JNS

LABORATORY INVESTIGATION

Activation of the NLRP3/IL-1β/MMP-9 pathway and intracranial aneurysm rupture associated with the depletion of ERα and Sirt1 in oophorectomized rats

Tadashi Yamaguchi, MD, Takeshi Miyamoto, MD, PhD, Eiji Shikata, MD, Izumi Yamaguchi, MD, PhD, Kenji Shimada, MD, PhD, Kenji Yagi, MD, PhD, Yoshiteru Tada, MD, PhD, Masaaki Korai, MD, PhD, Keiko T. Kitazato, MS, Yasuhisa Kanematsu, MD, PhD, and Yasushi Takagi, MD, PhD

Department of Neurosurgery, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan

OBJECTIVE Subarachnoid hemorrhage (SAH) due to intracranial aneurysm (IA) rupture is often a devastating event. Since the incidence of SAH increases especially in menopause, it is crucial to clarify the detailed pathogenesis of these events. The activation of vascular nucleotide-binding oligomerization domain–like receptor family pyrin domain–containing 3 (NLRP3) inflammasomes has been studied in ischemic stroke and cardiovascular disease. However, the role of NLRP3 in IA rupture still needs to be explained. The authors sought to test their hypothesis that, under estrogen-deficient conditions, activation of NLRP3 inflammasomes via downregulation of the estrogen receptor (ER) facilitates IA rupture.

METHODS Ten-week-old female Sprague Dawley rats with and without oophorectomy were subjected to hemodynamic changes and hypertension (OVX^+/HT and OVX^-/HT, respectively) and fed a high-salt diet. Separately, using human brain endothelial cells (HBECs) and human brain smooth muscle cells (HBSMCs), the authors tested the effect of NLRP3 under estrogen-free conditions and in the presence of estradiol or of ER agonists.

RESULTS In OVX^+/HT rats, the frequency of IA rupture was significantly higher than in OVX^-/HT rats ($p = 0.03$). In the left posterior cerebral artery prone to rupture in OVX^+/HT rats, the levels of the mRNAs encoding *ERα* and *Sirt1*, but not of that encoding *ERβ*, were decreased, and the levels of the mRNAs encoding *NLRP3*, interleukin-1β (*IL-1β*), and matrix metalloproteinase 9 (*MMP-9*) were elevated. Immunohistochemical analysis demonstrated that the expression profiles of these proteins correlated with their mRNA levels. Treatment with an ER modulator, bazedoxifene, normalized the expression profiles of these proteins and improved SAH-free survival. In HBECs and HBSMCs under estrogen-free conditions, the depletion of ERα and Sirt1 and the accumulation of NLRP3 were counteracted by exposure to estradiol or to an ERα agonist but not to an ERβ agonist.

CONCLUSIONS To the authors' knowledge, this work represents the first demonstration that, in an aneurysm model under estrogen-deficient conditions, the depletion of ERα and Sirt1 may contribute to activation of the NLRP3/IL-1β/MMP-9 pathway, facilitating the rupture of IAs in the estrogen-deficient rat IA rupture model.

https://thejns.org/doi/abs/10.3171/2022.4.JNS212945

KEYWORDS estrogen deficiency; estrogen receptor; intracranial aneurysm; NLRP3 inflammasome; Sirt1; vascular disorders

The rupture of intracranial aneurysms (IAs) is a major cause of subarachnoid hemorrhage (SAH). Epidemiological studies have shown that the incidence of IAs and SAH is higher in women, particularly those who are postmenopausal, than in men.[1,2] In a large cohort of patients with unruptured IAs, Morita et al.[3] found the condition to be more prevalent in women, in confirmation of the results reported by others.[4,5]

Estrogen exerts diverse vascular effects mediated mainly by estrogen receptor (ER)–α and ERβ.[6] Aging alters estrogen-mediated modulation of inflammatory biomarkers in women.[7] In our IA model mimicking aging-associated

ABBREVIATIONS Ang II = angiotensin II; BZA = bazedoxifene; DPN = diarylpropionitrile; eNOS = endothelial nitric oxide synthase; ER = estrogen receptor; HBEC = human brain endothelial cell; HBSMC = human brain smooth muscle cell; HT = hypertension; IA = intracranial aneurysm; IL-1β = interleukin-1β; MMP-9 = matrix metalloproteinase 9; OVX^+ = oophorectomized; OVX^- = nonoophorectomized; NLRP3 = nucleotide-binding oligomerization domain–like receptor family pyrin domain–containing 3; NOX4 = NADPH oxidase 4; PCA = posterior cerebral artery; PPT = propylpyrazoletriol; SAH = subarachnoid hemorrhage; Sirt1 = sirtuin 1.

SUBMITTED December 24, 2021. **ACCEPTED** April 14, 2022.
INCLUDE WHEN CITING Published online May 20, 2022; DOI: 10.3171/2022.4.JNS212945.

estrogen deficiency, the observed morphological changes at the left posterior cerebral artery (PCA) were shown to be associated with elevated mRNA levels and protein expression of interleukin-1β (IL-1β) and matrix metalloproteinase 9 (MMP-9),[8,9] indicating proinflammatory changes and higher vulnerability compared with other cerebral arteries. However, the pathological mechanisms leading to the upregulation of IL-1β and MMP-9 under estrogen-deficient conditions remain to be clarified in IA. The nucleotide-binding oligomerization domain–like receptor family pyrin domain–containing 3 (NLRP3) inflammasome is a multiprotein oligomer responsible for the activation of inflammatory responses. The NLRP3 inflammasome processes pro–IL-1β to its mature IL-1β form and induces the release of this cytokine, leading to inflammation and tissue damage.[10] Based on the high expression of IL-1β and MMP-9 in the vulnerable PCA in IA models,[8,9] we focused on the role of NLRP3. Although NLRP3 has been studied in ischemic stroke and cardiovascular disease[11,12] with and without kidney disease, there have been (to our knowledge) few studies of the role of NLRP3 in IA. Zhang et al.[13] documented that accumulation of NLRP3 was observed in ruptured and unruptured IAs in a study of 36 human surgical samples of cerebral aneurysms. However, details of the mechanisms underlying elevation of NLRP3 in ruptured and unruptured IAs remain unknown. Therefore, the data in that paper are associative and cannot be used to determine causation.

