consent was obtained from each subject, in accordance with the protocol approved by the Human Studies Committee of Nihon University.[15]

Biochemical analysis. Blood samples were obtained from subjects on the morning after a rest in the sitting position, and after at least 30 min without eating. In the clinical laboratory department of our university hospital, these blood samples were subjected to tests for plasma concentrations of total cholesterol and high-density lipoprotein cholesterol, and serum concentrations of creatinine and uric acid.[15]

Genotyping. As detailed data of SNPs in the *APE1/REF-1* gene on the Web site of HapMap were not clear, information on the allelic frequencies of SNPs registered on the Web site of the NCBI (National Center for Biotechnology Information) was used. We screened for SNPs among the NCBI data using a cut-off level of minor allele frequency ≥0.05 to identify useful genetic markers. However, there are a few SNPs in the *APE1/REF-1* gene with minor allele frequency >0.05. Therefore, we also studied rs3136814, which has minor allele frequency of almost 0.05 (0.044 in Asian populations according to NCBI Web site). Furthermore, we selected at least one SNP from each of the promoter, intron, and exon regions. rs1130409 (Asp148Glu (GAT→GAG)) is located in the amino-acid coding region in the human *APE1/REF-1* gene, is nonsynonymous, and has been studied previously.[16–18] Therefore, we selected rs1130409 for the present association study. Based on the above criteria, we selected rs1760944, rs3136814, rs17111967, rs3136817, and rs1130409 (Figure 1). We then examined the associations between EH and these five SNPs. Genotypes were determined using Assays-on-Demand kits (Applied Biosystems, Branchburg, NJ) together with TaqMan PCR (Applied Biosystems).[19] TaqMan SNP genotyping assays were also performed using the method of Taq amplification. In the 5′-nuclease assay, discrimination occurs during PCR, as the allele-specific fluorogenic probes are hybridized to the template and are cleaved by the 5′-nuclease activity of Taq polymerase. The probes contain a 3′-minor groove-binding group that hybridizes to single-stranded targets with increased sequence specificity when compared with ordinary DNA probes. This reduces nonspecific probe hybridization and results in low background fluorescence

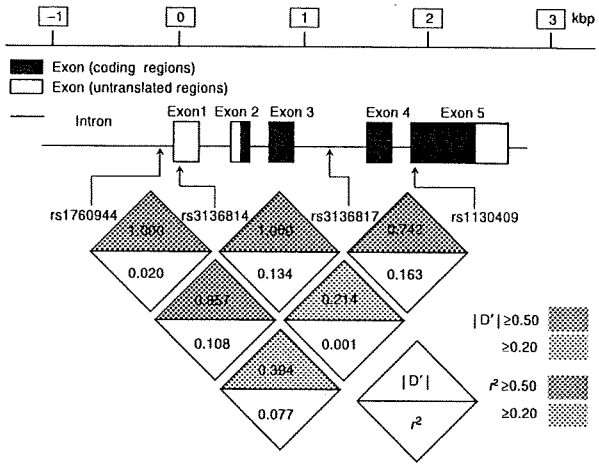

Figure 1 | Organization of the human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 (*APE1/REF-1*) gene with location of single-nucleotide polymorphisms (SNPs) used in the present association study, and pair-wise linkage disequilibrium (LD) in the *APE1/REF-1* gene, as evaluated by |D'| and r^2. Closed boxes indicate exons, and lines represent introns. Pair-wise LD values among the four marker pairs studied in the human *APE1/REF-1* gene were computed, and pairs in LD (|D'| ≥0.5 or r^2 ≥0.5) are shown as shaded values, and pairs in LD (|D'| ≥0.2 or r^2 ≥0.2) are light shaded values. kbp, kilobase pair.

during the 5′-nuclease PCR assay (TaqMan). Cleavage results in the increased emission of a reporter dye. Each 5′-nuclease assay requires two unlabeled PCR primers and two allele-specific probes. Each probe was labeled with two reporter dyes at the 5′-end; in this study, VIC and FAM were used as reporter dyes. Primers and probes in the TaqMan SNP genotyping assays (Applied Biosystems) were chosen from the information available on the Applied Biosystems Web site (http://www3.appliedbiosystems.com/AB_Home/index.htm).

PCR amplification was performed using 2.5 μl of TaqMan Universal Master Mix, No AmpErase UNG (2×) (Applied Biosystems) in 5-μl final reaction volumes, with 2 ng of DNA, 2.375 μl of ultrapure water, 0.079 μl of Tris-EDTA buffer (1×), 0.046 μl of TaqMan SNP genotyping assay mix (40×) containing primers at a final concentration of 331.2 nmol/l, and probes at a final concentration of 73.6 nmol/l. Thermal cycling conditions were 95 °C for 10 min, 50 cycles of 92 °C for 15 s, and finally 60 °C for 1 min, and was performed using the GeneAmp 9700 system (Applied Biosystems).[19]

Each 96-well plate contained 80 samples of an unknown genotype and four samples with no DNA but with reagents (control). Control samples without DNA were necessary in the SDS (Sequence Detection System) 7700 for signal processing, as outlined in the TaqMan Allelic Discrimination Guide (Applied Biosystems). Plates were read on the SDS 7700 instrument using the end-point analysis mode of the SDS, version 1.6.3, software package (Applied Biosystems). Genotypes were determined visually based on the dye-component fluorescent emission data depicted in the X–Y scatter plot of the SDS software. Genotypes were all determined automatically by the signal processing algorithms of the software. The results of each scoring method were saved in two separate output files for later comparison.[20]

Statistical analysis. Data are shown as means ± s.d. Hardy–Weinberg equilibrium was assessed using χ^2 analysis. The overall distribution of alleles was analyzed using 2 × 2 contingency tables, and the distribution of genotypes between EH patients and NT control subjects was analyzed using a two-sided Fisher exact test. To assess the contribution of confounding factors, multiple logistic regression analysis was performed.[21,22] Based on the genotype data of the genetic variations, linkage disequilibrium analysis and a haplotype-based case–control study were performed using the expectation maximization algorithm of the SNPAlyze software program, version 3.2 (Dynacom, Yokohama, Japan). Pair-wise linkage disequilibrium analysis was performed using SNP pairs. |D′| values ≥0.5 were used to assign SNP locations to one haplotype block. Tagged SNPs were selected by omitting one SNP from an SNP pair showing $r^2 \geq 0.25$ for each haplotype block. In this haplotype-based case–control study, haplotypes with a frequency of <0.02 were excluded. The association of haplotype frequencies with phenotype was assessed by χ^2 test. A probability level of $P < 0.05$ was considered to indicate statistical significance. Differences in clinical data between the EH and NT groups were assessed using analysis of variance followed by Fisher's protected least-significant difference test.[23] Diplotype construction was also analyzed by the SNPAlyze software program, version 3.2. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors.

RESULTS

Table 1 shows the clinical features of EH patients and NT control subjects. There were no significant differences in age, serum concentrations of creatinine, and plasma concentrations of high-density lipoprotein cholesterol and uric acid between the

Table 1 | Characteristics of study participants

	Total			Men			Women		
	NT	EH	*P* value	NT	EH	*P* value	NT	EH	*P* value
Number of subjects	266	265		171	172		95	93	
Age (years)	52.2 ± 10.3	51.0 ± 5.5	0.094	52.3 ± 7.3	50.9 ± 5.6	0.054	52.1 ± 14.3	51.2 ± 5.2	0.559
BMI (kg/m^2)	22.7 ± 3.3	24.6 ± 3.6	<0.001*	22.9 ± 3.3	24.7 ± 3.3	<0.001*	22.2 ± 3.3	24.5 ± 4.0	<0.001*
SBP (mm Hg)	112.3 ± 10.7	173.7 ± 20.2	<0.001*	112.8 ± 10.5	171.2 ± 18.9	<0.001*	111.2 ± 11.1	178.4 ± 21.7	<0.001*
DBP (mm Hg)	69.2 ± 8.5	106.2 ± 13.0	<0.001*	69.9 ± 8.0	106.5 ± 12.6	<0.001*	67.8 ± 9.2	105.7 ± 13.6	<0.001*
Pulse (beats/min)	73.5 ± 13.3	77.0 ± 14.9	0.014*	73.3 ± 14.4	76.7 ± 15.5	0.064	74.0 ± 11.0	77.7 ± 13.6	0.088
Creatinine (mg/dl)	0.83 ± 0.22	0.85 ± 0.25	0.281	0.90 ± 0.21	0.94 ± 0.23	0.151	0.69 ± 0.14	0.68 ± 0.18	0.701
Total cholesterol (mg/dl)	198.4 ± 46.2	208.6 ± 39.7	0.008*	193.2 ± 46.3	202.1 ± 39.3	0.065	207.8 ± 44.8	220.1 ± 38.0	0.049*
HDL cholesterol (mg/dl)	56.6 ± 17.7	56.5 ± 17.6	0.954	54.6 ± 16.7	52.8 ± 16.6	0.374	60.4 ± 18.9	62.9 ± 17.6	0.392
Uric acid (mg/dl)	5.41 ± 1.76	5.64 ± 1.60	0.135	5.86 ± 1.38	6.21 ± 1.49	0.030*	4.60 ± 2.08	4.59 ± 1.21	0.985
Hyperlipidemia (%)	18.0	25.7	0.034*	15.2	22.1	0.102	23.2	32.3	0.163
Diabetes (%)	2.6	10.2	<0.001*	2.3	11.6	<0.001*	3.2	7.5	0.182

Continuous variables are expressed as mean ± s.d. Categorical variables are expressed as percentages. *P* value for continuous variables was calculated by Mann–Whitney *U* test. The *P* value for categorical variable was calculated by Fisher's exact test.
BMI, body mass index; DBP, diastolic blood pressure; EH, essential hypertension; HDL, high-density lipoprotein; NT, normotension; SBP, systolic blood pressure.
*$P < 0.05$.

