

Abnormal accumulation of OFD1 in endometrial cancer with poor prognosis inhibits ciliogenesis

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Abstract. The aim of the present study was to examine primary cilia in endometrial tissue during the menstrual cycle and to clarify their morphological changes with different grades of endometrial cancer. Images of fluorescence immunostaining taken by confocal microscopy were used to count the number of primary cilia in normal endometrium and endometrioid carcinoma Grade 1 and Grade 3 specimens. To examine the association between autophagy and ciliogenesis in endometrioid carcinoma, the expression of p62/Sequestosome-1, a selective substrate for autophagy, and oral-facial-digital syndrome 1 protein (OFD1), a protein associated with ciliogenesis, were examined using images of fluorescence immunostaining taken by confocal microscopy. The level of p62 expression was confirmed by western blotting. In proliferative and secretory endometrial stromal cells, the percentage of cells that were ciliated was 7.2 and 32.7% (95% confidence interval=21.61-39.79; P<0.01), and the length of the primary cilia was 1.24 μ m and 2.34 μ m (0.92-1.26; P<0.01), respectively. In stromal cells of endometrioid carcinoma Grade 1 and Grade 3, the percentage of ciliated cells was 13.5 and 2.9% (7.89-15.05; P<0.001), and the length of the primary cilia was 2.02 and 1.14 μ m (0.76-0.99; P<0.001), respectively. In both normal menstrual cycle tissue and endometrial carcinomas, the percentage of primary cilia was lower and their length was

shorter in tissues with higher proliferative potential. The expression of OFD1 was significantly higher in Grade 3 compared with Grade 1 as indicated by quantifying the intensity of the fluorescence images (133-12248; P=0.046). To the best of our knowledge, this is the first study concerning the distribution of primary cilia in normal endometrium and endometrial cancer tissues. Overall, fewer ciliated cells in the highly malignant endometrial cancer tissues may be associated not only to the proliferation of cancer cells, but also to the excessive accumulation of OFD1 due to dysfunctional autophagy.

Introduction

Primary cilia are single, immotile organelles that are present in all mammalian cells. These structures protruding from the surface of the cell membrane function as extracellular signal receptors for mechanical and chemical stimuli (1). The primary cilium is an organelle that exists in the G₀/G₁ phase of the cell cycle (1). It has been reported that ciliogenesis, consisting of the assembly, degradation and disappearance of primary cilia, is regulated by intracellular and extracellular signaling and is involved in cell proliferation (2). The endometrium is a tissue that undergoes repeated proliferation and breakdown in response to ovarian steroid hormones during the menstrual cycle, and the state of primary cilia in this tissue undergoes change on a monthly basis (3).

Disorder in this ciliary cycle, leading to dysregulation of cell proliferation in various malignancies, has been thought to result in a loss or reduction of ciliated cells. To date, the changes associated with primary cilia have been reported in tumor tissues or cultured cells that originated from such tumors as glioblastoma (4), basal cell carcinoma (5), colorectal cancer (6), ovarian cancer (7), breast cancer (8), prostate cancer (9), renal cell carcinoma (10), pancreatic cancer (11) and cholangiocarcinoma (12). However, it has also been reported that inhibition of ciliogenesis suppressed tumor growth in medulloblastoma (13). Endometrial cancer is one of the most common gynecological cancers, and its incidence and mortality rates vary from country to country, perhaps due to environmental factors (14,15). Overall, ~20% of endometrial cancers are diagnosed as advanced cancers, which are

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difficult to treat with local therapy alone and are often resistant to systemic therapy, resulting in a poor prognosis (16). Histological classification of endometrial carcinoma includes endometrioid carcinoma, serous carcinoma, clear cell carcinoma, undifferentiated carcinoma, and so on (17). In Japan, endometrioid carcinoma accounts for 90% of all endometrial cancer (16). Endometrioid carcinoma is defined as Grade 1, 2 or 3 according to the degree of differentiation (16). Poorly differentiated Grade 3 is known clinically to be highly malignant. Although the state of motile cilia (18,19) in endometrial cancer has been observed previously, the state of primary cilia has not been reported as yet.

