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Estrogen Receptor β Is a Novel Therapeutic Target for Photoaging

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ABSTRACT

One of the many harmful factors faced by the skin is solar UV radiation, which damages skin by inducing chronic low-grade inflammation through increased expression of proinflammatory cytokines, metalloproteinases (MMPs) and cyclooxygenase-2 (COX-2). Estrogen receptors (ERs) α and β are ligand-dependent transcription factors that are expressed in skin, and an ERβ agonist has previously shown efficacy in vivo in models of pain and inflammation. Because ERβ does not carry the breast and uterine proliferation liabilities of ERα, we decided to explore the possibility of using ERβ as a target for photoaging. We show that ERβ-selective compounds suppressed the expression of cytokines and MMPs in activated keratinocytes and fibroblast-based in vitro models of photoaging. Furthermore, in activated dermal fibroblasts, ERβ-selective compounds also inhibited COX-2. These activities of ERβ ligands in skin cells correlated with the expression levels of ERβ and showed reversal by treatment with a potent synthetic ER antagonist. Furthermore, the pharmacology of ERβ-selective compound was observed in wild-type but not in skin cells obtained from ERβ knockout mice. Finally, we demonstrate that a synthetic ERβ agonist inhibited UV-induced photodamage and skin wrinkle formation in a murine model of photoaging. Therefore, the potential of an ERβ ligand to regulate multiple pathways underlying the cause of photoaging suggests ERβ to be a novel therapeutic target for the prevention and treatment of photoaging.

Photoaging results from the repetitive exposure of skin to damaging effects of solar UV radiation and is characterized by wrinkles, laxity, dryness, and mottled pigmentation. In recent years, some of the molecular mechanisms underlying the cause of photoaging have been described. The process of photoaging involves three cell types, namely keratinocytes, fibroblasts, and infiltrating neutrophils (Fisher et al., 2002; Rijken et al., 2005; Makrantonaki and Zouboulis, 2007). Within minutes of UVB radiation exposure, epidermal keratinocytes show an increased activation of transcription factors, activator protein 1 (AP-1), and nuclear factor-κB (NF-κB), resulting in high expression of matrix metalloproteinases (MMPs) and proinflammatory cytokines (Fisher et al., 1996). These cytokines and a UVA component of the UV radiation in turn activate dermal fibroblasts to secrete MMPs, which damage the collagen component of the dermal extracellular matrix (Fagot et al., 2004). Cytokines and chemotactic factors that are secreted by skin cells also recruit neutrophilic granulocytes to the dermis. These neutrophils further degrade dermal extracellular matrix by secreting MMPs and elastases, thus contributing to UV-mediated dermal collagen and elastin degradation (Rijken et al., 2005). Repetitive UV exposure leads to the accumulation of partially degraded extracellular matrix components in the dermis, resulting in wrinkle appearance. Therefore, a number of pathways and processes involved in photoaging could be targeted by potential therapeutic agents.

Estrogen receptors (ERα/NR3A1 and ERβ/NR3A2) are ligand-dependent transcription factors that belong to the nuclear receptor superfamily (Robinson-Rechavi et al., 2003).

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ABBREVIATIONS: AP-1, activator protein 1; MMP, metalloproteinase; COX-2, cyclooxygenase-2; ER, estrogen receptor; NHEK, normal human epidermal keratinocyte; LBD, ligand binding domain; NHDF, normal human dermal fibroblast; NF-κB, nuclear factor-κB; TNFα, tumor necrosis factor-α; HET, heterozygote; KO, knockout; TLDA, TaqMan Low Density Array; RT-PCR, reverse transcriptase-polymerase chain reaction; IL, interleukin; MED, minimal erythema dose; PPT, 4,4′,4″-(4-propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol; LXR, liver X receptor; Mapk1, mitogen-activated protein kinase 1 gene; ICI 182,780, fulvestrant.

Both ERα and ERβ are expressed in human epidermal keratinocytes and dermal fibroblasts, and their natural ligand, estrogen, has profound influence on the skin (Haczynski et al., 2004; Walker and Korach, 2004). It is important to note that estrogen has been shown to decrease aging and wrinkling of the skin when used therapeutically (Wolff et al., 2005). Estrogen prevents aging by increasing skin thickness, dermal collagen content, and water-holding capacity of postmenopausal skin. Estrogen increased skin moisture by strengthening epidermal barrier and enhancing the production of hydrophilic glycosaminoglycans in the dermis (Quatresooz and Pierard, 2007). Because ERα agonism is associated with unwanted breast and uterine side effects (Jordan, 2002), we decided to examine the possibility of using ERβ as a therapeutic target for the prevention and treatment of photoaging. Our notion that ERβ ligands could be used for photoaging was also strengthened by the in vivo efficacy observed with an ERβ compound in preclinical models of rheumatoid arthritis, inflammatory bowel disease, and inflammatory/chemical induced pain (Leventhal et al., 2006). ER ligands have been proposed to mediate their anti-inflammatory effects by inhibiting NF-κB-dependent gene expression via involvement of cofactor SRC-2 (Cvoro et al., 2006, 2008). In addition, topical estradiol has been shown to increase type I procollagen and decrease MMP1 expression in human epidermis (Rittié et al., 2008). Here, we demonstrate that ERβ ligands could interfere with various steps involved in the etiology of photoaging. We show that ERβ ligands inhibit the expression of proinflammatory cytokines and metalloproteinases in cell-based models of UVB-activated normal human epidermal keratinocytes (NHEKs) and cytokine-activated normal human dermal fibroblasts (NHDFs). We also demonstrate the efficacy of a synthetic ERβ ligand in a murine model of UV-induced photoaging. Our observations indicate that ERβ is a potential novel target for photoaging and could provide a new category of therapeutic agents for the prevention and treatment of this indication.