Table 2 | Genotype and allele distributions in NT and patients with EH

			NT	EH	*P* value
Number of participants			266	265	
Variants					
rs1760944	Genotype	G/G	82 (0.308)	91 (0.343)	
		G/T	126 (0.474)	124 (0.468)	
		T/T	58 (0.218)	50 (0.189)	0.584
	Allele	G	290 (0.545)	306 (0.577)	
		T	242 (0.455)	224 (0.423)	0.290
rs3136814	Genotype	A/A	254 (0.955)	247 (0.932)	
		A/C	12 (0.045)	18 (0.068)	
		C/C	0 (0)	0 (0)	0.255
	Allele	A	520 (0.977)	512 (0.966)	
		C	12 (0.023)	18 (0.034)	0.266
rs3136817	Genotype	C/C	194 (0.729)	190 (0.717)	
		C/T	66 (0.248)	68 (0.257)	
		T/T	6 (0.023)	7 (0.026)	0.929
	Allele	C	454 (0.853)	448 (0.845)	
		T	78 (0.147)	82 (0.155)	0.712
rs1130409	Genotype	G/G	38 (0.143)	47 (0.177)	
		G/T	119 (0.447)	131 (0.494)	
		T/T	109 (0.410)	87 (0.328)	0.136
	Allele	G	195 (0.367)	225 (0.425)	
		T	337 (0.633)	305 (0.575)	0.053

EH, essential hypertension; NT, normotension.

Table 3 | Combination of SNPs that showed significant difference

	Number of participants		Overall distribution		Distribution of individual haplotypes				
Combination of SNPs	NT	EH	χ^2	*P* value	Haplotype	NT	EH	χ^2	*P* value
rs1760944-rs3136817-rs1130409	266	265	15.72	0.008[a]	G-C-T	0.267	0.239	1.08	0.299
					T-C-T	0.358	0.316	2.10	0.147
					G-T-T	0.000	0.021	10.74	0.001[a]
					G-C-G	0.144	0.187	3.36	0.067
					T-C-G	0.105	0.104	0.00	0.990
					G-T-G	0.125	0.133	0.12	0.734

EH, essential hypertension; NT, normotension; SNP, single-nucleotide polymorphism.
[a]Significant difference.

two groups. However, body mass index, systolic blood pressure, diastolic blood pressure, pulse rate, plasma concentration of total cholesterol, and frequencies of hypercholesterolemia and diabetes differed significantly between the two groups.

Table 2 shows the distribution of genotypic and allelic frequencies of the SNPs in each group. rs1711967 was excluded because there was no heterogeneity; all of the participants were genotyped as C/C. The observed and expected genotypic frequencies in each SNP in all subjects and among both men and women in the NT group were in good agreement with the predicted Hardy–Weinberg equilibrium values (data not shown). There were no significant differences in the overall distribution of genotype or allele for all four SNPs between the EH and NT groups.

Linkage disequilibrium patterns are shown with their |D′| and r^2 values (Figure 1). Most |D′| values were large, thus indicating that all polymorphisms were located in one haplotype block. As the r^2 of all pairs of four SNPs was <0.25, we constructed a haplotype-based association study using rs1760944, rs3136814, rs3136817, and rs1130409.

Table 4 | Odds ratio (OR) and 95% confidence intervals (CIs) for each risk factor and haplotype associated with essential hypertension

Risk factor	OR	Total 95% CI	*P* value
G-T-T diplotype (heterozygote)	8.600	1.073–68.951	0.043[a]
Body mass index	1.165	1.009–1.236	<0.001[a]
Total cholesterol	1.004	0.998–1.009	0.176
Hyperlipidemia	3.929	1.564–9.870	0.004[a]
Diabetes	1.013	0.585–1.753	0.963

[a]Significant difference.

In the haplotype-based case–control study, 11 possible combinations of SNPs were predicted using these four SNPs. For these SNP combinations, Table 3 shows the combinations that give significant differences. The overall distribution was significantly different between the EH group and the control group ($\chi^2 = 15.72$, $P = 0.008$). After applying Bonferroni correction to the four SNPs, a P value of 0.008 was noted, thus indicating a significant difference ($P < 0.05/4$). The G-T-T haplotype constructed with the rs1760944-rs3136817-rs1130409 in the EH group had a significantly higher distribution than in the NT groups ($\chi^2 = 10.74$, $P = 0.001$). After applying Bonferroni correction using the eight haplotypes, a P value of 0.001 was noted, thus indicating a significant difference ($P < 0.05/8$).

Table 4 shows the results of logistic regression analysis. Logistic regression was performed using each of the risk factors and diplotypes associated with EH (Table 4). After adjustments for body mass index, total cholesterol, hypercholesterolemia, and diabetes, the frequency of the G-T-T diplotype (G-T-T heterozygote) was found to be significantly higher in EH patients than in NT subjects (OR = 8.600, 95% CI: 1.073–68.951, $P = 0.043$).

DISCUSSION

Although there have been reports showing that the *APE1/REF-1* gene is related to the pathophysiology of hypertension,[10,11] there have been no previous association studies regarding EH and *APE1/REF-1* gene. Thus, this is the first time a haplotype-based association study between the human *APE1/REF-1* gene and EH has been reported in the literature.

Among the SNPs used in the association study, rs1130409 (Asp148Glu (GAT→GAG)) is located in the amino-acid coding region in the human *APE1/REF-1* gene, is nonsynonymous, and has been studied previously.[16–18] Therefore, we selected rs1130409 for the present association study. Although there were no significant differences in the overall distribution of genotypic and allelic frequencies using all four SNPs between the EH and NT groups, the haplotype-based case–control study based on rs1760944-rsrs3136817-rs1130409 showed significant differences.

APE1/REF-1 is involved in the repair of DNA damage as well as in the reductive activation of transcriptional regulation of genes. Therefore, we investigated whether the endonuclease function, the reducing function, or both, are important in blood pressure. Jeon *et al.*[10] reported that expression of the redox-deficient mutant APE1/REF-1 (cysteine to alanine mutations at codons 65 and 93) did not result in increased e-NOS-catalyzed NO production, in contrast to wild-type APE1/REF-1. They also showed that the reducing function for APE1/REF-1 governed NO production, which is associated with hypertension. In their study, however, they did not experiment with APE1/REF-1 lacking only the DNA repair function. It is therefore unknown whether the endonuclease function is important for blood pressure. However, it has been reported that elevated levels of DNA repair enzyme are associated with human atherosclerosis.[24] Therefore, further studies are needed in order to clarify which factors are important for blood pressure control. Here, we performed a case–control association study using the rs1760944-rs3136817-rs1130409 haplotype (G-T-T haplotype), and we found that the G-T-T haplotype was significantly more frequent in the EH group than in the NT group (2.1% vs. 0.0%, $\chi^2 = 10.74$, $P = 0.001$), thus suggesting that this haplotype could be a genetic marker for EH, irrespective of whether the haplotype itself acts on the factors of EH: arterial pressure, NO production, and oxidative stress. It is possible that this mutation affects endonuclease activity, redox effector activity, or both in APE1/REF-1, which then affects vascular function.

Since the draft sequence of the human genome was completed in 2001, the methodologies and strategies for performing genetic research have changed markedly. SNPs are now used for the positional cloning of susceptibility genes after carrying out genome-wide scanning.[25] Haplotype analysis has changed particularly markedly. Recent studies have shown that the human genome has a haplotype block structure that can be divided into discrete blocks of limited haplotype diversity. In each block, a small fraction of SNPs, referred to as "tag SNPs," can be used to distinguish a large fraction of these haplotypes. These tag SNPs have the potential to be extremely useful for association studies, as they make it unnecessary to genotype all SNPs. Haplotype-based case–control studies are considered to be much more effective than marker-by-marker analyses.[26] In genes with multiple susceptibility alleles, particularly when the linkage disequilibrium between the polymorphisms is weak, a haplotype-based association study has advantages over an analysis based on individual polymorphisms.[27] In the present study, the haplotype-based case–control study was based on rs1760944-rs3136817-rs1130409, which exhibited a significant difference. Haplotype analysis has been used to successfully localize the susceptibility genes for some multifactorial diseases.[28,29] Based on such findings, we hypothesized that haplotype analysis would be useful in assessing the association between haplotypes and EH, and performed the present study, in which we attempted to use SNPs to establish the haplotypes of the EC-SOD gene. We found that the low-frequency haplotype in the EH group exhibited a significant difference in the haplotype-based case–control study. As several studies have indicated that this phenomenon may occur frequently, we believe that our results confirm the usefulness of case–control

studies in the examination of multifactorial diseases.[17,30] Although some case–control studies have identified gene variants associated with gender-specific susceptibility to EH,[31] we found no significant differences related to gender in the present study.

