We have demonstrated that LIGHT is overexpressed in RA synovial tissues and SF. LIGHT induced increased production of inflammatory cytokines, chemokines, and adhesion molecules through NF-κB activation, as well as proliferation of RA-FLS. These findings indicate that LIGHT signaling via LTßR plays an important role in the pathogenesis of RA by affecting key processes such as the proliferation and activation of RA-FLS. Therefore, regulation of LIGHT-LTßR signaling may represent a new therapeutic target for the treatment of RA.

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Yin Yang 1 induces transcriptional activity of p73 through cooperation with E2F1

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Abstract

The transcription factor p73 is a structural homologue of p53 and plays an important role in tumorigenesis, differentiation and development. However, the regulation of p73 pathway has not been wholly understood. Here we reported that YY1-silencing resulted in significant reductions in the activities of the p73 promoters and the endogenous p73 expression level, conversely, overexpression of YY1 could induce the activities of them. Furthermore, we showed that YY1 and E2F1 have synergistic effect on p73 promoter activity. The results of YY1-silencing and E2F1-silencing alone revealed that both factors are involved in the doxorubicin-induced activation of p73 promoter. Immunofluorescence staining and co-immunoprecipitation assays demonstrated that cooperation of YY1 and E2F1 is concomitant with physical interaction in nuclei. The results presented here suggested the cooperative transcriptional regulation of p73 by YY1 and E2F1, and might provide a new regulation mechanism by the YY1 network on tumorigenesis, differentiation and development.

Keywords: Yin Yang 1; E2F1; p73; Transcriptional activation; Synergistic effect

Yin Yang 1 (YY1) is a multifunctional transcription factor that exerts its effects on genes involved in various biological processes via its ability to initiate, activate, or repress transcription depending upon the context to which it binds, directly or indirectly via cofactor recruitments [1–3]. Today, YY1 is known to have a fundamental role in normal biological processes such as embryogenesis, differentiation, DNA replication, and cellular proliferation [3]. YY1-deficient embryos died at the time of implantation, and furthermore, its heterozygote knockout mice displayed significant growth retardation and neurological defects, indicating that YY1 plays an indispensable role in embryonic development and neuronal differentiation [4,5]. Recently, the physiologic significance of YY1 activity has also been reported to be associated with tumor biology [3,5,6]. It was known that YY1 is overexpressed in prostate cancer and sarcoma. Therefore, it is helpful to explain the cancer biological function of YY1 via studying its putative interactions with cell cycle regulators, death genes, as well as transcription factors and cofactors in the suppression or progression of various malignancies.

P73 has initially been known as a homologue of p53. They share relatively high sequence homology that includes an N-terminal transactivating (TA) domain and a central DNA binding domain. P73 has been reported to induce cell growth arrest and apoptosis in some cell lines irrespective of p53 status [7,8], and activate some p53 responsive genes, such as bax, cyclin G, IGF-BP3, and 14-3-3σ [9]. However, despite these similarities, p53 and p73 also show some fundamental differences in mechanisms and responses to DNA damage, and furthermore, in the phenotypes of their knockout mice. P73-null mice showed some defects in nervous system, which is not exhibited in p53-null mice

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[10,11]. On the other hand, p73-null mice do not develop spontaneous tumor formation while p53 knockout mice exhibit high susceptibility to tumorigenesis [3]. Furthermore, a very recent report showed that p73 could promote cell growth in a synergistic manner with the proto-oncogene c-Jun, and conversely, silencing p73 resulted in the reduction of growth rate and decrease of cyclin D1, indicating that p73 could act positively in tumorigenesis [12]. Therefore, p73 has a complex functions in biological processes including apoptosis, differentiation, tumorigenesis, and cell growth.

E2F1 has been identified as a regulation factor for p73 transcription. Pediconi et al. reported that responding to certain DNA damages, E2F1, but not E2F2, E2F3, or E2F4, can recruit the p73 promoter and efficiently and specifically activate its transactivation [13]. However, transcriptional regulation factors of the p73 promoter, excepting E2F1, remain unknown. Identification of other regulators that might control p73 is needed to further understand its functions and roles in tumorigenesis, differentiation and development.

Here, we identified a transcription factor, YY1, as a novel regulator of p73 transcription. Moreover, we showed that YY1 cooperates with E2F1 to induce the transcriptional activity of p73. Taken together, these findings not only shed new light on the nature of YY1's biological activities but also indicate the complex regulation mechanism of p73 in diverse biological processes.

Materials and methods

Cell cultures and chemicals. The U2OS, and HCT116 cells were obtained from the American Type Culture Collection; the SaOS2 cells were from Riken Cell Bank (Tsukuba, Japan). The U2OS and HEK293T cells were maintained in Dulbecco's modified Eagle's medium (Sigma–Aldrich) containing 10% fetal bovine serum (FBS, Invitrogen); The HCT116 and SaOS2 cells were maintained in McCoy's 5A medium (Invitrogen) containing 10% FBS or 15% FBS, respectively.

Plasmids and constructs. The long human p73-Luciferase reporter, which contains the p73 gene fragment −4052 to +438, was generously provided by Prof. Levrero (University of Rome 'La Sapienza', Rome, Italy) [13]. The short human p73-Luciferase reporter (−857 to +71) and the human p21-Luciferase reporter were constructed by inserting each PCR products into the BglII site and HindIII site of the pGL4 basic vector (Promega).

To generate the Flag-E2F1 expression vector, pcEF9-Flag-E2F1, the coding regions of human E2F1 were amplified by PCR and inserted into the HindIII and BamHI restriction sites of pcEF9 vector [14]. For the YY1 expression vector, the coding region of human YY1 was inserted into the pcDNA3 vector (Invitrogen). For construction of the HA-YY1 vector for use in immunofluorescence staining, the YY1 coding region in pcDNA3-YY1 was excised and inserted into the BamHI and EcoRI sites of pcDNA3-HA. To construct the Flag-YY1 expression vector for use in co-immunoprecipitation experiments, the Flag-YY1 coding region fragment was generated by PCR using pcDNA3-YY1 as a template, and the PCR product was again inserted into pcDNA3 vector predigested with BamHI and EcoRI.

RNA interference. To construct siRNA expression vectors, oligonucleotides with a hairpin, overhanging sequences and terminator were synthesized, annealed and then inserted into the BspMI sites of the pcENTRhU6 vector [15]. Based on the results of applying our algorithm [16], we identified the target sequences for YY1 and E2F1 genes: siYY1-1 (GCAAGAAGAGTTACCTCAG), siYY1-2 (GGCAGAATTTGCTAG AATG), siE2F1 (GGCTGGACCTGGAAACTGA). We used a T7 siRNA expression vector, which contains a stretch of 7 thymine (Ts) terminator sequences exactly downstream of the U6 promoter, as a control.

Transient transfection and luciferase assays. The U2OS and HCT116 cells were transfected with the different p73 firefly luciferase reporters along with indicated amounts of expression vectors and *Renilla* luciferase expression vector (pRL-SV40, Promega) for transfection normalization. For knockdown experiments, HCT116 and U2OS were transfected with siRNA expression vectors, and 24 h later selection was performed by puromycin. The selected cells were transfected with p73-luciferase reporters and pRL-SV40. FuGENE6 (Roche) were used for all transfections in U2OS cells, and Lipofectamine™ 2000 (Invitrogen) for HCT116 cells. Forty-eight hours after transfection, luciferase assays were performed in triplicate using the Dual Luciferase Assay System (Promega). All relative luciferase activities were determined by calculating the ratio between firefly and *Renilla* luciferase activities, and the results were shown as means ± SDs.

Real-time RT-PCR analysis. The total RNA was isolated using TRIZOL® Reagent (Invitrogen) and treated with DNase I (Qiagen) according to the manufacturer's instructions. The total RNA (1 μg) were then reverse-transcribed using a QuantiTect Reverse Transcription Kit (Qiagen). Real-time PCR was carried out using an Applied Biosystems 7500 system (Applied Biosystems) and QuantiTect® SYBR Green PCR Master Mix (Qiagen). All reactions were run in triplicate and expressional results were normalized to actin. Data were expressed as means ± SDs of triplicate wells. The primer sequences are available upon request.

Western blotting analysis. The cells were lysed in whole-cell extract buffer (50 mM Tris–HCl, pH 7.3, 10% glycerol, 250 mM sodium chloride, 2 mM EDTA, 0.1% Nonidet P-40, and 1 mM NaF) with protease inhibitors (complete cocktail; Roche), and the lysate samples proteins were electrophorated on 10% SDS–polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore). After blocking, the membrane was incubated with indicated primary antibodies, and then with horseradish peroxidase (HRP)-conjugated secondary antibody IgG (Amersham Biosciences). Detections were performed with ECL Plus™ reagent (Amersham Biosciences). Antibodies used for western blotting were a rabbit polyclonal anti-E2F1 antibody (C-20, Santa Cruz), a mouse monoclonal anti-YY1 antibody (H-10, Santa Cruz), and a rabbit monoclonal anti-actin antibody (Sigma–Aldrich).

Immunofluorescence staining. The U2OS cells were seeded on coverslips in 6-well plates and transfected with plasmids encoding HA-YY1 and E2F1. Forty-eight hours after transfection, cells were fixed for 20 min at room temperature with 10x PBS containing 4% paraformaldehyde, and permeabilized for 30 min in PBS containing 0.1% Triton X-100. After blocking, cover-slips were incubated at room temperature for 1 h in a 1:250 dilution of rat anti-HA and a 1:50 dilution of rabbit anti-E2F1 (Santa Cruz). Slides were then incubated for 1 h with a 1/1000 dilution of Alexa Fluor® 488 goat anti-rat IgG (H + L) and a 1/1000 dilution of Alexa Fluor® 568 goat anti-rabbit IgG (H + L) (Molecular Probes).