Autophagy is a self-cleaning pathway by which cells break down intracellular proteins and organelles. In previous years, autophagy has attracted attention as an important regulator of ciliogenesis and cilia length. Tang *et al* showed that autophagy promotes ciliogenesis through selective degradation of Oral-facial-digital syndrome 1 protein (OFD1) (20). Lee *et al* reported that genetic and pharmacological inhibition of autophagy in Hurthle cell carcinoma results in the formation and the elongation of primary cilia (21). In addition, Inami *et al* reported that p62/Sequestosome 1 (p62), which accumulates in hepatocellular carcinoma due to inhibition of autophagy, is involved in tumor growth by activating the Nrf2 signaling system (22). Therefore, the state of primary cilia and autophagy in cancers may act as determinants of the tumor grade and prognosis (23).

In this study, we examined primary cilia in tissues of normal endometrium, endometrioid carcinoma, and a typical histological type of endometrial cancer. We also analyzed the expression of OFD1, a ciliary protein, and p62, an autophagic protein, in tissues of endometrioid carcinoma Grade 1 and Grade 3 to elucidate the relationship between the state of primary cilia and autophagy in endometrioid carcinoma.

Materials and methods

Patients, study design and ethical approval. All patients underwent a total hysterectomy at Nagoya City University Hospital from January 2014 to December 2018. Normal endometrium of 4 patients in the proliferative phase, 5 patients in the secretory phase and 4 patients in the menopausal phase was used to examine differences in primary cilia at each phase of the menstrual cycle. Normal endometrium was obtained from patients who underwent a hysterectomy for cervical disease or ovarian cancer (Table SI). Obstetricians/gynecologists previously determined the menstrual cycle stage by histologically examining the specimens (24). We have postmenopausal specimens based on the medical records. For endometrial cancer, 17 patients with Grade 1 and 8 patients with Grade 3 endometrioid carcinoma were compared (Table SII). Pathologists in Nagoya City University Hospital were responsible for grading the endometrioid carcinomas. Medical information was collected regarding the patient's age, menstrual cycle, and previous medical history. For endometrial cancer patients, we obtained information on the stage of surgery, as well as recurrence or death following surgery to November 2021.

We examined the expression of primary cilia in normal endometrium and compared their number and length in

proliferative, secretory, and menopausal endometrium as well as between endometrioid carcinoma Grade 1 and Grade 3 by fluorescence immunostaining.

Next, we examined the expression of p62 and OFD1 in endometrioid carcinoma Grade 1 and Grade 3. The fluorescence intensity of OFD1 was quantified and compared.

This study was approved by the Medical Research Ethics Review Committee of the Nagoya City University Graduate School of Medicine. Written consent was obtained from each patient after being given an explanation of the purpose of the research.

Antibodies. The following primary antibodies were used: anti-acetylated-tubulin antibody (mouse monoclonal IgG_{2B}, Sigma, Cat# T7451, clone 6-11-B1; IF 1:500), anti- γ -tubulin antibody (mouse monoclonal IgG₁, Sigma, Cat#5326, clone GTU-88; IF 1:500), anti-Ki67 antibody (rabbit monoclonal IgG, Abcam, Cat#16667, IF 1:200), anti-SQSTM1/p62 antibody (mouse monoclonal IgG₁, Fitzgerald, Cat#10R5910, IF 1:200) and anti-OFD1 antibody (rabbit polyclonal IgG, NovusCat#32843, 1:200). The secondary antibodies used were: tetramethylrhodamine isothiocyanate (TRITC)-labeled goat anti-mouse IgG_{2B} (SouthernBiotech, Ca #1090-03), Alexa Fluor 633-labeled goat anti-mouse IgG₁ (Invitrogen, Ca #A21126) and Alexa Fluor 488-labeled goat anti-rabbit IgG(H+L). Rabbit IgG (Cell Signaling Technology, Ca#2729S) was used for a negative control of the primary antibody. Hoechst33342 (Invitrogen, Ca #H3570) was used for nuclear staining.