Although Bonferroni's correction is often used to cope with such a problem, it may yield too conservative conclusions because all of the tests are assumed to be independent.[32] For statistical analysis in a haplotype-based case–control study, we insist that Bonferroni correction should be basically applied to a number of SNPs because the selection of an SNP from many SNPs is independent, whereas a haplotype constructed with some alleles on the same chromosome is not thought to be independent in many cases. Therefore, we concluded that a number of haplotype combinations should not be applied to Bonferroni correction.[15]

In conclusion, the G-T-T haplotype may be a genetic marker for EH, and the human *APE1/REF-1* gene could be a susceptibility gene for EH in Japanese. Further studies need to be done in order to isolate the functional mutations in the human *APE1/REF-1* gene that modulate the process of atherosclerosis, and to evaluate the function of the APE1/REF-1 variants that are involved in the metabolism of the sex hormones.

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臨床研究

高血圧患者におけるロサルタンの尿酸排泄促進作用：24時間家庭蓄尿を用いた検討

宮田恵里　大田祐子　尾中宇蘭　丄橋卓也

国立病院機構九州医療センター 高血圧内科/臨床研究センター

はじめに

高尿酸血症は高血圧，肥満，脂質代謝異常，耐糖能異常などの生活習慣病と高率に合併することが知られている．高尿酸血症が危険因子の集積した状態と関連する指標なのか，それ自身が心血管病の危険因子であるのかについて多くの議論がなされてきたが，近年の大規模臨床試験の成績からは尿酸値自体が独立した心血管病の危険因子となることが報告されており[1)2)]，高尿酸血症を高率に合併する高血圧患者においても尿酸管理の重要性が強調されている．

「高血圧治療ガイドライン（JSH2009）」[3)]では尿酸に対して影響を与えない降圧薬としてCa拮抗薬やアンジオテンシン変換酵素（ACE）阻害薬，アンジオテンシンII受容体拮抗薬（ARB）を推奨している．ARBのなかでもロサルタンは尿酸排泄増加作用を有することが報告されており，ほかのARBと比較して高尿酸血症合併高血圧により適した薬剤であることが示唆されている．

そこで今回われわれはロサルタンの尿酸排泄促進作用に関して詳細に検討するため，高血圧外来患者を対象として，ロサルタンまたはそのほかのARBの投与前後の尿酸動態を24時間家庭蓄尿を用いて評価し比較検討をおこなった．

対象と方法

対象は当施設におけるARB未服用の高血圧患者73名（年齢61.3±10.7歳，男性29名，女性44名）で，ロサルタン（平均35 mg，n＝43）またはほかのARB（オルメサルタン：平均17 mg，n＝15，カンデサルタン：平均4.3 mg，n＝8，テルミサルタン：平均40 mg，n＝4，バルサルタン：平均53 mg，n＝3）を投与し，その前後（投与前：平均94日，投与後：平均175日）に24時間家庭蓄尿をおこなって尿酸クリアランス（CUA），尿酸クリアランス/クレアチニンクリアランス比（CUA/Ccr）を評価した．同時に血圧測定，血液検査をおこなってその変化を両群間で比較した．結果は平均±標準偏差で示し，統計にはpairedおよびunpaired-t検定また必要によりχ^2検定を用いた．

結　果

患者背景を**表1**に示す．ほかのARB群にくらべロサルタン群では女性の比率が有意に高率であったが，年齢，BMI，糖尿病の有病率などほかの背景要因に両群間で差異を認めなかった．

ARB投与前後の血圧，血液検査，24時間家庭蓄尿の変化を**表2**に示す．ロサルタン群では血圧は144±14/84±10 mmHgから137±10/80±9 mmHgへ，ほかのARB群では149±12/82±12 mmHgから133±11/74±11 mmHgへといずれも有意に低下した．ほかのARB群における降圧度はロサルタン群にくらべ有意に（$p<0.05$）大であった．血清尿酸値はロサルタン群で5.53±1.33から5.30±1.40 mg/dlと有意に（$p<0.05$）低下したのに対し，ほかのARB群では5.78±1.24から6.28±1.50 mg/dlと有意に（$p<0.01$）上昇し，ARB投与前後の血清尿酸値の変化は二群間で有意差を認めた（$p<0.01$）．尿酸クリアランス（CUA）はロサルタン群において6.4±2.7から7.0±3.1 ml/min（$p<0.05$）へ上昇したが，そのほかのARB群では5.9±1.7から5.4±1.8 ml/minと有意な変化を認めなかった．尿酸クリアランス/クレアチニンクリアランス比（UUA/UCr）は，

表 1. 患者背景

n	全体 73	ロサルタン 43	ほかの ARB 30
年齢	61.3±10.7	59.7±11.0	63.5±10.1
性別（男/女）	29/44	11/32	18/12**
BMI（kg/m²）	23.7±3.1	23.5±3.2	24.0±2.9
糖尿病（%）	21.9	23.3	20.0
ARB（新規/他薬からの変更）	49/24	27/16	22/8
併用降圧薬数	1.0±0.9	1.1±1.0	0.7±0.7
Ca 拮抗薬（%）	52.1	58.1	43.3
ACE 阻害薬（%）	17.8	20.9	13.3
β遮断薬（%）	16.4	20.9	10.0
α遮断薬（%）	5.5	7.0	3.3
利尿薬（%）	2.7	4.7	0
脂質異常症薬（%）	20.5	18.6	23.3
糖尿病薬（%）	5.5	7.0	6.7
尿酸産生阻害薬（%）	2.7	4.7	0

**p<0.01 vs ロサルタン

表 2. ARB 投与前後の各指標の変化

	ロサルタン		ほかの ARB	
	ARB 投与前	ARB 投与後	ARB 投与前	ARB 投与後
収縮期血圧（mmHg）	144±14	137±9.5*	149±12	133±11**
拡張期血圧（mmHg）	84±10	80±9.4	82±12	74±11**
総コレステロール（mg/dl）	211±28	210±32	223±36	205±30
中性脂肪（mg/dl）	131±81	139±93	194±218	153±96
HDL-コレステロール（mg/dl）	62±14	59±13**	53±14#	53±12
BUN（mg/dl）	16.2±4.6	16.7±4.3	14.9±3.0	15.6±4.7
クレアチニン（mg/dl）	0.75±0.21	0.74±0.21	0.74±0.15	0.80±0.21*
尿酸（mg/dl）	5.53±1.33	5.30±1.40*	5.78±1.24	6.28±1.50**
血糖（mg/dl）	110±34	108±27	116±39	107±25
尿中食塩（g/day）	9.2±3.2	9.4±3.5	8.7±3.0	9.3±3.7
尿中カリウム（g/day）	1.9±0.7	1.9±0.8	1.9±0.6	2.00±0.7
尿蛋白（g/day）	0.45±0.84	0.32±0.62	0.21±0.22	0.13±0.11
クレアチニンクリアランス（ml/min）	79.7±17.8	81.2±20.0	90.9±24.8#	82.5±21.2
尿酸クリアランス（CUA，ml/min）	6.4±2.7	7.0±3.1*	5.9±1.7	5.4±1.8
CUA/Ccr（%）	8.1±2.7	8.6±2.8	6.7±1.9#	6.8±2.0

#p<0.05 vs ロサルタン，*p<0.05，**p<0.01 vs 前値

ロサルタン群では 8.1±2.7 から 8.6±2.8%へと増加傾向を認めたが（p=0.13），そのほかの ARB 群では 6.7±1.9 から 6.8±2.0%と変化を認めなかった．血清尿酸値の変化は尿酸クリアランスの変化と r=−0.38 と有意（p<0.01）な負の相関を認めた（**図 1**）．とくにロサルタン服用者は尿酸クリアランスが増加し，血清尿酸値が低下する領域に多く分布していた．さらに血清尿酸値の低下を目的変数としてその規定要因について多変量回帰分析をおこなったところ，血清クレアチニン上昇（partial r=−0.57，p<0.01），ロサルタン投与（partial r=0.26，p<0.01）が，年齢，性別，BMI，血圧降下度とは独立した規定要因として検出された（**表 3**）．