Co-immunoprecipitation. HEK293T cells were transfected with 10 μg of pcDNA3-Flag-YY1 or pcDNA3 using Lipofectamine™ 2000. Transfected cells were harvested 48 h post-transfection for Immunoprecipitation–Western blotting. Cell lysates were solved in lysis buffer (10 mM HEPES, pH 7.5, 100 mM KCl, 0.1% NP-40) with protease inhibitors on ice for 30 min and then cleared by centrifugation at 15,000 rpm. The supernatants were incubated at 4°C for 1 h with protein G-beads in the presence of 3 μg of anti-flag monoclonal antibody. Then, the immunoprecipitated proteins were subjected to western blot analysis using anti-E2F1 antibody as described above.

Results and discussion

YY1-silencing reduces the transcriptional activity of p73

Although YY1 is well known as a p53 inhibitor, recent reports have implied that YY1 has p53-independent

pathway(s) for cell cycle regulation and apoptosis [5,6]. To elucidate novel pathways of YY1, we generated siRNA vectors, siYY1-1 and siYY1-2, targeted against two different sites of YY1 for knock-down experiments. The two siRNA vectors were used to ensure elimination of off-target effect of RNAi. Western blot analysis of YY1 siRNA vectors-transfected cells revealed that both siYY1-1 and siYY1-2 could significantly suppress the endogenous level of YY1 without affecting levels of E2F1 (Fig. 1A). Furthermore, using a p21 promoter/reporter construct, which is essentially dependent on p53, we confirmed the ability of the siYY1 vectors to functionally repress endogenous YY1, which resulted in the up-regulation of p53, and subsequently, the increase of p21 promoter/reporter activities (Supplemental Fig. 1). Thus, the siRNA vectors for YY1 used here were able to knockdown and block the endogenous function of YY1 effectively and specifically.

Next, using a p73 promoter/reporter construct, we found that in contrast to the case of the p21 promoter, YY1-silencing led to a significant reduction of p73 promoter activity in both HCT116 cells and U2OS (Fig. 1B and 1C). To further confirm this finding, we performed real-time RT-PCR using total RNA extracted from YY1-silenced U2OS cells. The results showed that the reduction of p73 mRNA level was observed in the YY1-silenced U2OS cells (Fig. 1D), indicating that in contrast with p53, knockdown of YY1 was able to reduce the transcriptional activity of p73. The results implied the novel regulation of p73 by YY1.

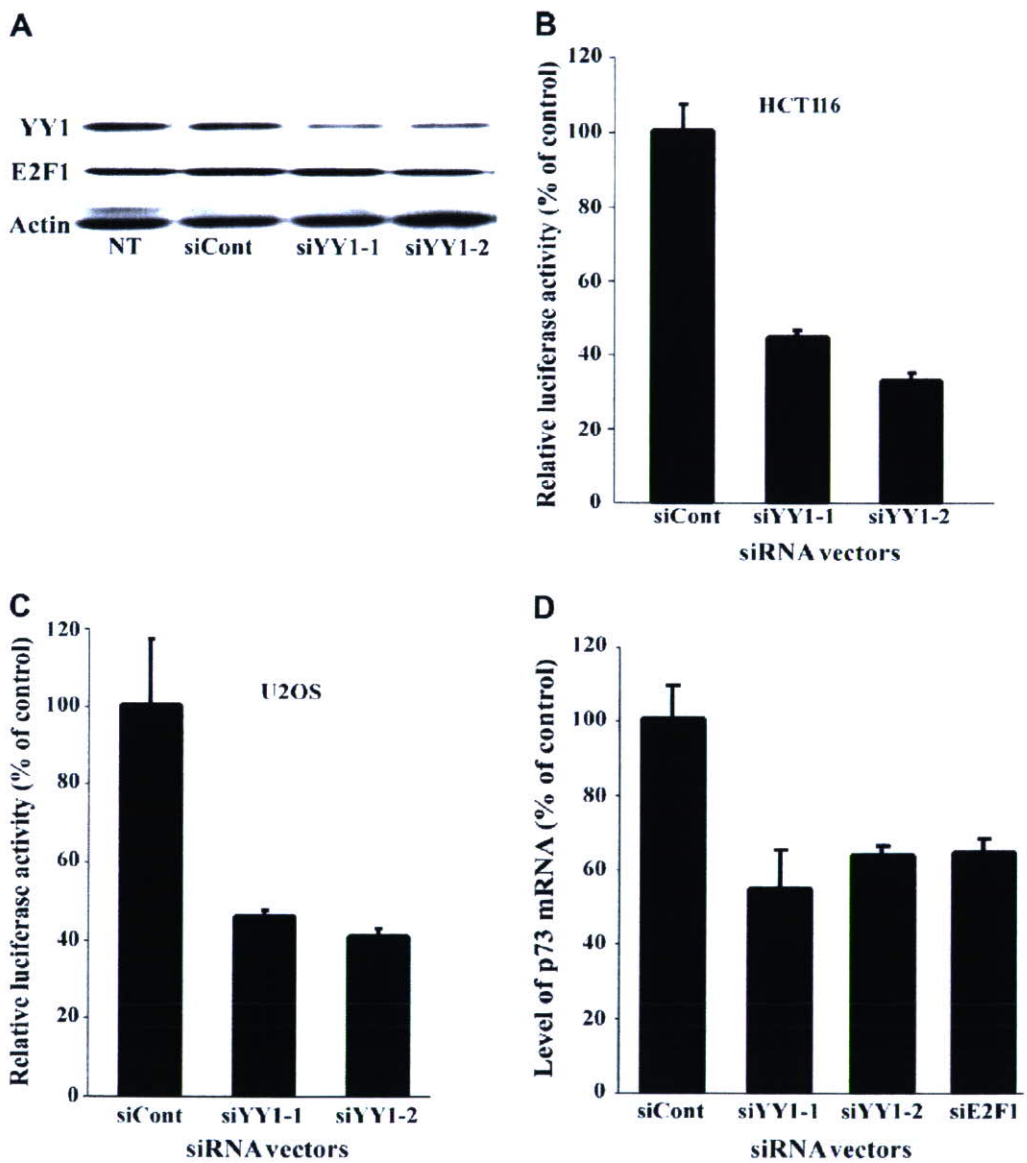

Fig. 1. The knockdown of YY1 reduces the transcriptional activity of p73. (A) HCT116 cells were transfected with siYY1-1 or siYY1-2 vectors or the siCont vector, the Western blotting was performed for detection of YY1 and E2F1. NT: non-transfected cells. (B–C) The effect of YY1-silencing on p73 promoter-driven transcription in HCT116 cells and U2OS cells. The indicated siRNA vectors-transfected HCT116 cells (B) and U2OS cells (C) were co-transfected with p73 luciferase (−4052/+438)/reporter and *Renilla* luciferase expression vector (pRL-SV40). Dual luciferase activity assay was performed 48 h after transfection. (D) Expression of p73 mRNA in YY1, E2F1-knocked down U2OS cells, determined by real-time RT-PCR analysis.

Effect of YY1 and E2F1 overexpression on the transcription activity of p73

Next, to assess the effect of YY1 overexpression and to confirm that of E2F1, a well-known p73 regulator, on p73 promoter activity, we generated a YY1-expression vector (pcDNA3-YY1) and an E2F1 expression vector (pcEF9-Flag-E2F1), and confirmed their expressions by Western blot analysis (Fig. 2A). Furthermore, the p53-inhibition activity of exogenous YY1 was also confirmed by p21 luciferase/reporter (Supplemental Fig. 2). Then, a different dose of the pcDNA3-YY1 was co-transfected into U2OS cells together with the two p73 reporters (Fig. 2B). The results were in agreement with those of knockdown experiments, as overexpression of YY1 led to the activation of the p73 promoter in a plasmid-dose dependent manner. Similar results were also obtained from SaOS2 cells, a p53 deficient human osteocarcinoma cell line (Fig. 2C), indicating that the YY1-induced p73 transcriptional activity is independent on p53 status. On the other hand, overexpressing E2F1 by co-transfecting E2F1 expression vector with the p73 promoter (−4052/+438)/reporter (Fig. 2D) or the p73 promoter (−857/+71)/reporter (Supplemental Fig. 3) resulted in the significant induction of both p73 reporters' activities, as shown previously [13]. Moreover, YY1 overexpression in U2OS cells resulted in the increase of endogenous p73 mRNA (Fig. 2E), similar with the effect of doxorubicin (Fig. 2F), which has been known to induce p73 activity via E2F1.