Materials and Methods

ERβ-Selective Compounds and Other Chemical Reagents. Test compounds used in this study were supplied by the Wyeth compound library (Princeton, NJ) that were either purchased or synthesized by Wyeth Medicinal Chemistry (Collegeville, PA). All other chemicals and reagents obtained from various vendors and used in this study were reagent grade or better.

Skin Cells and Cell Culture Conditions. NHEKs and NHDFs (Lonza Walkersville, Walkersville, MD), and human dermal fibroblasts cell lines, HFF and BJ-5ta (American Type Culture Collection, Manassas, VA), as well as human epidermal keratinocyte cell line KERTr (American Type Culture Collection) were cultured according to the manufacturers' recommendations. In general, cells were trypsinized and seeded on day 0, compound treatments were done on day 1 with and without UVB (8 mJ/cm^2; UV Stratalinker 2400; Stratagene, La Jolla, CA) or TNFα (1 ng/ml) activations, and cells were harvested on day 2 with lysis buffer (Applied Biosystems, Foster City, CA) directly added to the cultured cells after a phosphate-buffered saline wash. Cells either were used for RNA purification using an RNeasy RNA purification column (QIAGEN, Hilden, Germany) according to the manufacturer's protocol or were directly processed to cDNA using "Cell-to-cDNA" lysis buffer (Ambion, Austin, TX).

ERβ KO and Heterozygote Skin Cell Preparations. ERβ KO mice were generated at Wyeth Research (Collegeville, PA) as de-

scribed previously (Shughrue et al., 2002) and are not the same as the βER KO mice (Krege et al., 1998; Harris, 2007). Animal use was approved by the Institutional Animal Care and Use Committee of Wyeth Research and was conducted in accordance with Association for the Assessment and Accreditation of Laboratory Animal Care guidelines. Heterozygous females were intercrossed to the homozygous KO males to generate heterozygote (HET) and KO pups. Genotyping TaqMan assays with wild-type and KO-specific primer/probe sets were used to identify HET/KO pups. The primer/probe sequences are as follows: ERBWT forward primer, 5'-CTG GAA GGT GGG CCT GTT C; ERBWT reverse primer, 5'-TGG CAG TGG GTG GCT AAA G; ERBKO forward primer, 5'-ACA TTC TAC AGT CCT GCT GTG ATG A; ERBKO reverse primer, 5'-GGG TGT TGG GTC GTT TGT TC; ERBWT probe, 6-carboxyfluorescein-CCAAATGTGCTATGGCCAACTTCTGGAC-5-carboxytetramethylrhodamine; ERBKO probe, VIC-TCCCAGCAGCACCGGTAAC-TAGCTAAG-5-carboxytetramethylrhodamine (VIC is a product of Applied Biosystems). Skins from newborn mice (2–3 days old) were isolated and floated on 2.5 mg/ml dispase (Invitrogen, Carlsbad, CA) overnight at 4°C and separated into epidermal and dermal layers using small forceps. The epidermal and dermal layers were minced and subjected to several differential centrifugations, fractionations, and filtrations as described previously (Zheng et al., 2005). These cells were then cultured in Eagle's minimal essential medium containing fetal bovine serum (8%) in 24-well culture plates (day 0). Cells were treated with vehicle or ERβ-selective compounds on day 2, followed by isolation and purification of RNA on day 3 using RNeasy column (QIAGEN). Gene expression profiles were analyzed using TaqMan Low Density Array (TLDA) and individual TaqMan gene assays (Applied Biosystems).

TLDAs and Quantitative RT-PCR. The RNA or cDNA obtained from the compound treated cells were used in custom-designed TLDAs or individual TaqMan assays (Applied Biosystems, Foster City, CA) according to the vendor's protocols using ABI 7900HT real-time polymerase chain reaction machine. The level of expression was calculated based on the polymerase chain reaction cycle number (Ct), and the relative gene expression level was determined using the ΔΔC$_t$ method as described previously (Livak and Schmittgen, 2001). One TLDA was designed to contain oligonucleotide probe and primer pairs for TNFα, IL1β, IL6, IL8, MMP1, MMP3, MMP9, tissue inhibitor of metalloproteinases 1, DCN, collagen 1A1, CCL3, CCL4, CCL5, NOS2A, PTGS2, and 18S control gene (all from Applied Biosystems). Some of these individual gene assays from the list above were also purchased from Applied Biosystems and were used for confirmation or focused assay purposes. Other gene assays used in this study including human genes ERα and ERβ and mouse genes Mmp13, Ptgs2, mitogen-activated protein kinase 1 (Mapk1), Fbn1 were also purchased from Applied Biosystems.

