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).

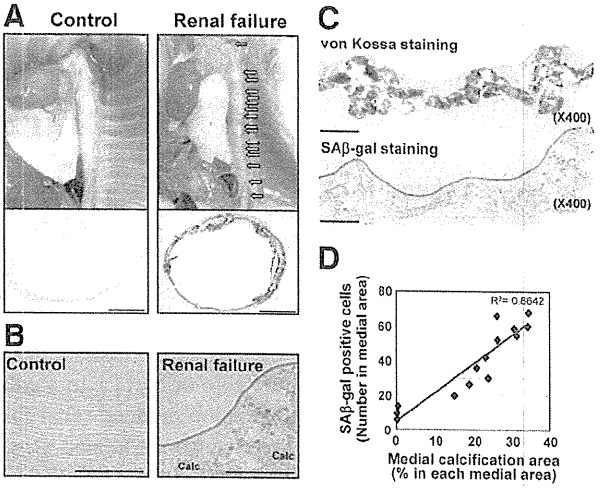

Figure 1. Presence of senescent vascular cells colocalized with calcification in aortic media of renal failure rats. A, Rats with severe renal failure had massive calcification throughout the aorta (right) compared with control rats (left) (n=5). Yellow arrows indicate calcified area. Morphological assessment 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

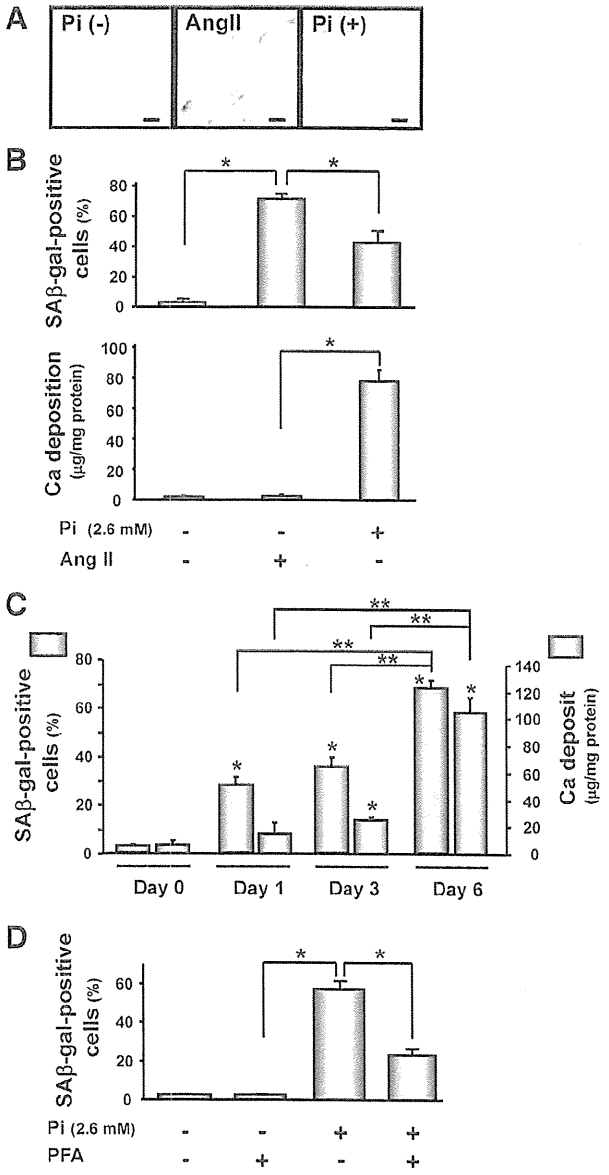

Figure 2. Inorganic phosphate (Pi) stimulation induces cellular senescence in vascular smooth muscle cells (SMCs) via its cotransporter. A, The effect of Pi on senescent transition in human aortic SMCs (HASMCs) was examined. Representative photographs showed that senescence-associated β-galactosidase (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-