On the other hand, the activation of sirtuin 1 (Sirt1), an NAD^+-dependent protein deacetylase, has been proven to be a key regulator of the inflammatory response in cardiovascular disease.[14] Sirt1 protein levels are decreased in several chronic inflammatory diseases and as part of the cellular response to inflammatory, metabolic, and oxidative stressors.[15] Indeed, chronic inflammation is associated with increased nuclear factor kappa B RelA/p65 activity, which secondarily leads to decreased Sirt1 levels.[16] The estrogen-Sirt1 axis plays a pivotal role in protecting arteries from menopause-induced senescence and atherosclerosis.[17] However, the mechanisms underlying regulation of the expression of NLRP3 and Sirt1, and how the levels of these proteins are associated with the expression of ERs, remain unclear in IA.

We hypothesized that, under estrogen-deficient conditions, the activation of the NLRP3 inflammasome due to depletion of Sirt1 may facilitate IA rupture. To better understand the mechanisms regulating the expression of ERs, NLRP3, and Sirt1, we employed a rat aneurysm model using animals that had undergone oophorectomy and those that had not undergone oophorectomy. In addition, oophorectomized rats were treated with an ER modulator to confirm the effects on NLRP3, ERα, and Sirt1. This work was complemented by in vitro studies using human brain vascular endothelial cells (HBECs) and human brain vascular smooth muscle cells (HBSMCs) grown under estrogen-free conditions in the presence or absence of ER modulators.

Methods

All animal experiments were approved by the ethics committee of the Institute of Tokushima University Graduate School and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Assessment and Treatment in the Intracranial Aneurysm Model

Animals were subjected to induction of IA as shown in Fig. 1A and the Online Appendix and as detailed elsewhere.[8] Ten-week-old Sprague Dawley female rats (230–260 g) were maintained on a high-salt diet. Furthermore, hemodynamic changes were induced by carotid artery ligation, and bilateral posterior renal arteries were ligated to induce hypertension (HT). To assess the role of oophorectomy-induced estrogen deficiency on the rupture of IAs, we randomly divided the animals into two groups. One group of rats (n = 24) was oophorectomized (OVX^+/HT rats) to decrease their plasma estradiol level;[18] the other group (n = 24) was not oophorectomized (OVX^-/HT rats). All OVX^+/HT and OVX^-/HT rats were observed from 6 to 19 weeks (mean of 90 days) after group assignment. Five rats in each group died within the 1st week of surgery-related issues and were excluded from this study. In each experimental group, judgment of the rupture was performed by an evaluator blinded to the group identity of the animal.

We next assessed whether treatment of these animals with bazedoxifene (BZA), an ER modulator, altered the protein expression of ERs, Sirt1, and NLRP3, as well as possible effects on the frequency of aneurysm rupture. Specifically, 36 rats were randomized and assigned to two groups at 6 weeks after oophorectomy; one group was treated with 1.0 mg/kg/day of BZA based on our previous study[19] and the other served as the vehicle control. Based on a previous report,[19] BZA was suspended in 5% gum arabic solution and administered orally once per day for 90 days.

Assessment in Cell Lines

For cell culture under estrogen-free conditions, we used phenol red–free minimum essential medium α supplemented with estrogen-depleted fetal bovine serum.[20] HBECs and HBSMCs were then treated for 24 hours with or without 17β-estradiol (10^{-7} M), in both the presence and absence of 10^{-6} M propylpyrazoletriol (PPT; Sigma H6036), diarylpropionitrile (DPN; Sigma H5915), or human angiotensin II (Ang II; Sigma A9525). The vehicle control consisted of complete medium (minimum essential medium α supplemented with fetal bovine serum) in the absence of 17β-estradiol, PPT, DPN, and Ang II. A detailed *Methods* section is provided in the Online Appendix.

Statistical Analysis

Statistical analyses were performed using Prism version 7.0 software (GraphPad). Fisher's exact test was used to analyze the incidence of aneurysm rupture. The SAH-free survival rate was analyzed using the log-rank test. Sequentially obtained data were analyzed using two-tailed one-way ANOVA followed by the Kruskal-Wallis test for multiple group comparisons. Data from two groups were