Murine Model of Photoaging. Five-week-old female albino hairless mice (Hos:HR-1) were obtained from the HOSHINO Laboratory Animals (Kotoh-cho, Japan). Animals had free access to food and water and were acclimated for 1 week before the study. Eight mice are allocated to each group (total six groups for each test compound). All experimental protocols were approved by Institutional Animal Care and Use Committee of Clinical Research Institute, Seoul National University Hospital (Seoul, Korea) (Association for the Assessment and Accreditation of Laboratory Animal Care-accredited facility). A UV irradiation device that included TL20W/12RS UV lamps (Philips, Eindhoven, the Netherlands) with an emission spectrum between 275 and 380 nm (peak, 310–315 nm) served as the UV source (Seo et al., 2001). A Kodacel filter (Eastman Kodak, Rochester, NY) was mounted 2 cm in front of the UV lamp to remove wavelengths of less than 290 nm (UVC). Irradiation intensity at the mouse skin surface was measured using a UV meter (Waldmann GmbH & Co., Villingen-Schwenningen, Germany). The irradiation intensity 20 cm from the light source was 0.5 mW/cm^2.

Initially, we measured the minimal erythema dose (MED) on

dorsal skin of mice. MED can be defined as the minimum amount of radiation exposure required to produce an erythema with sharp margins after 48 h. Mice were exposed to UV light three times per week (Monday, Wednesday, and Friday) for 6 weeks. The irradiation dose was increased weekly by 1 MED (1 MED = 100 mJ/cm^2) up to 4 MED and then maintained at 4 MED. UV irradiation was stopped after irradiation for the sixth week. ERB-041 or its vehicle (70% ethanol, 30% polyethylene glycol) was topically applied to the dorsal area (50 μl) after each exposure to UV irradiation (5 times a week). Skin wrinkle replica was made with a silicon rubber (Silflo Dental Impression Material, Flexico Developments, Stevenage, Hertford-shire, UK) from the backs of unstrained mice. Skin impressions were photographed using a coupling charge system video camera and analyzed by Skin-Visiometer SV 600 software (Courage + Khazaka electronic GmbH, Köln, Germany).

Results

Identification of ERβ as a Target for Photoaging. To evaluate ERβ as a target for photoaging, NHEKs were treated with either vehicle or a synthetic ERβ-selective ligand, WAY-200070 (Harris, 2007), for 24 h with or without UV radiation (8 mJ/cm^2). WAY-200070 is an ERβ-selective ligand, which bound to ERα and ERβ ligand binding domains (LBDs) with IC$_{50}$ (concentration of the ligand required for 50% displacement of the radiolabeled 17β-estradiol) values of 155 and 2.3 nM, respectively. In addition, it showed EC$_{50}$ (concentration of the ligand required for 50% increase in luciferase activity) values of 314 and 31 nM, respectively, in Gal4-ERα-LBD and Gal4-ERβ-LBD transactivation assays (Table 1) (Malamas et al., 2004; Harris, 2007). UV exposure induced the expression of MMPs, cytokines, and COX-2 in NHEKs and inhibited the expression of tissue inhibitor of metalloproteinases 1 and collagen 1A1 (Fig. 1). These results indicate that the UV exposure of keratinocytes induces in-flammatory and catabolic pathways, thus mimicking the eti-ology of photoaging. Because UV exposure of epidermal ke-ratinocytes is the first step involved in the etiology of photoaging, we examined the effect of WAY-200070 on UV-induced interleukins, MMPs, and COX-2 expression in NHEKs. It is interesting that WAY-200070 decreased the UVB-induced expression of MMP1, MMP3, IL1β, IL8, TNFα, IL6, and COX-2 in NHEKs (Fig. 2D). Because the etiology of photoaging also involves the activation of der-mal fibroblasts by cytokines produced from UV-activated epidermal keratinocytes, we next examined the effect of WAY-200070 on cytokines and MMP expression in TNFα-treated NHDFs. TNFα induced the expression of IL8, IL1β, COX-2, and MMP1 in NHDFs, which was signifi-cantly inhibited after treatment with WAY-200070 (Fig.

2E). Expression of IL6 and MMP3 was also induced in NHDFs, but WAY-200070 did not significantly suppress the expression as in NHEKs (Fig. 2E). The difference in the degree of suppression between NHEK and NHDF cells may be because the method of cytokine induction differed (UV versus TNFα) between the two cell types.