考 察

高尿酸血症は高血圧に高率に合併することが知られている．自施設の高血圧患者において高尿酸血症の合併頻度を検討したところ，心血管病に対するリスク閾値と報告されている男性 7.5 mg/dl，女性 6.2 mg/dl を超える者はそれぞれ 23%，16%に認めた[4]．さらに尿酸産生量，尿酸クリアランスで規定される高尿酸血症の病型分類をおこなうと，尿酸排泄低下型が 92.1%と高率であった．この結果はリスクが重積する高血圧患者において尿酸管理は心血管病予防の観点からも重要であること，さらに治療薬としては尿酸排泄促進作用をもつ薬剤の選択が望ましいことが示唆される．

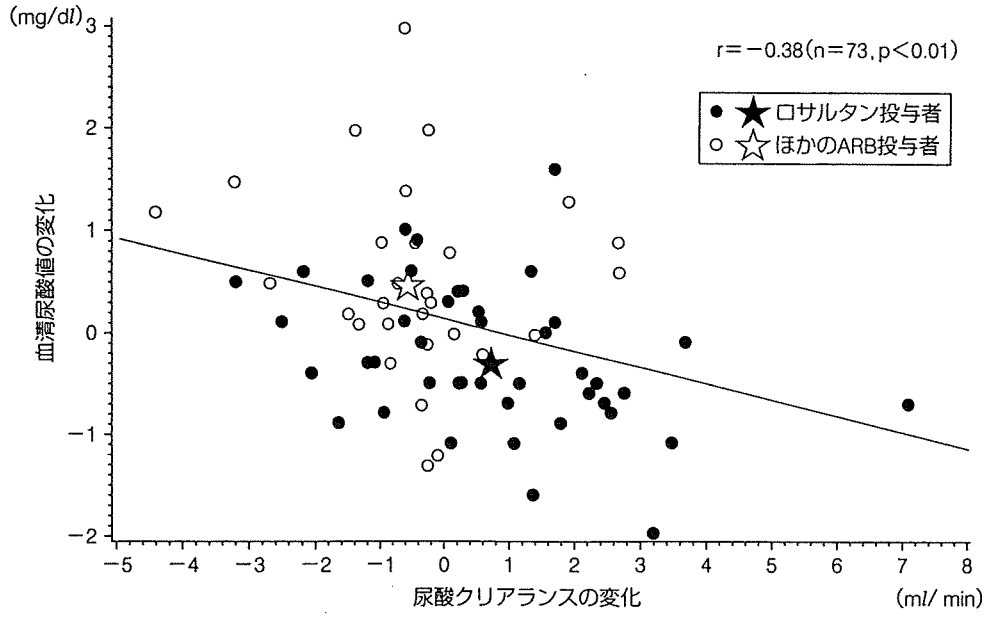

図 1. 尿酸クリアランスの変化と血清尿酸値の変化の関係

表 3. 血清尿酸値の低下に対する規定要因—多変量回帰分析—

variables	partial r	p
血清クレアチニンの上昇	−0.57	<0.01
ロサルタンの投与	0.26	<0.01
BMI	0.17	0.06
性別（女性）	0.15	0.09

独立変数：年齢，性別，BMI，血清クレアチニンの上昇，ロサルタン投与の有無，収縮期血圧の降圧度

現在，わが国において使用頻度の高い Ca 拮抗薬や ARB は尿酸に対して影響を与えず，とくにロサルタンは尿酸排泄増加作用があることから高尿酸血症合併高血圧には積極的使用が勧められている．ロサルタンによる尿酸排泄増加作用の機序については，腎近位尿細管に存在する尿酸トランスポーター（URAT1）の関与が報告されている[5]．URAT1 はその活性化により近位尿細管からの尿酸再吸収を促進することが明らかになっているが，ロサルタンは URAT1 活性を阻害して尿酸排泄を促進し，血清尿酸値低下作用を発揮すると考えられている．実際，ほかの ARB との比較研究において，ロサルタンが尿酸を有意に低下させたことが報告されている[6)~9)]．

今回のわれわれの成績は，ARB 投与前後の尿酸動態を 24 時間家庭蓄尿を用いて評価することにより，ロサルタンが有意な尿酸クリアランスの増加と血清尿酸値の低下をもたらすことを明らかにしたものである．本研究におけるほかの ARB 群の尿酸値上昇については，各 ARB のクラスエフェクトとして URAT1 の活性促進作用が報告されていること[10]，および有意な降圧による尿酸クリアランスの低下が要因と考えられるが，ARB の尿酸動態に対する影響については今後さらなる検討が必要である．

高血圧治療ガイドラインが提唱する厳格な血圧管理のためには降圧薬の併用療法，とくに利尿薬を積極的に利用することが推奨されているが，一方で，利尿薬投与による血清尿酸値などへの悪影響が懸念される．われわれは，ロサルタンが利尿薬使用による血清尿酸値上昇を軽度にとどめることを報告しており[11]，利尿薬と併用して用いる ARB としてロサルタンが尿酸管理の観点から有用性が高いと考えられる．

おわりに

ロサルタン投与により尿酸排泄の増加を伴った血清尿

酸値の有意な低下を認めた．高尿酸血症は心血管病の独立した危険因子であることが報告されており，ロサルタンは高尿酸血症合併高血圧患者の尿酸管理において有用性が高いことが示唆された．

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臨床研究

高血圧患者におけるロサルタンの尿酸排泄増加作用：他のARBからの変更例での検討

大田祐子　土橋卓也　宮田恵里

国立病院機構九州医療センター 高血圧内科/臨床研究センター

はじめに

高尿酸血症はほかの生活習慣病に合併することが多く[1]，高尿酸血症が独立した心血管病の危険因子であることが報告されていることから[2)~5)]，その管理が見直されている[6)]．降圧薬のなかでもアンジオテンシンⅡ受容体拮抗薬（ARB）のロサルタンは，ベンズブロマロンと同様に腎尿細管の尿酸トランスポーター（URAT1）阻害により，血清尿酸値を低下させると報告されている[7)8)]．そこで今回われわれは，ロサルタン以外のARB内服下の高尿酸血症合併高血圧患者の病型を評価し，ロサルタンへ変更後の尿酸病態を評価した．

対象と方法

対象はロサルタン以外のARB内服下の高尿酸血症合併高血圧外来患者28名｛平均年齢66±10歳，男性22名，女性6名，オルメサルタン（O群）17名，カンデサルタン（C群）7名，そのほか（T群）4名｝．血清尿酸値7 mg/d*l*以上もしくは尿酸降下薬服用者を高尿酸血症と定義し，試験期間中ARB以外の服薬は変更しなかった．随時尿中尿酸/クレアチニン（UUA/UCr）を用いて病型分類を評価し，ロサルタンへ変更後，尿酸動態の推移を評価した．結果は平均±標準偏差で示し，統計には対応のある*t*検定を用いた．患者に本研究についての詳細な説明をおこない同意を得た．

結　果

対象者の服用降圧薬はARB 100%，Ca拮抗薬75%，利尿薬18%，α遮断薬21%，β遮断薬36%であった（**表1**）．

表1．対象者の背景因子

症例数	28
男性/女性	22/6
年齢（歳）	66±10
降圧薬（%）	
ARB	100
Ca拮抗薬	75
利尿薬	18
α遮断薬	21
β遮断薬	36
高尿酸血症治療薬	16
ベンズブロマロン（平均37.5 mg）	12
アロプリノール（平均87.5 mg）	4

（平均±標準偏差）

また尿酸降下薬服用者は16人で，尿酸排泄促進薬服用者は12人，尿酸生成抑制薬服用者は4人であった．ロサルタン以外のARB服用時の全対象者の推定GFR（eGFR）は56±17 m*l*/min/1.73 m^2，血清尿酸値（UA）は6.9±1.3 mg/d*l*，UUA/UCrは0.39±0.16であった．ロサルタンへ変更後，平均1.7±1.2ヵ月の観察期間において，血圧は有意な変化を認めなかった（123±15/62±9 vs. 124±13/65±10 mmHg，n.s.）が，UAは有意な低下（6.9±1.3 vs. 6.0±1.1 mg/d*l*，$p<0.01$）を，UUA/UCrは有意な上昇を（0.39±0.16 vs. 0.56±0.30，$p<0.01$）認めた（**表2**）．尿酸動態の改善はeGFRによらず認められた．また，**図1**に示すようにO群とC群で尿酸動態の改善がみられたが，T群においては有意な変化がみられなかった（O群：UA；6.8±1.3 vs. 6.0±1.0 mg/d*l*，$p<0.01$，UUA/UCr；0.42±0.17 vs. 0.60±0.36，$p<0.05$，C群：UA；7.2±1.6 vs. 5.8±1.0 mg/d*l*，$p<0.01$，UUA/UCr；0.34±0.09 vs. 0.46±0.13，$p<0.05$，T群：UA；6.8±0.9 vs. 6.5±1.6 mg/d*l*，n.s.，UUA/UCr；0.37±0.19 vs. 0.57±0.20，n.s.），（**表3**）．また尿酸動態の改善は，尿酸排泄促進薬ベンズブロマロン投与の有無に関わらずみられた．