Collectively, the results from the knockdown and overexpression experiments for YY1 clearly identified the previously unsuspected role of YY1, i.e., the possibility that tumor activator gene YY1 up-regulates the transcriptional activity of p73. Recently, other evidences showed that spontaneous tumor formation did not develop in p73-null mice, and infrequent p73 mutations or overexpression of p73 protein was seen in a variety of tumors [17]; raising

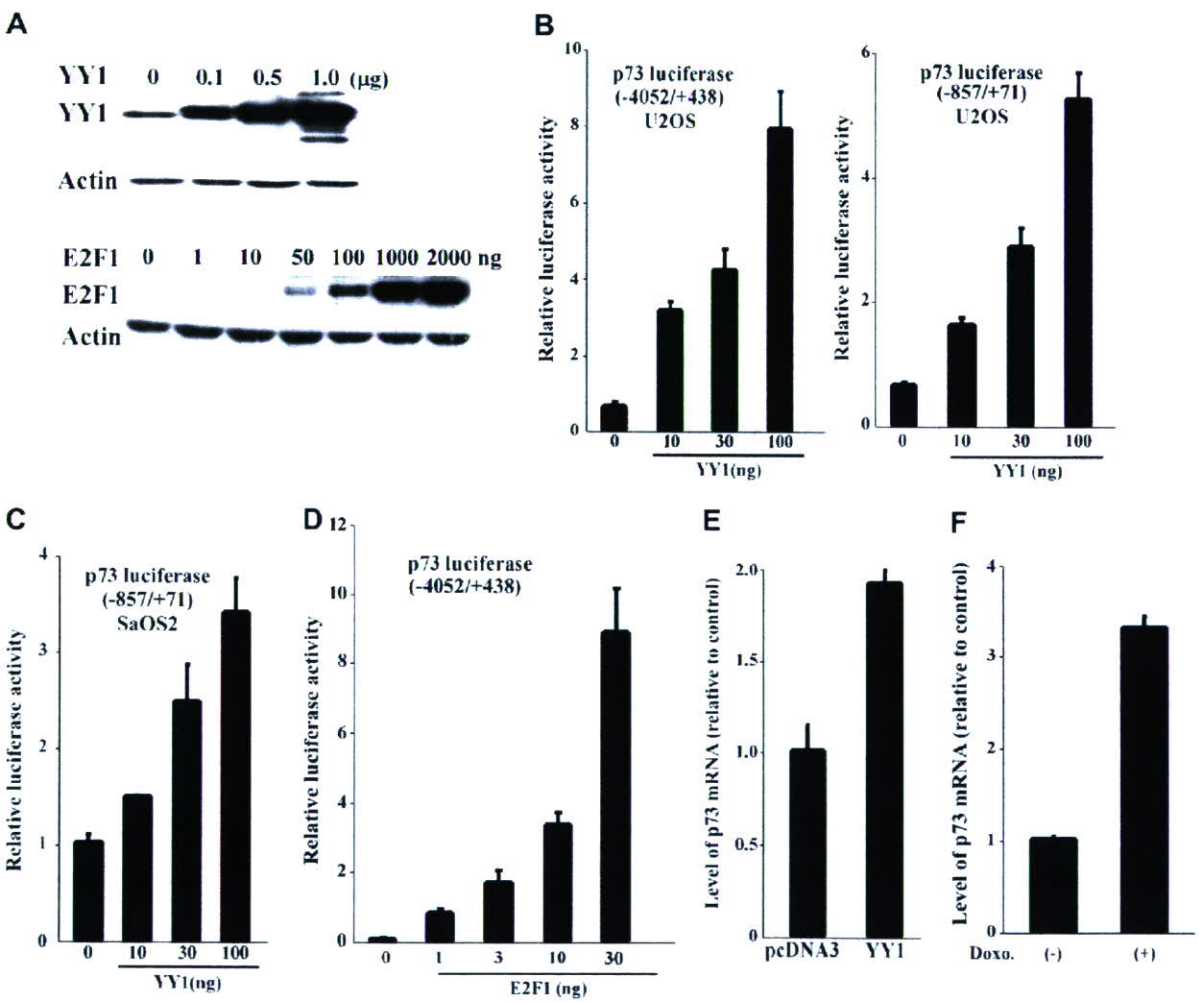

Fig. 2. The induction of p73 promoter activity by YY1 and E2F1. (A) Western blotting analysis of YY1 (upper panel) and E2F1 (lower panel) in HCT116 cells transfected with pcDNA3-YY1 or pcEF9-Flag-E2F1. (B) The effect of YY1 on the activities of p73 luciferase (−4052/+438)/reporter (left) or p73 luciferase (−857/+71)/reporter (right) in U2OS cells, determined by dual luciferase assay 48 h after transfection. (C) The effect of YY1 on the activities of p73 luciferase (−857/+71)/reporter in SaOS2 cells. (D) The effect of E2F1 on the activities of the p73 luciferase (−4052/+438)/reporter in HCT116 cells. (E) Expression of p73 mRNA in U2OS cells transfected with pcDNA3-YY1, determined by real-time RT-PCR analysis 12 h after transfection. (F) Expression of p73 mRNA in U2OS cells treated with doxorubicin (2 μM), determined by real-time RT-PCR analysis 24 h after treatment.

the question of the function of p73 as a tumor suppressor gene. A very recent work by Vikhanskaya et al. provided a potential answer to this question [12]. They found that p73 could promote cellular growth in a synergistic manner with the proto-oncogene c-Jun through AP-1 up-regulation, thus, p73 could positively act for tumorigenesis. Our results were consistent with their results. Moreover, it has also been reported that Yin Yang 1 is essential for oligodendrocyte progenitor differentiation and B-cell development [18,19]. Thus, our findings might also provide an important clue for unveiling molecular functions and mechanisms of YY1 and p73 pathway in neural differentiation and development. Therefore, we next addressed how YY1 regulates p73 gene transcription, specifically, whether there is a relationship between YY1 and E2F1 in regulating the transcriptional activity of p73.

The synergistic effect of YY1 and E2F1 on p73 transactivation

To examine whether there is cooperative regulation of p73 promoter activity by E2F1 and YY1, we performed co-transfection experiments using the p73 promoter (−4052/+438)/reporter, pcDNA3-YY1 and pEF9-E2F1 vectors. The U2OS cells co-transfected with both E2F1-expression and YY1-expression vectors showed a signification enhancement in the activity of p73 promoter compared to cells transfected with either of the vectors alone (Fig. 3A). Furthermore, in experiments with serial doses of YY1-expression vector under the constant presence of pEF9-E2F1, we found that in the presence of E2F1, the activity of p73 promoter increased in a dose-dependent manner with increasing amount of pcDNA-YY1 vector (Fig. 3B). These results demonstrated that YY1 could induce the transcriptional activity of p73 in a synergistic fashion with E2F1.

Previously, Schlisio et al. reported that E2F2 and E2F3, the members of E2F family, could interact with YY1 through mediation of the RYBP protein on the cdc6 promoter; they suggested that the interaction of E2Fs family with YY1 might determine the specificity of E2F2/E2F3 or E2F1 for different promoters [20]. In this study, we observed considerable cooperative transcriptional activation between E2F1 and YY1 on the p73 promoter. However, this functional interaction between YY1 and E2F1 might be not general, as the overexpression of YY1 did not show any significant activation of other well-defined E2F1-dependent promoters tested (DNMT1 and DHFR, data not shown), suggesting that the promoter specificity of E2F1 and E2F2/E2F3 is not simply determined by the

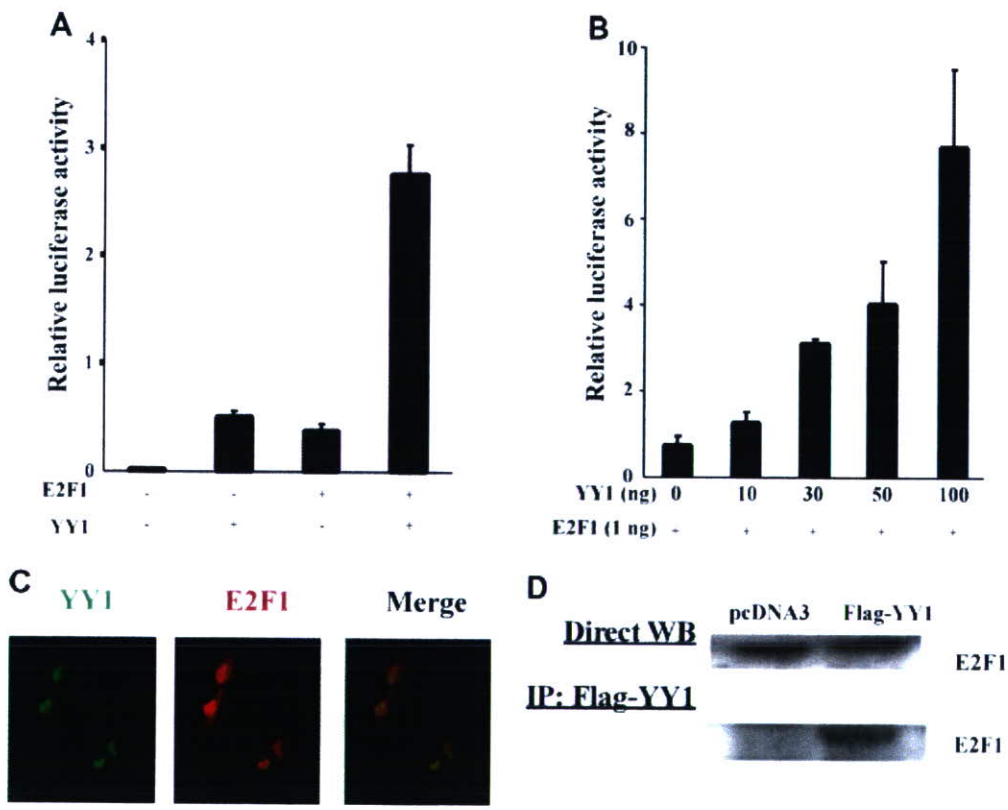

Fig. 3. The synergistic effect of YY1 and E2F1 on the transcriptional activation p73. (A) The activity of p73 luciferase (−4052/+438)/reporter in U2OS cells transfected with mock vector, pcDNA3-Flag-YY1 (50 ng), pcEF9-Flag-E2F1 (1 ng), or both pcDNA3-YY1 (50 ng) and pcEF9-Flag-E2F1 (1 ng). Dual luciferase assay was performed 48 h after transfection. (B) The activity of p73 luciferase (−4052/+438)/reporter in U2OS cells transfected with the indicated amounts of pcDNA3-YY1 and the constant amount of pcEF9-Flag-E2F1 (1 ng). (C) Immunofluorescence staining of YY1 and E2F1 in U2OS cells. (D) Co-immunoprecipitation of E2F1 and YY1 in HEK293T cells. Cells were lysed and pulled-down with anti-Flag antibody.

interaction with YY1, but might be determined by other undefined factors interacting with E2Fs and/or the epigenetic status of the promoters.