Validation of ERβ as a Target for Photoaging. Be-cause WAY-200070 is selective but not specific for ERβ, we next examined whether its potential antiphotoaging in vitro effects were specifically mediated via ERβ. To test this, KERTr keratinocytes were treated with WAY-200070 (1 μM) in the presence or absence of an ER-specific pan-antagonist, ICI 182,780 (1 μM) (Escande et al., 2006). Treatment of KERTr cells with WAY-200070 inhibited MMP1 expression in a dose-dependent manner, which was efficiently antago-nized by ICI 182,780 (Fig. 3A), suggesting that WAY-200070 mediated its effects through ER. To rule out the possibility that WAY-200070 mediated its effect through ERα, we next examined the effect of an ERα-selective ligand, PPT (1 μM) (Escande et al., 2006), on MMP1 expression in UV-activated NHEKs and TNFα-activated NHDFs. Unlike WAY-200070, PPT failed to inhibit the expression of MMP1 in activated NHEKs and NHDFs (Fig. 3B). In contrast to KERTr, NHEKs, and NHDFs, immortalized human fibroblasts cell lines, BJ and HFF, do not show significant expression of ERβ (Fig. 3C), and as expected, WAY-200070 did not inhibit the expression of cytokines/MMPs in these cells (data not shown). Finally, we examined the effect of WAY-200070 on responsive gene expression in UV-activated primary skin keratinocytes obtained from ERβ HET and KO mice (Shugh-rue et al., 2002; Ohnemus et al., 2005; Antal et al., 2008; Zhao et al., 2008). WAY-200070 inhibited the expression of Mmp13 (murine counterpart of human MMP1), and another ER-responsive gene, Mapk1, in ERβ heterozygote but not in KO keratinocytes (Fig. 3, D and E). Basal expression of Mmp13 was increased in the KO cells, suggesting that unliganded ERβ may influence Mmp13 expression. For both responsive genes, the loss of repression in the ERβ KO cells indicates that the effects of the ligand occur via ERβ. Taken together, these results indicate that WAY-200070 mediates its effects on skin cells through ERβ.

ERB-041 Is as Active as WAY-200070 in Skin Cells. ERB-041 is an ERβ-selective compound that bound to ERα and ERβ with IC$_{50}$ value of 1216 and 5.4 nM, respectively, thus demonstrating that it is 226-fold selective for the ERβ than ERα (Table 1) (Malamas et al., 2004; Harris, 2007).

TABLE 1
Binding affinity and the relative transactivation potency of selected skin active ERβ ligands

Data were extracted mainly from recent publications (Malamas et al., 2004; Harris, 2007) and confirmed with our unpublished internal testing.

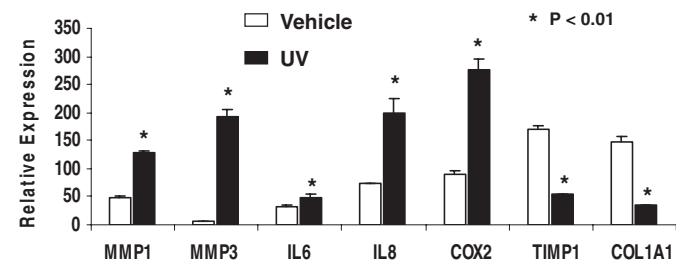|	ER Binding Affinity			ER Transactivation Assay (EC$_{50}$)		Base Structure
	ERβ	ERα	Ratio	ERβ	ERα	
	nM			*nM*		
17β-Estradiol	2	2	1	9	3	
WAY-200070	2.3	155	68	31	314	Benzoxazole
ERB-041	5.4	1216	226	290	>3000	Benzoxazole

Fig. 1. UV induces cytokines and MMP gene expression in human pri-mary keratinocytes. Relative gene expression levels of cytokines, MMPs, and other genes of interest in vehicle- (□) and UV-treated (8 mJ/cm^2, ■) NHEKs were measured using quantitative RT-PCR. Specifically, the expression of these genes was measured by using custom-made TLDAs, and the results were normalized to 18S RNA expression. p Values were determined by Student's t test.

Therefore, ERB-041 is more ERβ-selective than WAY-200070 in binding assays. ERB-041 has shown efficacy in vivo in preclinical models of rheumatoid arthritis, inflammatory bowel disease, endometriosis, and inflammatory-induced pain (Leventhal et al., 2006). We next compared the effects of ERB-041 (1 μM) with those of WAY-200070 (1 μM) on IL1β and MMP1 expression in skin cells, KERTr cells, and NHEKs. KERTr-immortalized keratinocytes expressed very high levels of IL1β and MMP1, which was significantly inhibited by both ERB-041 (3.6-fold) and WAY-200070 (7.8-fold) (Fig. 4A). Furthermore, ERB-041 also significantly inhibited the UV-induced IL1β and MMP1 expression in NHEKs, although it was less efficacious than WAY-200070 in these cells (Fig. 4B). Because ERB-041 showed better ERβ selectivity relative to WAY-200070 in binding assay, and it has shown efficacy in murine models of inflammation, we decided to use ERB-041 in a murine model of photoaging.

ERβ Ligand Inhibits Wrinkle Formation in Vivo. We next examined whether the anti-inflammatory/anticatabolic molecular changes observed with an ERβ compound in vitro translate into efficacy in a murine model of photoaging. Therefore, the effect of ERB-041 was examined in UV-induced wrinkle formation in a hairless albino mouse model (Chang et al., 2008). The experimental plan included two groups of mice. One group was mock-exposed [UV(−) group], and the second group of mice was exposed 3 times/week to UV treatment [UV(+) group]. The UV(−) group was further divided into two subgroups, wherein animals were treated topically with either vehicle or ERB-041 (10 mM) 5 times/week for 6 weeks. Likewise, the UV(+) group was divided into 4 subgroups, wherein mice were treated topically with vehicle or 0.1, 1.0, or 10 mM ERB-041 (Fig. 5A). UV exposure

of hairless mice for 6 weeks resulted in the appearance of prominent wrinkles in the UV(+) vehicle-treated group in comparison to mock-exposed groups, as shown by silicon rubber replicas of the mouse dorsal skin (Fig. 5A). Replica scans showed a quantitative increase in wrinkle score in the UV(+), vehicle-treated group relative to UV(−), mock-exposed group (Fig. 5B). It is remarkable that a statistically significant decrease in wrinkle score was observed at all three doses of the ERβ ligand compared with UV(+) vehicle-treated animals (Fig. 5, A and B). At 0.1 and 1 mM concentrations of ERB-041, wrinkle scores in the UV(+) groups were not significantly different ($p < 0.05$) from those of the UV(−) groups (Fig. 5B). With 10 mM ERB-041, wrinkling was reduced to a lesser extent; this high dose may have caused an off-target effect that limited its efficacy. These results indicate that the ERβ ligand inhibits the signs of photodamage in a murine model of photoaging.