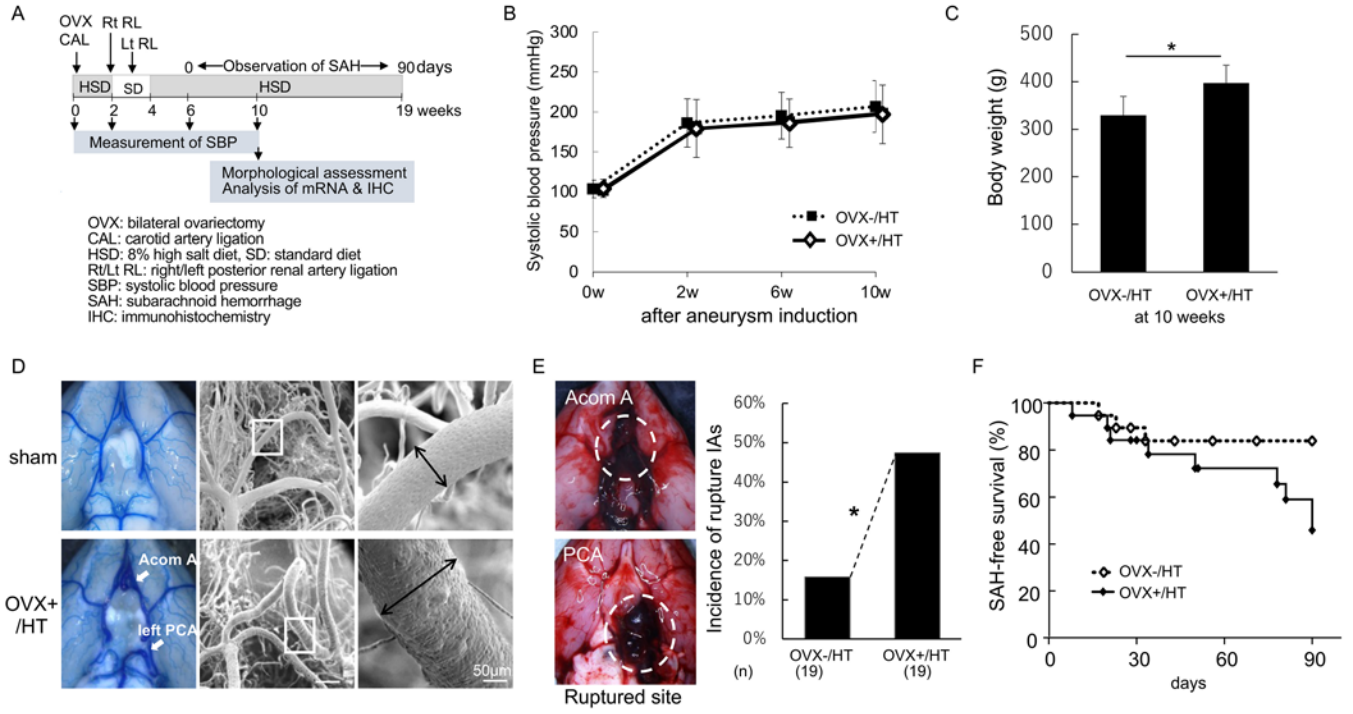

FIG. 1. Characteristics of IAs in OVX$^+$/HT and OVX$^-$/HT rats. **A:** Schematic of the experimental protocol. **B:** Systolic blood pressure before and 2, 6, and 10 weeks after aneurysm induction. **C:** Body weights recorded at 10 weeks after aneurysm induction (n = 16, each group). *p < 0.05, two-tailed unpaired Student t-test. Data are presented as mean ± SD. **D:** Representative vascular corrosion casts from sham-operated and OVX$^+$/HT rats. Note the formation of an unruptured IA, the irregular endothelial cell imprints, and enlargement of vessel at the left PCA in the OVX$^+$/HT rat. **E:** Ruptured aneurysms at the anterior communicating artery (Acom A) and PCA in an OVX$^+$/HT rat and the incidence of ruptured IAs in OVX$^-$/HT and OVX$^+$/HT rats. *p = 0.03, Fisher's exact test. **F:** SAH-free survival in OVX$^-$/HT and OVX$^+$/HT rats.

analyzed using a two-tailed unpaired Student t-test. Where relevant, data are presented as the mean ± SD. Differences were considered significant at $p < 0.05$.

Results

Increased NLRP3 Expression and Decreased ERα Expression at the Left PCA Prone to Rupture

As shown in Fig. 1A, 2 weeks after ligation of the carotid arteries, HT was induced, and the condition was maintained during the subsequent observation period (Fig. 1B). OVX$^+$/HT rats had higher body weights than OVX$^-$/HT rats (Fig. 1C). The left PCA of all OVX$^+$/HT rats demonstrated the irregular endothelial cell imprints and the vessel enlargement (Fig. 1D) reported in human ruptured saccular IAs.[21] Rupture in the left PCA was seen in 3 (16%) of 19 animals during the observation period between 8 and 60 days after the aneurysm-induction process (oophorectomy, hemodynamic changes, and HT) for 4 weeks in OVX$^+$/HT rats. On the other hand, ruptures in other vessels were seen during the 60- to 90-day observation period, including the anterior communicating artery in 3 animals (16%), the internal carotid artery in 2 animals (10%), and the middle cerebral artery in 1 animal (5%). Notably, these findings indicated that ruptures occurred earlier and at a higher frequency in the PCA than in the other arteries. However, it was difficult to count the correct number of unruptured IAs at sites prone to rupture. The incidence of aneurysm rupture was significantly higher (47% vs 16%, p = 0.03; Fig. 1E) and the SAH-free survival rate was lower in OVX$^+$/HT rats than in OVX$^-$/HT rats (Fig. 1F).

We examined the effect of estrogen deficiency on expression (at both the mRNA and protein levels) of the estrogen receptors ERα and ERβ as well as that of NLRP3, IL-1β, and MMP-9 in the rupture-prone vascular wall of the left PCA. Notably, the level of the mRNA encoding *ERα*, but not that of the mRNA encoding *ERβ*, was significantly decreased in OVX$^+$/HT rats compared with sham-operated rats. In contrast, the levels of the mRNAs encoding *NLRP3*, *IL-1β*, and *MMP-9* were significantly elevated in OVX$^+$/HT compared with OVX$^-$/HT rats (Fig. 2).

Elevation of NLRP3 Protein Level Is Associated With Decreased Sirt1 Protein Level Under Conditions of Estrogen Deficiency

To examine the role of estrogen deficiency in the accumulation of NLRP3, IL-1β, and MMP-9 and depletion of ERα, we focused on Sirt1, a protein that is known to attenuate the activation of inflammasomes.[14,15] The level of the *Sirt1* mRNA was significantly lower in OVX$^+$/HT rats than in the sham-operated rats (Fig. 3A), suggesting that the *Sirt1* transcript level is decreased in response to estrogen deficiency.