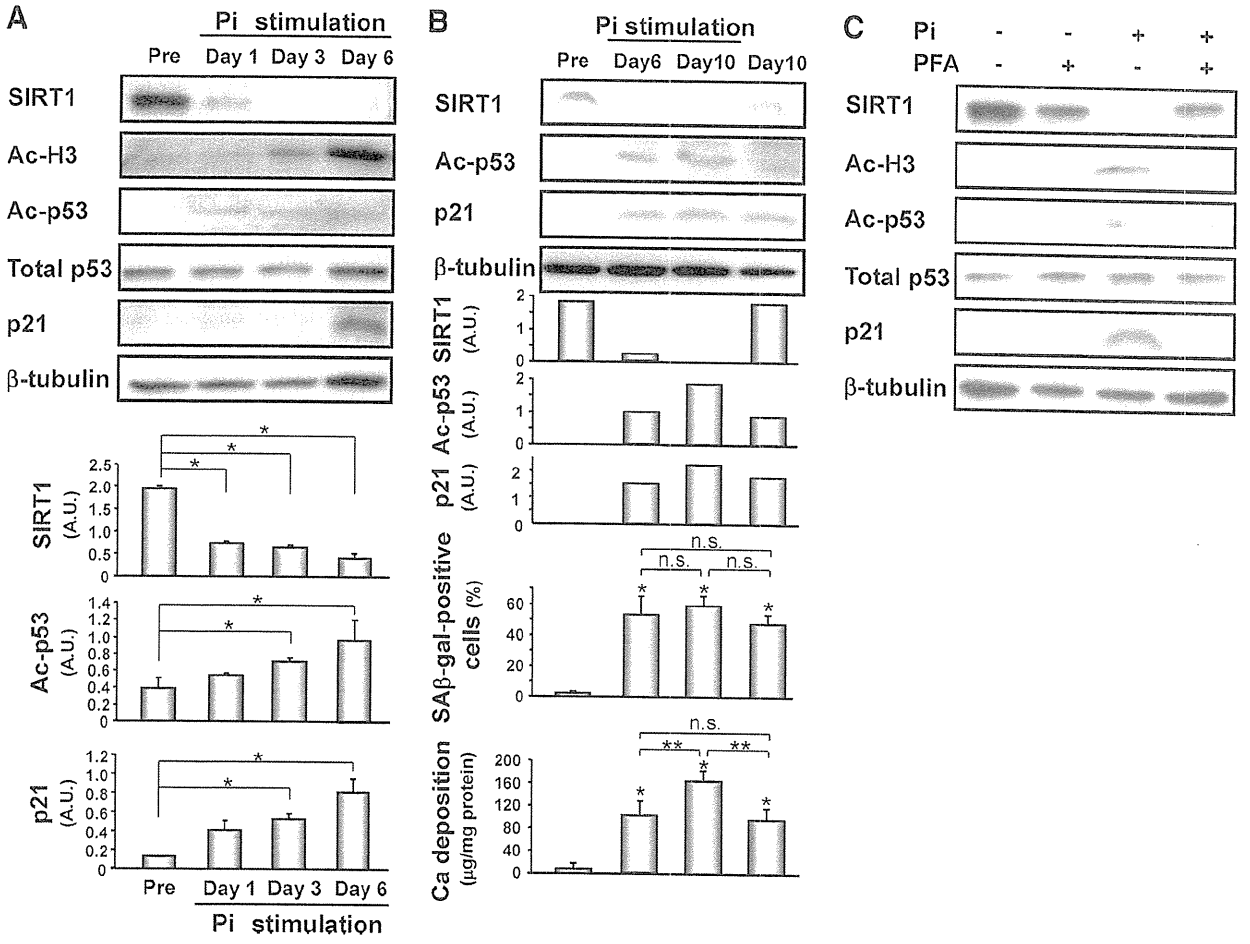

Figure 3. Inorganic phosphate (Pi) stimulation leads to sirtuin 1 (SIRT1) downregulation and subsequent p21 activation. A, The effect of Pi on SIRT1 expression and its downstream pathway was examined. Treatment of human aortic SMCs (HASMCs) with Pi (2.6 mmol/L) showed downregulation of SIRT1 expression, 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-

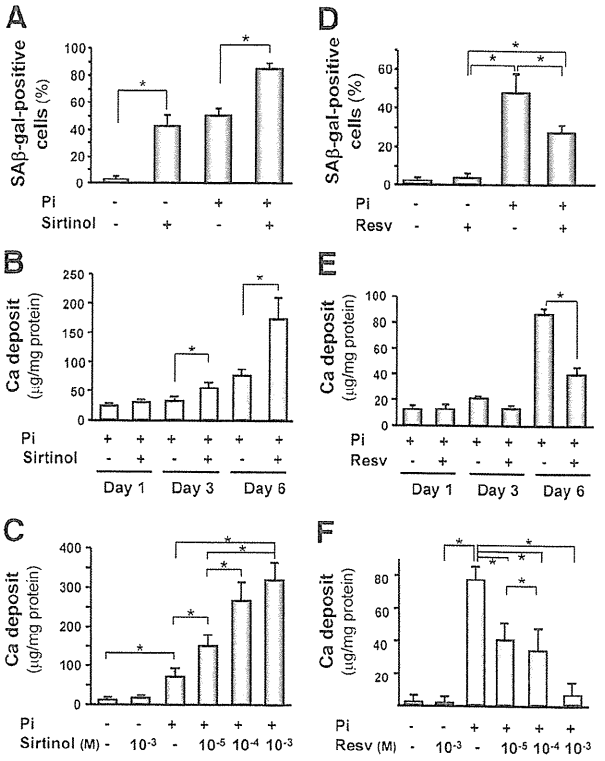

Figure 4. Modulation of sirtuin 1 (SIRT1) affects inorganic phosphate (Pi)–induced senescent phenotypic change and calcification in smooth muscle cells (SMCs). The effects of sirtinol (a chemical inhibitor of SIRT1 activity; A to C) and resveratrol (an activator of SIRT1; D to F) on Pi-induced senescent 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.