Discussion

An explosion in the population of aged individuals has resulted in an increase in demand for products that can diminish wrinkles and maintain the youthful appearance of the skin. Currently, natural (retinoic acid) and synthetic (tazarotene; Avage, Allergan Inc., Irvine, CA) retinoids are the only class of topical prescription agents that are approved by Food and Drug Administration for the treatment of photoaged skin (Goldfarb et al., 1990). However, retinoid use is associated with local skin side effects of irritation, erythema, burning, pruritus, and scaling (McGuire et al., 1988; Effendy et al., 1996). Other treatments, such as botulinum toxins and hyaluronic acid fillers, are injectable and painful, and they do
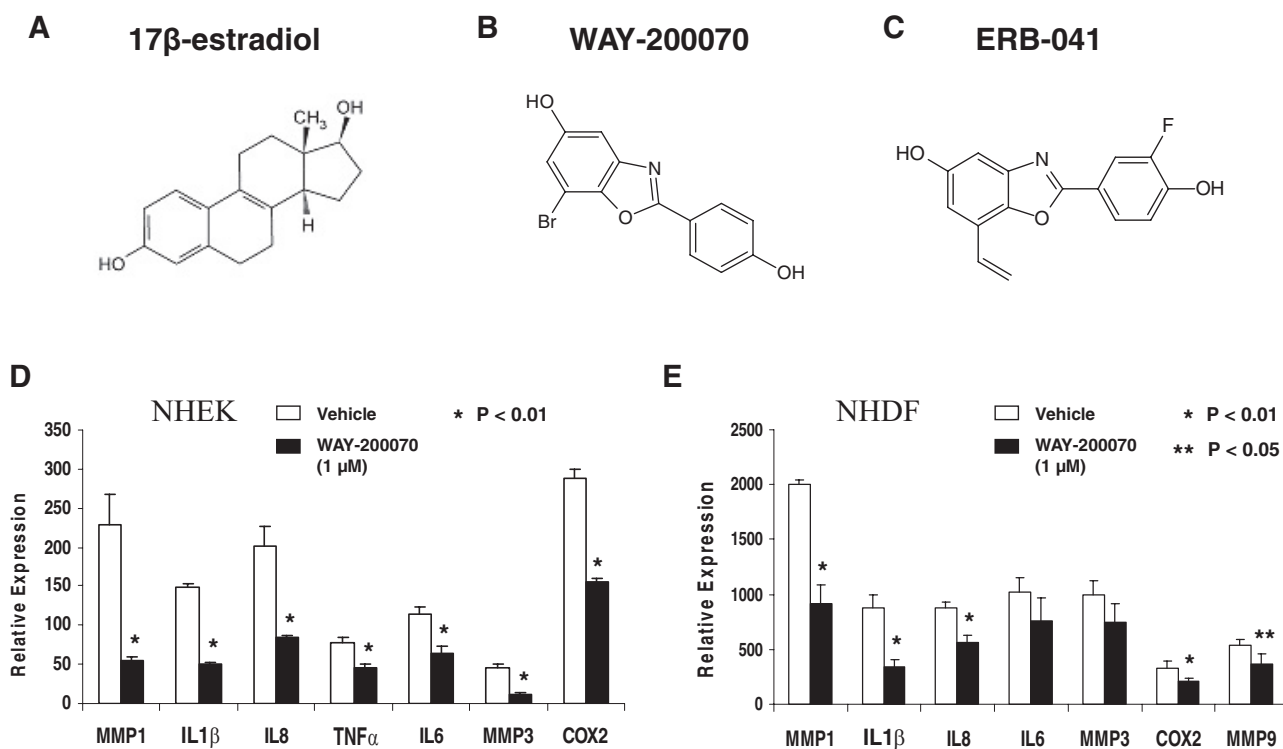
Fig. 2. ERβ ligand shows efficacy in keratinocyte and fibroblast-based in vitro models of photoaging. Structures of key compounds used in this study are shown: estradiol (A); WAY-200070 (B); and ERB-041 (C). WAY-200070 reverses the UV effect in the cell-based model of photoaging. The relative expression level of vehicle (□), 1 μM WAY-200070 (■)-treated UV-activated NHEK cells (D), and TNFα-treated NHDF cells (E) was measured by quantitative RT-PCR. p Values were determined by Student's t test.

not modulate the underlying etiological process of photoaging. Therefore, there is a need for safe and efficacious agents for the prevention and treatment of photoaging.