Immunostaining. After formalin fixation, paraffin-embedded tissue blocks were thinly sliced into 8 μ m pieces for immunostaining. For deparaffinization, paraffin-embedded tissue slides were heated in a dry incubator at 50°C for 10 min and sequentially washed with 100% xylene (4 times), 100% ethanol (2 times), 90% ethanol, 70% ethanol and pure water. All washing was performed at room temperature. Permeabilization was performed by heating in a microwave oven at 500W for 5 min with TrisEDTA (2 times). Tissue slides were blocked with phosphate buffered saline (PBS) with goat serum (5%) (Thermo Fisher, Cat#16210064) for 60 min at room temperature. Primary antibodies were incubated on the tissue slides overnight at 4°C. The slides were then washed 3 times with PBS. Secondary antibodies with Hoechst were incubated on the slides for 60 min at room temperature. Each slide was mounted with a cover glass (Matsunami, Cat# No1-s, 0.16-0.19 mm thickness) using Aqueous Permanent Mounting Medium (Diagnostic BioSystems, Cat#K002-xx).

Confocal microscopy. Images were acquired using a confocal laser scanning microscope (Olympus, FV3000). To reduce variation, we examined one slide per sample and at three locations on each slide. Images were taken every 0.63 μ m over a thickness of 5 μ m for Z stacks. Z images were projected using FV3000 software.

Image analysis. All image analyses were performed using ImageJ Fiji. We counted numbers of ciliated cells and Ki67-positive cells, and measured the length of primary cilia. OFD1 expression was quantified by fluorescence intensity.

Primary cilia were counted only in cells with both axonemes and basal bodies. The total number of stromal cells and the number of ciliated cells per field of view were counted. Each count was performed manually using an ImageJ Fiji cell counter. The lengths of the cilia were determined using a tool only for measuring the length of the axoneme, and the basal body was not included. The total number of cells per field of view and the number of Ki67-positive cells were counted. For quantification of the intensity, images obtained under the same staining and imaging conditions were used. We quantified the fluorescence intensity of OFD1 by setting the threshold of the intensity to 100-255 and binarizing it.

Protein extraction from FFPE blocks. Protein extraction from FFPE blocks was performed using a Qproteome FFPE Tissue kit (Qiagen, Ca#37623). We cut each block into 10 µm sections. For deparaffinization, we washed the sections with 100% xylene for 10 min (2 times), 100% ethanol for 10 min (2 times), 96% ethanol for 10 min (2 times), 70% ethanol for 10 min (2 times) and pure water for 30 sec. We transferred the tumor area only, as confirmed by HE staining, into a 1.5 ml collection tube using a needle. Samples and 100 µl of Extraction Buffer EXB Plus supplemented with beta-mercaptoethanol were incubated on ice for 5 min and mixed using a vortex mixer. These tubes were heated at 100°C for 20 min and then at 80°C for 2 h with a Thermomixer at 750 rpm. Concentrations of the extracted proteins were determined using a Pierce BCA protein assay kit (ThermoFisher, Ca#23227).

Western blotting. Proteins were separated on 12.5% SDS-PAGE and then transferred to a PVDF membrane. The membrane was blocked with 5% milk in PBST for 1 h at room temperature and then incubated with the following primary antibodies: anti-SQSTM1/p62 antibody (rabbit polyclonal IgG, Proteintech, Ca#18420, 1:1,000), anti-OFD1 antibody (rabbit polyclonal IgG, Proteintech, Ca#22851, 1:1,000) and anti-β-actin antibody (rabbit monoclonal IgG, Cell Signaling, Ca#4970, 1:1,000). Goat anti-rabbit IgG(H+L) (BIORAD Ca#1708241, 1:5,000) was used as the secondary antibody. The results were analyzed with an Amersham Imager 600 (GE Healthcare). We used β-actin as the index of the loaded protein content in each sample.

Statistical analysis. BellCurve for Excel (Social Survey Research Information Co., Ltd.) was used for statistical analysis. All data were expressed as mean values ± Standard Error (SE). One-way ANOVA followed by Bonferroni's multiple comparison test was used to compare three groups, and an unpaired t-test was used to compare two groups. Dot plots and Kaplan-Meier curves were also plotted using BellCurve for Excel. The Kaplan-Meier plotter (www.kmplot.com), an online database including gene expression data and clinical data, was used to examine OFD1 expression.

Results

The state of ciliated endometrial stromal cells during the menstrual cycle. Endometrial stromal cells with primary cilia were counted at proliferative and secretory phases of the menstrual cycle as well as at the menopausal phase.