表 2. 他の ARB からロサルタンへの変更に伴う各指標の変化

	他の ARB	ロサルタン
収縮期血圧（mmHg）	123±15	124±13
拡張期血圧（mmHg）	62±9	65±10
血清尿素窒素（mg/dl）	20±8	19±8
血清クレアチニン（mg/dl）	1.1±0.3	1.0±0.3*
推定 GFR（ml/分/1.73 m²）	56±17	59±17
血清尿酸（mg/dl）	6.9±1.3	6.0±1.1**
尿中尿酸/クレアチニン比	0.39±0.16	0.56±0.30**
尿 pH	6.0±0.5	6.1±0.6

（平均±標準偏差），*p<0.05，**p<0.01 vs. 他の ARB

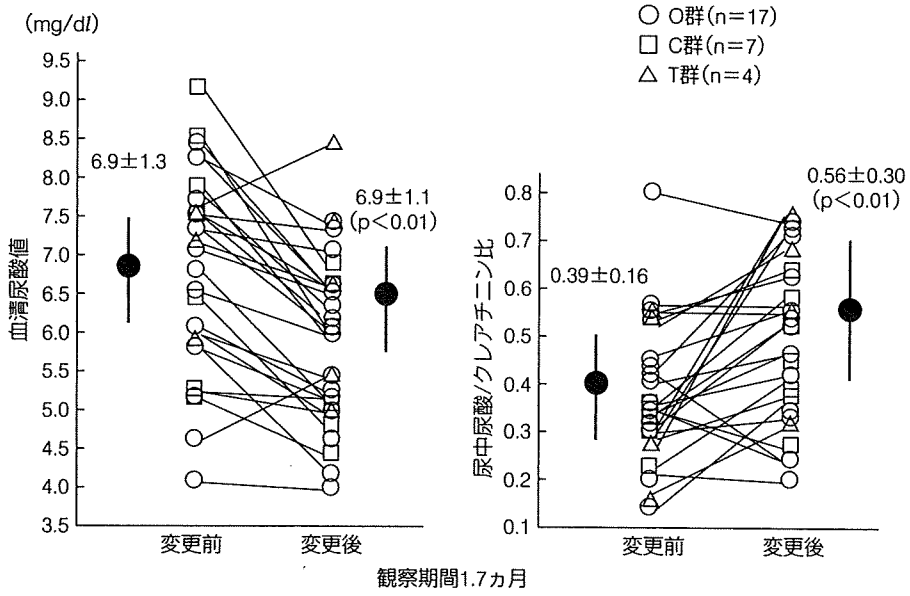

図 1. ロサルタン変更前後の ARB 別尿酸動態の変化
C 群：カンデサルタン
O 群：オルメサルタン
T 群：そのほかの ARB

表 3. ARB 別尿酸動態の変化（観察期間 1.7ヵ月）

ARB	尿酸 (mg/dl)		尿中尿酸/クレアチニン比	
	前	後	前	後
オルメサルタン（n=17）	6.8±1.3	6.0±1.0**	0.42±0.17	0.60±0.39*
カンデサルタン（n=7）	7.2±1.6	5.8±0.1**	0.34±0.1	0.46±0.13*
他の ARB（n=4）	6.8±0.6	6.5±1.6	0.37±0.19	0.57±2.0

（平均±標準偏差），*p<0.05，**p<0.01 vs. 前

考　察

高尿酸血症は肥満，高血圧，脂質異常症，耐糖能異常などの生活習慣病を合併することが多く[1]，われわれもメタボリック症候群合併高血圧患者で高頻度に高尿酸血症を合併することを報告している[9]．このように，高尿酸血症はマルチプルリスクファクター症候群を形成し，動脈硬化に深く関わると考えられ，最近，高尿酸血症が独立した心血管病の危険因子であることが報告されている[2)~5)]．

高尿酸血症はその機序により産生過剰型，排泄低下型，混合型に大別される．日本痛風・核酸代謝学会による高

尿酸血症・痛風の治療ガイドライン[6]にもとづいた高尿酸血症の病型分類は，尿酸産生量を尿中尿酸排泄量から，尿酸排泄能を尿酸クリアランスから求め判定する．しかしながら24時間尿での測定は必ずしも容易ではないため，簡便法として随時尿中の尿酸/クレアチニン比が有用とされている[10]．高血圧患者では尿酸排泄低下型高尿酸血症が主因であり，24時間蓄尿をおこなった当科通院中の高血圧患者における高尿酸血症の病型分類でも，産生過剰型は4.7%で，排泄低下型が92.1%と高値であった[11]．

尿酸はプリン体代謝の最終産物であり，その血中濃度は体内での産生と腎臓からの排泄によって決定される．降圧薬のなかでCa拮抗薬，ARB，ACE阻害薬，α遮断薬，カリウム保持性利尿薬は尿酸代謝に悪影響を及ぼさないと考えられている．とくにARBのロサルタンはベンズブロマロンと同様に腎尿細管に存在するURAT1を阻害して尿酸排泄を増加させ，血清尿酸値を平均0.7 mg/dl低下させることが報告されている[7][8]．本検討においてもロサルタン変更後，血清尿酸値は約0.9 mg/dl低下し，とくにカンデサルタン，オルメサルタンからの変更で尿酸動態が有意に改善した．カンデサルタン，オルメサルタン，バルサルタンではいずれもロサルタンと比較して相対的に血清尿酸値を増加させることが報告されている[12]~[15]．一方，そのほかのARB 4名のうち3名にテルミサルタンが投与されていたが，この群ではロサルタン変更後の尿酸動態に有意な変化がみられなかった．この理由として，症例数が少なかったこと，テルミサルタンがほかのARBにくらべ，尿酸を上昇させない可能性[7][16]が考えられる．高尿酸血症患者は，ほかの生活習慣病を合併している例が多く，心血管イベントの発症を予防するためにも尿酸値に配慮した降圧治療が望ましいと思われる．

なお本検討では症例数も少なく，ロサルタン変更平均1.7ヵ月後に判定しているため，今後も定期的な観察が必要であると思われる．

おわりに

高血圧患者において高尿酸血症の合併は多く，そのほとんどが尿酸排泄低下型と考えられるため，高尿酸血症合併高血圧には尿酸排泄促進作用のあるロサルタンを用いた治療が有用と思われる．

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REVIEW ARTICLE

Silent information regulator, Sirtuin 1, and age-related diseases

Li Zeng,[1] Rui Chen,[2,4] Fengxia Liang,[3,5] Hiroshi Tsuchiya,[1] Hiroshi Murai,[1] Takeshi Nakahashi,[1] Kunimitsu Iwai,[1] Takashi Takahashi,[2,6] Tsugiyasu Kanda[2] and Shigeto Morimoto[1]

[1]Geriatric Medicine, [2]General Medicine, and [3]Endocrinology, Kanazawa Medical University, Ishikawa, [6]Division of Internal Medicine, Tama-Hokubu Medical Center, Tokyo Metropolitan Health and Medical Treatment Corporation, Tokyo, Japan; and [4]Department of Traditional Chinese Medicine, Union Hospital Affiliated to Huazhong University of Science and Technology, [5]Department of Acupuncture and Moxibustion, Hubei College of Traditional Chinese Medicine, Wuhan, China

Sirtuin 1 (SIRT1), a member of the silent information regulator 2 in mammals, has recently been found to be involved in age-related diseases, such as cancer, metabolic diseases, cardiovascular disease, neurodegenerative diseases, osteoporosis and chronic obstructive pulmonary disease (COPD), mainly through deacetylation of substrates such as p53, forkhead box class O, peroxisome proliferator activated receptor γ co-activator 1α, and nuclear factor-κB. It is widely reported that SIRT1 can promote not only carcinogenesis but also metastasis and insulin resistance, andhave beneficial effects in metabolic diseases, mediate high-density lipoprotein synthesis and regulate endothelial nitric oxide to protect against cardiovascular disease, have a cardioprotective role in heart failure, protect against neurodegenerative pathological changes, promote osteoblast differentiation, and also play a pivotal role as an anti-inflammatory mediator in COPD. However, there are controversial results suggesting that SIRT1 has an effect in protecting against DNA damage and accumulation of mutations, and preventing tumorigenesis. In addition, a high level of SIRT1 can induce cardiomyopathy and even heart failure. This article reviews recent developments relating to these issues.

Keywords: age-related disease, forkhead box class O (FOXO), nuclear factor-κB (NF-κB), p53, Sirtuin 1 (SIRT1).