This article provides the first evidence demonstrating ERβ as a novel target for the prevention and the potential treatment of photoaging. In this study, we demonstrate that ERβ-selective ligands exhibit molecular signatures of a potential photoaging therapeutic, because a synthetic specific agonist inhibited the expression of proinflammatory cytokines MMPs and COX-2 in activated skin cells (Fig. 2). The broad anti-inflammatory potential of an ERβ-selective compound did translate into efficacy in vivo, because it inhibited UV-induced wrinkle formation in a murine model of photoaging (Fig. 5).

Natural and synthetic estrogens have long been recognized for their ability to rejuvenate human skin. These estrogens were included in many skin care products; however, no clear mechanisms of action were defined. A recent study showed that estrogen cream failed to protect sun-damaged skin in a clinic (Rittié et al., 2008). In this study, no significant changes in collagen production were observed in women or men after estradiol treatment of photoaged forearm or face skin. This is consistent with our unpublished results using estradiol in our in vitro photoaging model, wherein we found no significant reversal of the cytokine and MMP gene expression induced by UV, as well as no significant induction of procollagen synthesis. Likewise, in our in vitro assays, most ERβ ligands did not significantly increase procollagen ex-
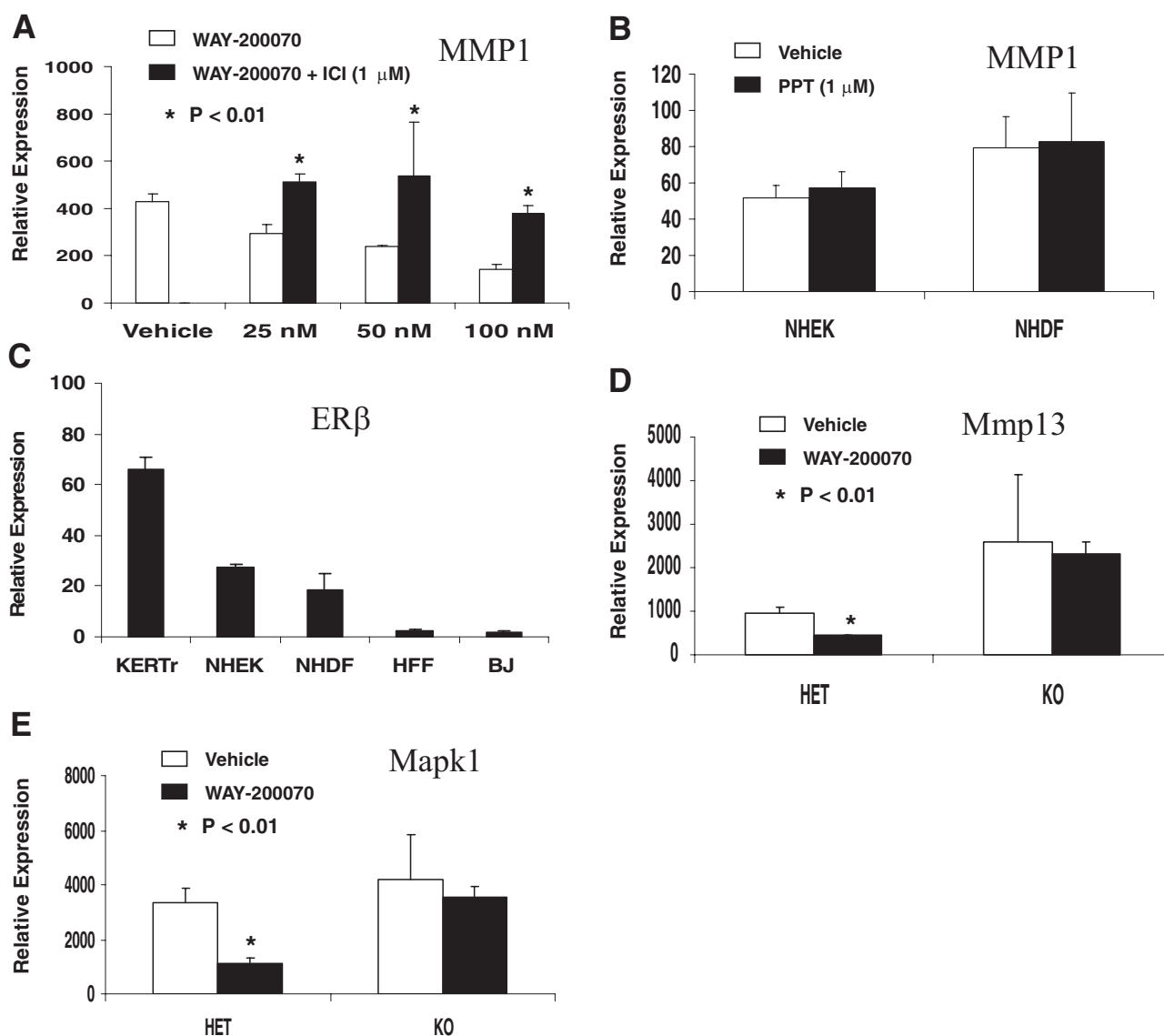
Fig. 3. Validation of ERβ as a target of photoaging. A, ICI 182,780 blocks ERβ ligand effect on MMP1 gene expression in the keratinocyte cell line KERTr. The effect of WAY-200070 (0, 25, 50, and 100 nM) on MMP1 gene expression in the absence (□) and presence (■) of ICI 182,780 (1 μM) in KERTr cells is shown. The relative expression was measured by quantitative RT-PCR, and the results were normalized to 18S RNA expression. B, the effect of PPT in TNFα-treated NHDF and UV-treated NHEK cells. A potent ERα agonist, PPT (1 μM), did not repress MMP1 expression in NHEKs and NHDFs. Cells were treated with PPT for 24 h. RNA was prepared, and MMP1 expression was analyzed by quantitative RT-PCR. C, relative gene expression of ERβ in human skin cells. ERβ expression was analyzed in keratinocytes KERTr, NHEK, and dermal fibroblasts NHDF, HFF, and BJ cells by quantitative RT-PCR. D and E, ERβ KO abolishes the suppression effect of ERβ ligands on Mmp13 and Mapk1 gene expression, respectively. ERβ KO and HET mouse epidermal keratinocytes were isolated as described under *Materials and Methods*. These ex vivo-cultured keratinocytes were activated by UV and incubated in the presence and absence of WAY-200070 (1 μM) (■) for 24 h. The relative gene expression levels for Mmp13 (D) and Mapk1 (E) were measured using quantitative RT-PCR. *p* Values were determined by Student's *t* test.

