question on polypharmacy. Number of comorbidities was significantly associated with age ($r = 0.32$, $P < 0.0001$) and with the number of drugs ($r = 0.62$, $P < 0.0001$).

Next, on multivariate analyses, the questionnaire asking "whether taking five or more drugs" were excluded from the fall risk index and the simple screening test. Therefore, the fall risk index was analyzed by a total of 21 items and the simple screening test by a total of 11 points in this analysis. To evaluate the association of four fall risk indices with comorbidities and drugs, all the variables that were significantly associated in either one of four univariate analyses were entered into the model. As shown in Table 3, the number of drugs was the only factor which was significantly associated with all four indices, independent of age, sex and other variables. Because each disease variable or drug variable might have affected the number of comorbidities or the number of drugs in this analysis, we just compared the number of comorbidities and the number of drugs to exclude the double count in next analysis. As shown in Table 4, the number of drugs was significantly associated with all of the four fall risk indices independent of age, sex and the number of comorbidities, while the number of comorbidies was inversely associated with history of falls and simple screening test. As shown in Figure 1, the association of the number of drugs with

Table 3 Multivariate analysis of association between risk factor variables and four fall indices: history of falls in a year, fall risk index, simple screening test, one leg standing test

		History of fall in a year (No = 0/Yes = 1) Odds ratio (95% CI)	Fall risk index (21 items)† β	Simple screening test (11 points)† β	One-leg standing test (s) β
Age		1.00 (0.96–1.05)	0.073	0.127	−0.370***
Female	(No = 0/Yes = 1)	2.36 (1.12–5.00)*	0.199**	0.197**	−0.149*
Hypertension	(No = 0/Yes = 1)	1.87 (0.61–5.76)	0.166	0.218*	−0.110
Osteoporosis	(No = 0/Yes = 1)	0.67 (0.28–1.60)	0.093	0.027	0.023
History of stroke	(No = 0/Yes = 1)	1.43 (0.38–5.45)	0.080	0.032	−0.083
Number of comorbities		0.60 (0.38–0.95)*	−0.062	−0.237*	−0.024
Antihypertensives					
	(No = 0/Yes = 1)	0.52 (0.18–1.54)	−0.141	−0.158	0.142
Aspirin	(No = 0/Yes = 1)	1.59 (0.72–3.50)	0.053	0.046	0.002
Bisphosphonates	(No = 0/Yes = 1)	2.27 (0.73–7.07)	0.055	0.105	0.033
Hypnotics	(No = 0/Yes = 1)	0.84 (0.33–2.15)	0.094	−0.018	0.084
Number of drugs		1.24 (1.07–1.45)*	0.247**	0.335***	−0.250**

*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$. Logistic regression analysis was performed for history of fall in a year, and multiple regression analysis for the remaining three. The risk factor variables used in these multivariate analyses were those associated in either of the four univariate analysis significantly. †The questionnaire asking "whether taking five or more drugs" were excluded from the scores in this analysis. Therefore, fall risk index were analyzed by a total of 21 items and simple screening test by a total of 11 points. CI, confidence interval; β, standardized regression coefficient.
[Table 3 amended after online publication date September 27, 2011]

Table 4 Multivariate analysis of association between number of comorbidities and drugs with four fall indices: history of falls in a year, fall risk index, simple screening test, one-leg standing test

	History of fall in a year (No = 0/Yes = 1) Odds ratio (95% CI)	Fall-risk index (21 items)† β	Simple screening test (11 points)† β	One-leg standing test (s) β
Age	1.00 (0.96–1.05)	0.101	0.115	−0.376***
Female (No = 0/Yes = 1)	1.73 (0.90–3.34)	0.207**	0.191**	−0.110
Number of comorbidities	0.63 (0.45–0.89)*	0.073	−0.137	−0.034
Number of drugs	1.23 (1.08–1.41)*	0.223*	0.316***	−0.233**

*$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$. Logistic regression analysis was performed for history of fall in a year, and multiple regression analysis for the remaining three. †The questionnaire asking "whether taking five or more drugs" were excluded from the scores in this analysis. Therefore, fall risk index was analyzed by a total of 21 items and simple screening test by a total of 11 points. CI, confidence interval; β, standardized regression coefficient.

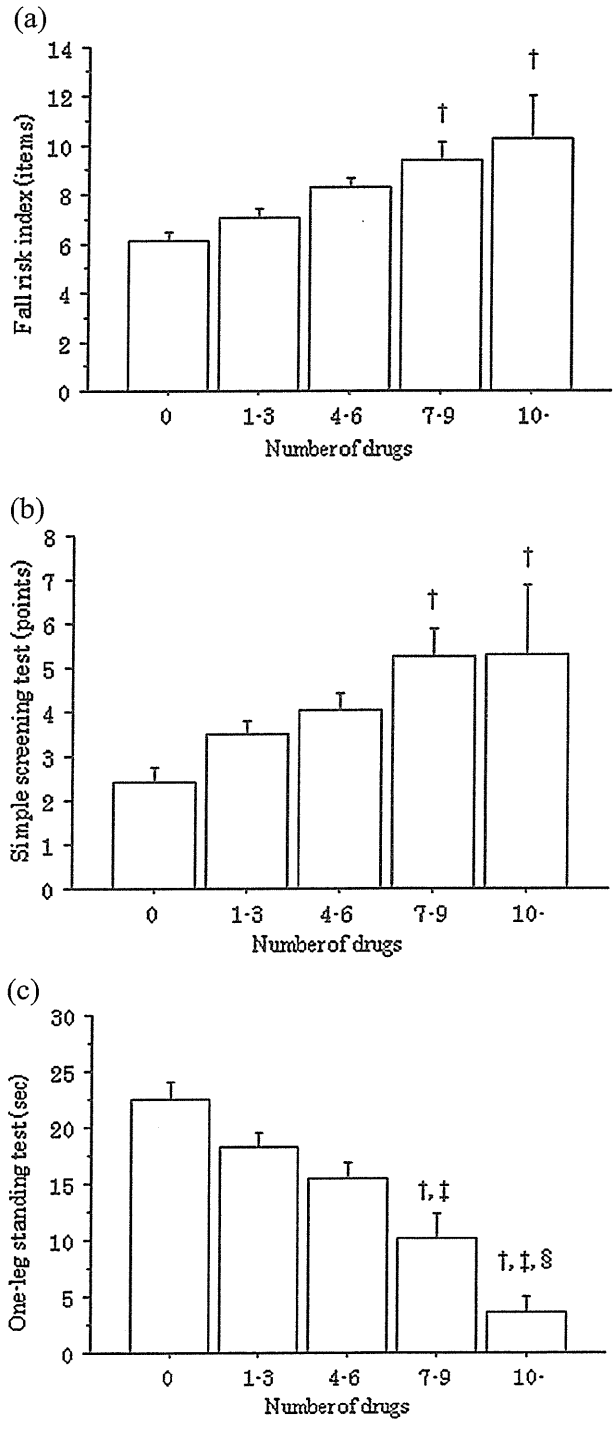

Figure 1 Averages of fall risk according to the number of drugs. (a) Fall risk index excluding the questionnaire concerning polypharmacy. (b) Simple screening test excluding the questionnaire concerning polypharmacy. (c) Duration time of one-leg standing test. The differences between the number of the drugs were compared through ANOVA, $P < 0.0001$ for (a), $P < 0.005$ for (b), $P < 0.0001$ for (c). For post-hoc analysis, $^{\dagger}P < 0.05$ vs 0 drug; $^{\ddagger}P < 0.05$ vs 1–3 drugs; $^{\S}P < 0.05$ vs 4–6 drugs. Values are expressed as mean ± standard error.

fall predicting score, simple screening test and duration time of one-leg standing test was stepwise.

Discussion

Epidemiological studies have assessed the risk of falls in community-dwelling people, but not in geriatric outpatients, who are likely to fall and need special consideration for the treatment of their illness. This cross-sectional study investigated the association between comorbidities, medications and fall risks in Japanese elderly outpatients and found that all four indices were significantly associated with the number of drugs. Because polypharmacy is frequently seen in patients with multiple comorbidities, this study compared the impact of the number of drugs with that of the number of comorbidities on fall risk, and found the significance of polypharmacy as fall risk in elderly outpatients.

In the present study, the number of comorbidities was inversely associated with the history of fall in the past year and with an 11-point simple screening test in the multivariate analysis. The reason is unclear; however, there are some speculations about this. None of the patients with four or more comorbidities ($n = 19$, 79.4 ± 5.2 years old) had history of fall in the past year. This accounts for the lower points of the simple screening test in these patients, because the history of fall consists of 5 points out of a total of 11 points in the simple screening test. So the question is why they had lower frequency of falling experiences, although they are at higher risk of falls according to fall risk index and one-leg standing test (9.6 ± 3.8 items and 8.6 ± 9.4 s, respectively). These patients may take care not to fall in their daily lives because of their consciousness of fall risk or frailty, or maybe due to elevated vigilance of caregivers and their constant physical assistances. They might have simply forgotten their fall experiences due to subclinical cognitive impairment, although demented patients were not included in this study. It is also possible that the patients who had more comorbidities and had fallen did not meet our inclusion criteria because of their recent injurious falls or their severe conditions.

Several medications and comorbidities have been reported as risks of fall.[6,7,13–19] Among these, diabetes,[9,10] imsonia,[13] hypnotics[13–15] and antihypertensive use[8] were not significantly associated with fall risk in our study. Only 20 patients (40.8% of diabetic patients) were prescribed hypoglycemic agents such as sulfonylurea ($n = 17$) or insulin ($n = 3$) in this study. Because hypoglycemia is considered to be the main cause of accidental falls in diabetic patients, relatively less prescription of hypoglycemic agents might have affected our result. The patients who were prescribed hypnotics tended to be at higher risk of falls in univariate analysis, which did show statistical significance. Also, antihypertensives such as diuretics are reported to increase the fall risk.[8] No

association between these drugs and fall risk in our study might be due to the small sample size. Other drugs such as major tranquillizers,[14] antidepressants[17,18] and antiparkinsonians[19] might increase fall risk; however, very few patients used these drugs in this study.

There are some other limitations. First, the causal relationship of the associations observed in this study is unknown because of the cross-sectional design. Polypharmacy has been regarded as a risk in several aspects in elderly patients. Previous studies have shown that adverse drug events were seen more frequently in the polypharmacy patients during their stay in the geriatric inpatient ward,[20] and polypharmacy was one of the important predictors for postdischarge mortality in elderly patients after emergent hospitalization.[21] Because patients with multiple diseases and in severer conditions are likely to take more medications, we used the number of comorbidities in analysis as fall risk variables. However, it is still unclear whether polypharmacy is a risk of falls independent of severity of each comorbidity. Interventional studies to reduce the number of drugs are needed to clarify the causal relationship between polypharmacy and fall risk. Second, this study did not evaluate the fall itself. The validity of four indices used in this study is well established as fall risk markers. However, prospective studies which evaluate the incidence of fall should be carried out in the future. Third, although the included subjects were receiving the same prescriptions for more than 1 month, the exact duration of each drug use or polypharmacy was not assessed in this study. Consequently, the long-term adverse effects over months or years seen in elderly patients should be more precisely investigated.

In summary, this study demonstrated that geriatric outpatients with polypharmacy were at higher risk of falls, consistent with the previous studies conducted in community-dwelling elderly. Our finding may add new information on pharmacotherapy in elderly patients with chronic diseases. Prospective studies and intervention studies examining the effect of drug reduction are needed in the future.