Introduction

Sirtuin 1 (SIRT1), the family member with the greatest homology to the silent information regulator 2 (Sir2), has recently been pinpointed as one good candidate to regulate the process of caloric restriction, a beneficial regimen for aged people to increase the resistance to oxidative and stress, inhibit fat storage, ameliorate neurodegeneration and so on, thereby mitigating disease processes in many tissues and extend lifespan.[1] Its gene encodes a nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylase (HDAC).[2] Several lines of evidence suggest that the SIRT1 protein plays a role in regulating different cellular processes through deacetylation of important substrates such as p53,

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Correspondence: Professor Shigeto Morimoto MD PhD, Department of Geriatric Medicine, Kanazawa Medical University, 1-1 Daigaku, Uchinada-machi, Kahoku-gun, Ishikawa 920-0293, Japan. Email: shigeto@kanazawa-med.ac.jp

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Table 1 Substrates of Sirtuin 1 and their main effects on age-related diseases

Substrate	Regulation type	Main effects	Reference
Ku70	+	DNA repair activity/suppression of apoptosis	3,4
hTERT	–	Suppression of cell growth	5
p53	–	Inhibition of p53-dependent apoptosis	6,7
FOXO-3	+/–	Stress resistance/reduction of apoptosis	8
NF-κB	–	Promotion of carcinogenesis etc.	9,10
FOXO-1	+/–	Metastasis/carcinogenesis	11,12
PGC-1α	+	Promotion of gluconeogenesis	13
IRS-2	+	Regulation of insulin signaling pathway	14
UCP-2	–	Enhanced insulin secretion by pancreas	15
LXR	+	Promotion of HDL synthesis	16
eNOS	+	Regulation of NO and vascular tone	17
PARP	–	Protection of cardiac myocytes in heart failure	18,19

+, Positive regulation; –, negative regulation. hTERT, human telomerase reverse transcriptase; FOXO, forkhead box class O transcription factor; NF-κB, nuclear factor-κB; PGC-1α, peroxisome proliferator activated receptor γ co-activator 1α; IRS-2, insulin receptor substrate 2; UCP-2, uncoupling protein-2; LXR, liver X receptors; HDL, high-density lipoprotein; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; PARP, poly(ADP-ribose) polymerase.

forkhead box class O (FOXO) transcription factors, peroxisome proliferator activated receptor (PPAR)γ co-activator 1α (PGC-1α), nuclear factor (NF)-κB and others, which are closely linked to some age-related diseases (Table 1). This paper reviews the published work dealing with issues surrounding these effects and the mechanisms of actions of SIRT1 in age-related diseases such as cancer, metabolic diseases, cardiovascular disease, neurodegenerative diseases, osteoporosis and chronic obstructive pulmonary disease (COPD).

Cancer

Cancer is an age-related disease caused mainly by age-related accumulation of gene mutations due to errors during DNA replication. In addition, tumor growth mainly depends on loss of control of differentiation and inhibition of apoptosis. Normal expression of SIRT1 has a protective effect against DNA damage, enhancing DNA repair capacity and guarding against accumulation of mutations, to prevent tumorigenesis.[20] However, overexpression of SIRT1, such as in human prostate cancer cells[21] and colon carcinoma,[22] which allows rapid proliferation and loss of checkpoints, promotes continued propagation of the progress of cancer. In order to clarify the effect of SIRT1 in cancer cells in detail, several aspects should be considered carefully.

Beneficial effects in carcinogenesis

Sirtuin 1 has a protective effect on the limited replicative lifespan, protecting against DNA damage, enhancing DNA repair capacity and guarding against accumulation of mutations and against genomic instability through its normal expression. Loss of SIRT1 expression, activity or regulation can bypass replicative senescence, allow cell division to proceed without the proper repair of DNA, and promote accumulation of mutations and genomic instability, leading to tumor development.[20]

Its protective effect against DNA damage is associated with Ku70, a heterodimer of a polypeptide of approximately 70 kDa that binds strongly to DNA double-strand breaks. Its homolog, yKu70, promotes genomic stability both by promoting accurate DNA repair and by serving as a barrier to error-prone repair processes.[23] Once DNA is damaged, SIRT1 physically complexes with Ku70, leading to subsequent deacetylation and DNA repair activity to prevent the formation of tumor cell.[3]

Human telomerase reverse transcriptase (hTERT) is a catalytic subunit of the mammalian telomerase. It is the first tumor antigen identified to have a global expression in more than 85% of human cancer cells and its continuing expression is necessary to the oncogenic process.[24] Narala *et al.*[5] found that there was a small increase in hTERT mRNA level and a significant increase in levels of hTERT protein in some cell lineages when SIRT1 was inhibited. Therefore, SIRT1 might act as a growth suppressor cooperating with hTERT. Nevertheless, it is still uncertain whether SIRT1 cooperates with hTERT to suppress the cell growth in tumor cells.

Hence, resveratrol, a plant polyphenol that stimulates SIRT1 activity, was purified and shown to have cancer chemopreventive activity in assays representing three major stages of carcinogenesis. It was found to act as an antioxidant, antimutagen and anti-initiation.[25] This was supported by the study of Bickenbach *et al.*[26] who found that resveratrol can activate the radio-

and chemo-inducible cancer gene therapy vector Ad.Egr.TNF, a replication-deficient adenovirus that expresses human tumor necrosis factor-α (TNF-α) under control of the Egr-1 promoter, which suggested its anti-neoplasia activity.

Adverse effects on tumor growth

In several human cancers, overexpression of SIRT1 has been found to stimulate rapid proliferation and promote continued propagation.[21,22] This adverse effect is mainly associated with the negative regulation of apoptosis-dependent factors and the promotion of neovascularization in tumors.

The most widely known substrate of SIRT1 is p53, a tumor suppressor with a critical role in cancer cell-cycle regulation and apoptosis that responds to various biological signals. The key role of p53 in regulating cell proliferation and stress response is highlighted by inactivation or loss-of-function of p53 in over 50% of human tumors.[27] It regulates expression of genes, including p21, p53-upregulated modulator of apoptosis and Bax, to initiate cell-cycle arrest, senescence or apoptosis. SIRT1 binds to and deacetylates p53 on lysine 382 (K379 in mouse p53), thereby negatively regulating p53-mediated transcriptional activation, repressing p53-dependent apoptosis and promoting tumor growth.[6,7]

Forkhead box class O transcription factors, including FOXO-1, FOXO-3a, FOXO-4 and FOXO-6, respond to DNA damage and oxidative stress and regulate expression of cell-cycle, DNA repair and apoptosis genes.[28] FOXO proteins can regulate cell fate by modulating the expression of genes involved in apoptosis, cell-cycle transitions, DNA repair, and oxidative stress, as well as cell differentiation in cancer, after regulation by phosphorylation and acetylation.[29] During the process of tumor growth, SIRT1 appears to shift FOXO-induced responses away from apoptosis towards cell survival by deacetylation.[8]

Nuclear factor-κB is a dimeric transcription factor that regulates the expression of numerous genes controlling immune and inflammatory responses, cell proliferation, differentiation and apoptosis. Recent *in vivo* data have indicated that inhibition of NF-κB in hepatocytes may actually promote hepatocarcinogenesis.[30,31] In addition, SIRT1 can inhibit NF-κB-mediated transcription through interacting with transducin-like enhancer of split-1.[9] Moreover, gankyrin is an oncoprotein commonly overexpressed in some human carcinomas. It directly binds to RelA (p65), one of the NF-κB subunits. In human uterine cancer HeLa and embryonic kidney 293 cells, overexpression of gankyrin suppresses the basal as well as TNF-α-induced transcriptional activity of NF-κB, whereas downregulation of gankyrin can increase it. Importantly, the inhibitory effect of gankyrin is abrogated by nicotinamide as well as downregulation of SIRT1.[10]

E2F1 also has important roles in regulating cell proliferation and apoptosis in neoplasia, mainly by stimulating the transcription of several genes in the apoptotic pathway.[32] Similarly to p53, E2F1 is stabilized and activated by DNA damage.[33] Once DNA is damaged, E2F1 is overexpressed to induce premature S-phase entry and often results in apoptosis.[34] SIRT1 is a direct transcriptional target of E2F1 and its expression protects cells from death by regulating E2F1.[35]

Though Ku 70 can promote genomic stability and prevent the change from normal cells to cancer cells, Ku70 can also suppress Bax-mediated apoptosis. The acetylation level of Ku70 is regulated by the result of a dynamic equilibrium between the activity of acetyltransferases and the opposing deacetylases. According to the report of Cohen *et al.*,[4] treatments by increasing Ku70 acetylation, either by treating cells with SIRT1 inhibitor nicotinamide or by overexpressing acetyltransferases CBP or PCAF, are capable of abrogating the ability of endogenous Ku70 to suppress Bax-mediated apoptosis. Hence, SIRT1 might increase the anti-apoptotic ability of endogenous Ku70 and play as harmful role in the treatment of tumor.