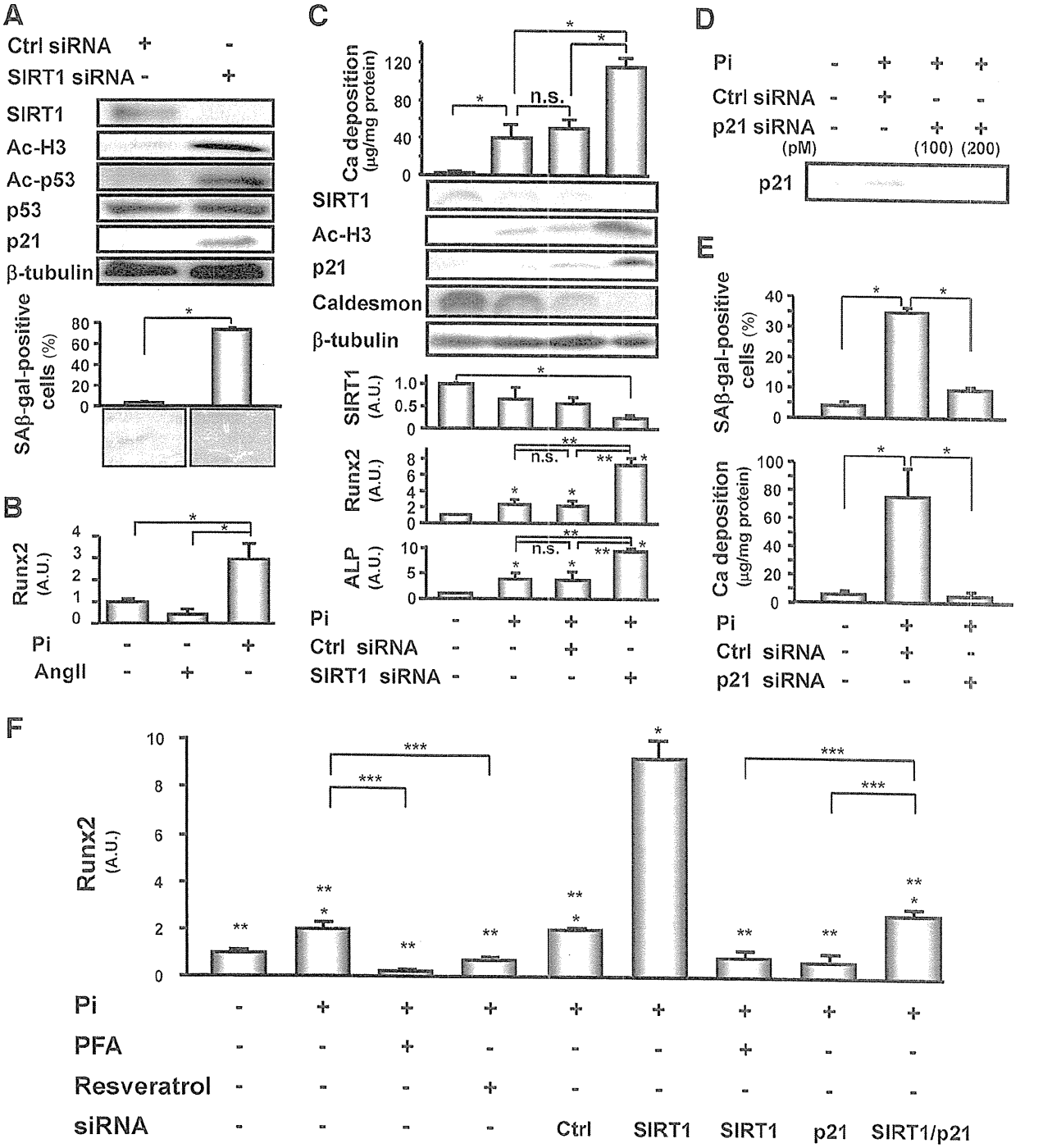

Figure 5. Augmentation of senescence-related smooth muscle cell (SMC) calcification by sirtuin 1 (SIRT1) knockdown in association with osteoblastic phenotypic change and prevention of inorganic phosphate (Pi)–induced changes by p21 knockdown. A, To achieve SIRT1 knockdown in human aortic SMCs (HASMCs), 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).

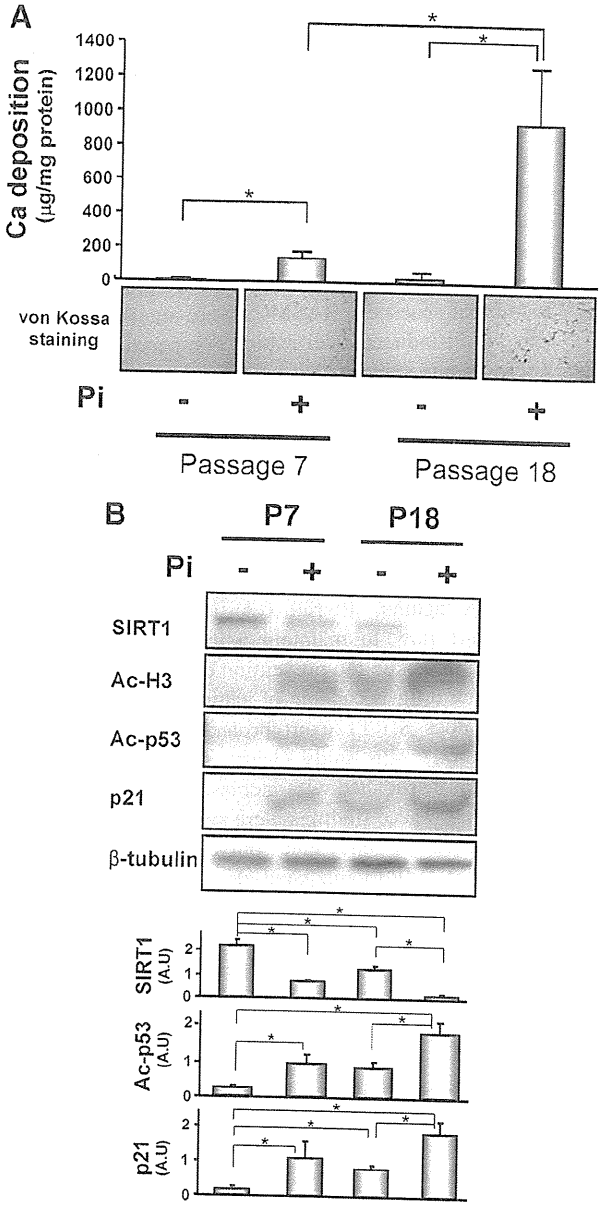

Figure 6. High sensitivity of smooth muscle cells (SMCs) with replicative senescence to inorganic phosphate (Pi)–induced calcification. The effects of replicative senescence in human aortic SMCs (HASMCs) on Pi-induced calcification (A) and sirtuin 1 (SIRT1)-related molecules (B) were also evaluated. 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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Author Contributions: Annweiler had full access to the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses. Study concept and design: Annweiler and Beauchet. Acquisition of data: Calès, Redureau, and Abraham. Analysis and interpretation of data: Abraham, Calès, Redureau, Annweiler, and Beauchet. Drafting of the manuscript: Annweiler, Abraham, Beauchet, Calès, and Redureau. Critical revision of the manuscript for important intellectual content: Fantino and De Decker. Statistical expertise: Annweiler. Administrative, technical, or material support: Beauchet. Study supervision: Annweiler.