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Appendix I. 22 items of fall-predicting score (questionnaire)

Q1. Have you fallen during the last 12 months?	Yes, 1; No, 0.
Q2. Have you tripped during the last 12 months?	Yes, 1; No, 0.
Q3. Can you climb stairs without help?	Yes, 0; No, 1.
Q4. Do you feel your walking speed has declined recently?	Yes, 1; No, 0.
Q5. Can you cross a road within the green signal interval?	Yes, 0; No, 1.
Q6. Can you walk 1 km without stopping?	Yes, 0; No, 1.
Q7. Can you stand on one foot for about five seconds?	Yes, 0; No, 1.
Q8. Do you use a stick when you walk?	Yes, 1; No, 0.
Q9. Can you squeeze a towel tightly?	Yes, 0; No, 1.
Q10. Do you feel dizzy at times?	Yes, 1; No, 0.
Q11. Is your back bent?	Yes, 1; No, 0.
Q12. Do you have knee pain?	Yes, 1; No, 0.
Q13. Do you have a problem with your vision?	Yes, 1; No, 0.
Q14. Do you have a hearing problem?	Yes, 1; No, 0.
Q15. Do you think you are forgetful?	Yes, 1; No, 0.
Q16. Do you feel anxious about falling when you walk?	Yes, 1; No, 0.
Q17. Do you take five or more prescribed medicines?	Yes, 1; No, 0.
Q18. Do you feel unsafe because your home is dark?	Yes, 1; No, 0.
Q19. Are there any obstacles in your house?	Yes, 1; No, 0.
Q20. Is there any difference in level within your home?	Yes, 1; No, 0.
Q21. Do you have to use stairs in daily living?	Yes, 1; No, 0.
Q22. Do you have to walk on a steep slope around your house?	Yes, 1; No, 0.

Appendix II. Simple screening test for risk of falls

Q1. Have you fallen during the last 12 months?	Yes, 5 points; No, 0.
Q2. Do you feel your walking speed has declined recently?	Yes, 2 points; No, 0.
Q3. Do you use a cane when you walk?	Yes, 2 points; No, 0.
Q4. Is your back bent?	Yes, 2 points; No, 0.
Q5. Do you take five or more prescribed medicines?	Yes, 2 points; No, 0.

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OBSTRUCTIVE SLEEP APNEA EXACERBATES ENDOTHELIAL DYSFUNCTION IN PEOPLE WITH METABOLIC SYNDROME

To the Editor: The process of aging can be found in a variety of organs, frequently overlapping in the metabolic, cardiopulmonary, and nervous systems. A recent study showed that visceral fat accumulation is associated with metabolic risk factor clustering in older adults.[1] Obstructive sleep apnea (OSA) and metabolic syndrome (MetS) are well known as risk factors for cardiovascular disease and comorbid disorders in obese and older adults,[2] but whether OSA affects vascular endothelial dysfunction, a surrogate marker of cardiovascular disease,[3] in people with MetS has not been determined. Flow-mediated dilation (FMD) of the brachial artery, an indicator of endothelial vasomotor function, was therefore examined in people with MetS with or without OSA.

Forty-nine consecutive overweight subjects (body mass index $\geq 25.0\,kg/m^2$, aged 35–69) who were referred for medical examinations were enrolled and categorized into three groups; with MetS but not OSA (MetS group, n = 21), with MetS and OSA (MetS+OSA group, n = 14), and with no metabolic risk factors but overweight (control group, n = 14). MetS was defined using the International Diabetes Federation criteria and OSA using polysomnography. Participants who had some risk factors but did not meet the criteria for MetS and those who declined to undergo polysomnography were excluded. Blood sampling and measurement of FMD were performed early in the morning after an overnight fast. FMD was measured using ultrasound as percentage change in brachial artery diameter as previously described.[4]

The MetS and MetS+OSA groups had significantly lower plasma high-density lipoprotein cholesterol (HDL-C) (41.9 ± 9.4 and 40.7 ± 5.9 vs 57.9 ± 12.5 mg/dL, $P < .001$) and higher triglycerides (192.2 ± 57.7 and 157.3 ± 52.4 vs 104.1 ± 34.4 mg/dL, $P = .008$) and glycosylated hemoglobin ($5.71 \pm 0.87\%$ and $5.81 \pm 0.90\%$ vs $4.80 \pm 0.38\%$, $P = .001$) than the control group. Although the apnea-hypopnea index was 34.0 ± 13.6 events per hour in MetS+OSA group, in contrast to 3.1 ± 1.6 events in the MetS group ($P < .001$), there were no significant differences between the MetS and MetS+OSA groups in terms of cardiovascular risk factors, including age, body mass index, waist circumference, blood pressure, low-density lipoprotein cholesterol (LDL-C), and homeostasis model assessment of insulin resistance (data not shown).

The control group had a significantly lower increase in percentage of FMD (%FMD) than the other two groups. Moreover, %FMD in the MetS and OSA group was significantly lower than that in the MetS group (Figure 1), whereas nitroglycerine-induced endothelium-independent dilation was comparable between the groups ($15.0 \pm 4.2\%$ control, $13.5 \pm 3.2\%$ MetS, $11.5 \pm 3.5\%$ MetS+OSA). On multiple regression analysis, OSA (yes = 1, no = 0) was significantly related to %FMD, independent of age, waist circumference, systolic blood pressure, LDL-C, HDL-C, triglycerides, fasting plasma glucose, and smoking ($\beta = -0.324$, $P = .04$). The results of other multiple regression models were similar (data not shown).

It has been shown that continuous positive airway pressure treatment improves endothelial vasomotor function with no influence on metabolic risk factors,[5,6] indicating that vascular endothelial dysfunction in people with OSA is attributable to OSA-induced hypoxia. These findings imply that OSA is an additional risk factor in people with MetS. Consistent with the present results, it has been reported that OSA is independently associated with carotid intima-media thickness and pulse wave velocity, other

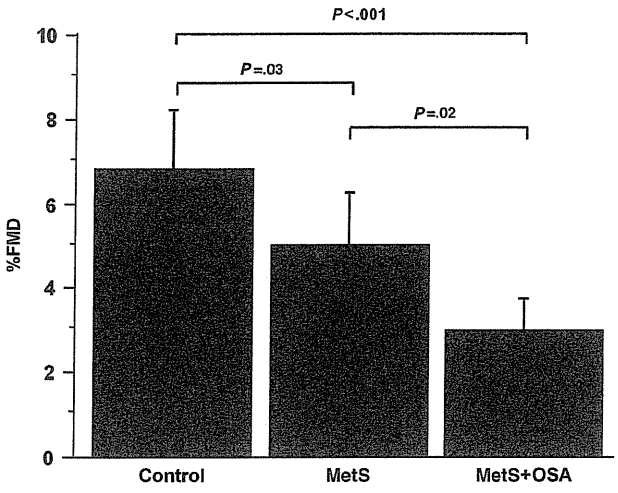

Figure 1. Increase in percentage of flow-mediated diameter (%FMD) of the brachial artery in control overweight subjects (control, n = 14), patients with metabolic syndrome (MetS, n = 21), and patients with MetS and obstructive sleep apnea (MetS+OSA, n = 14). Data are shown as means ± standard deviations.

markers of atherosclerosis, in people with MetS.[7] In conclusion, the results of the current study suggest that OSA exacerbates endothelial dysfunction in people with MetS, possibly leading to greater risk of cardiovascular disease.

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COMMENTS/RESPONSES

RELEVANT OUTCOMES IN INTERVENTION TRIALS FOR SARCOPENIA

To the Editor: We read with interest the paper by Brass and Sietsema on drug development to treat sarcopenia.[1] The authors raise important points to consider when designing clinical trials addressing sarcopenia-related outcomes.

As they state, a universally accepted definition for sarcopenia needs to be established. The difficulty encountered in doing so is a direct result of the complexity of the problem. The European Working Group for Sarcopenia in Older Persons (EWGSOP) has recently developed and published a practical clinical definition and consensus diagnostic criteria for age-related sarcopenia[2] that several international scientific societies, namely the European Geriatric Medicine Society (EUGMS), the European Society for Clinical Nutrition and Metabolism (ESPEN), the International Association of Gerontology and Geriatrics—European Region, and the International Association of Nutrition and Aging, have endorsed. In line with Brass and Sietsema's suggestion, the EWGSOP advocates a definition that allows chronic disease, besides aging per se, to contribute to sarcopenia.

For the diagnosis of sarcopenia, EWGSOP recommends using the presence of low muscle mass and reduced muscle function (strength or performance) and variously applies these characteristics to further define such conceptual stages as presarcopenia, sarcopenia, and severe sarcopenia. EWGSOP also reviewed a wide range of tools that can be used to measure the specific variables of muscle mass, muscle strength (e.g., hand grip), and physical performance (e.g., gait speed). The report summarizes currently available data defining sarcopenia cutoff points according to age and sex; suggests an algorithm for sarcopenia case finding in older individuals based on measurements of gait speed, grip strength, and muscle mass; and presents a list of suggested primary and secondary outcome domains for research.

In their review, Brass and Sietsema emphasize the standards that trials should meet to establish efficacy. They point out that efficacy should be measured according to meaningful clinically relevant end points and that surrogate markers of benefit will not be sufficient to validate Food and Drug Administration (FDA) approval. This is a complex issue for sarcopenia, because it fulfills criteria for a geriatric syndrome and is thus characterized by a complex interplay

Sirtuin 1 Retards Hyperphosphatemia-Induced Calcification of Vascular Smooth Muscle Cells

Aya Takemura, Katsuya Iijima, Hidetaka Ota, Bo-Kyung Son, Yuki Ito, Sumito Ogawa, Masato Eto, Masahiro Akishita, Yasuyoshi Ouchi

Objective—Arterial calcification is associated with cardiovascular disease as a complication of advanced atherosclerosis. Aged vascular cells manifest some morphological features of a senescent phenotype. Recent studies have demonstrated that mammalian sirtuin 1 (SIRT1), a histone deacetylase, is an exciting target for cardiovascular disease management. Here, we investigated the role of SIRT1 in a calcification model of vascular smooth muscle cells (SMCs).

Methods and Results—In adenine-induced renal failure rats with hyperphosphatemia, massive calcification was induced in the aortic media. Senescence-associated β-galactosidase (SAβ-gal) activity, a marker of cellular senescence, in medial SMCs was significantly increased, and its induction was positively associated with the degree of calcification. In cultured SMCs, inorganic phosphate (Pi) stimulation dose-dependently increased SAβ-gal-positive cells, and Pi-induced senescence was associated with downregulation of SIRT1 expression, leading to p21 activation. The activation via SIRT1 downregulation was blunted by inhibition of Pi cotransporter. Activation of SIRT1 by resveratrol significantly reduced the senescence-associated calcification. Conversely, SIRT1 knockdown by small interfering RNA accelerated the Pi-induced SMC senescence and subsequent calcification. In addition, SIRT1 knockdown induced phenotypic change from a differentiated state to osteoblast-like cells. The senescence-related SMC calcification was completely prevented by p21 knockdown. In addition to Pi-induced premature senescence, SMCs with replicative senescence were also more sensitive to Pi-induced calcification compared with young SMCs, and this finding was attributable to augmented p21 expression.