Neovascularization is one of the important characteristics of neoplasia. SIRT1 is highly expressed in the vasculature during blood vessel growth in tumors, where it promotes the angiogenic activity of endothelial cells. Loss of SIRT1 function blocks sprouting angiogenesis and branching morphogenesis of endothelial cells, with consequent downregulation of genes involved in blood vessel development and vascular remodeling. Disruption of SIRT1 gene expression in zebrafish and mice results in defective blood vessel formation and blunts ischemia-induced neovascularization.[36]

Harmful effects in metastasis

Sirtuin 1 plays a very important role in metastasis, mainly by activating FOXO-1 to induce transcription of vascular endothelial growth factor-C (VEGF-C). VEGF-C has been identified as being involved in lymph node metastasis of several cancers including colorectal cancer, human pancreatic endocrine tumors, esophageal carcinoma, head and neck squamous cell carcinoma, uterine cervical cancer, primary non-small-cell lung cancer, gastric carcinoma, and laryngeal squamous carcinoma. FOXO-1 is a potential transcription factor for VEGF-C and is activated by SIRT1.[11]

Now that SIRT1 has been shown to have such adverse effects on tumor growth and metastasis, SIRT1-specific inhibitors may be useful chemotherapeutic agents for some SIRT1-dependent tumors. Cambinol, a SIRT1 inhibitor, inactivates the critical

oncogene B-cell lymphoma 6 protein (BCL6) in Burkitt's lymphoma cells by promoting its acetylation, and leads to induction of apoptosis. In mouse xenograft models, cambinol alone was effective specifically against tumors expressing BCL6. Interestingly, inhibition of SIRT1 by cambinol sensitizes cells to DNA-damage-induced apoptosis independently of p53.[37] BML-210, another specific SIRT1 inhibitor, can abrogate FOXO-1-dependent VEGF-C transcription, which is beneficial for inhibiting metastasis.[11] Nevertheless, SIRT1-specific inhibitors have not yet been used in the clinical treatment of carcinoma, perhaps mainly because of SIRT1's protective effect against DNA damage and gene mutation.

Metabolic diseases

In low and middle income countries, the majority of people with diabetes are in the age range of 45–64 years, according to World Health Organization reports. Other metabolic diseases are mostly associated with age-related changes of body function as well, for instance, the imbalance of food intake and energy expenditure, which results in abdominal fat accumulation, causing insulin resistance. It is known that the SIRT1 protein level is increased after fasting and returns to nearly the control level upon re-feeding.[13] Resveratrol can protect mice against diet-induced obesity.[38] All these findings indicate that SIRT1 may be a regulator of energy and metabolic homeostasis, and may even regulate some key points in age-related metabolic diseases, such as insulin resistance.

To define the role of SIRT1, Milne *et al.*[39] identified and characterized novel small molecule activators of SIRT1 both *in vitro* and *in vivo*. These SIRT1 activators ablated insulin resistance and diabetes in diet-induced obese mice fed a high-fat diet and in diabetic $Lep^{ob/ob}$ mice. In addition, these new SIRT1 activators ameliorated the metabolic disturbances in Zucker *fa/fa* rats. Moreover, SIRT1 activators improved glucose homeostasis and insulin sensitivity in key metabolic tissues including liver, muscle and fat.

The mechanisms of SIRT1 in ameliorating insulin resistance and improving glucose and lipid homeostasis are mainly associated with PGC-1α, PPARγ and FOXO-1. PGC-1α is a key regulator of glucose production in the liver through activation of the entire gluconeogenic pathway.[40–42] SIRT1 induces gluconeogenic genes and hepatic glucose output through PGC-1α. In addition, SIRT1 modulates the effects of PGC-1α repression of glycolytic genes in response to fasting. Thus, SIRT1 acts as a modulator of PGC-1α in regulating glucose homeostasis.[13] Moreover, SIRT1 and PPARγ bind to the same DNA sequences and SIRT1 is a co-repressor of PPARγ. Adipogenesis in a cell model, 3T3-L1, with lipid accumulation is promoted by the nuclear receptor PPARγ. SIRT1 acts a negative modulator of adipogenesis in this cell model by docking with the PPARγ cofactor, nuclear receptor co-repressor (NcoR).[43] FOXO-1 is not only involved in insulin's inhibition of hepatic glucose production and stimulation of β-cell proliferation in insulin-resistant mice,[44] but also plays an important role in coupling insulin signaling to adipocyte differentiation.[45] The regulation of adipocyte differentiation by FOXO-1 can potentially affect insulin sensitivity by regulating adipocyte size.[45] Furthermore, FOXO-1 and PGC-1α interact in insulin-regulated gluconeogenesis.[41] SIRT1 binds FOXO-1, decreases its acetylation and inhibits its transcriptional activity,[12] which might be another pathway for intervention in metabolic diseases.

As a critical component of overall energy homeostasis, the insulin signaling pathway has been well studied and the key steps have been characterized.[46] The insulin signaling pathway is initiated by auto-tyrosine phosphorylation of the insulin receptor upon insulin binding, and subsequently tyrosine phosphorylation of several key adaptor proteins including insulin receptor substrate 1 (IRS-1) and IRS-2. The phosphorylated IRS proteins further transmit insulin signaling to downstream events, mainly through two kinase cascades, the mitogen-activated protein kinase cascade and the phosphatidylinositol 3-kinase-Akt cascade. SIRT1 protein may, through regulation of the acetylation level of IRS-2 protein, directly regulate insulin-induced IRS-2 tyrosine phosphorylation and its downstream Akt activation.[14]

Insulin secretion by pancreatic β cell plays a very important role in the pathophysiology of type 2 diabetes. An age-associated impairment of β-cell function has also been demonstrated in rodents.[47] Increasing SIRT1 dosage or activity in pancreatic β cells can provide life-long beneficial effects of enhanced β-cell function on glucose homeostasis in the process of aging. One of the mechanisms is that SIRT1 mediates the repression of uncoupling protein (UCP)2 expression and thereby increases adenosine triphosphate (ATP) content, resulting in the enhancement of glucose-stimulated insulin secretion.[15]

Additionally, adiponectin is secreted by adipose tissue in response to metabolic effectors in order to sensitize the liver and muscle to insulin. Reduced circulating levels of adiponectin that usually accompany obesity contribute to the associated insulin resistance.[48] Adiponectin secretion is regulated by SIRT1. A lower level of SIRT1 increases adiponectin transcription by activating FOXO-1 and enhancing FOXO-1 and CCAAT/enhancer binding protein (C/EBP)α interaction in adipocytes in patients with obesity or type 2 diabetes.[49] However, in the study of Qiang *et al.*, the secretion of high molecular weight adiponectin was decreased by the treatment of SIRT1 activator resveratrol, whereas it was enhanced by SIRT1 inhibitor nicotinamide.[50]

Heart disease

Cardiovascular disease

Sirtuin 1 has protective effects against cardiovascular disease. Genetic analysis of SIRT1 haplotypes revealed a tendency for decreased cardiovascular mortality in haplotype 2 carriers.[51] This observation is in accordance with other genetic and clinical studies. It is well known that plasma high-density lipoprotein (HDL) level is inversely associated with risk of cardiovascular events, as HDL-mediated reverse cholesterol transport can protect against atherosclerosis by clearing excess cholesterol from arterial cells.[52,53] SIRT1 activates transcription of the liver X receptors (LXR) target gene encoding the ATP-binding cassette transporter A1, which mediates HDL synthesis, reverses cholesterol transport and decreases the risk of atherosclerosis and cardiovascular events.[16]

Moreover, SIRT1 plays a fundamental role in regulating endothelial nitric oxide (NO) and endothelium-dependent vascular tone by deacetylating endothelial nitric oxide synthase (eNOS), which is closely associated with blood pressure. SIRT1 and eNOS co-localize and co-precipitate in endothelial cells, and SIRT1 deacetylates eNOS, stimulating eNOS activity and increasing endothelial NO. Inhibition of SIRT1 in the endothelium of arteries inhibits endothelium-dependent vasodilation and decreases bioavailable NO.[54] On the contrary, resveratrol, a SIRT1 activator, can activate eNOS,[17] improve endothelial function, prevent elevation of blood pressure and restore vascular eNOS activity in animal models of endothelial dysfunction.[55]

Heart failure

Increasing lines of evidence suggest that the balance between growth and death of cardiac myocytes plays an important role in determining long-term cardiac function in heart failure patients.[56] SIRT1 plays a very important role in cardiac development and the growth of myocardial cells to maintain cardiac function. In SIRT1-null embryos, developmental defects in the heart have been observed in a previous study,[57] while in wild-type embryos, the expression pattern of SIRT1 protein and mRNA is high during cardiogenesis in embryogenesis.[58] Moreover, in isolated neonatal cardiomyocytes, inhibitors of SIRT1 activity cause a moderate increase in basal cell death and upregulation of the expression of the hypertrophy-associated gene, atrial natriuretic factor, even though cell size actually decreases. Likewise, an increased SIRT1 level protects myocytes from serum starvation-associated cell death, while also increasing overall cell size.[59]

In addition, overexpression of SIRT1 in the heart of dogs with heart failure protected cardiac myocytes from apoptosis in response to serum starvation and significantly increased the size of cardiac myocytes, which suggest that endogenous SIRT1 plays an essential role in mediating cell survival and maintaining modest hypertrophy.[60] Hence, an increase in SIRT1 expression may have a cardioprotective role in pathological hearts.