To further clarify the relationship among the expres-

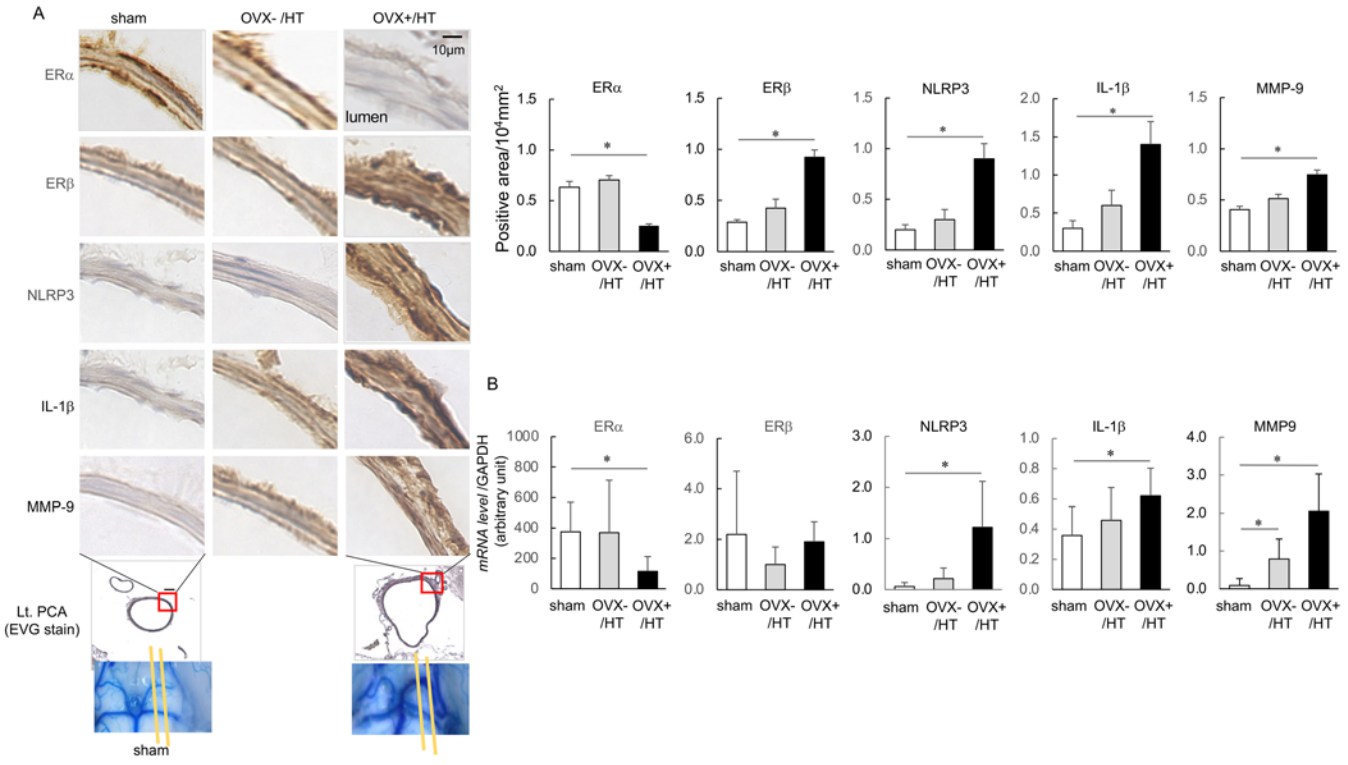

FIG. 2. Changes in the expression (at the mRNA and protein levels) of *ERα*, *NLRP3*, *IL-1β*, and *MMP-9* in the left PCA prone to rupture. **A:** Elastica van Gieson (EVG) staining and representative immunohistochemistry results following staining for ERα, ERβ, NLRP3, IL-1β, and MMP-9. Sections were cut vertically at the left PCA as shown by the *yellow lines*. The *red boxes* denote the portion of the PCA that is prone to rupture. Note disruption of internal elastic lamina in the intima and the degenerated matrix at the PCA in the media of the OVX^+/HT rat. Positive area was analyzed by BZ-X710 (Keyence). **B:** The levels of the mRNAs encoding *ERα*, *ERβ*, *NLRP3*, *IL-1β*, and *MMP-9* in the left PCA OVX^+/HT and OVX^-/HT rats at 10 weeks after IA induction; values in sham-operated rats are provided for comparison. Each column of data indicates mean ± SD. *$p < 0.05$, one-way ANOVA followed by the Kruskal-Wallis test (6 rats in each group).

sion of ERα, ERβ, Sirt1, and NLRP3 in PCAs prone to rupture, we used HBSMCs and HBECs cultured in the presence and absence of estrogen. In HBSMCs cultured under estrogen-free conditions (Fig. 3B), the protein expression of ERα and Sirt1 was decreased, and that of ERβ (column data not shown) and NLRP3 was increased, compared with cells cultured in the presence of estrogen. Young and Davisson[22] showed that, in the central nervous system, Ang II contributes to a hypertensive state. Therefore, we treated HBSMCs with 10^{-6} M Ang II to mimic hypertensive conditions, as described in our previous study.[20] However, the protein expression of Sirt1 and NLRP3 was not affected by Ang II, in either the presence or absence of estrogen (Fig. 3B), indicating the dominant effects of estrogen even in the presence of Ang II or HT.

Downregulation of ERα and Sirt1 by Estrogen Deficiency Leads to Activation of NLRP3, IL-1β, and MMP-9 in HBECs and HBSMCs

Given that Sirt1 is associated with oxidative stress,[23] we assessed the relationships among the protein levels of Sirt1, endothelial nitric oxide synthase (eNOS), NADPH oxidase 4 (NOX4), and NLRP3 in HBECs in the absence of estrogen. Under estrogen-free conditions, depletion of ERα, but not of ERβ, was associated with a decrease in eNOS and Sirt1 protein levels and an increase in NOX4 and NLRP3 protein levels (Figs. 3C and 4A). These cells also exhibited elevated levels of IL-1β and MMP-9 proteins (Fig. 3D), consistent with the increase in NLRP3.

In HBECs grown under estrogen-free conditions, we further confirmed that treatment with 17β-estradiol counteracted the decrease in ERα, Sirt1, and eNOS levels and attenuated the increase in NOX4 and NLRP3 levels (Fig. 4A). In HBSMCs cultured under estrogen-free conditions, we also observed a decrease in ERα and Sirt1 protein levels and an increase in NLRP3, IL-1β, and MMP-9 protein levels (compared with those in HBSMCs grown in the presence of estrogen) (Fig. 4B).

Again, treatment with 17β-estradiol counteracted the decreases in ERα and Sirt1 protein levels and the increases in NLRP3, IL-1β, and MMP-9 protein levels otherwise seen under estrogen-free conditions (Fig. 4B). These findings in HBECs and HBSMCs may imitate the cerebral vascular wall prone to rupture under estrogen deficiency, suggesting that the depletion of Sirt1 and ERα leads to oxidative stress, resulting in the activation of the NLRP3/IL-1β/MMP-9 pathway in the vascular wall (Fig. 4C). The depletion of NLRP3 and the accumulation of Sirt1 in response to ERα may be crucial to prevent the rupture of IAs under estrogen-deficient conditions.