Conclusion—SIRT1 plays an essential role in preventing hyperphosphatemia-induced arterial calcification via inhibition of osteoblastic transdifferentiation. In addition, Pi-induced SMC calcification may be associated with both premature and replicative cellular senescence. (***Arterioscler Thromb Vasc Biol.* 2011;31:2054-2062.)**

Key Words: cellular senescence ■ hyperphosphatemia ■ longevity gene SIRT1 ■ vascular calcification ■ vascular smooth muscle cell

Atherosclerotic vascular damage associated with aging manifests several features, namely atherosis, sclerosis, and calcific change, finally leading to cardiovascular events. These pathological changes result in arterial wall thickening (localized morphological changes) and arterial stiffening (functional changes).[1] Arterial calcification makes the management of hemodynamics more difficult in the elderly, because ectopic calcium deposition in the aorta and arteries contributes to vessel wall stiffening and loss of elastic recoil.[2] These pathological conditions result in unstable hemodynamic consequences, finally leading to a decline in end-organ perfusion and subsequent ischemic events. Recently, several reports have demonstrated that aortic calcification detectable on chest X-ray examination is a strong predictor of future cardiovascular events beyond traditional risk factors.[3]

Arterial calcification is anatomically separated into two types, intimal and medial calcification.[4] Intimal calcification, which is seen as patchy scattered deposits only occurring within atherosclerotic plaques, is shown to be associated with plaque vulnerability.[5] On the other hand, medial calcification, which is frequently seen in the elderly and in diabetes and chronic renal failure, is observed as continuous linear deposits along the internal elastic lamina.[6] Advanced atherosclerosis with both types of calcified lesions is the consequence of overlapping pathological mechanisms.

Ectopic calcification in the vasculature has been shown to result from passive precipitation of calcium with aging and osteoporosis, the so-called calcium shift theory, as a previous hypothesis.[7] However, accumulating recent evidence has shown it to be attributable to an active "cell-mediated process" resembling osteogenesis in bone rather than passive mineral precipitation in vascular smooth muscle cells (SMCs).[8,9]

Silent information regulator-2 (Sir2), an NAD^+-dependent HDAC, is highly conserved in organisms ranging from Archaea

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to humans.[10] In yeast, Sir2 has been shown to play critical roles in DNA repair, stress resistance, and longevity. Mammalian sirtuin 1 (SIRT1), the closest homolog of Sir2, regulates the cell cycle, apoptosis, and metabolism by interacting with a number of molecules, including p53, promyelocytic leukemia protein, Foxo, Ku70, and peroxisome proliferator-activated receptor-γ.[11] A previous study has shown that SIRT1 antagonizes p53-mediated premature senescence in mouse embryo fibroblasts.[12] In addition, we have recently demonstrated that SIRT1 inhibits oxidative stress-induced premature senescence in vascular endothelial cells.[13] However, the detailed mechanism of how SIRT1 affects vascular SMC senescence and arterial calcification remains unclear.

In this study, we hypothesized that SIRT1 plays an important role in preventing arterial calcification due to renal failure, in association with modulation of cellular senescence. Here, we demonstrated the protective potential of SIRT1 against hyperphosphatemia-induced premature and replicative senescence and subsequent calcification in SMCs.

Methods

Aortic Calcification in Renal Failure Rats

Renal failure was induced in rats by a 0.75% adenine-containing diet as previously described.[14] All procedures and animal care were in accordance with the Guide for the Care and Use of Laboratory Animals of the University of Tokyo. Detailed methods are described in the supplemental materials, available online at http://atvb.ahajournals.org.

Induction of SMC Calcification

Primary human aortic SMCs (HASMCs) were treated with a pathological concentration of inorganic phosphate (Pi) up to 3.2 mmol/L in culture medium as previously described.[29] To quantitatively measure Pi-induced calcification, two distinct experiments were performed as previously described[14]: (1) intracellular calcium deposition as determined by *o*-cresolphthalein complexone method, and (2) visualization of mineralization as determined by von Kossa staining, Detailed methods are described in the supplemental materials.

Senescence-Associated β-Galactosidase Staining

To assess senescent changes in the phenotype of cultured HASMCs or aortic medial cells of rats, staining for senescence-associated β-galactosidase (SAβ-gal), a well-established biomarker of cellular senescence, was performed. Detailed methods are described in the supplemental materials.

Knockdown of SIRT1 or p21 by Small Interfering RNA

HASMCs were transfected with 200 pmol/L small interfering RNA (siRNA) for SIRT1, $p21^{WAF1/CIP1}$, or both. Detailed methods are described in the supplemental materials.

Real-Time Polymerase Chain Reaction Analysis: Osteoblastic Markers

To examine whether Pi stimulation induces change to an osteoblastic phenotype, the expression of Runx-2/Cbfa-1 and alkaline phosphatase, which are well known to be representative osteoblastic markers, was checked using real time-polymerase chain reaction analysis. In addition, the effect of knockdown of SIRT1, p21, or both by siRNA on the osteoblastic phenotypic change in HASMCs was examined. Primer sequences are shown in Supplemental Figure I.

Results

Association of Senescent Vascular Cells With Aortic Medial Calcification in Renal Failure Rats

The adenine-fed rats had severe renal failure, with a huge increase in serum creatinine (3.0±0.9 mg/dL in renal failure rats versus 0.3±0.0 mg/dL in control rats), similar to a previous report.[14] The renal failure rats showed an approximately 2.0-fold increase in serum phosphorus (18.9±4.7 mg/dL) compared with control rats (9.8±0.9 mg/dL). Histological assessment using von Kossa staining showed that the aorta in renal failure rats had extensive linear calcification, which was localized in the aortic media, resembling the typical Mönckeberg's pattern (Figure 1A). Numerous SAβ-gal positive cells were found in the aortic media of renal failure rats, whereas the aortic wall in control rats did not contain senescent cells (Figure 1B). The senescent cells were mainly localized to the calcified area and its surrounding area, which was defined as the area not stained black by von Kossa staining. Quantitative assessment showed that the number of senescent cells with high SAβ-gal activity was positively correlated with the calcified area in the aortic media (Figure 1C).

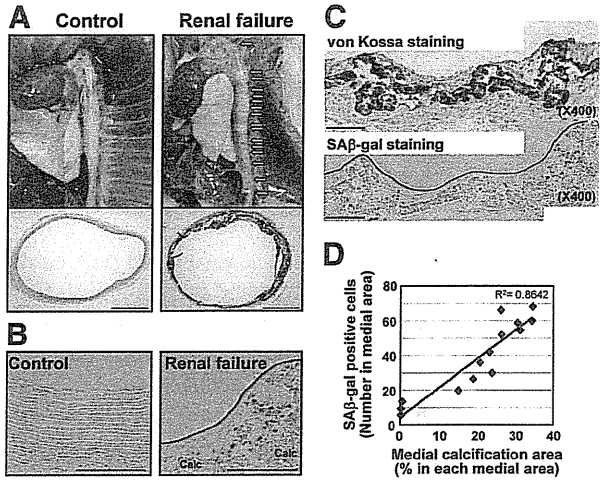

Figure 1. Presence of senescent vascular cells colocalized with calcification in aortic media of renal failure rats. A, Rats with severe renal failure had massive calcification throughout the aorta (right) compared with control rats (left) (n=5). Yellow arrows indicate calcified area. Morphological assessment by von Kossa staining showed extensive calcification in the aortic media of renal failure rats. Scale bar=500 μm. B, Senescent vascular cells (senescence-associated β-galactosidase [SAβ-gal]-positive: blue) were significantly detected throughout the calcified area (Calc) in renal failure rats, whereas these senescent cells were not present in control rats. Scale bar=100 μm. C, Localized association between calcification and senescent cells is shown in renal failure rats. SAβ-gal-positive cells were frequently found in areas with marked calcification. D, The association of the number of SAβ-gal-positive cells with the calcified area in each photograph was evaluated. The senescent cell number was linearly correlated with the area of calcification in the aortic media of renal failure rats (calcified area in media: percentage).

Pi Induces Cellular Senescence in Cultured SMCs

On the basis of our results obtained from animal experiments, we hypothesized that senescent SMCs in the aortic media are strongly associated with the development of arterial calcification. Therefore, the effect of excessive Pi stimulation (2.6 mmol/L) on cellular senescence in cultured SMCs was examined. SAβ-gal-positive senescent HASMCs were significantly induced by not only angiotensin II (Ang II) but also Pi

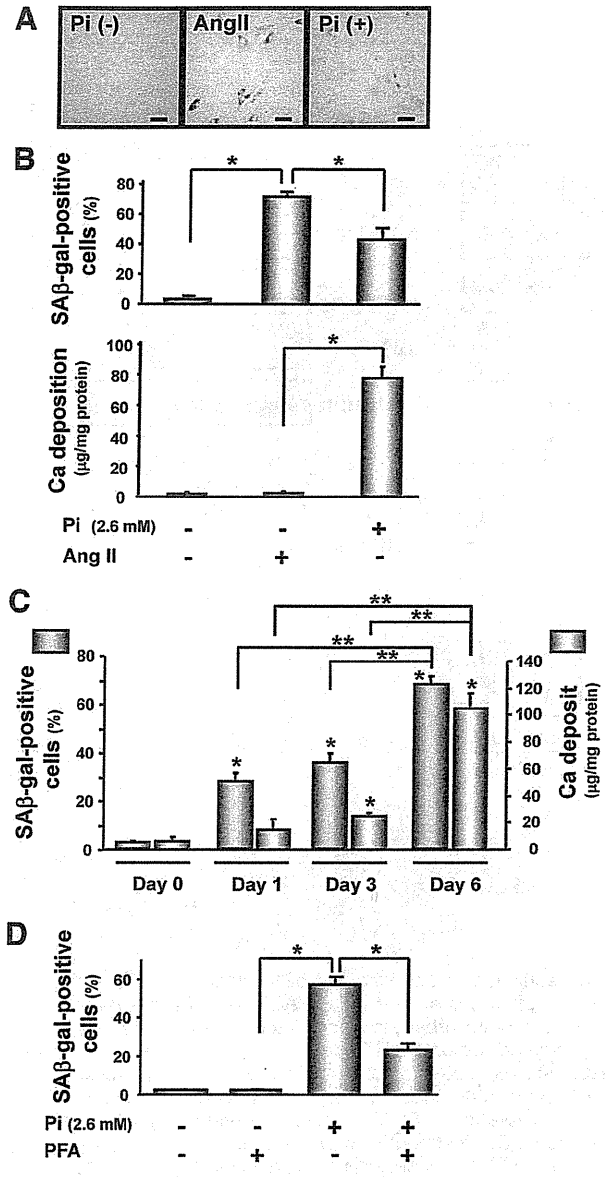

Figure 2. Inorganic phosphate (Pi) stimulation induces cellular senescence in vascular smooth muscle cells (SMCs) via its cotransporter. A, The effect of Pi on senescent transition in human aortic SMCs (HASMCs) was examined. Representative photographs showed that senescence-associated β-galactosidase (SAβ-gal) activity (blue) in cells was significantly induced by not only angiotensin II (Ang II: 10 pmol/L, as a positive control) but also Pi stimulation (2.6 mmol/L). B, The number of senescent cells was significantly increased by not only Ang II but also Pi. Calcium deposition was significantly increased by Pi; however, calcification in HASMCs was not induced by Ang II alone in the absence of Pi. C, Senescent cells were significantly increased by Pi stimulation even on day 1; however, a statistically significant increase in calcium deposition was found from day 3 and later. D, Inhibition of the phosphate cotransporter Na-dependent phosphate cotransporter by the inhibitor phosphonoformic acid (PFA) (100 μmol/L) reduced SAβ-gal activity, which was increased by Pi (2.6 mmol/L) in HASMCs. Each experiment was performed al least 3 times.

stimulation (Figure 2A). Notably, Pi stimulation increased calcium deposition; however, Ang II alone did not (Figure 2B). It suggests that high-dose Pi condition, but not stress by Ang II alone, is indispensable to induce SMC calcification. These findings also suggest that intracellular Pi influx at least is essential to induce this SMC calcification model.