Co-localization and interaction between YY1 and E2F1

To understand the cellular and molecular mechanisms of the synergistic effects between YY1 and E2F1 on p73 promoter activation, we first examined the subcellular localization of YY1 and E2F1. As shown in Fig. 3C, YY1 was largely colocalized with E2F1 in the nucleus. Next, we examined the physical interactions between YY1 and E2F1 by performing co-immunoprecipitation experiments. The results showed that E2F1 immunoprecipitated in cells transfected with the Flag-YY1-expression vector, whereas no band was detected in cells transfected with the control vector (Fig. 3D). These observations demonstrated the direct physical association between YY1 and E2F1.

Role of YY1 in DNA damage-induced transcriptional activity of p73 promoter

As reported previously, the p73 promoter was activated E2F1-dependently by doxorubicin, a DNA damaging agent [13]. Therefore, we examined whether YY1 contributed to doxorubicin-induced p73 transcriptional activation. YY1-silenced cells showed significant reduction in the activation of the p73 promoter induced by doxorubicin (Fig. 4A, B), which was similar to that of E2F1-silencing cells.

Furthermore, p73 promoter activation by doxorubicin treatment has been reported to be critically dependent on

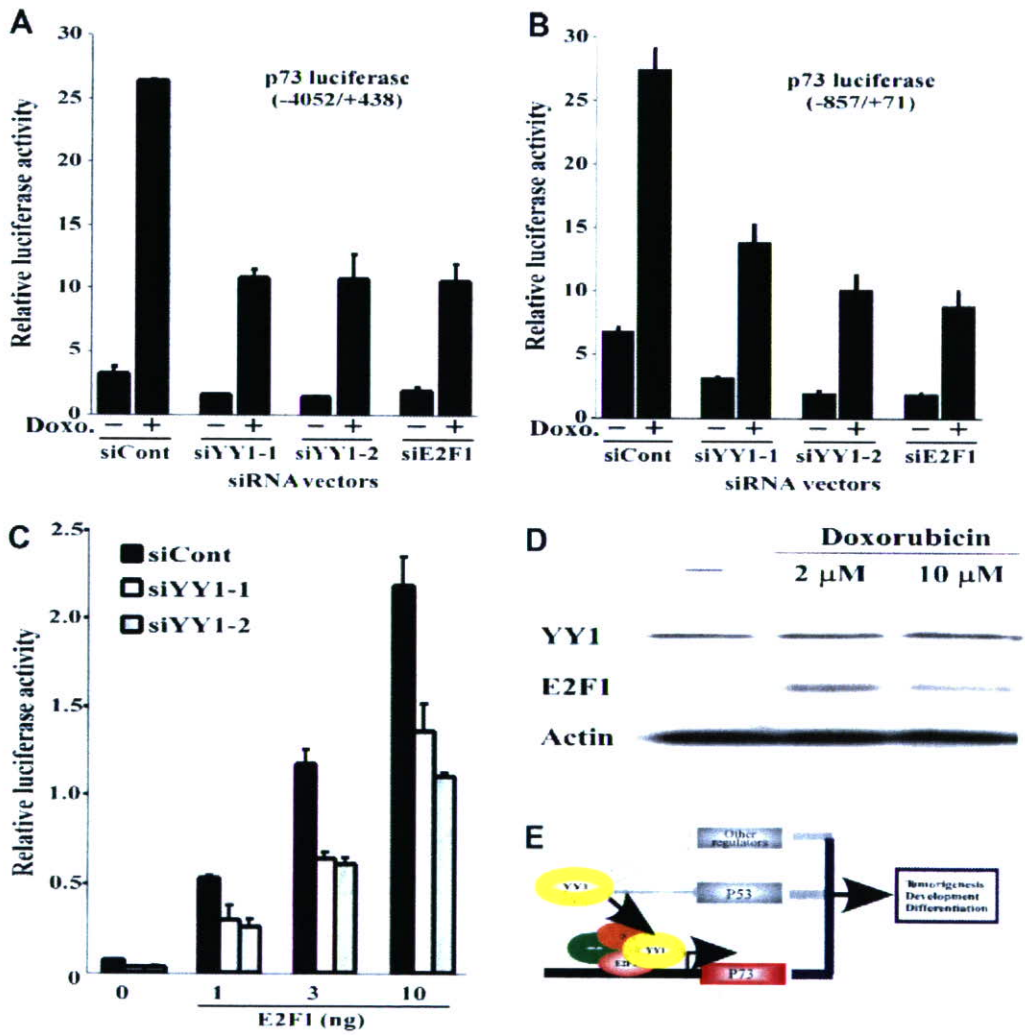

Fig. 4. Involvement of YY1 in transcriptional activation of p73 induced by doxorubicin. (A, B) The activity of p73 promoter in the YY1-silenced U2OS cells under doxorubicin treatment. siYY1-1, siYY1-2 or siE2F1 vectors-transfected U2OS cells were co-transfected with p73 luciferase (−4052/+438)/reporter (A) or (−857/+71)/reporter (B) and pRL-SV40. Twenty-four hours after transfection, U2OS cells were untreated or treated with doxorubicin (2 μM), and 24 h later, dual luciferase assay was performed. (C) The effect of YY1-silencing on p73 promoter activity induced by E2F1 in U2OS cells. The indicated siRNA vectors-transfected U2OS cells were co-transfected with p73 luciferase (−4052/+438)/reporter and increasing amount of pcEF9-Flag-E2F1. (D) Western blot analysis of E2F1 and YY1 in HCT116 cells treated by doxorubicin. (E) A model of the co-regulation of YY1 and E2F1 on p73.

E2F1 [13]. Based on the interaction of E2F1 and YY1, we next tested whether p73 promoter activation by E2F1 overexpression also showed the YY1 dependency. The YY1-knocked down cells were co-transfected with serial doses of E2F1-expression vectors and the p73 reporter vector. As shown in Fig. 4C, in YY1-silenced cells, the p73 promoter activation induced by E2F1 was significantly reduced, which was a comparable suppressive effect as that of doxorubicin-treated cells, suggesting that the YY1 signal might be constitutive during doxorubicin treatment. Together with the results of Western blot analysis (Fig. 4D), which revealed that only endogenous E2F1, not YY1 expression was induced by doxorubicin treatment, these data indicated that cooperative action between the constitutive YY1 and the inducible E2F1 contributes to the activation of the p73 promoter under the treatment with doxorubicin. Furthermore, YY1-silencing did not affect the expression of endogenous E2F1 (Fig. 1A), which also strongly suggested that YY1 induces the transcriptional activity of p73 by cooperation with E2F1, not via increasing E2F1.

Taken together, our results uncovered a novel function of YY1 on E2F1-mediated p73 regulation. We summarized the model in Fig. 4E. The fact that YY1 affects p53 family members opened up an attractive possibility that needs further investigation, that is, YY1 might function as a key integrator or modulator of various pathways in the network that includes p53 family members and regulators concerned with cancer progression, development and differentiation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.10.145.

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Photodynamic Therapy for Corneal Neovascularization Using Polymeric Micelles Encapsulating Dendrimer Porphyrins

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PURPOSE. To investigate the accumulation of new photosensitizers (PSs), dendrimer porphyrin (DP, free DP), and DP encapsulation into polymeric micelles (DP-micelle) and the efficacy of photodynamic therapy (PDT) in an experimental corneal neovascularization model in mice.

METHODS. Corneal neovascularization was induced by suturing 10-0 nylon 1 mm away from the limbal vessel in C57BL6/J mice. To determine the accumulation of free DP and DP-micelle, 10 mg/kg free DP or DP-micelle was administered by intravenous injection 4 days after suture placement. Mice were killed 1, 4, 24, and 168 hours after the injection of PS. Twenty-four hours after the administration of free DP or DP-micelle, mice were treated with a diode laser of 438-nm wavelength at 10 or 50 J/cm^2. Fluorescein angiography was performed before and 7 days after irradiation, and the area of corneal neovascularization was quantified.

RESULTS. Free DP and DP-micelle accumulated in the neovascularized area 1 hour to 24 hours after administration. Fluorescence of DP was weaker than that of DP-micelle. Neither DP-micelle nor DP could be detected in normal limbal vasculature. In the PDT experiments using PS, mean residual rates of corneal neovascularization were 10.1% in the mice treated with DP-micelle and 21.6% in the mice treated with free DP at 10 J/cm^2 ($P < 0.01$). At 50 J/cm^2, mean residual rates of corneal neovascularization were 10.6% in the mice treated with DP-micelle and 13.7% in the mice treated with free DP ($P > 0.05$). Although corneal neovascularization in PDT-treated mice exhibited significant regression compared with the control group, significant energy-related vessel regression with increasing laser energy could not be observed.

CONCLUSIONS. PDT with DP-micelle and free DP can provide efficacious treatment of corneal neovascularization. (*Invest Ophthalmol Vis Sci.* 2008;49:894-899) DOI:10.1167/iovs.07-0389

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Corneal neovascularization is a major sight-threatening condition and is caused by infections, inflammation, degenerative disorders, and long-time contact lens wear.[1] This major ocular complication can lead to corneal scarring, edema, lipidic deposition, and inflammation that may not only compromise visual acuity but also decrease the success rate of subsequent penetrating keratoplasty.[2] In the clinical setting, topical corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) remain the principal primary treatment for suppressing proliferating corneal vessels.[3] However, in corneas in which vessels have been established for extended periods, corticosteroid and NSAID treatment are ineffective. Although laser photocoagulation for corneal neovascularization has been reported,[4-7] this method achieves an inadequate effect because of the high incidence of recanalization and thermal damage to adjacent tissue.[4] The discovery of the many factors involved in corneal neovascularization and their mechanisms of action has been followed by efforts to develop new drugs specifically targeting these molecules. For example, therapy targeting vascular endothelial growth factor (VEGF) looks promising for the treatment of corneal neovascularization.[8] However, the agents tested thus far have yet to become available clinically.