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PULMONARY FEATURES ASSOCIATED WITH BEING UNDERWEIGHT IN OLDER MEN

To the Editor: Body mass index (BMI) is one of the most potent prognostic markers in chronic obstructive pulmonary disease (COPD),[1] but it is unclear whether low body weight worsens the symptoms, respiratory function, and prognosis of COPD or only reflects one aspect of advanced COPD.

It is difficult for people with COPD to differentiate the effects of being underweight on pulmonary symptoms and function from the worsening of COPD itself, because a cachexic state is frequently associated with the advance of COPD. One effective approach to this uncertainty would be to assess the respiratory function and symptoms of people with severely low body weight due to causes not associated with respiratory function.[2] These observations would indicate whether nutritional therapy could mitigate respiratory symptoms and dysfunction in people with COPD.

This report presents a case of an 85-year-old man who underwent total gastrectomy and cholecystectomy because of stomach cancer. He had never smoked and had shown no shortness of breath during exercise. He had mild anemia and was diagnosed with stomach cancer. No major postoperative complications occurred, and he left the hospital 12 days after the surgery, but he had severe loss of appetite and his BMI decreased from 25.0 kg/m^2 before the surgery to 17.2 kg/m^2 5 months after the surgery. Although no pulmonary complications were detected on chest computed tomography, he complained of shortness of breath during light exercise corresponding to Medical Research Council (MRC) dyspnea scale Grade 4. His vital capacity (VC) decreased from 3.04 L before surgery to 1.96 L after, and his forced expiratory volume in 1 second (FEV_1)/VC ratio paradoxically increased from 62.8% to 93.9%, masking the preexisting mild obstructive pulmonary disorder. His residual volume to total lung capacity (RV/TLC) ratio reached 62.6% after the surgery, whereas TLC was 94.0% of the predicted value. No hypoxia was observed despite the severe shortness of breath.

The pulmonary features of older outpatients with severely low body weight without respiratory diseases were

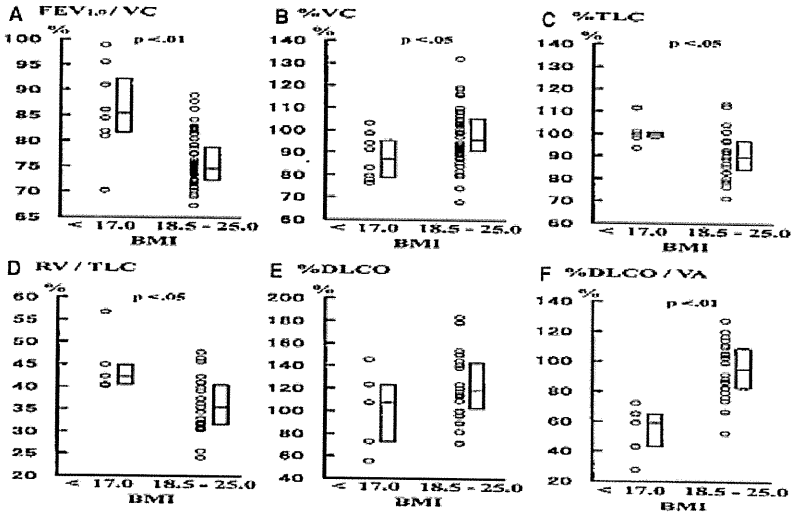

Figure 1. Pulmonary function of underweight patients and normal-weight patients. Ratio of forced expiratory volume in 1 second to VC (FEV_1/VC), percentage of VC (%VC), percentage of TLC (%TLC), residual volume to total lung capacity (RV/TLC) ratio, percentage of diffusing capacity of carbon monoxide (%DLCO), and %DLCO to alveolar volume (%DLCO/VA) of older men with a body mass index (BMI) less than 17.0 kg/m^2 were compared with those of older men with a BMI of 18.5 to 25.0 kg/m^2. Each circular point indicates measured individual data. The boxes indicate the 25th percentile, median, and 75th percentile values. The FEV_1/VC ratio, %TLC, and the RV/TLC ratio were significantly higher, and %VC and %DLCO/VA were significantly lower in the men with BMI < 17.0 kg/m^2.

also investigated. The study population included men aged 65 and older who visited the Department of Geriatric Medicine at the University of Tokyo Hospital from September to October 2008 and men aged 65 and older who visited the Department of Respiratory Medicine at the University of Tokyo Hospital in October 2009. Spirometry had been performed according to Japanese Respiratory Society guidelines.[3] The ethics committee in the medical department at the University of Tokyo approved this study protocol.

The study excluded patients with a FEV_1/FVC ratio of less than 70% or other pulmonary complications observable on chest radiographs or documented in medical records, such as pulmonary malignancies, marked sequelae of pulmonary tuberculosis, interstitial pneumonia, massive pleural effusion, active pneumonia, acute or refractory heart failure, history of lobectomy, and bronchial asthma. Angina pectoris, palpitation, and paroxysmal dyspnea were excluded from the evaluations of respiratory symptoms.

Respiratory symptom and function data were obtained from eight older men with a BMI less than 17.0 kg/m^2. The cause of low body weight of these men were depression (n = 2), a past history of partial or total gastrectomy (n = 3), a past history of ulcerative colitis (n = 1), and no specific diseases (n = 2). Five had shortness of breath when walking up a slight hill, and two complained of shortness of breath even during light exercise, corresponding to MRC dyspnea scale Grades 3 to 4, as observed in the presented case. None had signs of hypoxia at rest.