In addition, to determine how many days after the initiation of Pi stimulation the cells showed a senescent phenotype and subsequent calcification, the time-dependent effects of Pi stimulation on both SAβ-gal activity and calcium deposition were examined. As shown in Figure 2C, SAβ-gal-positive cells were significantly increased by Pi stimulation even on day 1, although calcium deposition was not markedly increased at the same time point. A statistically significant increase in calcium deposition was found from day 3 and later. Cotreatment with phosphonoformic acid, an inhibitor of Na-dependent phosphate cotransporter (NPC), showed significant inhibition of Pi-induced senescence (Figure 2D). Our previous report showed that treatment with PFA completely inhibited Pi-induced SMC calcification,[15] suggesting the importance of increased intracellular influx of phosphate in Pi-induced SMC senescence.

Downregulation of SIRT1 by Pi

Treatment of HASMCs with Pi caused downregulation of SIRT1 expression in a time-dependent manner (Figure 3A). The decline was dependent on Pi concentration (data not shown). An increase in acetylation of both substrates of SIRT1, histone-3 and p53 (a nonhistone substrate), was found according to the decline in SIRT1 deacetylase activity. In addition, expression of p21, a downstream molecule of p53, was significantly induced by Pi as well. Quantitative assessment showed that an increase in these expression levels of acetylated (Ac)-p53 and p21 on day 3 and day 6 was statistically significant compared with the pretreatment levels, suggesting that downregulation of SIRT1 activity may mediate the subsequent increase in Ac-p53 and p21 expression.

To address whether SIRT1 downregulation-related SMC senescence and calcification are reversible or not, the effects of continuation or termination of high-dose Pi were examined. As shown in Figure 3B, the continuation of Pi up to day 10 was associated with SIRT1 downregulation and subsequent upregulation of Ac-p53 and p21, leading to induction of senescence-related calcification. However, the slight increase in senescent cells was not statistically significant, although calcification was significantly induced. Of note, the Pi-induced downregulation of SIRT1 was almost completely reversed by withdrawal (termination) of Pi stimulation (exchange of Pi from 2.6 mmol/L to 1.4 mmol/L as a normal level on day 6) as shown in Figure 3B. According to the restoration of SIRT1, levels of both Ac-p53 and p21 were also decreased without more progression. In addition, termination of Pi showed no progression of senescence-related calcification; however, preexisting senescent cells and calcification on day 6 continued without regression.

Next, NPC inhibition by PFA completely blunted Pi-induced SIRT1 downregulation and subsequent activation of its downstream p53/p21 pathway (Figure 3C).

Regulation of SIRT1 Modulates Pi-Induced SMC Senescence and Calcification

The effects of modulation of SIRT1 activity on Pi-induced cellular senescence were investigated. First, sirtinol, a chem-

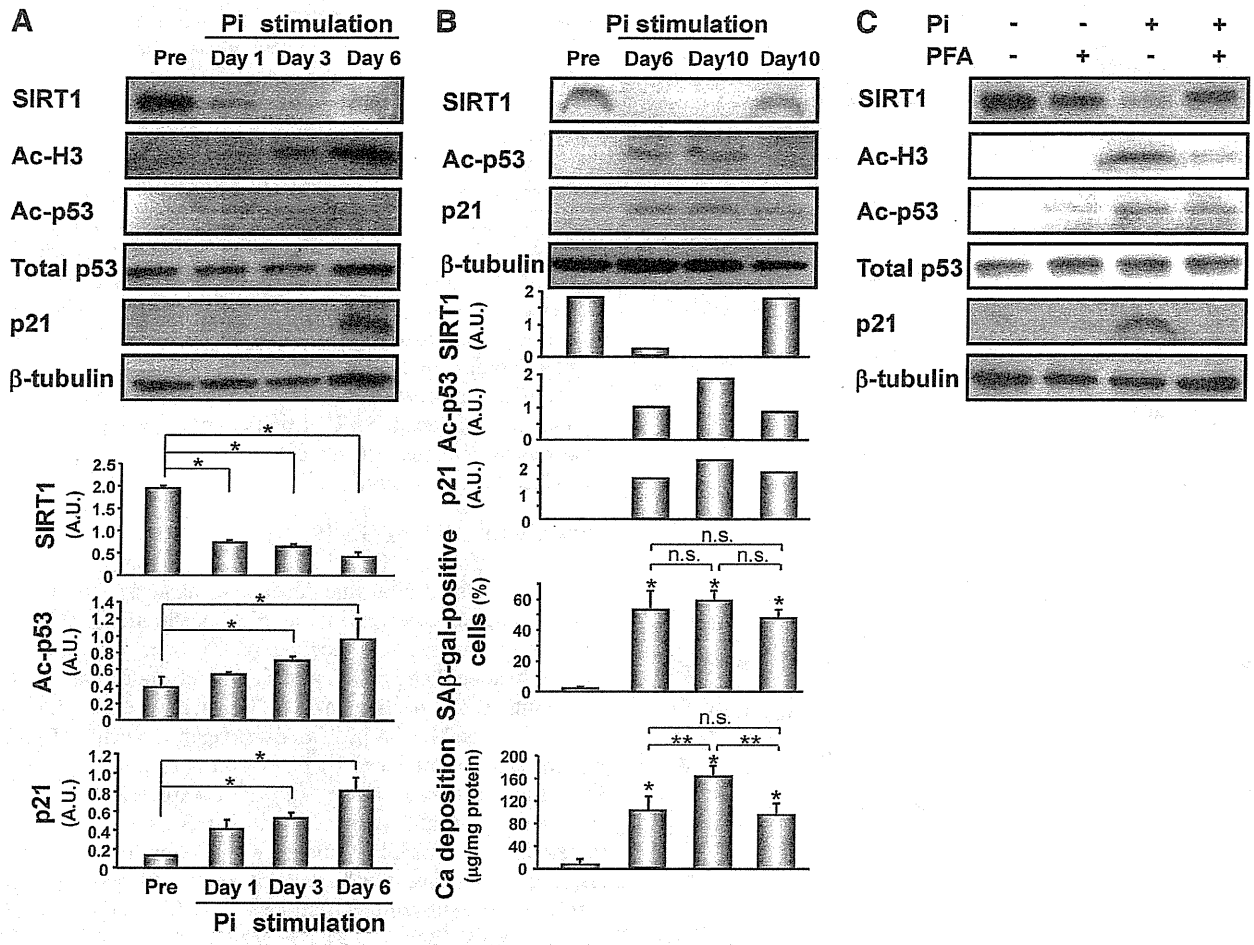

Figure 3. Inorganic phosphate (Pi) stimulation leads to sirtuin 1 (SIRT1) downregulation and subsequent p21 activation. A, The effect of Pi on SIRT1 expression and its downstream pathway was examined. Treatment of human aortic SMCs (HASMCs) with Pi (2.6 mmol/L) showed downregulation of SIRT1 expression, leading to an increase in acetylation of its substrates (acetylated [Ac]-H3 and Ac-p53) and p21 expression. Bottom: Quantitative analysis showed that Pi gradually induced not only SIRT1 downregulation but also upregulation of Ac-p53 and p21. B, To address whether SIRT1 downregulation-related senescence and subsequent calcification are reversible, the effects of continuation or termination of high-dose Pi were examined. As shown in 4th lane from left, termination (on day 6) of Pi showed no progression of senescence-related calcification in association with restoration of SIRT1, whereas continuation (up to day 10, 3rd lane from left) of Pi stimulation showed further progression of calcification. C, Treatment with phosphonoformic acid (PFA), a Na-dependent phosphate cotransporter inhibitor, completely reversed Pi-induced SIRT1 downregulation. A decline in Ac-H3 and Ac-p53 reflected the restoration of SIRT1 deacetylase activity. Pi-induced p21 activation was significantly inhibited by inhibition of Pi transport.

ical inhibitor of SIRT1, induced an increase in SAβ-gal-positive cells even under a normal Pi (1.4 mmol/L), and the increased number of senescent cells induced by Pi was significantly augmented by sirtinol (Figure 4A). Sirtinol dose-dependently augmented Pi-induced calcification, although no augmentation was found under a normal Pi (Figure 4B and 4C). Conversely, treatment with resveratrol, an activator of SIRT1, significantly reduced both Pi-induced senescent transition and calcification in a dose-dependent manner (Figure 4D to 4F).

Second, complete knockdown of SIRT1 by siRNA caused a significant increase in acetylation of both substrates (histone-3 and p53) and p21 expression (Figure 5A). Similarly to sirtinol, SIRT1 inhibition by siRNA also augmented not only senescent transition (Figure 5A, bottom) but also calcium deposition (Figure 5C, top).

Although stimulation with Ang II alone could increase the number of SAβ-gal-positive cells, it did not increase calcium deposition. To understand the mechanism of these discrepant phenomena, the effect of Ang II alone on osteoblastic phenotypic change was examined. Ang II alone did not increase the expression of Runx2 in the absence of Pi stimulation, unlike Pi stimulation (Figure 5B).

To understand the detailed mechanism by which SIRT1 modulates senescence-related calcification, the effect of SIRT1 on phenotypic change in HASMCs was examined. Pi inhibited the expression of caldesmon, a differentiated SMC lineage marker, and complete knockdown of SIRT1 augmented the Pi-induced partial downregulation of caldesmon (Figure 5C, middle). In contrast, real-time polymerase chain reaction analysis showed that Pi induced the expression of two representative osteoblastic markers, Runx-2/Cbfa-1 and alkaline phosphatase (Figure 5C, bottom) with statistical significance. In addition, complete knockdown of SIRT1 using siRNA significantly accelerated the Pi-induced os-

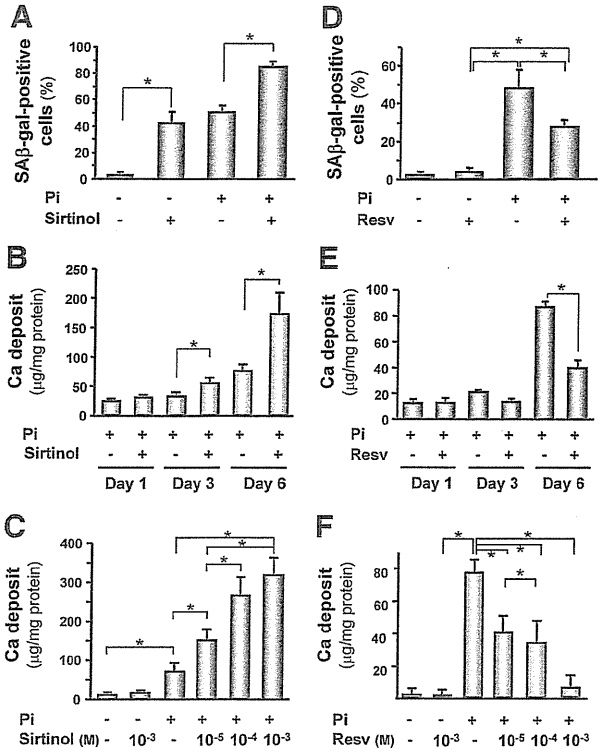

Figure 4. Modulation of sirtuin 1 (SIRT1) affects inorganic phosphate (Pi)–induced senescent phenotypic change and calcification in smooth muscle cells (SMCs). The effects of sirtinol (a chemical inhibitor of SIRT1 activity; A to C) and resveratrol (an activator of SIRT1; D to F) on Pi-induced senescent phenotypic change and calcification were examined (n=6). A, SIRT1 inhibition by sirtinol (10 μmol/L) showed an increase in the number of senescence-associated β-galactosidase (SAβ-gal)-positive cells even without Pi stimulation. The increase in Pi-induced senescence was significantly augmented by sirtinol. Sirtinol augmented Pi-induced calcium deposition in human aortic SMCs (HASMCs) in a time-dependent (B) and dose-dependent manner on day 6 (C). Conversely, treatment with resveratrol (Resv; 10 μmol/L) showed a reduction of the Pi-induced senescent phenotype (D) and calcification (E). The inhibitory effect of resveratrol on calcification was dose dependent (F).

teoblastic phenotypic change, suggesting that modulation of SIRT1 is associated with osteoblastic phenotypic change in SMCs.