Photodynamic therapy (PDT) has been introduced recently as a novel treatment for corneal neovascularization.[9] In this therapy, a photosensitizer (PS) is injected systemically and accumulates in newly formed vessels; it is then activated by mild laser excitement to liberate cytotoxic reactive oxygen species (ROS) that selectively occlude the target vessels. Although benzoporphyrin (verteporfin), a PS, is used for choroidal and corneal neovascularization clinically,[9] nonspecific binding activities of verteporfin induce skin phototoxicity in bright conditions, and patients must remain in the dark for 48 hours after injection of this drug. Hence, innovative PS should be developed for realizing safe and effective PDT.

Specific delivery of a PS to the neovasculature site might be a promising way to achieve safe and effective PDT for corneal neovascularization. Drug vehicles such as liposomes can be used for this purpose; however, the self-quenching effect of PS caused by aggregate formation could decrease the efficiency of ROS production. To solve this problem, we have recently developed dendrimer porphyrin (DP) as a novel PS for drug delivery (Fig. 1A).[10] It is assumed that the dendritic framework of DP might prevent the interactions of the center dye molecules, thereby achieving efficient ROS production even at extremely high concentrations. Indeed, encapsulation of DP into polymeric micelles (DP-micelle), which are characterized by the polyion complex core surrounded by poly(ethylene glycol) (PEG) palisades (Fig. 1C), resulted in remarkably increased photocytotoxicity.[11,12] We previously reported the review and general introduction of drug delivery of these PSs in corneal neovascularization.[13] In this study, to demonstrate the potential usefulness of DP and DP-micelle for PDT of corneal neovascularization, we investigated the accumulation of those PS

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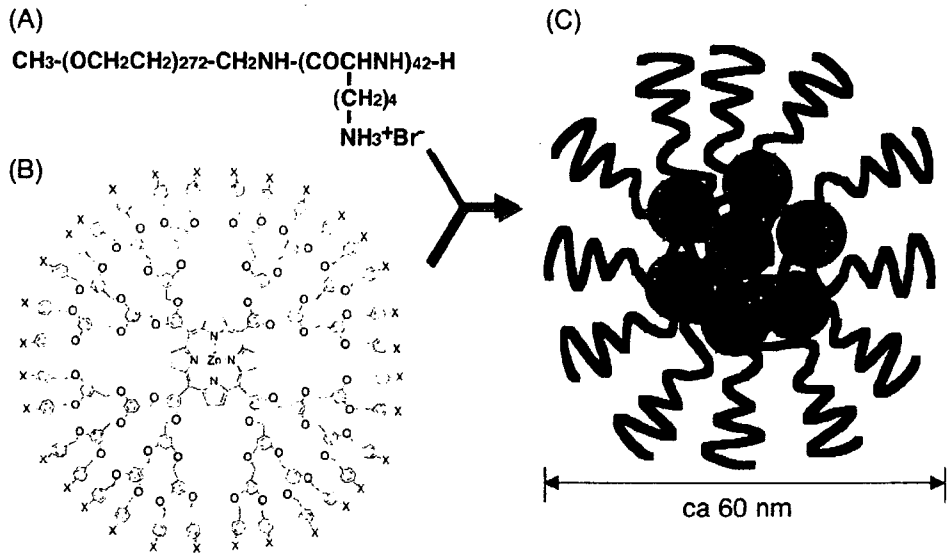

FIGURE 1. Schematic structure of polymeric micelle encapsulating DP. Chemical structures of poly(ethylene glycol) (PEG)-*b*-poly(L-lysine) (**A**) and ionic dendrimer porphyrin (X = COO^-) (DP) (**B**). DP-micelle is spontaneously formed through the electrostatic interaction between PEG-*b*-poly(L-lysine) and DP (**C**).

formulations and their efficacy of PDT in an experimental corneal neovascularization model in mice.

MATERIALS AND METHODS

Animals and Experimental Corneal Neovascularization

Eight-week-old male C57 BJ/6 mice were maintained with free access to food and water. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were placed under general anesthesia by the administration (1.5 mL/kg) of a mixture of ketamine hydrochloride (Ketalar; Sankyo, Tokyo, Japan) and xylazine hydrochloride (Celactal; Bayer, Tokyo, Japan). Corneal neovascularization was induced by suturing 10-0 nylon 1 mm away from limbal vessel under microscopy. Erythromycin ophthalmic ointment was instilled immediately after the procedure.

Photosensitizers

In this study, a third-generation aryl ether dendrimer zinc porphyrin with 32 carboxyl groups on the periphery (DP) and polymeric micelles composed of the DP and PEG-*b*-poly(L-lysine) (DP-micelle) were used for PDT as a PS formulation (Fig. 1). The DP-micelle was prepared according to a previous report.[11] Both DP and DP-micelle have a maximum excitation wavelength at 433 nm. DP-micelle showed 130- to 280-fold higher photocytotoxicity against murine Lewis lung carcinoma cells compared with free DP.[11]

Accumulation of DP and DP-Micelle in Corneal Neovascularization Lesions

Four days after suture placement, DP or DP-micelle was administered by intravenous injection at the dose of 10 mg/kg, again under general anesthesia. Mice were killed 1, 4, 24, and 168 hours after the injection of PS. Before the kill, mice received intravenous BS-1 lectin conjugated with FITC (500 μg/g; Vector Laboratories, Burlingame, CA) to trace the corneal neovascularization area. Corneas were excised and flat-mounted on glass slides. Accumulations of DP or DP-micelle in vascularized areas were observed by fluorescence microscopy (Leica, Deerfield, IL) using 436-nm excitation wavelength. Fluorescence intensities were calculated using NIH Image software and were normalized by traced vascularized areas.

Photodynamic Therapy

Twenty-four hours after DP-micelle or free DP, 38 mice were treated with a diode laser (in-house built laser equipment; Topcon, Tokyo, Japan) of 438-nm wavelength at 500 mW/cm^2 for 20 or 100 seconds, for a total dose of 10 or 50 J/cm^2. The spot size was 1 mm in diameter. As controls, six mice with corneal neovascularization were irradiated without administration of photosensitizers (total dose, 50 J/cm^2). Fluorescein angiography was performed before and 7 days after treatment, and the area of corneal neovascularization was quantified using NIH Image software before and 7 days after irradiation. We defined residual ratio as follows: Residual ratio = (neovascularization area 7 days after irradiation/neovascularization area before irradiation) × 100%.

RESULTS

Accumulation of DP and DP-Micelle in Corneal Neovascularization Lesions

One hour after the administration of DP-micelle, fluorescence started to accumulate in the neovascularized area and increased until 24 hours after administration (Figs. 2A-C). DP-micelle accumulation decreased but continued after 168 hours (data not shown). After DP administration, fluorescence of DP was observed in the corneal neovascularization area, but it was weaker than that of DP-micelle group (Figs. 2D-F) and disappeared by 168 hours (data not shown). Figure 3 shows the time course of normalized fluorescence intensities of DP in the neovascularized area. DP-micelle intensities were significantly higher than those of free DP 1 hour (n = 7/each condition), 4 hours (n = 6/each condition), and 24 hours (n = 6/each condition) after administration (P = 0.032, 0.047, 0.0066; Mann-Whitney U test). Neither DP-micelle nor free DP could be detected microscopically in normal limbal vasculature or other ocular tissue such as iris, retina, and conjunctiva (data not shown).

Photodynamic Therapy

Figure 4 is a series of fluorescence angiographic images of corneal neovascularization before and 7 days after PDT in the control, DP-micelle, and DP groups. As shown in Figure 4A, there was no effect of irradiation in the control (n = 6). Seven days after irradiation at 10 J/cm^2, the mean residual ratio of