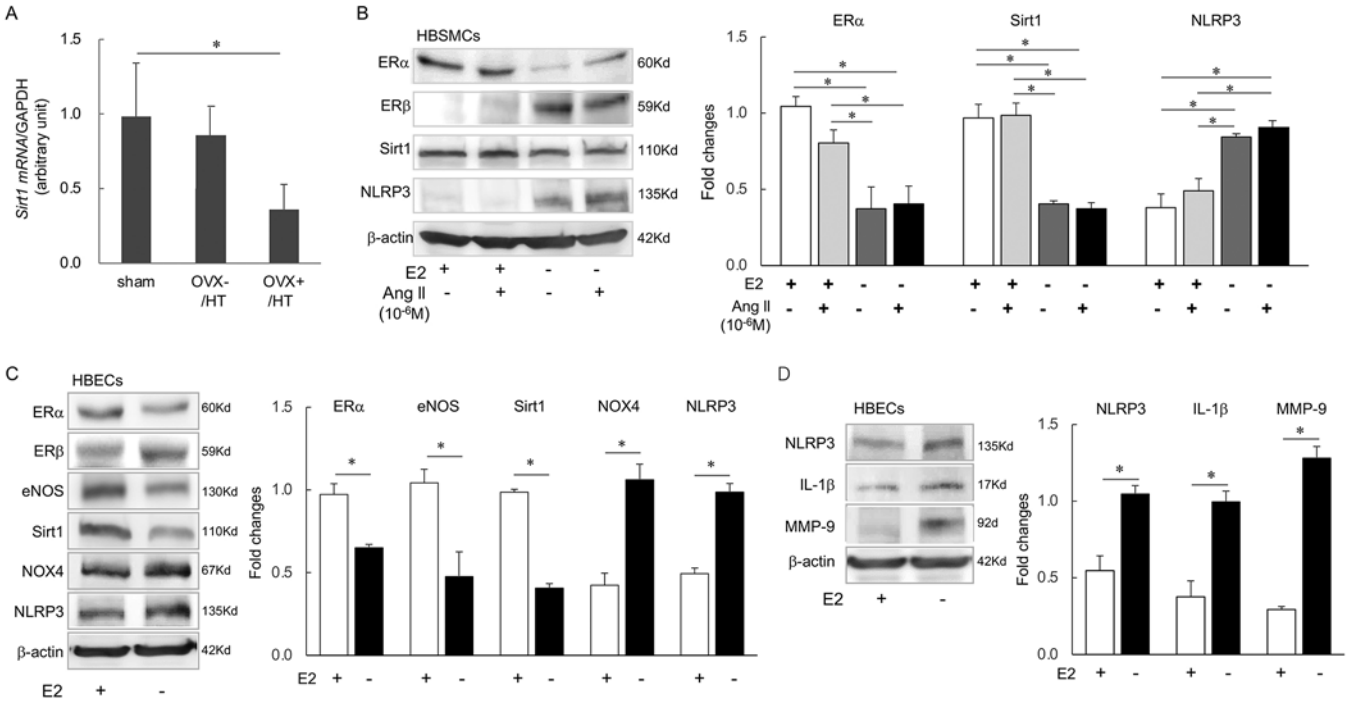

FIG. 3. Effects of estrogen deficiency in cultures of HBECs and HBSMCs. **A:** The level of the mRNA encoding *Sirt1* at the left PCA in IA model rats subjected to OVX^-/HT or OVX^+/HT, and in sham-operated rats. *$p < 0.05$, one-way ANOVA followed by the Kruskal-Wallis test (6 rats in each group). **B:** Western blot analysis of ERα, Sirt1, and NLRP3 protein levels in HBSMCs treated with or without Ang II (10^{-6} M) under estrogen-free (E2−) conditions. **C:** The expression of ERα, eNOS, Sirt1, NOX4, and NLRP3 proteins in HBECs cultured with or without estrogen, as quantified from the Western blot analysis. **D:** The expression of NLRP3, IL-1β, and MMP-9 in HBECs cultured with or without estrogen, as quantified from the Western blot analysis. Each experiment was repeated four times; values are presented as mean ± SD. *$p < 0.05$, Student t-test and one-way ANOVA followed by the Kruskal-Wallis test. d = dalton; Kd = kilodalton.

ERα Modulator Counteracts the Decreased ERα and Sirt1 Levels and Elevated NLRP3 Levels Seen Under Estrogen-Deficient Conditions

To assess whether exposure to ER agonists counteracts the decreased ERα and Sirt1 levels and increased NLRP3 levels seen under estrogen-free conditions, we tested the effects of two separate molecules, an ERα-specific agonist (PPT) and an ERβ-specific agonist (DPN). Under estrogen-free conditions, the exposure of HBECs and HBSMCs to PPT reversed the effect of estrogen deficiency on the levels of Sirt1 and NLRP3 proteins (Fig. 5A and B), while exposure to DPN did not (data not shown).

In a further experiment, we examined (in the rat IA model) the effects of treatment with BZA, a selective modulator of ER, on ERα, Sirt1, and NLRP3 levels. We previously observed that treatment with 1 mg/kg BZA resulted in decreases in the levels of the mRNAs encoding *IL-1β* and *MMP-9*.[19] However, in that previous study, we did not address the detailed mechanism of this effect, including the possible involvement of NLRP3. Therefore, in the present work, we treated OVX^+/HT rats by oral administration of 1 mg/kg of BZA. There was no difference in blood pressure between groups dosed with and without BZA (data not shown). However, BZA counteracted the depletion of ERα and Sirt1 protein levels and the accumulation of NLRP3 protein and normalized the increase in vascular diameter otherwise seen in OVX^+/HT rats (Fig. 5C). Notably, treatment with BZA also resulted in increased SAH-free survival in the rat IA rupture model (Fig. 5D). Thus, the elevation of ERα and Sirt1 protein levels by exposure to an ER modulator appeared to contribute to the inhibition of the IA rupture in this animal model.

Discussion

In this study, we documented a relationship between upregulation of the NLRP3/IL-1β/MMP-9 pathway and downregulation of ERα and Sirt1 at the left PCA, a site prone to rupture in the estrogen-deficient rat IA rupture model. We also demonstrated that the observed decreases in ERα and Sirt1, in contrast to the activation of the NLRP3/IL-1β/MMP-9 pathway at the mRNA and protein levels, were dependent on estrogen deficiency but were not affected by exposure to Ang II. These findings suggest that the estrogen deficiency–induced depletion of ERα and Sirt1 may activate the NLRP3 inflammasome separately from any effects of HT. To further confirm these relationships, we used HBECs and HBSMCs. In HBECs cultured in estrogen-free medium, the decreased expression of ERα and Sirt1 presumably elicits accumulation of NOX4 protein and depletion of eNOS protein, resulting in the generation of reactive oxygen species and the accumulation of NLRP3, IL-1β, and MMP-9. These changes were abrogated by supplementation of the growth medium