Inhibition of Senescence-Related Calcification in SMCs by p21 Knockdown

To address the association of p21 with senescence-related calcification, knockdown of p21 using siRNA was performed. Treatment of p21 siRNA (up to 200 pmol/L) completely inhibited p21 (Figure 5D). p21 knockdown completely inhibited Pi-induced senescence and subsequent calcification (Figure 5E).

Regulation of NPC-Mediated Runx2 Expression by SIRT1/p21 Pathway

As the next step, the role of SIRT1 in NPC-mediated Runx2/Cbfa1 expression was examined. First, complete knockdown of SIRT1 did not show any change in both osteoblastic markers, Runx2 and alkaline phosphatase, in a normal Pi (Supplemental Figure I). As shown in Figure 5F, Pi-induced Runx2 was significantly blunted by PFA, an NPC inhibitor. SIRT1 activation by resveratrol inhibited Pi-induced Runx2 activation. The Runx2 induction was augmented by knockdown of SIRT1 by siRNA, and the activation was completely inhibited by PFA. Surprisingly, Runx2 activation was strongly inhibited by knockdown of p21 alone. In addition, the inhibition of Runx2 induction by double knockdown of SIRT1 and p21 was less than that by single knockdown of SIRT1.

To address a difference in senescent induction by Pi or Ang II, immunohistological assessment of SIRT1 in HASMCs was examined (Supplemental Figure II). Although SIRT1 was predominantly localized in nucleus without Pi, the translocation of SIRT1 to cytoplasm was observed after Pi stimulation for 24 hours, and its expression disappeared in both areas on day 6. In contrast, Ang II stimulation did not show the dynamic translocation.

High Sensitivity of SMCs With Replicative Senescence to Pi-Induced Calcification

Not only Pi-induced "premature senescence" in HASMCs but also the effects of Pi on "replicative senescence" were evaluated. Senescent cells (passage 18) were more sensitive to Pi-induced calcification compared with young cells (passage 7) (Figure 6A). SIRT1 expression was downregulated in senescent cells compared with young cells, and the downregulation was significantly augmented by Pi stimulation (Figure 6B, top). In parallel with this finding, senescent cells showed an increase in Ac-p53 and p21 expression. Statistical analyses using densitometric measurement showed that (1) downregulation of SIRT1 and upregulation of Ac-53 and p21 were augmented by replicative senescence, and (2) Pi inhibited the SIRT1-p21 pathway even in cells with replicative senescence (passage 18) (Figure 6B, bottom).

Discussion

Vascular aging, leading to cardiovascular disease, manifests complex and diverse vascular changes (eg, impairment of distensibility due to loss of arterial elasticity).[1,16] Arterial wall stiffness resulting from ectopic calcification is a complication of advanced atherosclerosis and makes the management of hemodynamics more difficult in the elderly. Few reports have addressed whether cellular senescence is associated with SMC calcification. This study showed the importance of SIRT1, a longevity gene, in arterial calcification in association with cellular senescence.

First, our data obtained from animal experiments clearly showed the association of senescent SMCs with aortic medial calcification in the renal failure rats with hyperphosphatemia. Senescent cells showed significant colocalization with calcium deposition. Intriguingly, numerous senescent cells could be detected before microscopic calcification occurred at 4 weeks after the start of renal failure induction (data not shown), suggesting that the transition to a senescent phenotype in medial SMCs may be associated with the initiation and progression of calcification. Therefore, hyperphosphatemia, a potent uremic factor, may be a stimulator to induce senescent phenotypic transition of medial SMCs.

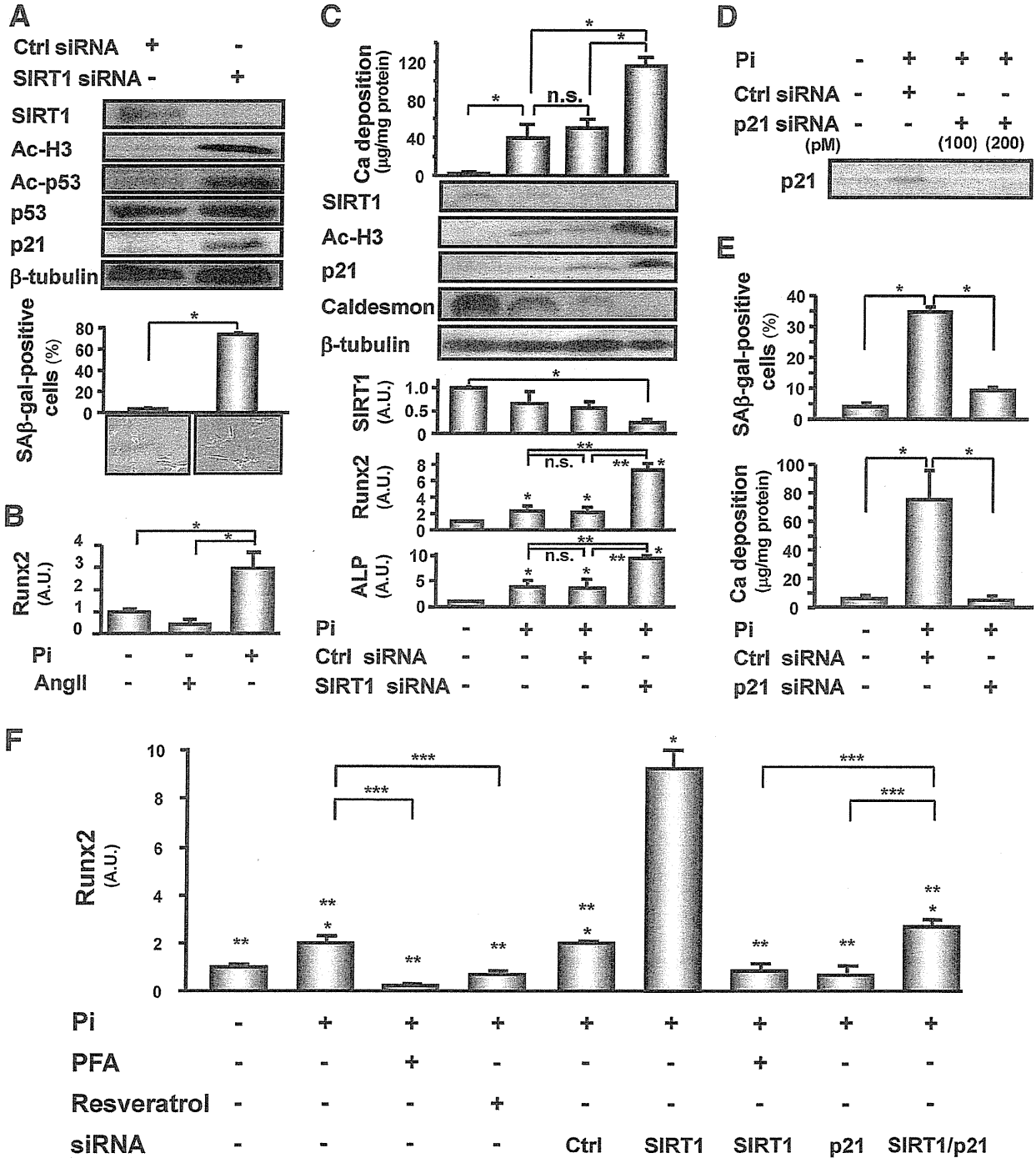

Figure 5. Augmentation of senescence-related smooth muscle cell (SMC) calcification by sirtuin 1 (SIRT1) knockdown in association with osteoblastic phenotypic change and prevention of inorganic phosphate (Pi)–induced changes by p21 knockdown. A, To achieve SIRT1 knockdown in human aortic SMCs (HASMCs), small interfering RNA (siRNA) was simultaneously administered at the start of Pi stimulation (2.6 mmol/L). Complete inhibition of SIRT1 showed a significant increase in acetylation of both substrates (acetylated [Ac]-H3 and Ac-p53), p21 expression and senescence-associated β-galactosidase (SAβ-gal)-positive cells. B, Angiotensin II (Ang II) alone (10 pmol/L) did not increase the expression of Runx2 in the absence of Pi stimulation, unlike Pi stimulation. C, top: SIRT1 knockdown by siRNA significantly accelerated Pi-induced calcification (n=6), whereas control (Ctrl) siRNA did not. C, middle and bottom: Western blots showed that Pi partially inhibited the expression of a differentiated SMC marker, caldesmon, and complete knockdown of SIRT1 by siRNA augmented its downregulation. Real-time polymerase chain reaction analysis showed that Pi induced the expression of Runx-2 and alkaline phosphatase (ALP). Complete knockdown of SIRT1 significantly accelerated the Pi-induced osteoblastic markers. A.U. indicates arbitrary units. *$P<0.05$. D and E, Knockdown of p21 by siRNA (200 pmol/L) significantly reduced the senescent phenotypic change and subsequent calcification (n=6). F, The role of SIRT1/p21 axis in Na-dependent phosphate cotransporter-mediated Runx2 expression was evaluated. Augmentation of Pi-induced Runx2 expression by SIRT1 knockdown was significantly inhibited by double knockdown of SIRT1 and p21. *$P<0.05$ vs control without Pi stimulation (left column), **$P<0.05$ vs Pi-stimulated cells with SIRT1 siRNA (sixth column from left).