pression. However, the suppression of MMPs by ERβ ligands, especially MMP1, is likely to translate into the reduction of collagen degradation induced by UV exposure. The positive effect of ERB-041 in the in vivo murine photoaging model (Fig. 5) supports the idea that suppression of MMPs may lead to a reduction in collagen degradation and eventually the prevention of wrinkle formation. Furthermore, the inability of estradiol to suppress MMP1 in our in vitro photoaging model may explain why it did not improve sun-exposed human skin in the cited study (Rittié et al., 2008). The data presented in this article point to ERβ and not ERα as the receptor responsible for mediating the antiphotoaging effect.

Because ERβ ligands suppress several cytokines and MMP1, their anti-inflammatory effect is likely to involve NF-κB pathways. Because retinoic acid, a Food and Drug Administration-approved antiphotoaging retinoid, mainly suppresses AP-1-responsive metalloproteinases, collagenase,

stromelysin-1, and 92-kDa gelatinase (Fisher et al., 1996) and not NF-κB-responsive cytokines (K. Chang and S. Nagpal, unpublished observations), retinoids may exert their antiphotoaging effects via inhibition of AP-1-dependent gene expression (Herrlich and Ponta, 1994; Boehm et al., 1995; Nagpal et al., 1995). Therefore, if successfully developed and approved, ERβ ligands will become a novel class of antiphotoaging agents. It has been postulated that the anti-inflammatory potential of ERα/ERβ is tissue/cell type-specific (Brandenberger et al., 1997). ERα plays a more important role in the uterus, whereas ERβ seems to play important roles in ovary, cardiovascular system, and brain (Brandenberger et al., 1997). Although much evidence points to the clear involvement of an NF-κB-mediated mechanism for the anti-inflammatory activities of ERs, most of this evidence also suggests the involvement of other cofactors (Guzeloglu-Kayisli et al., 2008). For example, ER ligands have been
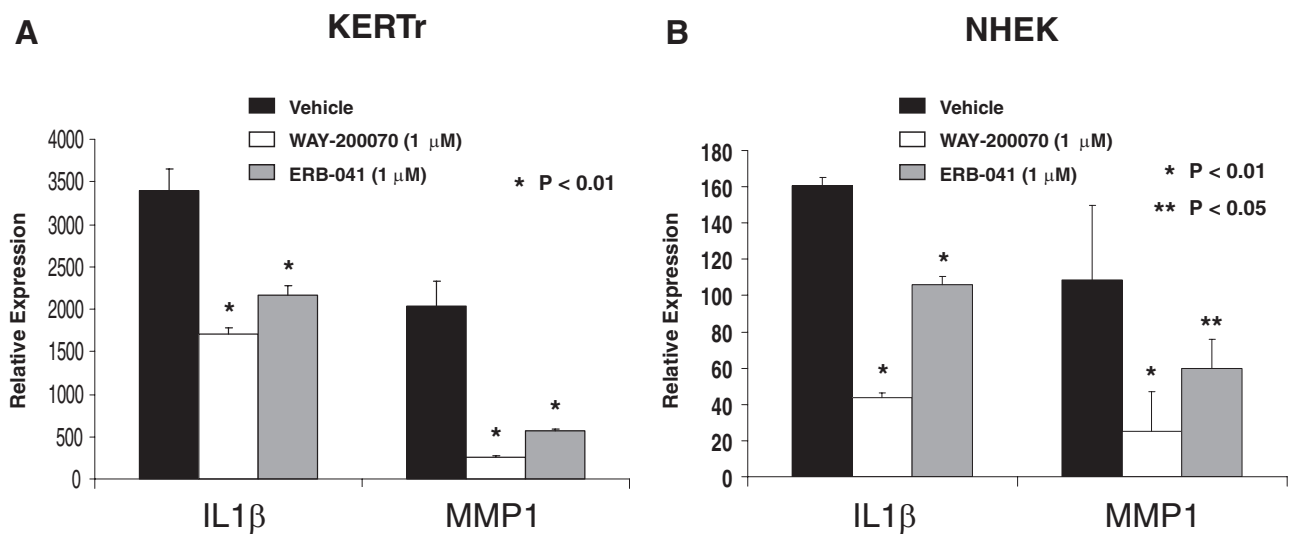
Fig. 4. Inhibition of IL1β and MMP1 gene expression in human keratinocytes by ERB-041 and WAY-200070. Human keratinocyte cell line KERTr cells (A) or NHEKs (B) were treated with vehicle (■), 1 μM WAY-200070 (□), or 1 μM ERB-041 (▨) for 24 h. The relative gene expression levels for IL1β and MMP1 were measured using quantitative RT-PCR as described under *Materials and Methods*. *p* Values were determined by Student's *t* test.
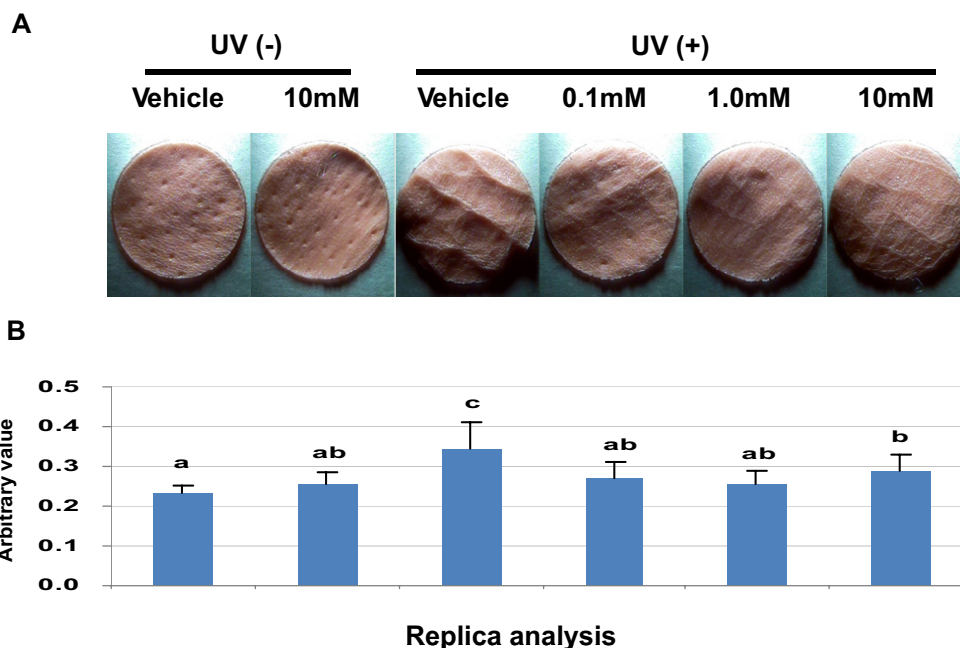