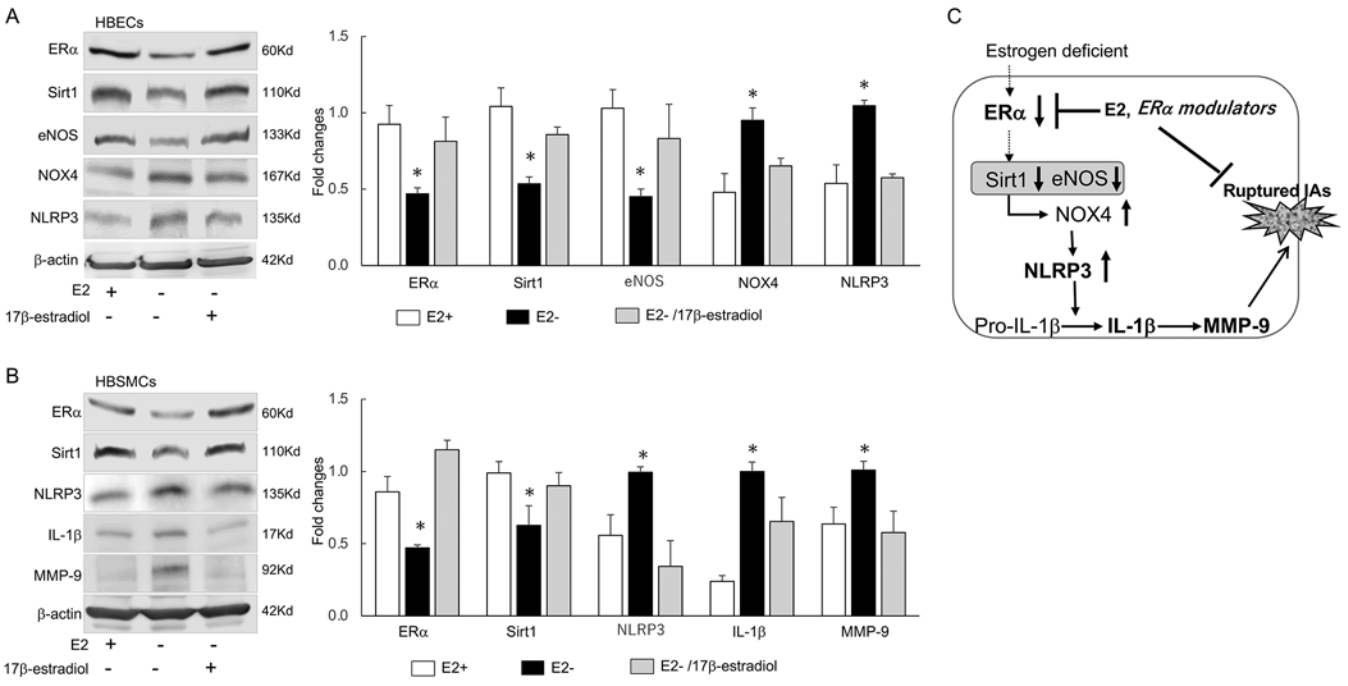

FIG. 4. Effects of 17β-estradiol on protein expression in HBECs and HBSMCs cultured under estrogen-free (E2−) conditions. **A:** Western blot analysis in HBECs. The addition of 17β-estradiol (10^{-7} M) counteracts the decreased expression of ERα, Sirt1, and eNOS and the increased expression of NOX4 and NLRP3 seen in cells grown in the absence of estrogen. **B:** Western blot analysis in HBSMCs. The addition of 17β-estradiol (10^{-7} M) counteracts the decreased expression of ERα, Sirt1, and eNOS and the increased expression of NLRP3, IL-1β, and MMP-9 seen in cells grown in the absence of estrogen. Each experiment was repeated four times; values are presented as mean ± SD. *$p < 0.05$, one-way ANOVA followed by the Kruskal-Wallis test. **C:** Schematic figure summarizing the postulated effects of estrogen deficiency in the IA wall that is prone to rupture.

with estrogen or with the ERα-specific agonist PPT, but not with the ERβ-specific agonist DPN (data not shown). Taken together, these results suggest that IA rupture may be attributable, at least in part, to the upregulation of the NLRP3/IL-1β/MMP-9 pathway through the decrease of ERα and Sirt1 expression due to estrogen deficiency.

ERα and ERβ regulate distinct sets of genes and mediate various effects on different cells and tissues.[24] The relationship between NLRP3 and ERs differs depending on pathway organization.[25–28] In a preliminary clinical study, Zhang et al.[13] reported accumulation of NLRP3 protein in the vascular wall of ruptured IAs, but no subsequent studies (to our knowledge) have evaluated the activation of NLRP3 in the context of ER expression in IAs.

In our in vitro study using HBECs and HBSMCs, treatment with an ERα agonist exerted beneficial effects similar to those seen with estrogen. Novella et al.[7] reported that estradiol exposure results in decreased expression of most inflammatory cytokines in the early stages of menopause, suggesting that the effect of estrogens is time dependent. In the present study, estrogen deficiency was induced by oophorectomy, which may (at the earliest time points after oophorectomy) impose effects similar to those of perimenopause.

Our study demonstrated the depletion of ERα and the accumulation of NLRP3 at the rupture-prone PCA, resulting in an increase in IL-1β and MMP-9 in the rat IA rupture model as well as in HBECs and HBSMCs cultured under estrogen-deficient conditions. These effects were accompanied by a decrease in Sirt1 protein levels and an increase in the levels of NOX4, IL-1β, and MMP-9 proteins, consistent with the results seen in other studies. Specifically, the activation of Sirt1 was shown to inhibit the expression of NLRP3 in human umbilical vein endothelial cells.[29] Additionally, according to Gorenne et al.,[30] Sirt1 in human vascular smooth muscle cells protects against DNA damage and medial degeneration and inhibits atherosclerosis. We hypothesize that a decrease in Sirt1 protein levels facilitates the activation of NLRP3, thereby inducing the accumulation of IL-1β and MMP-9, leading in turn to IA rupture.

Administration of 17β-estradiol restored aortic Sirt1 expression, activated eNOS, and retarded oophorectomy-induced arterial senescence and atherosclerotic development. BZA, an ER modulator, also potentiated Sirt1 expression while inhibiting arterial senescence and atherosclerotic development.[17] Thus, administration of estrogen or BZA counteracts the deleterious changes otherwise induced by Sirt1 expression. In a separate study, our group has shown that BZA inhibits the rupture of IAs at the left PCA, an effect that is accompanied by decreases in the levels of the mRNA encoding *IL-1β* and *MMP-9* in OVX^+/HT rats.[19] In the present study, we demonstrated that the expression of ERα and Sirt1 is significantly decreased in OVX^+/HT animals, in contrast to the activation of NLRP3 at the vascular wall prone to rupture. Furthermore, treatment of IA model animals with BZA resulted in increases in vascular ERα and Sirt1 and decreases in NLRP3, leading to an improvement in SAH-free survival. These findings support the hy-