Median ages (range) of patients with proliferative, secretory, and menopausal endometrium were 49 (45-50), 46 (40-47), and 60.5 (52-69) years (Table SI), respectively. Primary cilia were visualized by fluorescence staining using acetylated α-tubulin antibody for the axonemes of primary cilia and γ-tubulin antibody for the basal bodies (Fig. 1A; arrows). The numbers of nuclei of proliferating, secretory and menopausal endometrial stromal cells were 6,387 (n=4; 782-2074), 5,612 (n=5; 545-1936) and 13,651 (n=5; 1127-4595), respectively. The median rate of endometrial stromal cells with primary cilia was 7.22% (7.47-8.66), 32.70% (29.22-55.78), and 20.56% (16.36-32.13) (Fig. 1B). Bonferroni test results showed statistically significant differences between each group (Table I). The same images were used to measure the length of primary cilia on endometrial stromal cells at proliferative and secretory phases (Fig. 1C) and the average length was 1.24 µm (n=191) and 2.34 µm (n=812), respectively. An unpaired t-test showed a statistically significant difference between these two groups (95% confidence interval (CI)=0.92-1.27, P<0.001) (Fig. 1D).

The state of primary cilia on Grade 1 and Grade 3 endometrioid carcinoma cells. The median ages (range) of patients with Grade 1 and Grade 3 endometrioid carcinoma was 51 (44-71) and 60 (47-76), respectively (Table II). All Grade 1 patients had Stage I or II early-stage cancer, while 5 out of 8 Grade 3 patients had Stage III or IV advanced cancer. During the observation period, there were no recurrences or deaths in any of the Grade 1 patients, whereas 3 patients died after recurrence in the Grade 3 group. Kaplan-Meier curves for the survival rates of patients of both groups are shown in Fig. 2A. Primary cilia of endometrioid carcinoma Grade 1 and Grade 3 were visualized with fluorescence immunostaining as shown in Fig. 2B. In Grade 3, the number of primary cilia was counted in the less-differentiated, solid portion. Numbers of nuclei of endometrioid carcinoma cells of Grade 1 and Grade 3 were 9,554 (n=17; 193-1190) and 5,615 (n=8; 340-985), respectively, and the median rates of ciliated cells were 13.50% (3.11-31.79) and 2.91% (0.43-4.37). An unpaired t-test showed that the differences were statistically significant (95% CI=7.89-15.05, P<0.001) (Fig. 2C). Fluorescence immunostaining was performed using Ki67 antibody as a proliferative marker for human tumor cells (Fig. 2D). Ki67-positive rates of Grade 1 and Grade 3 were shown with a dot plot (Fig. 2E). Nuclei of Grade 1 and Grade 3 numbered 7,245 (n=5; 419-569) and 5,147 (n=5; 340-985). An unpaired t-test showed that the differences were statistically significant (95% CI=11.17-19.35, P<0.001). On measuring the lengths of primary cilia on Grade 1 and Grade 3 cells (Fig. 2F), the mean length of Grade 1 was 2.02 µm (n=372) and that of Grade 3 was 1.14 µm (n=225) (Fig. 2G). An unpaired t-test showed that this was a statistically significant difference (95% CI=0.76-0.99, P<0.001) (Fig. 2G).

The expression of OFD1 in endometrioid carcinoma Grade 1 and Grade 3. The inhibition of autophagy induces the accumulation of p62, which is an autophagy receptor. As previously reported, fluorescence immunostaining with p62 antibody showed that the accumulation of p62 in Grade 3 endometrioid carcinoma cells was more than that of Grade 1 (Fig. 3A). In addition, the expression of p62 was confirmed by western blotting with proteins extracted from the same