Data were also obtained from 50 older men with a BMI between 18.5 and 25.0 kg/m^2 (normal weight) without evident pulmonary disease. Only four had shortness of breath during exercise ($P = .001$, Fisher exact test), and none had severe shortness of breath corresponding to MRC dyspnea scale Grade 3 or greater.

The pulmonary function data of the older underweight men without pulmonary diseases (BMI < 17.0 kg/m^2) were compared with those of the older normal-weight men without pulmonary diseases (BMI 18.5–25.0 kg/m^2) using the Mann-Whitney *U* test. DLCO/VA is the ratio of diffusing capacity of carbon monoxide to alveolar volume. Percentage of VC (%VC), percentage of TLC (%TLC), and the percentage of DLCO/VA (%DLCO/VA) were determined by calculating the percentage of individual data to the predicted values; %TLC ($P = .04$) and RV/TLC ratios ($P = .02$) were significantly higher in the underweight men than in those who were normal weight (Figure 1). The %VC and %DLCO/VA was significantly lower in the underweight men than in those who were normal weight (%VC, $P = .04$; %DLCO/VA, $P = .001$; Figure 1). The FEV_1/VC ratio was paradoxically greater in the underweight men, maybe because of the lower VC and higher RV/TLC ratios ($P = .003$).

Being severely underweight involves weakened muscular strength. The higher RV/TLC ratios, lower VC, and higher FEV_1/VC ratios of the older underweight men could be due to the weakened respiratory muscles, especially expiratory muscles.[4] Higher %TLC and lower %DLCO/VA without a concomitant DLCO decrease would indicate lung hyperinflation in the older underweight men. These findings are also consistent with reported lung pathology in starved animals, which shows endogenous alveolar loss without necrosis or inflammation.[5]

These findings support the clinical benefit of promoting nutritional therapy to avoid excessive weight loss in people with COPD and older adults.[6] Further prospective studies evaluating the changes in respiratory symptoms and

function associated with low body weight or alterations in body composition are warranted.

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LIVING WITH STAIRS: FUNCTIONING IN A LARGE COHORT OF OLDER AUSTRALIAN ADULTS

To the Editor: Stair climbing has been encouraged as a health promotion measure in the general population,[1] and health benefits have been demonstrated in young people,[2] but these benefits may not extend to older adults, and living in a building with stairs may prove challenging for older adults. The difficulty that older adults experience in climbing up or down stairs has been associated with a number of chronic conditions, including hypertension, arthritis, and depression.[3] Living with stairs may increase the risk of fall-related injury or death. Nonfatal injuries on stairs are common in older persons and are more likely to result in hospitalization than accidents in younger people; 10% of fall-related deaths occur as a consequence of falls on stairs.[4] For older adults with no partner, managing stairs could be problematic, because many receive support in activities of daily living from their spouses.[5] This is potentially more germane for women, who tend to outlive their spouses. This study aimed to examine health-related difficulties with stairs in a large, prospective cohort study of older adults. The hypothesis was that older adults with a chronic condition and without a partner would report greater difficulty in managing stairs.

METHODS

Data were obtained from the Men, Women and Ageing (MWA) project, which incorporates data from two population-based longitudinal studies that began in 1996: the 1921 to 1926 birth cohort of the Australian Longitudinal Study on Women's Health (ALSWH) and the Perth Health in Men Study (HIMS). Detailed methods for both studies have been described elsewhere.[6,7] The human research ethics committees of the University of Newcastle and the University of Queensland approved the research protocol for the ALSWH. The ethics committee of the University of Western Australia approved the HIMS research protocol.

This analysis is based on data drawn from the fifth ALSWH survey and the third HIMS survey, both conducted in 2008. Participants in HIMS live in an urban area (Perth, Western Australia), whereas ALSWH participants are a national sample including rural and regional areas. To eliminate potential confounding related to area of residence, analyses for this study used data from ALSWH urban residents only. At the time of the 2008 surveys, the women were aged 82 to 87, and the men were aged 77 to 91. For this analysis, the age range of 82 to 87 was used for women (n = 2,421) and men (n = 1,072).

Measures

Difficulty in managing stairs was measured according to a question drawn from the Medical Outcomes Study 36-item Short Form,[8] which asks "Does your health now limit you in these activities? If so, how much: climbing several flights of stairs; climbing one flight of stairs." Responses were yes, limited a lot; yes, limited a little; or no, not limited at all.

Participants reported whether they had ever been diagnosed with any of the following chronic medical conditions: arthritis, osteoporosis, chronic obstructory pulmo-

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第14回認知症を語る会

開催日：2011年2月26日(土)　15：00～
場　所：エーザイ株式会社東京コミュニケーションオフィス
世話人：大庭　建三(日本医科大学老年内科)

講演Ⅰ
生活習慣病の治療薬と認知症

座長：国立長寿医療研究センター　鳥羽　研二
演者：国立長寿医療研究センターもの忘れセンター　櫻井　孝

講演Ⅱ
アルツハイマー病に対するfull stage治療
―ドネペジル増量タイミングと近未来の治療法―

座長：杏林大学医学部高齢医学　神崎　恒一
演者：昭和大学横浜市北部病院神経内科　福井　俊哉

講演Ⅲ
認知症包括的医療の課題―発症前診断から終末期まで―

座長：日本医科大学老年内科　大庭　建三
演者：東北大学加齢医学研究所脳科学研究部門老年医学分野　荒井　啓行

講演I

生活習慣病の治療薬と認知症

櫻井　孝*

1 はじめに

認知症は増えているが，注目すべきことは有病率も増加しているということである．現在の有病率は10～12%程度である．有病率が増加する原因として，生活習慣の変化に伴い認知症の危険因子となる疾患自身が増えてきたという可能性もある．残念ながら，認知症の根本治療薬はなく，生活習慣の介入が非常に重要な課題であると考えられる．