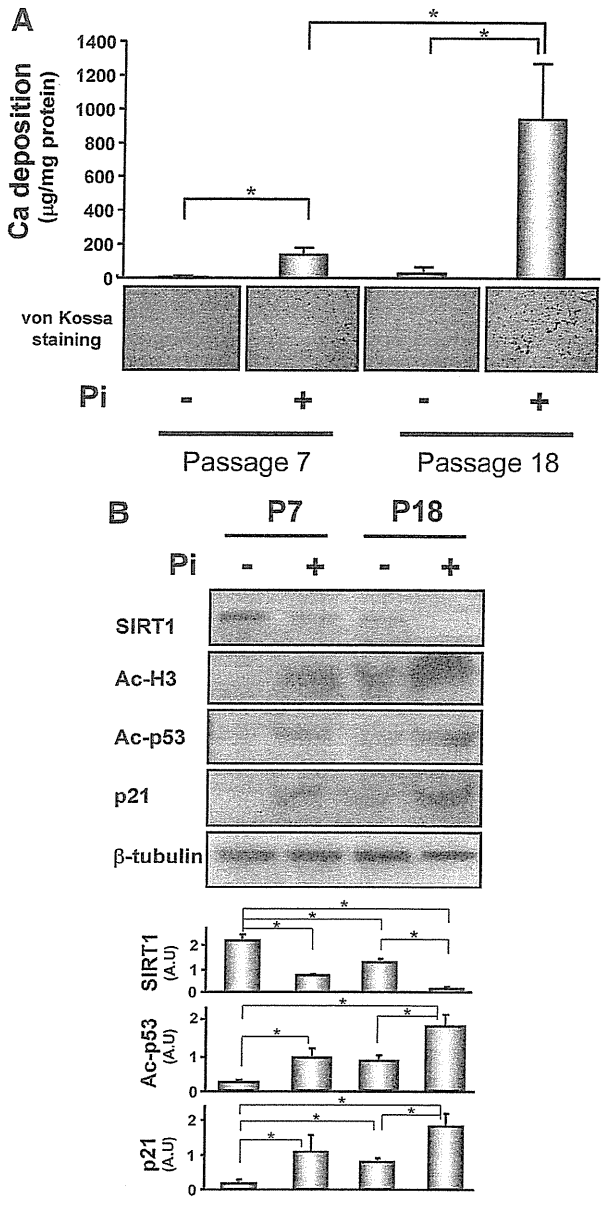

Figure 6. High sensitivity of smooth muscle cells (SMCs) with replicative senescence to inorganic phosphate (Pi)–induced calcification. The effects of replicative senescence in human aortic SMCs (HASMCs) on Pi-induced calcification (A) and sirtuin 1 (SIRT1)-related molecules (B) were also evaluated. A, Senescent cells (passage 18 [P18]) were more sensitive to Pi-induced calcification compared with young cells (passage 7 [P7]) (n=6). Representative photographs of von Kossa staining (bottom) show strong induction of calcium deposition by Pi (2.6 mmol/L). B, Senescent HASMCs (P18) showed a decline in SIRT1 expression and an increase in p21 expression compared with young cells (P7). Pi stimulation of senescent cells significantly inhibited SIRT1 expression and accelerated the increase in p21 and acetylated (Ac)-p53. Densitometric analysis confirmed these more sensitive responses in senescent cells. A.U. indicates arbitrary units. *$P<0.05$.

Second, we also confirmed the association of Pi-induced SMC senescence with calcification in in vitro experiments. Senescent SMCs were significantly increased by Pi even on day 1, although calcium deposition was not markedly increased at the same time point. A statistically significant increase in calcium deposition was found from day 3 and later. Considering these data, we hypothesize that (1) calcium deposition may be more readily induced in senescent cells compared with nonsenescent cells, and (2) Pi-induced senescent change is observed earlier than calcium deposition. In other words, senescent transition associated with Runx2 induction may lead to progressive calcification.

Senescent SMCs were associated with the SIRT1-related p53/p21 pathway, based on the findings that SIRT1 knockdown augmented not only cellular senescence but also calcification. In addition, p21 knockdown completely inhibited senescence-related calcification induced by Pi. This raises the question of how cellular senescence in SMCs is associated with calcification. Our experiments to understand the detailed mechanisms by which SIRT1 modulates senescence-related calcification showed that Pi-induced SIRT1 downregulation led to the phenotypic change from a differentiated state to osteoblast-like cells in SMCs. It has been reported that Pi induces osteoblastic change, in which NPC plays a role in inducing Runx2/Cbfa-1 expression, in SMCs.[17] As the next step, to determine how SIRT1 regulates NPC-mediated Runx2 expression, we examined the effects of knockdown of SIRT1, p21, or both by siRNA on Pi-induced Runx2 expression. Our data shown in Figure 5F suggested that (1) NPC plays an essential role in Pi-induced Runx2 expression, (2) SIRT1 has an inhibitory effect on NPC-mediated Runx2 expression, (3) knockdown of p21 alone ameliorates Runx2 induction, and (4) p21-related osteoblastic change is at least in part dependent on SIRT1.

There is now the new question of how SIRT1 regulates Runx2 regulation. A report by Jeon[18] has shown that acetylation of Runx2 itself is important in osteoblast differentiation, and it is downregulated by HDAC activities. Based on this evidence, SIRT1, 1 of the HDACs, may be able to deacetylate Runx2, leading to inhibition of Runx2-related osteoblastic transition in SMCs. Therefore, the inhibition of SIRT1 by hyperphosphatemia may lead to Runx2 activation via its hyperacetylation. Further investigation of the detailed mechanism of the SIRT1/p21/osteoblastic gene axis is needed. These data clearly suggest that SIRT1 activation may inhibit the hyperphosphatemia-induced osteoblastic phenotypic change of SMCs, and the degree of change may be dependent on SIRT1 expression level. It is possible that the inhibition of SIRT1 expression by Pi alone is "partial," because complete downregulation of SIRT1 by siRNA worsened the dynamic phenotypic change compared with Pi only. We have already shown that tumor necrosis factor-α, a potent atherogenic cytokine, augmented Pi-induced SMC calcification, as previously described.[19] In addition, tumor necrosis factor-α significantly decreased Pi-induced SIRT1 downregulation further (data not shown). According to these results, we currently hypothesize that hyperphosphatemia induces SIRT1 downregulation and subsequent osteoblastic phenotypic change in SMCs, leading to calcification, and these changes are worsened by some harmful atherogenic factors, which decrease SIRT1 expression/activity further. These results provide a new insight, showing that SIRT1 plays an essential role in the prevention of arterial calcification and that the beneficial effect may be associated with an inhibition in Pi-induced SMC senescent transition.

In addition, Ang II did not increase calcium deposition, although the stimulation increased the number of senescent cells. Of note, Ang II alone did not increase Runx2 expression in the absence of Pi (Figure 5B). This result suggests that SMC senescence shows two different features: one is SAβ-gal-positive cells with an increase in Runx2 and the other is SAβ-gal-positive cells without. First, it has recently been reported that SMCs with replicative senescence, rather than the cells without senescence, show hypersensitivity in response to induction of calcification with the more induction of osteoblastic markers,[20] suggesting that the induction of osteoblastic transdifferentiation is strongly associated with the senescent change in SMCs. In addition, the translocation of SIRT1 to cytoplasm was observed after Pi stimulation for 24 hours, although SIRT1 predominantly localized in nucleus without Pi. In contrast, Ang II did not show the dynamic translocation. Thinking about the mechanism for regulating the activity of HDACs, including SIRT1, recent several reports show the importance of their coordinated shuttling between nucleus and cytoplasm. A report demonstrates that HDAC7, an HDAC, represses the transcriptional activity of Runx2 and that osteogenic stimuli induce export of HDAC7 from nucleus, leading to a decline in the repressive potentials of HDAC7 for Runx2.[21] On the basis of our findings and a previous report, the reason that stimulation with Ang II alone did not induce Runx2 expression and subsequently SMC calcification may in part depend on the difference of SIRT1 translocation after stimulation. Therefore, we strongly hypothesize that in the senescent SMCs with upregulation of p21, Pi stimulation, but not Ang II stimulation, may activate Runx2 via at least two phenomena, the hyperacetylation of Runx2 by SIRT1 downregulation and the dynamic SIRT1 translocation, leading to marked osteoblastic transdifferentiation and subsequent calcification. In addition, we have another hypothesis. In general, it has been shown that high-dose Pi navigates release of matrix vesicles from SMCs in parallel with osteoblastic transdifferentiation. The vesicles play an essential role in the initiation of hydroxyapatite aggregation, so-called nucleation. Accumulating recent reports show that the nanocrystal formation as an initial step under hyperphosphatemia accelerates the harmful cascade of osteoblastic transdifferentiation in SMCs via endocytosis.[22,23] Maybe Ang II alone does not induce the nanocrystal formation and the cascade of osteoblastic change. Therefore, we explain that the difference of senescent phenotypic changes in SMCs between both stimulations, Pi and Ang II alone, may depend on (1) SIRT1 translocation and (2) nanocrystal formation to accelerate calcification. Further investigation to address the detailed mechanisms by which SIRT1 regulates osteoblastic transdifferentiation in SMCs under the cellular senescence is needed.

Are SIRT1 downregulation-related SMC senescence and subsequent calcification reversible or not? To answer this question, the effects of continuation or termination of high-dose Pi were examined. As shown in Figure 3B, termination (on day 6) of Pi showed no progression of senescence-related calcification in association with the restoration of SIRT1, whereas continuation (up to day 10) of Pi stimulation showed further progression of calcification. It is suggested that a therapeutic strategy to manage hyperphosphatemia to the normal range of serum phosphate concentration may lead to at least termination of progressive calcification via reversal of SIRT1 activity.

Cellular senescence has been shown to have two features: not only stress-induced premature senescence but also replicative senescence, indicating a limited number of divisions in culture.[24] In fact, both endothelial cells and SMCs derived from human atherosclerotic plaques show a senescent phenotype earlier than do cells from normal vessels.[25] Notably, we found that senescent HASMCs were significantly more sensitive to Pi-induced calcification compared with young cells. These results suggest that calcium deposition may be more readily induced in arterial medial SMCs with replicative senescence. This insight may explain the mechanisms by which arterial calcification occurs in the elderly more frequently than in the young population. Therefore, these observations support our hypothesis that arterial calcification is accelerated by both senescent types (premature and replicative senescence) in SMCs. To explore new therapeutic strategies against arterial calcification, it is essential to investigate how to maintain a higher SIRT1 level in the vasculature, leading to prevention of medial SMC senescence and which drug is capable of achieving it.

How does SIRT1 exert protective effects against SMC calcification? This study clearly showed that inhibition of SIRT1 was associated with increases in both Ac-p53 and p21 expression. These findings were significantly induced by not only replicative senescence but also Pi-induced premature senescence. SIRT1-mediated deacetylation of p53 inhibits p53-dependent transactivation of target genes, including p21. A report showed that a decline in cellular deacetylase activity increases the half-life of endogenous p53,[26] suggesting that p53 acetylation is also associated with p53 stabilization. Therefore, the increased Ac-p53 by Pi-induced SIRT1 downregulation may induce SMC senescence because of a decline in degradation of p53, leading to calcification. In addition, p53 itself can inhibit SIRT1 transcription because the SIRT1 promoter has two response elements to p53.[27] Further investigation to address how the SIRT1-p53 negative regulatory pathway is associated with SMC calcification is needed.

On the other hand, regarding p21 activation, it is reported that inhibition of p21 expression in the vasculature significantly attenuates cellular senescence, leading to prevention of atherosclerosis.[28] This evidence suggests a pivotal role of p21 in the development of atherosclerosis. p21 activation has been shown to be regulated by a pathway that is p53 dependent, p53 independent, or both. Okamoto et al have demonstrated that inhibition of HDAC by trichostatin A showed activation of p21 promoter activity by the Sp1 site even in vascular SMCs, and the induction of p21 was independent of the p53 pathway.[29] The p21 transcriptional activation in response to HDAC inhibitors was mediated by histone hyperacetylation in its promoter region. Based on these findings, Pi-induced p21 activation via SIRT1 downregulation may be in part involved in a p53-independent pathway, leading to a senescent phenotype of SMCs. Further investigation exploring which molecule activates the p21 promoter under hyperphosphatemia is needed.