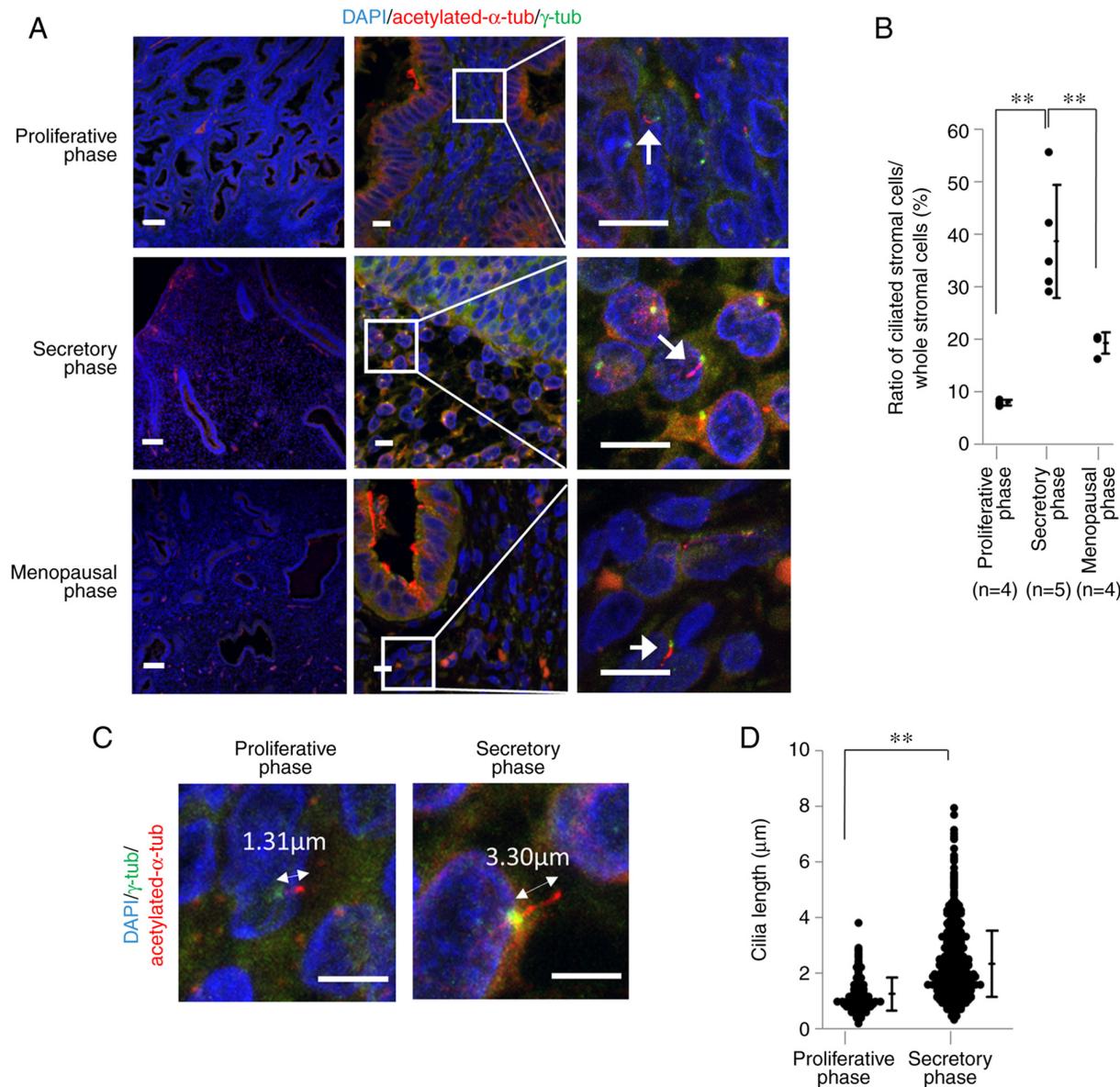


Figure 1. State of primary cilia on endometrial stromal cells is dynamic during the menstrual cycle. (A) Proliferative, secretory and menopausal endometrium stained with acetylated- α -tubulin antibody for ciliary axonemes (stained red), γ -tubulin antibody for centrosomes (stained green) and Hoechst for nuclei (stained blue). In the right panels, the structure with a single axoneme extending from the centrosome is a primary cilium (indicated with arrow). Scale bars: Left panels, 100 μm ; center and right panels, 10 μm . (B) Ratio of ciliated stromal cells per whole stromal cells at each menstrual phase. (C) Primary cilium lengths on endometrial stromal cells in the proliferative and secretory phase. The double-headed arrow indicates the length of the axoneme measured as the length of the cilia (scale bar, 5 μm). (D) Dot plot of primary cilium lengths in the proliferative (n=191) and secretory phases (n=812). The center bars represent the average, and the error bars represent standard deviations. **P<0.01. Tub, tubulin.