認知症の危険因子は様々あるが，今回は，インスリン抵抗性を中心に述べる．

2 インスリン抵抗性と認知症

インスリン抵抗性，高インスリン血症を来す代表的な疾患は肥満，メタボリックシンドローム，糖尿病である．

肥満と認知症の関連は，長期研究によると肥満がアルツハイマー型認知症(AD)や血管性認知症のリスクを上昇するというデータがある．一方，BMIと認知症の関連はU字カーブであるというデータもあり，一定の結論には至っていない．

メタボリックシンドロームと認知症の関連については，炎症の強いメタボリックシンドロームでは，認知機能の低下が大きいという発表があった[1]．また，Kuopio studyでは，ADとメタボリックシンドロームとの関連が示され，背景として高インスリン血症や炎症，高レプチンなどが影響するといわれている[2]．

糖尿病とADの関係を示すメタ解析では，ADおよび血管性認知症の発症リスクは1.5～2.0倍を示している．ただし年齢依存性があり，若年層や85歳以上の超高齢者では関連は明らかでない．

以上より，インスリン抵抗性は認知症と関連があるように考えられる．

わが国の久山町研究では，糖負荷試験を行っていた135名の剖検脳を解析し，老人斑と耐糖能障害の指標の関連を報告した．その結果，神経原線維変化と耐糖能異常との関連はなかったが，老人斑と，負荷後2時間の血糖，fasting insulin，およびインスリン抵抗性の指標であるHOMA-IRとは関連があることが示されている[3]．

脳内ではインスリンは少量作られるが，その多くは末梢からの移行によって賄われる．脳内のインスリン受容体は海馬や大脳皮質に広く分布しており，様々な作用を発現することが知られている．

高インスリン血症とADに関する仮説を示す(図1)．急性高インスリン血症では，脳内へのインスリンの移行は増加する．ところが，慢性高インスリン血症ではインスリン輸送体がdown regulationを受け，脳内へのインスリンの移行が減少するといわれている．結果，インスリンの脳内シグナルが低下し，本来インスリ

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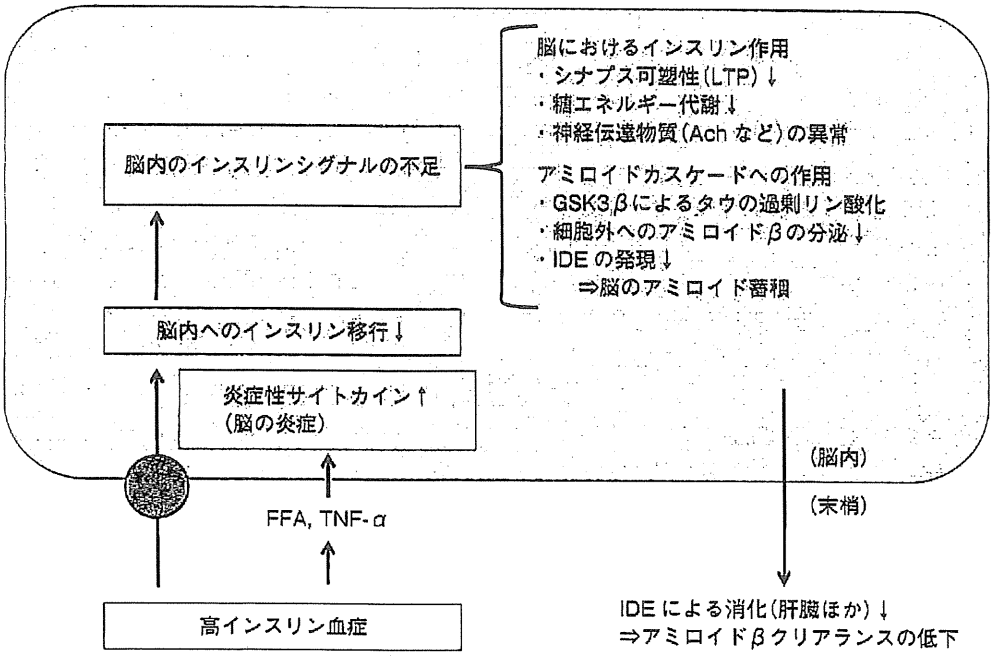

図1　高インスリン血症とアルツハイマー病

ン作用であるシナプスの可塑性，エネルギー糖代謝，神経伝達物質などの合成が低下する．Amyloid cascade にも作用し，インスリンシグナルの下流である GSK-3β が活性化し，*tau* タンパクの過剰リン酸化が進む．

また，脳内から体循環中にアミロイドは流出しているが，肝臓で消化される．この際にインスリン分解酵素によって分解されるが，高インスリン血症があると拮抗阻害されると考えられている．

さらに高インスリン血症では，炎症性のサイトカインが分泌され，脳内の炎症性サイトカインも増加し，神経障害を来すとされている．

細胞レベルで考えると，神経細胞でも様々なインスリンシグナリングが明らかになってきた．ところが，Aβ が併存すると，インスリン受容体の数が細胞内にトランスロケーションし，細胞表面のインスリン受容体が減少し，インスリンシグナルが低下することも基礎研究で示されている．