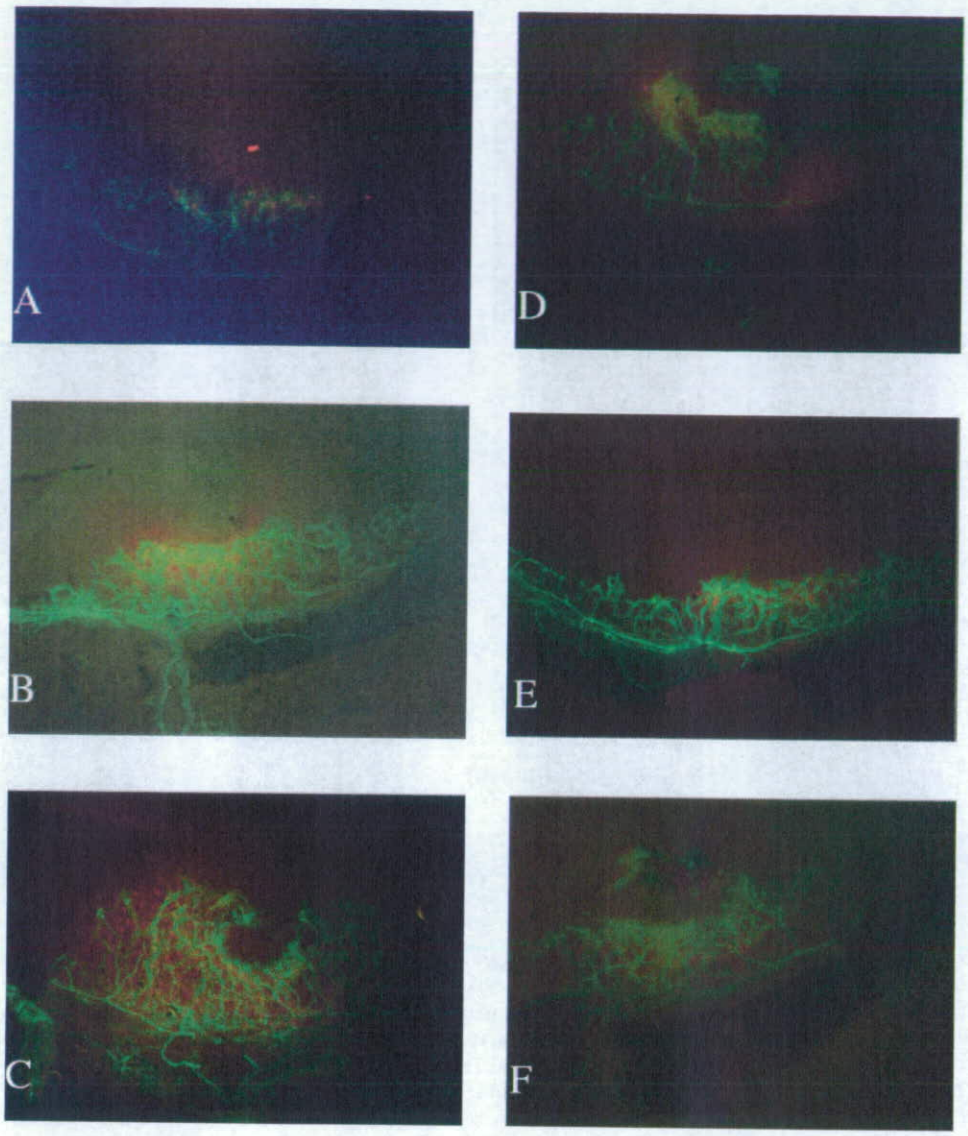

FIGURE 2. Accumulation of DP and DP-micelle to neovascularized area. The flatmounts of the neovascularization area were observed under a fluorescent microscope 1 hour (**A**), 4 hours (**B**), and 24 hours (**C**) after administration of DP-micelle and 1 hour (**D**), 4 hours (**E**), and 24 hours (**F**) hours after injection of DP. Vessels were stained with BS-1 lectin conjugated with FITC. Fluorescence of DP was observed 1 hour after administration (**A**, **D**) and enhanced until 24 hours after.

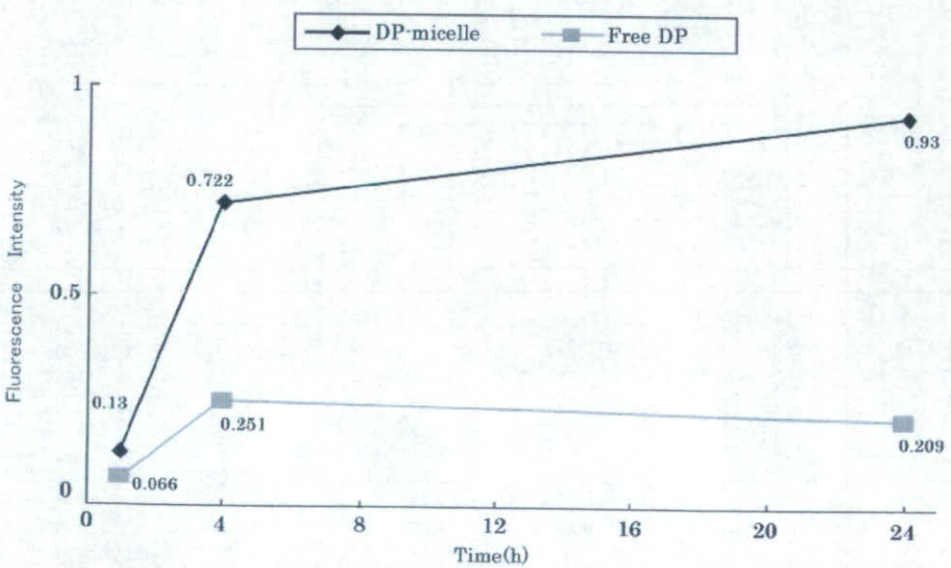

FIGURE 3. Time course of fluorescence intensity. Fluorescence intensity of DP in cornea after administration of DP-micelle or free DP. Fluorescence of DP was detected in the corneas of the DP-micelle group as early as 1 hour, and intensity reached a peak 24 hours after intravenous administration, whereas the intensity in the free DP group was significantly lower than that in the DP-micelle group.

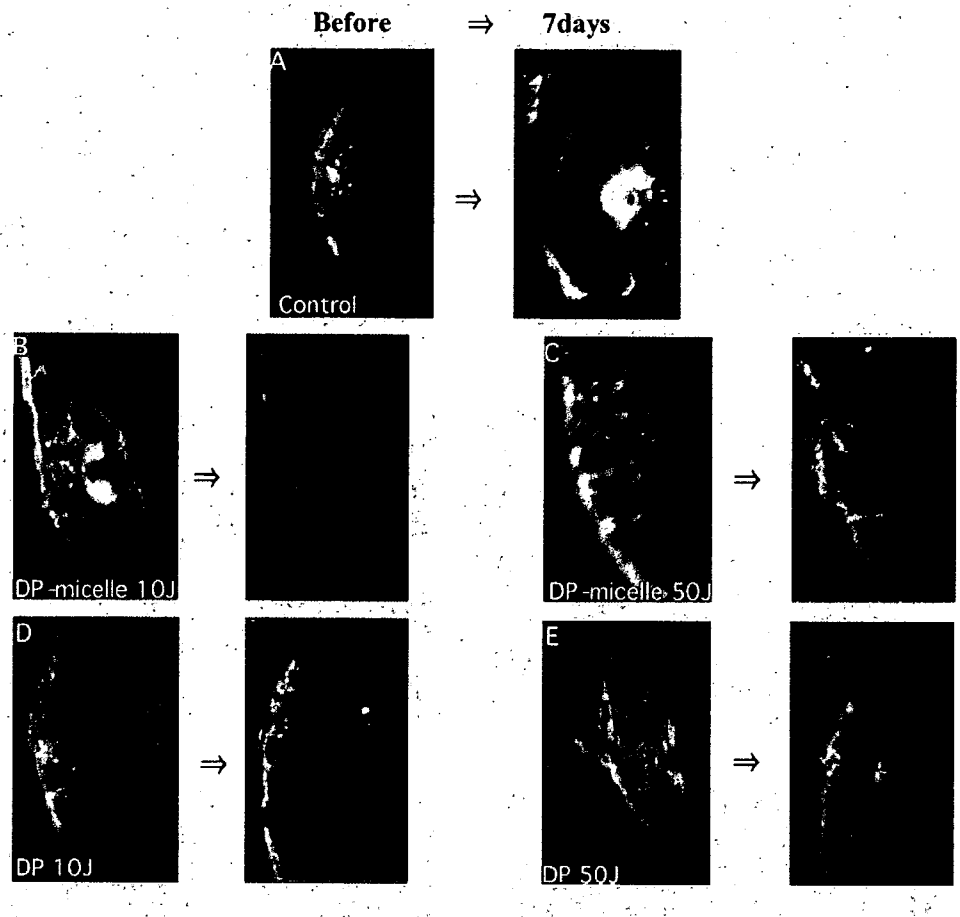

FIGURE 4. Neovascularization regression after PDT. (A) Control. No regression was observed without micelle or DP administration. (B) Irradiation at 10 J/cm^2 with DP-micelle. (C) 50 J/cm^2 with DP-micelle. (D) Irradiation at 10 J/cm^2 with DP. (E) Irradiation at 50 J/cm^2 with DP. (B–E) In each group, significant regression of neovascularization was observed.

corneal neovascularization was 10.1% in the mice treated with DP-micelle (n = 9) and 21.6% in the mice treated with free DP (n = 10; Fig. 5). The residual ratio of mice treated with DP-micelle was significantly higher than that of mice treated with free DP ($P < 0.01$; Mann-Whitney U test). Seven days after irradiation at 50 J/cm^2, the mean residual area of corneal neovascularization was 10.6% in the mice treated with DP-micelle (n = 10) and 13.7% in the mice treated with free DP (n = 9; $P > 0.05$; Mann-Whitney U test; Fig. 5). Histologic examination after PDT showed no injury on corneal tissue (data not shown).