Conclusion

We showed that SIRT1 exerts a protective role in hyperphosphatemia-based arterial calcification via inhibition of osteoblastic transdifferentiation, in association with cross-talk between calcification and cellular senescence. This ability of SIRT1 may orchestrate an analogous protective/longevity paradigm even in vascular SMCs, leading to maintenance of healthy elasticity of the arterial wall. Strategies to maintain a higher level of SIRT1 activity may provide novel therapeutic opportunities for the prevention of arterial calcification.

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Disclosures

None.

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特 集 ■ 生物学的精神医学の進歩

認知症 ── AD-FTD スペクトラムを中心に

Advances in Biological Psychiatry Research on Dementia: AD-FTLD Spectrum

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Masatoshi Takeda*

Abstract

Neurodegenerative dementia, including Alzheimer disease (AD) and frontotemporal lobar degeneration (FTLD), is one of the main target areas for research in biological psychiatry. In this review, the historical view, present situation, and further development in dementia research have been discussed from the viewpoint of biological psychiatry.

Considering the rapidly increasing number of dementia patients in Japan, the importance of dementia in clinical psychiatry service will keep increasing in the near future. Biological as well as psychosocial knowledge is required to elucidate the mechanism underlying dementia.

Although the molecular mechanism underlying the pathological features of AD has not yet been fully elucidated, it can be placed under the concept of the AD-FTLD spectrum, in which loss of function of an important gene may result in accumulation of insoluble proteins inside and outside neurons. To develop disease-modifying drugs for AD and FTLD, elucidation of pathological events that occur earlier than abnormal protein deposition is essential. Early diagnosis and early intervention are important for overcoming these neurodegenerative dementia.

Key words : Alzheimer disease, frontotemporal lobar degeneration, disease-modifying drug, amyloid beta protein, progranulin

はじめに

認知症は，記憶障害と判断力障害のために，職業上および日常生活上に著しい障害を呈する疾患と定義される。認知症の患者数は，全世界で2,430万人で，毎年460万人の新しい患者が発生している[1]。認知症は，その患者数の多さ，障害の大きさ，罹病期間の長さからみて，人類が取り組むべき「最大の悪性疾患」とも言える。わが国において，現時点での認知症患者数は約150万人であるが，平均余命の延長とともに増加し続けており，2050年には350万人に達すると見込まれる。

認知症は，生物学的精神医学にとって重要な疾患の1つであることは言うまでもない。また，今後も認知症は，生物学的精神医学および精神医学全体にとって重要な疾患であり続けるであろう。その理由は，①わが国の認知症患者がこれからも増加し続けること，②認知症は，冒頭に掲げた定義（下線部）に示されるように，生物学的視点だけでは不十分であり，認知症の心理・社会的要因への理解が求められることにある。本稿では，このような認知症全体への精神医学の関与について述べた後に，代表的な認知症である，アルツハイマー病（Alzheimer disease：AD），前頭側頭型認知症（frontotemporal dementia：FTD）について概説する。

Ⅰ．認知症患者数の増加

わが国は，平均寿命（83歳；2010年），高齢者の比率

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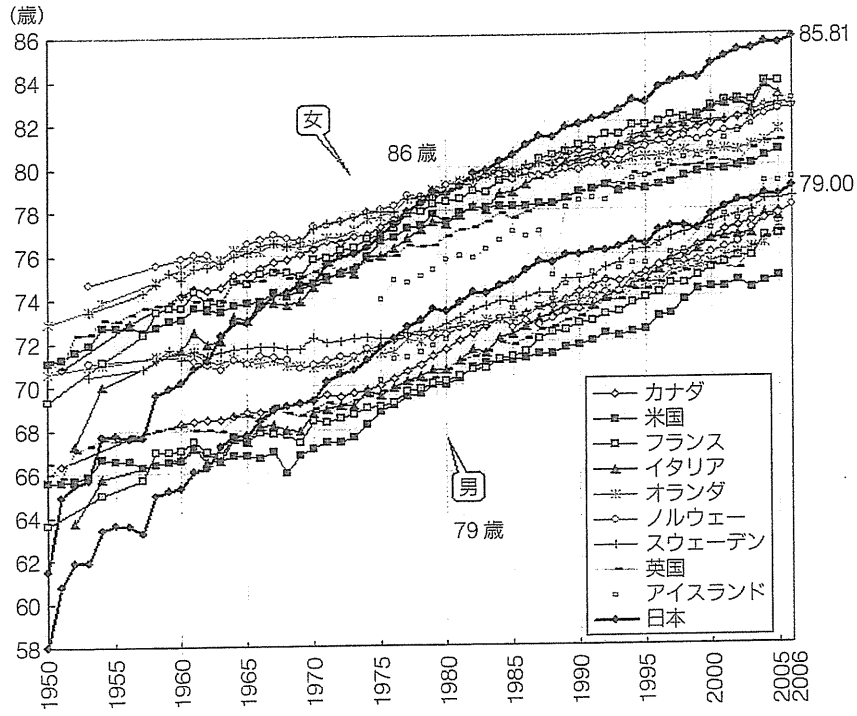

Fig. 1　主要先進国における平均寿命の推移

(65歳以上が23%；2010年)，後期高齢者の比率（75歳以上が10%；2010年)，百歳超長寿者数（40.399人；2009年）のいずれでみても世界一の高齢社会であるが，1番の問題は社会の高齢化が世界一のスピードで進行したことにある。欧米諸国が50〜100年かかって高齢化社会（aging society：高齢者が人口の7%）から高齢社会（aged society：高齢者人口が14%）へと移行したのに対して，わが国は1970年（7%）から1994年（14%）とわずか24年間で高齢社会に移行し，さらに2006年には高齢者人口が20%を超える超高齢社会（super-aged society）となった。このような意味から，わが国は長寿社会のトップランナーであり，欧米諸国と比較してもより深刻な事態に直面していると言える。超高齢社会に関しては欧米にモデルがなく，わが国は独自の視点からこの問題を解決していかなければならない。

日本社会の急速な高齢化は，戦後の急激な平均寿命の延長と出生数の低下による。Fig. 1にわが国の平均寿命の年次推移を示す。第二次大戦直後は男女ともに50歳代であったが，経済成長期に合わせて平均寿命は大幅に伸長し，今や日本人の平均寿命は83歳となり，世界第1位に伸長した。女性の平均寿命（85.91歳；2006年）は世界一であり，男性（79.0歳；2006年）はアイスランドについで第2位である。この平均寿命の延長は，高齢期における死亡者数の減少と周産期死亡率の減少とによる。言葉を換えて言うと，平均寿命の延長は最大寿命の延長によるものではなく，多くの人が最大寿命近くまで生存するようになったことによる。

一方，わが国の出生数は年々低下しており，合計特殊出生率は戦前は4〜5程度あったものが，1974年に人口を維持するのに必要とされる2.08を割り込み，それ以降30年間は低下し続け，2005年には1.26にまで低下した。それ以降の合計特殊出生率はやや持ち直しているものの2008年度は1.37という数字であり，人口維持のために必要とされる2.08にはるかに及ばない。このような出生数の低下により，2005年には死亡数が出生数を上回り，わが国の総人口は減少に転じた。

少子高齢社会はわが国が直面している大きな社会問題であり，田舎や大都市近郊のニュータウンにおけるコミュニティの高齢化，高齢者の核家族化，単身高齢者の増加など多くの社会問題を提起している。このような状況を考えると，認知症は医学だけでなく介護・福祉を含めた社会のシステムの問題として取り組むべきだろう。

Ⅱ．社会的な疾患である認知症

認知症は極めて人間的かつ社会的な疾患である。認知症の最大のリスクは加齢であり，90歳以上の高齢者では約半数が認知症になる。同世代の人の半数が認知症ということになると，これははたして疾患として扱ってよいのかという議論も起こってこよう。認知症は，その有病

率の高さから非常にありふれた社会的な疾患ということができる。

ヒトの行動は多彩でありそのライフスタイルなど複雑な行動様式については個人差が大きく，個人差は加齢とともに大きくなる。この加齢による個人差は認知機能の低下についてもあてはまり，認知症の症状経過，重症度の推移についても個人差が大きい。そして，この個人差は社会的条件により規定されている。症状の多彩さ，個人差は，社会的コンテクストを考慮して初めて理解できるものであり，このような意味からも認知症は極めて社会的な疾患ということができる。

前述したように，認知症は，記憶障害と判断力障害により，社会的・職業的能力に障害をきたすことにより診断される。すなわち，認知症とは社会生活能力の障害を意味する。社会生活能力の障害は，個人の能力と社会の許容度のバランスにより決定される。個人の能力が高くても，社会の許容度が低ければ，社会生活能力は障害される。逆に，個人の能力が低くても社会の許容度が高ければ，その人の社会生活能力は障害されない場合も多い。このような意味で，認知症は極めて社会的な疾患である。

Ⅲ．軽度認知障害

人の記憶力は加齢とともに低下する。以前は，生理的良性もの忘れ（physiological benign forgetfulness; Kral, 1962），あるいは，加齢に伴う記憶障害（age-associated memory impairment：AAMI）（Crook, 1986）と呼ばれ，このような記憶力低下は正常な脳加齢変化の表現とみなされていた時期があった。しかしながら近年では，軽度認知障害（mild cognitive impairment：MCI）（Petersen, 1997）の概念のもとに病的状態として理解されるようになった。MCIの基準は，①記憶力低下の訴えがあり，②検査により客観的な記憶力低下が認められ，③記憶以外の認知機能は正常であり，④日常生活動作（activities of daily living：ADL）が正常であり，⑤認知症ではないこと，と規定されていた。そして，MCIの人は一般人口と比較して認知症に移行する比率が高いことから，認知症の前段階として理解されるようになった。しかしながら，実際の臨床場面では，上記のMCI診断基準に基づいて判断すると，ADの前段階，うつ病，血管性認知症の前段階，FTDの前段階など多くの病態が含まれることとなり，MCIは臨床概念としては不十分な所が多かった。MCIのほかにも認知症の前段階を示す状態として，加齢に伴う認知力低下（age-associated cognitive decline：AACD）（Levy, 1994），

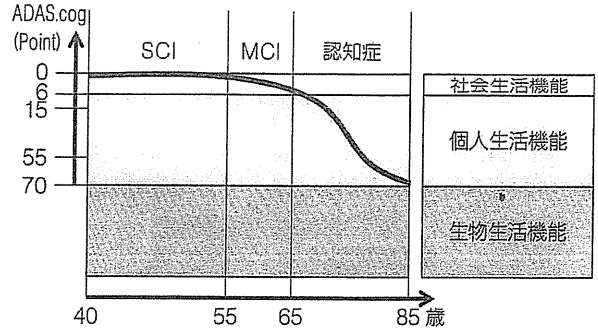

Fig. 2　SCI，MCI，認知症と生活機能障害

年齢に相関した認知力低下（APA, 1994），軽度認知症（mild cognitive decline：MCD）（WHO, 1993）などの概念も提出されているが，いずれの用語も，その意味するところは，社会生活機能は維持されており，認知症とは言えない前段階を規定しようとする概念である。