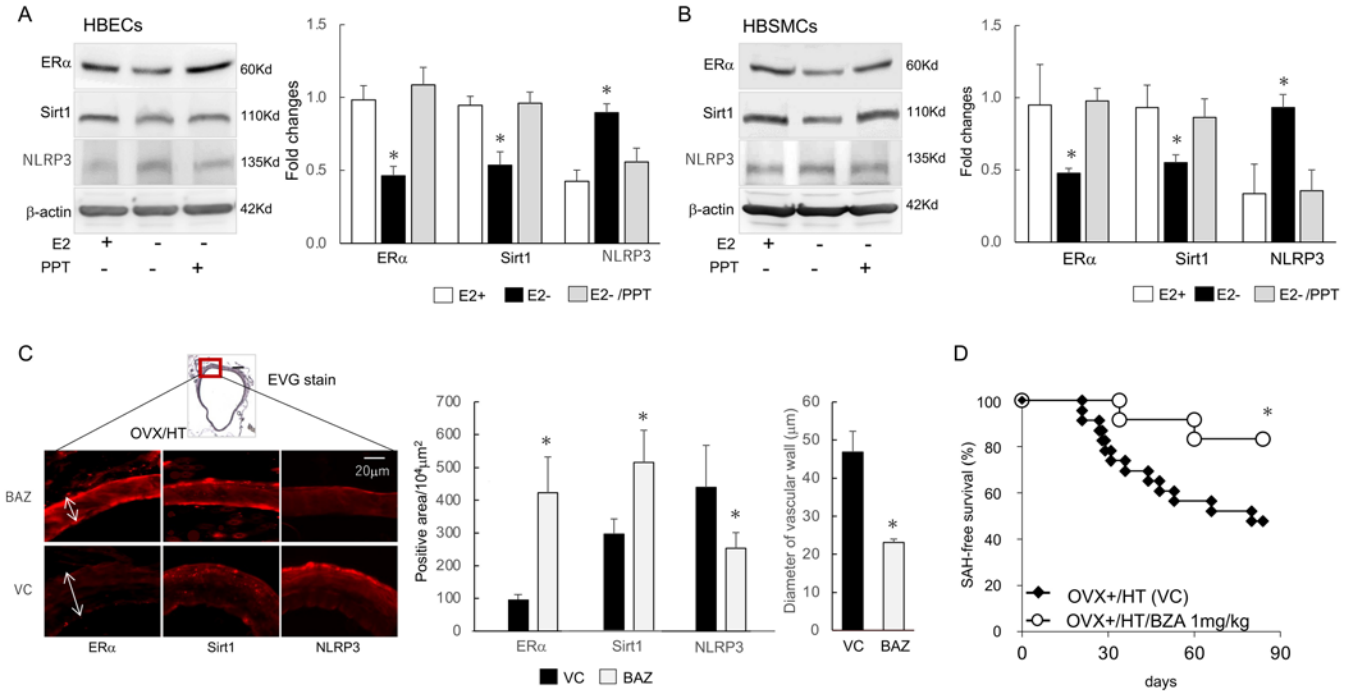

FIG. 5. Effects of ER modulators in HBECs and HBSMCs and the vascular wall. **A and B:** Representative Western blot analysis of ERα, Sirt1, and NLRP3 in HBECs (A) and HBSMCs (B) cultured with or without ERα agonist PPT (10^{-6} M). Each experiment was repeated four times; values are presented as mean ± SD. *p < 0.05, one-way ANOVA followed by Kruskal-Wallis test. **C:** Representative immunohistochemistry of ERα, Sirt1, and NLRP3, and changes in diameter of vascular wall, as assessed in vehicle control (VC) and animals treated with ER modulator BZA (1.0 mg/kg/day). All rats harbored IAs prone to rupture. **C:** Treatment with BZA increases SAH-free survival in a rat model of IA rupture. *p < 0.05, log-rank test.

pothesis that IA rupture is associated, at least in part, with the activation of NLRP3 following the depletion of ERα in a rat IA model. Consistent with our conjecture, Laser et al.[31] reported that in a mouse model, the activation of ERα suppresses the formation of abdominal aortic aneurysms and decreases the expression of vascular MMP-2 and -9.

Our study has some limitations. We found that the accumulation of NLRP3 protein was associated with decreased levels of ER and of Sirt1 in the PCA prone to rupture in oophorectomized rats. We previously determined the mRNA level of *IL-1β* and *MMP-9* in animals dosed with BZA, but this was not assessed in the present study. In addition, we did not examine the expression of IL-1β and MMP-9 in HBECs and HBSMCs subjected to PPT in the in vitro study. Although the in vitro studies using HBECs and HBSMCs partially mimicked estrogen-deficient conditions, these in vitro models do not exactly reproduce the conditions that would be seen in a clinical setting. In addition, we were able to count the number of ruptured aneurysms, but not the number of unruptured IAs, because unruptured IAs are prone to rupture. Furthermore, given that the pathogenesis of unruptured and ruptured IAs is multifactorial, we cannot rule out contributions to aneurysm ruptures by mechanisms not addressed in our study.

Conclusions

This work is the first study (to our knowledge) to show a relationship between NLRP3 activation and IA rupture associated with the depletion of ERα and Sirt1 under estrogen-deficient conditions. We postulate that the depletion of ERα and Sirt1 elicits the activation of NLRP3 via oxidative stress, leading to the elevation of IL-1β and MMP-9 levels, and subsequently to vascular damage and IA rupture. Optimal management of these risk factors may be needed to prevent the rupture of IAs in postmenopausal patients.

Acknowledgments

We thank Emiko Nishikawa for her excellent technical support. This work was supported by JSPS Kakenhi Grants-in-Aid for Scientific Research (C) nos. 17K10832 and 20K09391 to Y. Kanematsu, no. 17K10831 to Y. Tada, no. 18K16562 to M. Korai, and no. 20K09326 to K. Shimada.

References

1. Algra AM, Lindgren A, Vergouwen MDI, et al. Procedural clinical complications, case-fatality risks, and risk factors in endovascular and neurosurgical treatment of unruptured intracranial aneurysms: a systematic review and meta-analysis. *JAMA Neurol*. 2019;76(3):282-293.
2. de Rooij NK, Linn FH, van der Plas JA, Algra A, Rinkel GJ. Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and time trends. *J Neurol Neurosurg Psychiatry*. 2007;78(12):1365-1372.
3. Morita A, Kirino T, Hashi K, et al. The natural course of unruptured cerebral aneurysms in a Japanese cohort. *N Engl J Med*. 2012;366(26):2474-2482.
4. Backes D, Rinkel GJE, Greving JP, et al. ELAPSS score for prediction of risk of growth of unruptured intracranial aneurysms. *Neurology*. 2017;88(17):1600-1606.
5. Greving JP, Wermer MJ, Brown RD Jr, et al. Development