DISCUSSION

PDT potentially represents a new approach for the treatment of neovascular disease and tumor.[14] The successful treatment of choroidal neovascularization by PDT opens the possibility of treating other neovascular diseases of the eye, including the cornea, in a similar manner.[15–17] Indeed, several past studies revealed that PDT was efficacious for the treatment of corneal neovascularization.[18–21]

One of ideal characteristics of PS is selectivity to neovasculature. In this study, DP-micelle, a newly developed PS

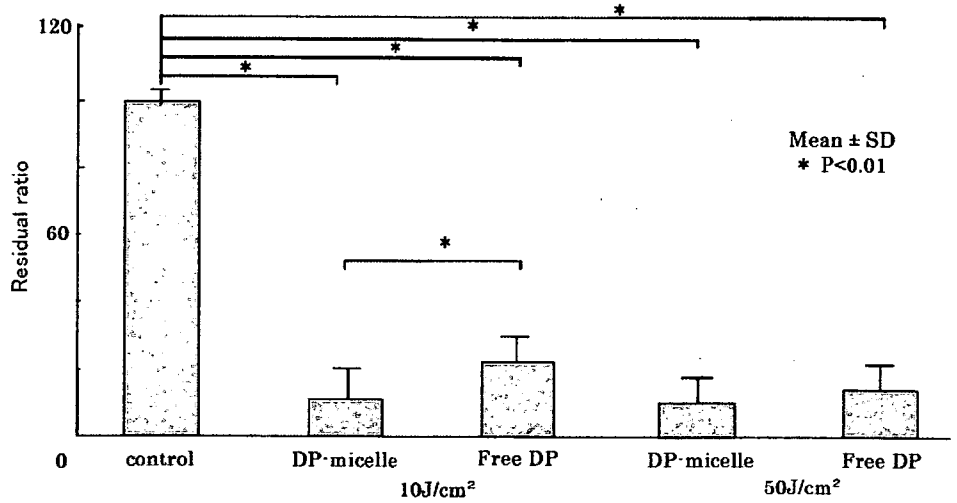

FIGURE 5. Residual ratio of corneal neovascularization 7 days after PDT. Residual ratio of corneal neovascularization 7 days after PDT with DP-micelle or DP or with no administration. Residual ratios were 10.1% with irradiation at 10 J/cm^2 and micelle, 21.6% with irradiation at 10 J/cm^2 and DP, 10.6% with irradiation at 50 J/cm^2 and micelle, and 13.7% with irradiation at 10 J/cm^2 and DP. Error bars indicate SD.

FIGURE 6. Long-term effect of PDT. (A) Before irradiation. (B) Seven days after irradiation at 10 J/cm^2 with micelle. Significant regression of neovascularization was observed. (C) Sixty-three days after irradiation. Little recanalization of neovascularization was observed, but a few matured vessels were not occluded.

formulation, was accumulated only in pathologic vascularized areas. On the other hand, DP-micelle and free DP were not detectable in normal vessel areas, including other ocular vessels (data not shown). The high selectivity of these PSs to neovascularization should result in minimal side effects in the adjacent normal corneal structure. Indeed, slit lamp examination and histologic observation showed no alterations by PDT to the surrounding structure, including epithelium, stroma, and endothelium (data not shown).

Macromolecular compounds can accumulate and prolong their retention in the perivascular regions of solid tumors to a greater extent than in normal tissues because newly formed vessels in solid tumors exhibit higher substance permeability than in normal tissues and because lymph systems in tumor tissue are incomplete. This effect is known as the enhanced permeability retention (EPR) effect.[22] We have demonstrated that polymeric micelles with diameters of several tens of nanometers with narrow distribution can accumulate in a solid tumor though the EPR effect.[23–25]

As do solid tumors, corneal neovascularization sites appear to have high permeability and incomplete lymph systems. Hence, we assume that DP-micelle can accumulate selectively in corneal neovascularization lesions.

Another ideal characteristic in PDT is high photocytotoxicity with lower dark cytotoxicity. As reported earlier, the laser energy of PDT for corneal neovascularization with verteporphin, a PS used clinically for choroidal neovascularization, was 150 J/cm^2 to create long-lasting clinical regression of corneal neovasculature.[19] This is three times the amount needed to create regression of choroidal neovascularization. PDT with DP-micelle required only 10 J/cm^2 for long-lasting regression of neovascularization. Most PSs have hydrophobic properties resulting in their self-quenching from aggregation, decreasing photooxidation efficacy to achieve successful PDT. Dendrimer photosensitizers are designed to prevent the aggregation of core PS even in the micellar core. In addition to the EPR effect, this property might have led to a higher neovascularization regression rate in our study than in past studies.

This study indicates PDT with DP-micelle and free DP can provide efficacious treatment of corneal neovascularization. It is important to observe and examine the long-term effects in the future. We observed 2 months after irradiation with PIC micelle at laser energies of 10 J/cm^2. As shown in Figure 6, most of the corneal neovasculature was not recanalized, though a few matured vessels remained over 2 months. In addition, polymeric micelles can encapsulate a variety of drugs, including hydrophobic substances, nucleic acids, and proteins in the core[23]; therefore, they have great potential for effective drug delivery targeting to corneal neovascularization.

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シンポジウム「運動器の10年」変形性関節症の病態解明・診断・治療の新世紀

変形性関節症の疫学研究*

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緒　言

変形性関節症(osteoarthritis: OA)は関節に非炎症性，進行性に骨形成性の変化を来し，疼痛によって日常生活に不都合を来す疾患である．OAは加齢とともに発生が増加するため，高齢化が急速に進み続けるわが国においては，その患者数は今後も増加し続けることは明らかである．平成16年の厚生労働省国民生活基礎調査の結果を見ると，高齢者が要支援になる原因としては老衰についで本疾患が挙げられ，疾病の中では最も多いと報告されている．すなわちOAはきわめて多くの高齢者の生活の質(quality of life: QOL)を低下させることによって，その健康寿命を短縮し，さらに医療費の高騰，労働力の低下の一因となっていることが明らかである．

しかしながら，本疾患は慢性に進行し経過が長いことから発生の日時の特定に困難がつきまとう．そのためOAの予防に必要な疫学指標を推定するためには一般住民の集団を設定して，集団全体について検診を行う必要がある．このような研究デザイン上の困難と制約のために，患者数がきわめて多く，予防のニーズが大きいと考えられるにもかかわらず，本疾患を目的疾患とした疫学研究の報告は十分とは言えない．

著者らは国際共同研究の一環として，従来よりOAの頻度と危険因子の研究を行ってきた．本稿ではその結果からOAの頻度と危険因子について述べ，最後に昨年開始され明らかになってきた大規模疫学研究を紹介したい．

Key words: Osteoarthritis, Prevalence, Risk factors, Population-based cohort study

*Epidemiology of Osteoarthritis in Japan

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1. OAの頻度

OAの有病率を明らかにするためには特性を明らかにした集団を設定し，X線による悉皆調査を行う必要がある．著者らは和歌山県の山村部にコホートを設定し，その頻度を推定した．

1) L/S OAの有病率[1)]

【対象と方法】著者らは和歌山県山村部で40-79歳の全住民1543人を対象として，コホートを設定した．この集団から40-79歳の各年代男女各50人，計400人をランダムに選び，骨粗鬆症予防検診を行った．骨粗鬆症検診の際に撮影された腰椎X線フィルムを，対象者の許可を得て熟練した整形外科医が1人で読影し，Kellgren-Lawrence(KL)の分類を用いてOAの頻度を求め，これを同様の調査を行っている英国ハートフォードシャー住民の頻度と比較した．

【結果】60歳以上の骨粗鬆症予防検診参加者を対象としたため，解析対象者は男性98人，女性99人となった．KLの分類を用いたL/S OAの頻度を図に示す(図1)．これから見ると，男性ではグレード2が最も多く，女性ではグレード0/1が最も多かった．この結果，L/S OAにおいては女性よりも男性の方がグレードが高い

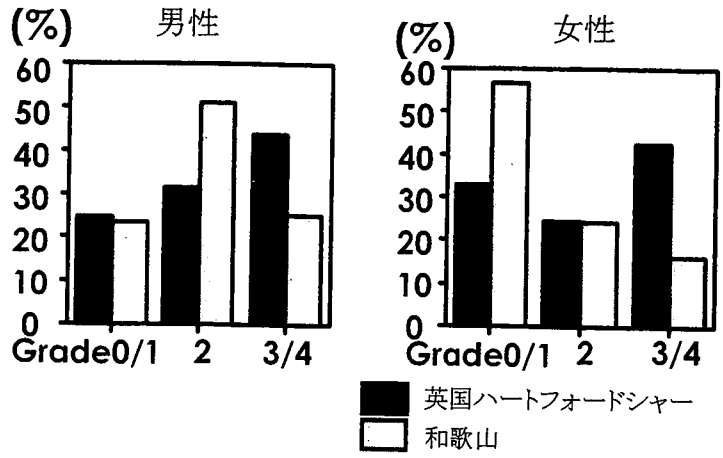

図1 K/L 分類の分布

ことが明らかになった．英国住民と比較すると，男女ともに，L/S のグレードは明らかに英国の方が重症であることがわかった．

2) Hip OA の有病率[2)]

【対象と方法】前述の和歌山県山村部における 40-79 歳の各年代男女各 50 人，計 400 人のランダムサンプリングによる骨粗鬆症予防検診参加者から，撮影された股関節 X 線フィルムを読影する許可を得て，熟練した整形外科医が 1 人で読影し，KL 分類の変法である Croft 分類[3)]を用いて Hip OA の頻度を求めた．

【結果】Hip OA の頻度調査においても L/S OA 調査と同様和歌山県山村部の骨粗鬆症予防検診受診者のうち 60 歳以上の男女としたため，解析対象者は男性 99 人，女性 99 人となった．この集団の股関節 X 線読影において Croft の分類で 3 度以上を重症 OA とし，その頻度を求めたところ，女性では 2%に認められたが，男性では重症 OA はいなかった．

2. OA の危険因子

疾病を予防するためには，まずその目的疾患の頻度を明らかにした後に，コホート研究や症例対照調査などの分析疫学的手法を用いて，疾病の危険因子，予防因子を明らかにすることが有効である．

著者らは，記述疫学的方法で得た結果を生かし，OA の発症要因に迫るために，Hip OA および Knee OA の症例対照研究を実施した．

1) Hip OA の危険因子[4)]

【対象と方法】和歌山県北部の和歌山市，有田市の 5 つの病院の協力を得て本調査を行った．まず症例を調査の 1 年以内に対象病院を受診した 45 歳以上の男女で，整形外科医により Hip OA と診断され，かつ調査期間中に人工股関節全置換術の適応と診断されているものと定義した．対照は 1 症例につき 1 人とし，症例と性，年齢(±2 歳)をあわせ，症例の住所における住民台帳よりランダムに抽出した．