Ⅳ．主観的認知機能障害

最近は，さらにMCIの前段階として主観的認知機能障害（subjective cognitive impairment：SCI）の概念が言われるようになった。このような関係を図示するとFig. 2のようになろう。SCIとは主観的には記憶障害があるが，その記憶障害は一般人口との比較ではなく個人の以前の状態との低下を意味する。もちろん判断力や社会生活障害が認められない。40歳代以降の多くの人は，20〜30代の自分と比較して記憶力が低下していることを体験している。このような客観的な指標において記憶障害を明瞭にすることができない状態でも，その人の若い時の記憶力と比較すれば，明らかに記憶力は低下しているものであり，このような状態をSCIと呼ぶ。この時期は，広い意味では脳の老化と関連するプロセスであろうが，必ずしも生物学的に十分には規定されていない。しかしながら，アミロイドβ（amyloid β：Aβ）沈着や神経原線維変化（neurofibrillary tangle：NFT）の形成はおそらくこの時期に始まっていると考えられており，今後の生物学的な検討が最も必要とされている時期である。

Ⅴ．生涯を通じた認知機能の変化

ヒトの認知機能の発達は，大脳神経細胞のプルーニング，ミエリン化などの生物学的過程により出生後から思春期まで持続しており，20歳代後半に認知機能レベルが最大に達するとされている。乳児期・幼児期・学童期・

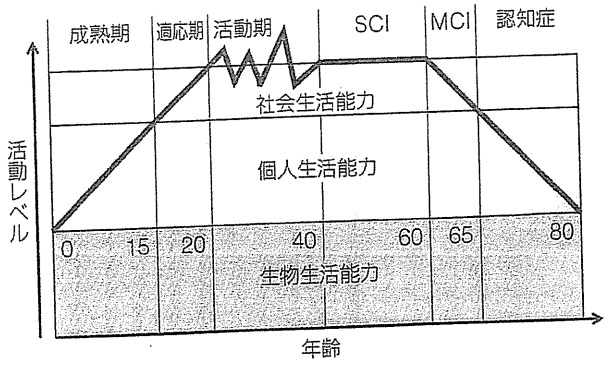

Fig. 3　生涯を通じた認知機能の変化

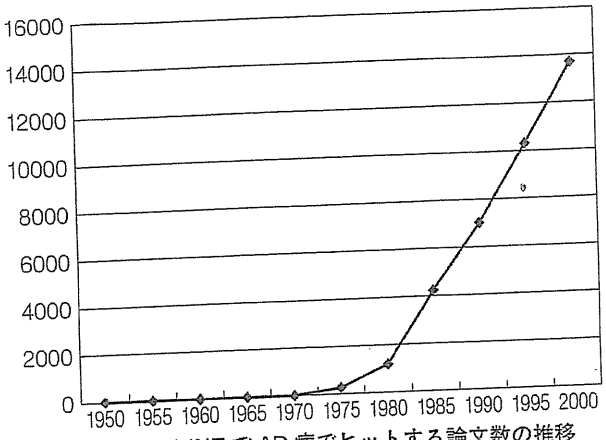

Fig. 4　MEDLINE で AD 病でヒットする論文数の推移

思春期を通じての経験や教育を通じてヒトの認知機能は発達する。そして成人期となり社会的活動を維持している時期には，一定のレベルを維持するが，疾患あるいは精神障害などにより一時的に低下する場合がある。そしてSCI，MCIの時期を経て，一部の者については認知症が発症するものと考えられる（Fig. 3）。認知症診療の要点は，早期診断と早期介入に尽きる。このような観点から言うと，SCIの時期に，認知症に移行する可能性を見出しそれに対応して介入することが肝要と考えられる。

Ⅵ. 代表的な変性認知症

ADは，認知症の50～70%を占める最も頻度の高い一次変性性認知症である。AD発症の危険因子として，高齢，女性，家族歴，頭部外傷，教育歴が挙げられるが，なかでも年齢は最大のリスクである。65歳以上になると，ADの有病率は年齢とともに急激に増加する。その後，5歳ごとに2倍ずつの増加を示し，85歳以上においては40%にも達する[2]。

ADは，記銘力障害で発症し，次第に，失語・失行・失認・遂行機能障害などの認知機能障害と判断力の低下を呈する。びまん性レビー小体病（dementia with Lewy body：DLB）は，65歳以上においてはADについで頻度が高い。DLBは，変動する認知機能，具体的な幻視，パーキンソン症状が特徴であり，大脳皮質神経細胞に出現するレビー小体が特徴的である。前頭側頭葉性変性症（front temporal lober degeneration：FTLD）は65歳以下ではADについで頻度が高い[3]。人格変化，脱抑制，行動異常と言語障害とが前景となり，初期において記憶は保持される。FTDは昔から初老期認知症として知られているピック病を含む上位概念であり，その疾患分類についてさまざまな議論がなされてきた。FTLDを大きくFTD，進行性非流暢性失語症（progressive non-fluent aphasia：PA），意味性認知症（somatic dementia：SD）に区分し，FTDの下位分類として，前頭葉変性型（front lobar degeneration：FLD），ピック病型，運動ニューロン型（motor neuron degeneration：MND）に分けることが多い。

Ⅶ. AD研究の進歩

ADの第1症例がAlois Alzheimerにより発表されたのは1906年であった。クレペリンの精神医学教科書第8版に記載された「アルツハイマー病」は，当初から初老期発症の特殊な認知症であり，その後長い間ADは老年期認知症とは区別して扱われてきた。

ADの病態研究が大きく進展したのは1980年代になってからであり，神経病理学的研究に加えて生化学的研究手法が導入された頃である。Fig. 4にMEDLINE検索でヒットした「アルツハイマー病」に関する論文数の推移を示すが，1980年から急激な増加を示すことがわかる。

NFTは電子顕微鏡では2本の線維がねじり合わさった特徴的な構造（paired helical filaments：PHF）として観察され，1980年代になってAD脳内では正常な線維性蛋白が特徴的な不溶性構造物を形成することが基本的な病理変化であると考えられるようになった。そして，NFTの構成蛋白のための研究がすすめられ，マイクロチュブル，ニューロフィラメント，マイクロフィラメントなどの細胞骨格蛋白の研究と合わせて，その同定がすすめられた[4]。そして，1986年にPHFの構成蛋白はリン酸化タウであることが明らかにされた[5-7]。この頃から，老人斑・NFT・神経細胞脱落の3徴を特徴とするADの病理は老年期認知症の病理と区別できないことから，両者を合わせてADあるいはアルツハイマー型認知症と

Table 1　1980代以降のAD病研究における大きな発見

1983	AD病脳内の不溶性蛋白[4]
1986	NFTの構成蛋白（タウ）の同定[5-7]
1987	アミロイド前駆体蛋白（APP）のクローニング[8]
1991	APP変異（V717I）の同定[9]
1993	AD病のリスクとしてのアポリポ蛋白E4[10]
1995	プレセニリン1の同定[11]
1995	プレセニリン2の同定[12]
1999	γセクレターゼとしてのプレセニリン複合体の同定[13]
1998	FTDP-17におけるタウ変異の発見[14]
1999	アミロイド抗体療法の提唱[15]
2004	「痴呆」の用語が廃止され「認知症」の提唱
2006	プログラニューリンの同定[16]

称するようになった。

続いて老人斑中心部に沈着するAβのアミノ酸配列が明らかにされ，その部分配列を基にしてアミロイド前駆体蛋白（amyloid precursor protein：APP）遺伝子が21番染色体上に同定された[8]。老人斑コアや血管壁に沈着しているAβ蛋白は，APPから切り出されることが明らかになり，そのプロセシングの研究が精力的に推し進められ，その後のAD研究は，Aβの病理，タウの病理を中心に推し進められてきた。AD研究における1980年代以降の大きな業績を年表にまとめて示す（Table 1）。

Ⅷ. ADとFTLDの遺伝子

多くの神経変性疾患の病態解明は，遺伝子が特定されている家族性発症型の知見をもとにして進められてきた。ADは遺伝的には複雑かつ雑多な疾患である（Table 2）。約10%が若齢発症型であり，そのうち約60%は常染色体優性遺伝性を示す。これまで，APP[17]，プレセニリン1（presenilin 1：PSEN1）[18]，プレセニリン2（PSEN2）[19]の変異が家族性AD（familial AD：FAD）の原因遺伝子として同定されている。ADの基本病理過程は，アミロイドカスケード仮説により説明されることが多い。APPからβセクレターゼ（BACE 1），およびγセクレターゼによりAβが切り出されるのであるが，PSENはニカストリン，Aph-1，Pen-2とともにγセクレターゼ複合体を形成する。PSENは，その分子構造の中にγセクレターゼ活性を担っている最も重要な分子である[20]。γセクレターゼにより，アミノ酸数の異なるいくつかのAβが切り出されるが，Aβ40は最も大量にあり，Aβ42は重合しやすく神経細胞毒性を有する。

FADにおいて，Aβの切り出しに関与する基質（APP）およびプロテアーゼ（PSEN1，PSEN2）に変異が見出さ

Table 2　ADの原因

原因	頻度
染色体異常（ダウン症候群）	＜1%
家族性	～25%
若齢発症型（AD1，AD3，AD4）	＜2%
高齢発症型（AD2）	15～25%
孤発性（遺伝要因と環境要因の相互作用による発症を含む）	～75%

れたことから，アミロイドカスケード仮説は広く受け入れられるようになった[21]。この仮説では，脳内Aβの産生，プロセシング，排泄の異常により，Aβの沈着が起こり，Aβ沈着とそれに引き続くNFT形成，神経細胞の変性が惹起され，ADの病理過程が惹起されると考えられている。

高齢発症ADの大部分（75%）は孤発性であるが，一部分には家族性の発症も知られている[22]。高齢発症ADの発症にはアポリポ蛋白E4（APOE）の関与が示されている[23]。APOEε4アリルを1本持つ人ではAD発症のリスクが3倍に，2本持つ人では15倍に上昇する[24]。APOEε4アリルは，AD発症のリスクを高めるだけでなく，ADの発症年齢を引き下げること[25]，治療に対する反応性が悪いことなどが報告されている[26]。

APOEは脂質代謝に関与する蛋白であるが，ADの発症機構については十分に解明されていない。APOEは，コレステロール輸送，酸化ストレス，突起伸長，タウリン酸化，Aβの重合・代謝への関与が推定されており，APOEε4アリルを有すると，Aβ沈着量が増加すること[27]，APOEε4アリルを有する健常高齢者においてもAβの量が増加していることが知られている[28]。

FTDは50歳代，60歳代の若齢発症が多い。AD以上に遺伝性に発症することが多く，FTLDの約40～50%は家族性とされている。この10年間にFTLDの発症に関与する遺伝子が相次いで同定された[29]。まず，家族性のFTDの一亜型でパーキンソニズムを呈し17番染色体上の遺伝子部位と強い連鎖を示す家系（FTDP-17）の解析から，1998年にタウ（MAPT）の変異が見出された[30]。これは，FTLDを特徴づける抗タウ抗体陽性の封入体（FTLD-tau）の形成にタウの変異が関与していることを示したものであり，ADにおけるアミロイドカスケードとの関係で言うと，Aβの関与がなくても，NFTや神経細胞変性が起こり得ることを示した新しい知見であった。FTLDを特徴づける病理学的変化としてタウ陽性の封入体（FTLD-tau）とユビキチン陽性かつタウ陰性の封入体（FTLD-U）とがあることが知られており，その後