Pillai *et al.*[18] reported more evidence for the protective effect of SIRT1 against heart failure. Poly(ADP-ribose) polymerase-1 (PARP) is a multifunctional DNA-bound enzyme located in the nuclei of various cells, including cardiac myocytes. Robust activation of PARP by oxidative stress and other factors has been demonstrated to be a major cause of myocyte cell death contributing to heart failure. In both cultured cardiac myocytes and failing hearts, increased activity of PARP was associated with reduced SIRT1 deacetylase activity, and myocyte cell death induced by PARP activation was prevented only when SIRT1 was intact, which indicates that SIRT1 is a beneficial factor leading to cardiac myocyte protection in heart failure.[19]

Nevertheless, the opposite result was reported, that a high level of SIRT1 can induce cardiomyopathy and even heart failure, possibly through induction of mitochondrial dysfunction in the heart *in vivo*.[61] The mechanism may be linked to NAD^+ consumption. A high level of SIRT1 causes depletion of NAD^+ which is required for mitochondrial respiration. Depletion of NAD^+ could lead to deficiency of ATP and, consequently, myocardial cellular dysfunction and eventual cardiac cell death. Hence, although stimulation of SIRT1 may be considered as anti-aging therapy for the heart, careful evaluation regarding the dosage seems essential to best use the therapeutic potential of SIRT1.

Neurodegenerative diseases

Neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease, Huntington's disease and amyotrophic lateral sclerosis are increasingly prevalent in aging societies because of a progressive loss of neurons with age. Recent studies demonstrated that activation of SIRT1 could attenuate neuronal degeneration and death in animal models of neurodegenerative disease and exert a neuroprotective effect.

According to the study of Kim *et al.*,[62] SIRT1 is not only enriched in the nucleus but also localized in the cytoplasm in AD patients and mouse models, acting as a protective response to neurodegenerative conditions. Its activating molecule, resveratrol, can slow *in vitro* neuron death as well as *in vivo* neurodegeneration. In a mouse model of Parkinson's disease, SIRT1 also protects against neurodegenerative pathological changes.[63] The mechanisms of the neuroprotection by SIRT1 may be associated with its downregulation of the pro-apoptotic factors, p53,[7,64] FOXO[8,65] and NF-κB,[66] and the deacetylation and activation of PGC-1α.[62]

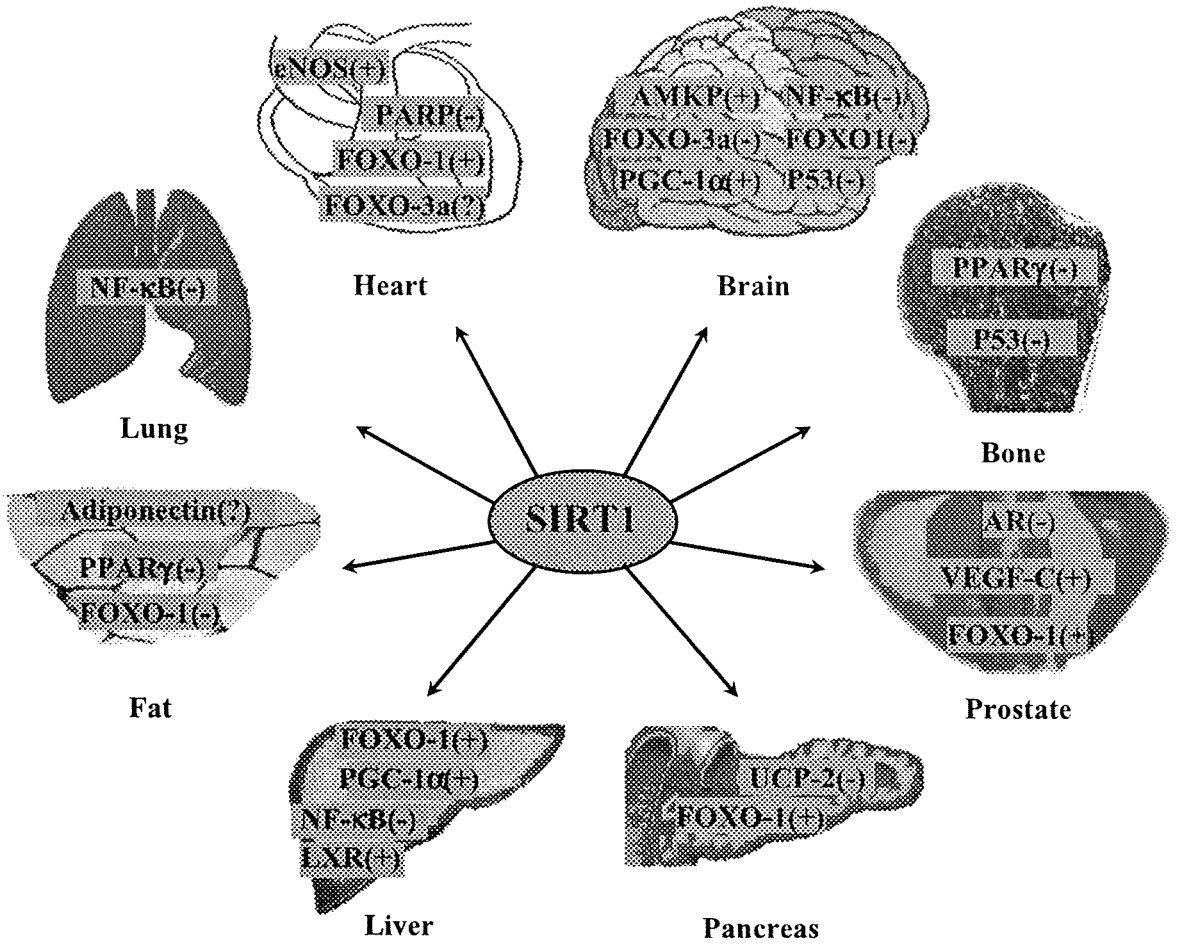

Figure 1 Different substrates of Sirtuin 1 (SIRT1) in different target organs. SIRT1 plays an important role in age-related diseases, such as cancer, metabolic diseases, cardiovascular disease and neurodegenerative diseases, mainly through deacetylation of substrates such as p53, FOXO, PGC-1α and NF-κB. AMPK, adenosine monophosphate-activated protein kinase; AR, androgen receptor; eNOS, endothelial nitric oxide synthase; FOXO, forkhead box class O transcription factor; LXR, liver X receptors; NF-κB, nuclear factor κB; PARP, poly (ADP-ribose) polymerase-1; PPARγ, peroxisome proliferator activated receptor γ; PGC-1α, PPARγ co-activator 1α; UCP-2, uncoupling protein-2; VEGF-C, vascular endothelial growth factor-C. +, Positively regulated by SIRT1; –, negatively regulated by SIRT1; ?, uncertain regulation type by SIRT1.

Furthermore, SIRT1 may be involved in the axonal protection observed in the wallerian strain of mice, which have a translocation that increases the level of the NAD biosynthetic enzyme nicotinamide mononucleotide adenylyl-transferase 1 and renders peripheral axons more stable after a neuronal insult. One study showed that the effects of NAD and the wallerian strain are dependent on SIRT1, leading to the conclusion that SIRT1 is a neuroprotective factor.[67]

There have been many studies focusing on mitochondrial dysfunction in the etiology and pathogenesis of neurodegenerative diseases.[68–70] SIRT1 acts as a functional regulator of PGC-1α that induces a metabolic gene transcription program of mitochondrial fatty acid oxidation and promotes mitochondrial function in skeletal muscle.[71] Its activator, resveratrol, can also improve mitochondrial function through the SIRT1/PGC-1α pathway in muscle.[38] However, the modulation of the SIRT1/PGC-1 pathway in the central nervous system is not well documented, and requires further study.

Osteoporosis

Osteoporosis is widespread in elderly people, and age-related deficiency of osteoblast differentiation is one well-known pathogenetic mechanism. Inhibiting

adipocyte formation and promoting osteoblast differentiation to enhance bone formation is a promising therapy for osteoporosis.[72] PPARγ is an important regulator of adipocyte differentiation. p53 is one of the key factors in osteoblast differentiation. Both of their activities are regulated by SIRT1. Activation of SIRT1 in mesenchymal stem cells can decrease adipocyte and increase osteoblast differentiation.[73]

COPD

Chronic obstructive pulmonary disease is also a major cause of disability, morbidity and mortality in elderly patients. It is characterized by progressive and largely irreversible airflow limitation, which is associated with an abnormal inflammatory response in the lung.[74] Increased NF-κB activation and acetylation of histone proteins have been identified as important inducers of local secretion of pro-inflammatory cytokines. SIRT1 is an important protein involved in deacetylation of histone proteins and negatively regulates NF-κB activation to decrease pro-inflammatory cytokine release.[75]

Conclusions

The core role of SIRT1 in age-related diseases is associated with different substrates in different organs (Fig. 1), which regulate carcinogenesis, metastasis, metabolic homeostasis, anti-inflammatory effects, vascular tone, cardiac function, neurodegeneration and others. Sometimes, SIRT1 has dual effects on its substrate, such as FOXO-3[8] and FOXO-1,[11,12] and induces conflicting promotion. Additional insights into the biological actions of SIRT1 are required to identify the precise roles of different members of its substrate family in different age-related diseases, especially *in vivo*. Then, its inhibitors or activators can be safely used for the treatment of age-related diseases.

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