Fig. 5. Topical application of ERB-041 shows efficacy in a murine model of photoaging. UV radiation effect on hairless mouse skin of various concentrations (0.1, 1, and 10 mM) of ERB-041 after 6 weeks of topical treatment is shown. The mice were either mock-irradiated [UV(−)] or exposed to UV radiation [UV(+)] three times a week. The UV dose was increased weekly by 1 MED (1 MED = 130 mJ/cm2) to a maximum of 4 MED and subsequently maintained at this level for the remainder of the study period. Wrinkle formation was assessed after 6 weeks of UV exposure by preparing skin replicas (A) and measured quantitatively by computer scanning of the replicas (B). Representative photographs taken at week 6 are shown. Values represent the mean ± S.E.M. (*n* = 8). Bars marked with "a" represent groups that were statistically significantly different (*p* < 0.05) from those with "b" or "c." The bars with "ab" represent groups that were not significantly different (*p* < 0.05) from those with either "a" or "b."

proposed to mediate their anti-inflammatory effects by inhibiting NF-κB-dependent gene expression via involvement of cofactor steroid receptor coactivator 2 (Cvoro et al., 2006, 2008). Binding of our ERβ ligands may allow a more effective interaction of ERβ with SRC-2, thus providing more potent ERβ-mediated anti-inflammatory activity than estradiol. Based on our findings, we conclude that ERβ, but not ERα, plays a major role in reversing the process of photoaging in skin, and its antiphotoaging activity could potentially involve cofactor recruitment or NF-κB-mediated mechanism, or both.

Our recent publication showed that treatment with an LXR ligand significantly prevented UV-induced skin thickening as well as reducing skin wrinkling (Chang et al., 2008). In the current study, although the ERβ ligand was more effective than an LXR agonist in suppressing MMP1 and cytokine gene expression in vitro, it did not show a similar effect on skin thickness (data not shown). The efficacy of LXR ligands in reducing UV-induced skin thickening may lie in their ability to promote lipid synthesis and transport, consequently supporting barrier function. The potent anti-inflammatory activity of ERβ ligands, together with the lipogenicity of LXR ligands, suggests that the combination of these two nuclear receptor ligands might be most effective for preventing and treating photoaging.

In summary, we have identified ERβ as a target for photoaging and have shown that selective ligands could reverse the in vitro photoaging process, at least at the molecular level. A proof of concept study using ERB-041 showed a statistically significant reversal of wrinkle formation in a murine photoaging model (Fig. 5), warranting further development of this target for the prevention and treatment of photoaging.

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