of the PHASES score for prediction of risk of rupture of intracranial aneurysms: a pooled analysis of six prospective cohort studies. *Lancet Neurol*. 2014;13(1):59-66.
6. Miller VM, Duckles SP. Vascular actions of estrogens: functional implications. *Pharmacol Rev*. 2008;60(2):210-241.
7. Novella S, Heras M, Hermenegildo C, Dantas AP. Effects of estrogen on vascular inflammation: a matter of timing. *Arterioscler Thromb Vasc Biol*. 2012;32(8):2035-2042.
8. Miyamoto T, Kung DK, Kitazato KT, et al. Site-specific elevation of interleukin-1β and matrix metalloproteinase-9 in the Willis circle by hemodynamic changes is associated with rupture in a novel rat cerebral aneurysm model. *J Cereb Blood Flow Metab*. 2017;37(8):2795-2805.
9. Yamaguchi T, Miyamoto T, Kitazato KT, et al. Time-dependent and site-dependent morphological changes in rupture-prone arteries: ovariectomized rat intracranial aneurysm model. *J Neurosurg*. 2020;133(5):1486-1494.
10. Usui F, Shirasuna K, Kimura H, et al. Inflammasome activation by mitochondrial oxidative stress in macrophages leads to the development of angiotensin II-induced aortic aneurysm. *Arterioscler Thromb Vasc Biol*. 2015;35(1):127-136.
11. Cicolari S, Catapano AL, Magni P. Inflammaging and neurodegenerative diseases: role of NLRP3 inflammasome activation in brain atherosclerotic vascular disease. *Mech Ageing Dev*. 2021;195:111467.
12. Ridker PM, MacFadyen JG, Glynn RJ, et al. Inhibition of interleukin-1β by canakinumab and cardiovascular outcomes in patients with chronic kidney disease. *J Am Coll Cardiol*. 2018;71(21):2405-2414.
13. Zhang D, Yan H, Hu Y, Zhuang Z, Yu Z, Hang C. Increased expression of NLRP3 inflammasome in wall of ruptured and unruptured human cerebral aneurysms: preliminary results. *J Stroke Cerebrovasc Dis*. 2015;24(5):972-979.
14. Winnik S, Auwerx J, Sinclair DA, Matter CM. Protective effects of sirtuins in cardiovascular diseases: from bench to bedside. *Eur Heart J*. 2015;36(48):3404-3412.
15. Singh V, Ubaid S. Role of silent information regulator 1 (SIRT1) in regulating oxidative stress and inflammation. *Inflammation*. 2020;43(5):1589-1598.
16. Yang SR, Wright J, Bauter M, Seweryniak K, Kode A, Rahman I. Sirtuin regulates cigarette smoke-induced proinflammatory mediator release via RelA/p65 NF-kappaB in macrophages in vitro and in rat lungs in vivo: implications for chronic inflammation and aging. *Am J Physiol Lung Cell Mol Physiol*. 2007;292(2):L567-L576.
17. Sasaki Y, Ikeda Y, Miyauchi T, Uchikado Y, Akasaki Y, Ohishi M. Estrogen-SIRT1 axis plays a pivotal role in protecting arteries against menopause-induced senescence and atherosclerosis. *J Atheroscler Thromb*. 2020;27(1):47-59.
18. Jamous MA, Nagahiro S, Kitazato KT, Satomi J, Satoh K. Role of estrogen deficiency in the formation and progression of cerebral aneurysms. Part I: experimental study of the effect of oophorectomy in rats. *J Neurosurg*. 2005;103(6):1046-1051.
19. Maekawa H, Tada Y, Yagi K, et al. Bazedoxifene, a selective estrogen receptor modulator, reduces cerebral aneurysm rupture in Ovariectomized rats. *J Neuroinflammation*. 2017;14(1):197.
20. Tamura T, Jamous MA, Kitazato KT, et al. Endothelial damage due to impaired nitric oxide bioavailability triggers cerebral aneurysm formation in female rats. *J Hypertens*. 2009;27(6):1284-1292.
21. Frösen J, Tulamo R, Paetau A, et al. Saccular intracranial aneurysm: pathology and mechanisms. *Acta Neuropathol*. 2012;123(6):773-786.
22. Young CN, Davisson RL. Angiotensin-II, the brain, and hypertension: an update. *Hypertension*. 2015;66(5):920-926.
23. Khan M, Ullah R, Rehman SU, et al. 17β-Estradiol modulates SIRT1 and halts oxidative stress-mediated cognitive impairment in a male aging mouse model. *Cells*. 2019;8(8):928.
24. Leitman DC, Paruthiyil S, Vivar OI, et al. Regulation of specific target genes and biological responses by estrogen receptor subtype agonists. *Curr Opin Pharmacol*. 2010;10(6):629-636.
25. de Rivero Vaccari JP, Patel HH, Brand FJ III, Perez-Pinzon MA, Bramlett HM, Raval AP. Estrogen receptor beta signaling alters cellular inflammasomes activity after global cerebral ischemia in reproductively senescence female rats. *J Neurochem*. 2016;136(3):492-496.
26. Liu S, Fan W, Gao X, et al. Estrogen receptor alpha regulates the Wnt/β-catenin signaling pathway in colon cancer by targeting the NOD-like receptors. *Cell Signal*. 2019;61:86-92.
27. Han SJ, Jung SY, Wu SP, et al. Estrogen receptor β modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. *Cell*. 2015;163(4):960-974.
28. Xu Y, Sheng H, Bao Q, Wang Y, Lu J, Ni X. NLRP3 inflammasome activation mediates estrogen deficiency-induced depression- and anxiety-like behavior and hippocampal inflammation in mice. *Brain Behav Immun*. 2016;56:175-186.
29. Li Y, Yang X, He Y, et al. Negative regulation of NLRP3 inflammasome by SIRT1 in vascular endothelial cells. *Immunobiology*. 2017;222(3):552-561.
30. Gorenne I, Kumar S, Gray K, et al. Vascular smooth muscle cell sirtuin 1 protects against DNA damage and inhibits atherosclerosis. *Circulation*. 2013;127(3):386-396.
31. Laser A, Ghosh A, Roelofs K, et al. Increased estrogen receptor alpha in experimental aortic aneurysms in females compared with males. *J Surg Res*. 2014;186(1):467-474.

Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: T Yamaguchi, Shikata, I Yamaguchi. Acquisition of data: T Yamaguchi. Analysis and interpretation of data: T Yamaguchi. Drafting the article: T Yamaguchi. Critically revising the article: Miyamoto, Kitazato, Takagi. Approved the final version of the manuscript on behalf of all authors: T Yamaguchi. Statistical analysis: T Yamaguchi. Administrative/technical/material support: T Yamaguchi, Shikata, I Yamaguchi, Shimada, Yagi, Tada, Korai, Kitazato, Kanematsu, Takagi. Study supervision: Kitazato, Takagi.

Supplemental Information

Online-Only Content

Supplemental material is available with the online version of the article.

Online Appendix. https://thejns.org/doi/suppl/10.3171/2022.4.JNS212945.

Previous Presentations

This work was presented in poster format at the 11th World Stroke Congress, Montreal, Canada, October 17–20, 2018, and at the International Stroke Conference 2019, Honolulu, Hawaii, February 6–8, 2019.

Correspondence

Tadashi Yamaguchi: Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan. tadashi117117@hotmail.co.jp.