インスリン抵抗性を改善する薬剤として，ビグアナイド，RA 系降圧薬，α-blocker などがある．ペルオキシゾーム増殖剤応答性受容体(PPAR)は，核内受容体のスーパーファミリーの1つで，体内および食品に依存する低分子の脂溶性生理活性物質などをリガンドとし，神経細胞内にも多く分布していることが知られている．α と γ のサブタイプがあり，α のアゴニストは高トリグリセリド(TG)血症時に用いられるフィブラート系の薬剤である．一方，γ のアゴニストがチアゾリジンである．チアゾリジンは経口投与により髄液中まで移行することが確認されている．

高インスリン血症と AD との関連から，インスリン抵抗性改善薬が AD の進展を抑制する可能性が示唆される．

3　症例提示

1．症例1

81歳女性．肥満，高血圧，脂質異常を合併したメタボリックシンドローム型高齢者糖尿病である．もの忘れを主訴に来院され，神経心理，画像検査を行い，probable AD と診断した．

糖尿病に関しては，インスリン抵抗性が強いため，ビグアナイド系薬を処方した．当初HbA1cが8.5%近くあったが徐々に低下した．6カ月の経過期間において，MMSE 24点，HDS-R 20点を維持できていた．しかしその後，血糖が悪化し，1年後にはMMSE，HDS-Rともに4～5点程度低下した．この時点でピオグリタゾンの投与を開始したところ，血糖が急速に改善し，同時に認知機能の改善が認められた．MMSE，HDS-Rともに4点以上の回復があった．

しかし本例では，認知機能の改善がピオグリタゾンの作用によるものか，糖代謝の改善によるものかは特定できなかった．

2．症例2

79歳女性．2型糖尿病とADと診断されている．本例の特徴は，HbA1cが6～7%の間で厳格にコントロールされているという点である．

ドネペジル投与後ピオグリタゾンを投与したところ，ADASが20点から14点に改善した．そこでピオグリタゾン30 mgに増量したところ，数カ月後には下肢浮腫が認められ，ピオグリタゾンを中止した．本例では，ピオグリタゾンが脳機能を改善させたと考えられた．

4 AD治療薬としてのインスリン抵抗性改善薬

インスリン抵抗性改善薬のAD治療における有効性は，様々な文献で報告されている[4-6]．これらの文献で筆者が着目した点は，HbA1cが6.5～7.0%で，罹病期間が約3年，発症年齢が75歳以前の糖尿病であるという患者群の特性である．すなわち，インスリン使用者，重症糖尿病患者は除外され，比較的軽度で高齢発症の糖尿病患者を解析しているという点が重要であると考える．

ロシグリタゾンでの研究結果は様々あるが，観察期間が24～54週と比較的短いため，長期間における効果をみることが重要な課題である．

軽度認知障害(MCI)を合併した2型糖尿病で，ロシグリタゾンを投与し，36週追跡した結果，認知障害の進行を抑制したという報告がある[7]．また一方，糖尿病のないAD患者にピオグリタゾンを投与し，1.5年追いかけた結果，効果はなかったという[8]．これらの結果から，長期効果はまだ一定の結論には至っていない．

チアゾリジンは脳血管にも作用する．ピオグリタゾンは脳卒中の二次予防で，プラセボに対し，47%程度発症率を減少させた．ただし，この解析では脳卒中の細かな病型の分類は検討されていない．脳血管障害を合併したADに対する効果を考えるときには，small vessel diseaseに対する効果を考えなければならない．

ADの進展ステージをみると，認知症として発症した時点で既に脳内には多くの病理変化が生じており，神経変性も起こしている．すなわちADは長期に及ぶ疾患であり，インスリン抵抗性改善薬はより早期のADで効果を示すものと考えられる．認知症の進行した例ではその作用は限定的かもしれない．

ビグアナイドがインスリン抵抗性を改善することはよく知られているが，脳機能に対する臨床試験に関する論文は検索した範囲ではなかった．基礎論文で，メトフォルミンを用いると神経細胞内のAβ40と42が増えてくるというデータがある[9]．ビグアナイドはAMPキナーゼを介して耐糖能障害を改善する．運動でもAMPキナーゼは改善する．一方で，運動により認知能は改善することも知られており，ビグアナイドの中枢に及ぼす作用については，さらに検討が必要であろう．

2型糖尿病において，低血糖は認知症のリスクとなる．またHbA1cの上昇も認知症のリスクを上昇させる．さらに高齢糖尿病患者における血糖の変動が悪いとされている．24時間血糖モニタリングしMMSEとの相関を調べたところ，明確な逆相関を示した(**図2**)[10]．われわれの基礎研究においても，血糖の変動が大きいとADモデルでは神経変性が増加することがわかった．海外からの報告では，低血糖から血糖を補正するときにNADPH oxidaseに伴う酸化ストレスが細胞死を起こすとしている．

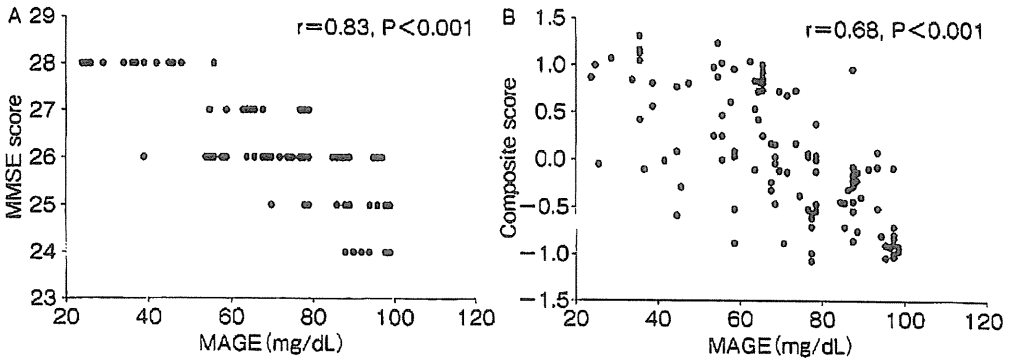

図2　高齢者糖尿病における血糖の変動と認知機能(文献10より引用)