調査は症例，対照とも同一の調査者が訪問し，本人の承諾をとった後に，対面聞き取り調査を行った．調査項目は職業，移動手段，スポーツ，趣味，たばことアルコールの摂取頻度，関節障害の有無，運動障害の有無，既往歴，月経状況など約 60 項目となった．分析は conditional regression analysis により行った．

【結果】表 1 に症例の身体的および X 線読影結果の特徴を示す．この結果から Hip OA の症例においては，男性が圧倒的に少ないことがわかった．症例の年齢は 63.8 歳であり，その 98.3%が KL グレード 3 以上の重症 OA であった．症例と対照の背景要因を比較すると，社会経済指標の 1 つである最終学歴(卒業時年齢)，および，過去の膝痛の有無に有意差が見られたが，体格に差は認められなかった(表 2)．職業上の動作の Hip OA 発生に対するリスクを求めたところ，荷重のかかる仕事が有意にリスクが高いことがわかった(表 2)．この関連は最終学歴と過去の膝痛を補正しても認められた．

2) Knee OA の危険因子[5),6)]

【対象と方法】和歌山県の和歌山市，有田市，大阪府泉南市の 6 つの病院の協力を得て本調査を行った．症例の定義は，調査の 1 年以内に対象病院を受診した 45 歳以上の男女で，整形外科医により KL 3 度以上の

表1 Hip OA 対象者の背景要因

	総数	男性	女性
症例数	114	11	103
年齢(標準偏差)[歳]	63.8(10.9)	61.0(10.0)	64.1(11.0)
股関節の部位			
一側性(%)	70(61.4)	4(36.4)	66(64.1)
両側性(%)	44(38.6)	7(63.6)	37(35.9)
Minimum joint space(% 1 mm 以下)	96.5	100	96
K/L グレード 3 以上	98.3	100	98

表2 Hip OA の危険因子

危険因子	カテゴリー	オッズ比(95% CI)
Body Mass Index (kg/m^2) †	軽い	1
	中程度	0.8(0.4-1.5)
	重い	1.0(0.5-1.9)
最終学歴(卒業時年齢) [歳]	高い(18 歳以上)	1
	中程度(15-17 歳)	1.4(0.8-2.5)
	若い(14 歳以下)	2.4(1.1-5.0)
過去の膝痛	はい vs いいえ	2.3(1.3-4.3)
最初の仕事		
10 キロ以上の荷物を持ち上げる	はい vs いいえ	1.4(0.8-2.7)
25 キロ以上の荷物を持ち上げる	はい vs いいえ	3.6(1.3-9.7)
50 キロ以上の荷物を持ち上げる	はい vs いいえ	5.4(1.2-25.4)

*$p<0.05$
†3 分位で比較

Knee OA と診断されているものとした．対照は 1 症例につき 1 人とし，症例と性，年齢(±2 歳)をあわせ，症例の住所における住民台帳よりランダムに抽出した．調査は症例，対照とも同一の調査者が訪問し，本人の承諾をとった後に，対面聞き取り調査を行った．調査項目は Hip OA と同様，職業，移動手段，スポーツ，趣味，たばことアルコールの摂取頻度，関節障害の有無，運動障害の有無，既往歴，月経状況など約 60 項目であった．分析は conditional logistic regression analysis により行った．

【結果】表 3 に症例と対照の身体的特徴と背景要因を示す．Knee OA においても集められた症例の男女比では男性が少なかった．症例は対照よりも体重が重く，体格指数である body mass index (BMI kg/m^2) は有意に高かった．Conditional logistic regression analysis によって，Knee OA の危険因子を求めたところ，体重，膝のけが，職業上の動作が関連していることがわかった．職業について詳しく解析を行うと，男性では，はじめて就いた職業が肉体をよく使う労働であることがリスクを上昇させていた．女性では就業期間が長いほどオッズ比は有意に高くなっていた(表 4)．

3. 大規模疫学研究 ROAD

前述の著者らが行ってきた OA を目的疾患とした疫学研究はいずれも小規模なものであり，研究方法，特にサンプリング方法には細心の注意を払ったつもりではあるものの，この結果を一般化することには問題が多いと考えられた．しかしその一方，2010 年にはわが国の人口の 25%以上が 65 歳以上となると推測され，要支援の対象となる原因疾患の第 1 位が OA であることを考え合わせる[7]と OA の予防対策はまさに待ったなしの状況にある．

そこで著者らは，わが国の一般住民における OA の有病率・発生率などの基本的疫学指標を明らかにし，その危険因子となる生活習慣を同定すること，さらに OA の経過，各治療別の経過に影響を及ぼす要因について

表3　Knee OA の症例と対照の背景要因

	男性		女性	
	症例	対照	症例	対照
患者数	37	37	101	101
年齢(歳)	70.0±6.6	70.1±7.0	73.3±9.8	73.3±9.8
体重(kg)	64.1±10.7*	59.3±8.7	53.8±10.1**	49.7±8.6
身長(cm)	162.5±6.9	163.0±6.7	148.8±6.3	149.6±6.9
Body Mass Index(kg/m²)	24.2±3.4*	22.4±3.8	24.3±3.9***	22.1±3.0
喫煙率(%)	16(43.2)	15(40.5)	9(8.9)	5(5.0)
飲酒率(週5回以上，%)	20(54.1)	22(59.5)	10(9.9)	5(5.0)

平均 ±標準偏差
(　　)内%
*p<0.05，症例 vs 対照

表4　Knee OA の危険因子

男性	カテゴリー	補正後オッズ比(95%CI)
過去の最大体重†	中程度 vs 軽い	1.25(0.29-5.35)
	重い vs 軽い	6.01(1.18-30.5)*
いずれかの膝のけが	あり vs なし	6.25(1.13-34.5)*
職業上の要因	はじめて就いた仕事が肉体的労働‡(vs 他の職種)	6.20(1.40-27.5)*
女性	**カテゴリー**	**補正後オッズ比(95%CI)**
過去の最大体重§	中程度 vs 軽い	3.13(0.94-10.48)
	重い vs 軽い	4.42(1.17-16.64)*
いずれかの膝のけが	あり vs なし	6.84(2.35-19.94)***
移動	ほぼ毎日自転車に乗るのが12カ月以上(vs 未満)	1.77(0.66-4.78)
職業上の要因	はじめて就いた仕事で2時間以上座る(vs 未満)	0.35(0.15-0.84)*
	現在までについた仕事の数(vs 1つ)	0.92(0.67-1.25)
	総就労年数(1年)	1.05(1.01-1.08)**

各リスク要因はお互いに補正された．
95%CI: 95% 信頼区間
†体重は3分位で比較．男性では，軽い: 61.0 kg 未満，中程度: 61.0 kg 以上 72.0 kg 未満，重い: 72.0 kg 以下．女性では，軽い: 55.0 kg 未満，中程度 55.0 kg 以上 62.0 kg 未満，重い: 62.0 kg 以上
‡ここでは肉体的労働を工場，建築，農業，漁業と定義した．
*p<0.05，**p<0.01，***p<0.001

明らかにすることを目的として，大規模臨床統合データベースの設立を企画した．この一連の研究活動をROAD(Research on Osteoarthritis Against Disability)プロジェクトと名付けた．ROADは，特徴の異なる地域の一般住民計5000人と，OA患者5000人を10年間追跡する2本立てのコホート研究であり，詳細なゲノム情報と臨床情報をともに含む統合データベースの構築を目指している．

ここではすでに研究が進んでいる2つの地域コホートの結果から，OAの有病率を推定したので報告する[8]．

【対象と方法】都市部コホートとして，東京都板橋区において2005年1月-12月に骨関節疾患予防検診として60歳以上の住民を対象に問診票調査，運動機能調

査，および腰椎・膝X線撮影を行った．また，山村部コホートとして，和歌山県日高川町において40歳以上の住民を対象として2005年11月-2006年2月に板橋区と同様の検診を行った．X線画像はKL法を用いて整形外科医が読影し，グレード2以上をOA変化ありとした．

【結果】都市部コホートでは現在1300人の検診が終了しており，X線の解析については1030人(男性355人，女性675人：平均年齢76.9歳)が終了した．山村部の検診には864人の参加を認めたが，40歳未満の9人を除き，855人(男性317人，女性538人：平均年齢69.3歳)の結果を解析した．この結果，両地域における50歳以上の住民の腰椎にOA変化を認めるものの割合(有病率)は，男性で81%，女性で68%であることがわかった．また，左右いずれかの膝にOA変化を認めるものをKnee OAと定義すると，Knee OAの有病率は男性で54%，女性で75%となった．これを平成16年度の年齢別人口構成にあてはめてみると，全国で男性1770万人，女性1530万人，総計3300万人がL/S OA，男性1240万人，女性1840万人，総計3080万人がKnee OAであると推定された．これはX線での診断結果からの推定であるため，症状が顕在化した患者数というわけではないが，潜在患者数としても従来の試算よりもはるかに高い数字であった．

おわりに

本稿では，著者らが行ってきたOAに関する疫学研究について述べ，その欠点をふまえた上で開始されたROADプロジェクトの概要を紹介した．ROADは従来のOA研究にはなかった規模の人的，物的環境を背景としており，本プロジェクトによってOAの疫学指標が確立し，その発症要因の解明と治療の標的分子が同定できれば，焦眉の課題であるOAの根本的な予防・治療法の実現も視野に入ってくる．これは，高齢者QOLの改善，健康寿命の延伸につながるのみならず，医療経済的にも大きな意味を持つと考える．しかしこのプロジェクトはまだ始動したばかりであり，今後多くの専門家や現場の方々とのネットワークを強化し，その指導と協力を得て，研究の精度をあげ，継続拡大させていきたい．

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