つまり認知症を合併した糖尿病の血糖管理では，低血糖，過剰な高血糖を避け，しかも血糖の変動を最小にすることが望ましいと思われる．では認知症を合併した高齢者糖尿病で，どのような薬剤が適切であるかを次に述べたい．

5 DDP-Ⅳ阻害薬の台頭

最近非常に注目されているのがDDP-Ⅳ阻害薬である．DDP-Ⅳ阻害薬には，食事の度に上下動する血糖値を平坦化する作用がある．

インクレチンは糖尿病治療の新しい扉を開けた．インクレチンは脳腸管ペプチドで，小腸のL細胞，K細胞から分泌され，膵臓に働いて，インスリンの分泌を調整する．血糖値が低い場合にはインスリン分泌を抑制するが，血糖値が高い場合，β細胞から分泌されるインスリンが増えるという生体にとっては合目的なペプチドである．

このインクレチンファミリーの受容体は全身に分布しており，心臓に対する臓器保護，肝臓での糖産生抑制など様々な働きをしている．脳では食欲抑制という本来の働きが知られている．神経細胞にGLP-1が受容体に働くとcAMP kinase，PKA，pI3-kinaseなどのシグナルが活性化され，様々な細胞保護的な機能を発現することが知られている．

インクレチン関連の神経細胞での作用として，神経アポトーシスの抑制，軸索突起の成長を加速することが報告されている．

さらに注目すべきことに，インクレチンは，神経細胞内のAβの量を低下させるという報告もみられる．

これらの知見を踏まえ，GLPのアナログであるエキセナチド(注射薬)がAD治療に有効である可能性が期待されており，米国では既に治験が始まっている．

チアゾリジン，インクレチン関連薬はADの根本治療ではないが，ADの進展予防という意味では今後期待される．

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質疑応答

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(発言順)

鳥羽　どうもありがとうございました．何かご質問，ご意見はございませんか．

山田　国立長寿医療研究センターには，認知症の行動・心理症状(BPSD)を改善させる病棟があると伺っております．医師の科の構成と，BPSDが強い場合はどの科の医師がどのようなことを行っているのでしょうか．

櫻井　外来ではそれぞれの外来担当医が診ていますが，コントロールの難しい症例は精神科の医師に相談しています．

入院は2月現在，まだ稼働していないのですが，システムとしては，たとえば癌の患者が入院してきた場合，腫瘍科の医師と2人体制をとるようになっています．

何よりBPSDでは，介護力が大事になってきますので，介護力に関する教育システムを作っている段階です．

山田　認知症の方は，基本的に住み慣れた地域で普通の生活ができることを目標に治療するものだと思います．入院することでBPSDが改善をするということは，在宅や外来が難しい方というイメージがあるのですが，今後の病棟戦略についてお聞かせいただけないでしょうか．

櫻井　確かに在宅や施設で安心して暮らしていただくというのは一番よいことだと思います．ですから，短期間入院していただいて，帰っていただくということが大原則です．

鳥羽　認知症疾患センターには地域型と中核型がありますが，当院は地域型です．ですから個室がなく，重症の神経疾患の場合はタイアップしている精神疾患の専門病院にお願いしています．あくまでBPSDと身体疾患がある場合，身体疾患を主体として診ています．ただし，当院にも精神科の医師がいますので，軽症から中等症までは十分対応できるという仕組みです．

ほかにいかがですか．

岩本　以前，ピオグリタゾンを使った抗認知症効果に関する先生のご講演を拝聴しました．おそらく糖尿病を診られている医師は，糖尿病に対して手替え，品替え，様々な治療をしながら，認知症の患者を診ているということで，母集団が異なる印象があります．

先生のところで行ったピオグリタゾンを使った対照群に対して，認知症が先なのか，糖尿病が先なのかといった患者のインフォメーションがいただければありがたいと思います．

櫻井　われわれが診ていた患者は，10～20年くらい糖尿病を患っていた方の中で認知症を合併された方がほとんどです．ですから，まずは血管合併症があり血糖値も悪化したような方で，家族の協力が得られる方です．

岩本　もう1点，糖尿病があると，糖尿病がない方に比べ認知機能の低下が早いという成績と，むしろ軽いといった経過のレポートがあるのです．この点について，何かデータはお持ちでしょうか．

櫻井　その点はよくわからないというのが現状だと思いますが，よくよく考えますと，血管が悪いのか，アミロイド代謝異常に伴う代謝障害が悪いのかというところにいきつくように思います．糖尿病では，アミロイド代謝異常の方は軽度だ，血管障害の方がより重きがあるという

ようなことが一番多いのではないかと考えています．血管障害の強い方なのか，アミロイド代謝異常の強い方なのかというところをきちんと分けて考える必要があるのではないかと考えています．

鳥羽　最後のところは非常に本質的なところなので，ご意見のありそうな方も表情でうかがえましたが，よろしいですか．

荒井　少数例なのですが，われわれは糖尿病のある方とない方で，アミロイドのイメージングというのを行ったことがあります．少数例のためはっきりしたたことは申し上げられませんが，蓄積量は，認知機能も一定のところでとると変わらないのです．糖尿病のない方とある方で同じくらいの色素沈着をしているので，その意味づけまではわからないのですが，そういうことがイメージングを使うと少しわかるという話です．

鳥羽　ありがとうございました．

まだいろいろあると思いますが，また意見交換会のときにしたいと思います．櫻井先生，ありがとうございました．