FFPE samples as the ones used for immunostaining (Fig. S1). p62 was highly expressed in Grade 3 carcinoma compared to Grade 1 or normal tissue. Both overexpression and a defect in OFD1, a ciliary protein, induced abnormal primary cilia or a loss of primary cilia (20,25). The inhibition of autophagy led to the accumulation of OFD1 in the cytoplasm (20). OFD1 accumulated in Grade 3 cells more than in Grade 1 (Fig. 3B). The Kaplan-Meier plotter showed that the patients with high *OFD1* expression tended to have a poor prognosis (Fig. 3D). For each image, the mean of the values of *OFD1* intensity per three areas was dot plotted (Fig. 3C, 95%CI=133-12248, P=0.046), and the relationship between the overexpression of *OFD1* and primary cilia was examined. Fluorescence immunostaining for *OFD1* with acetylated- α -tubulin antibodies was

performed using tissues of normal endometrium, and Grade 1 and 3 endometrioid carcinoma (Fig. 3E). In normal endometrial stromal cells, *OFD1* was localized at the basal bodies of primary cilia. In Grade 1, abnormal overexpression of *OFD1* was found in cells with short axonemes, while in Grade 3, the accumulation of *OFD1* was even greater and fewer primary cilia were observed.

Discussion

In the present study, we found that the state of primary cilia on endometrial stromal cells changes during the normal menstrual cycle and the number of cells with longer primary cilia is highest in the secretory phase. We also observed more

Table I. Differences in percentages of primary cilia of endometrial stromal cells in proliferative, secretory and menopausal phases.

Group A	Group B	Mean difference	Standard Error	P-value	95% CI	
					Lower limit	Upper limit
PP	SP	30.70	4.64	<0.01	21.61	39.79
PP	MP	11.37	4.89	0.12	1.79	20.95
SP	MP	19.33	4.64	<0.01	10.24	28.42

PP, proliferative phase (N=4); SP, secretory phase (N=5); MP, menopausal phase (N=4); CI, confidence interval.

endometrioid carcinoma cells without primary cilia or with shorter primary cilia in Grade 3 compared to Grade 1. In addition, we found excessive accumulation of p62 and OFD1, which possibly inhibit primary ciliogenesis, in endometrioid carcinoma cells that were highly malignant (Fig. 3F).

Primary cilia are organelles that appear during the quiescent phase of the cell cycle (26). Disassembly of primary cilia is thought to trigger re-entry of quiescent cells into the mitotic cycle and induce proliferation (27). Indeed, the excess expression of aurora A kinase (AURA), which is known as a ciliolysis-associated protein, has been reported in ovarian cancer (7). However, Ki67-negative cells were found in endometrioid cancer cells without primary cilia, indicating that ciliogenesis in the stromal cells of endometrial carcinoma may be regulated in a manner independent of the cell cycle. Therefore, this study focused on the failure of autophagy in order to elucidate a mechanism for abnormal primary ciliogenesis in endometrial carcinoma. The relationship between malignant tumors and autophagy has recently received attention. The excessive expression of p62, which represents the failure of autophagy, has been reported in various malignant tumors such as liver (22,28), breast (29), kidney (30), colon (31), ovary (32), as well as uterus (33). In this study, we also found the excessive expression of p62 in these types of endometrioid carcinoma. Since we found an abnormal accumulation of OFD1, whose proper degradation by autophagy around the centrosome is necessary for the formation of primary cilia (13), the abnormal expression of OFD1 induced by dysfunction of autophagy in Grade 3 seems to cause a loss of primary cilia or shorter primary cilia in Ki67-negative endometrioid carcinoma cells in the quiescent phase of the cell cycle. The data showing the poor prognosis of endometrial cancer patients with a high expression of OFD1 also support our model. Interestingly, OFD1 expression was also increased in human papilloma virus (HPV)-positive oropharyngeal carcinoma compared with HPV-negative carcinoma and was associated with tumor proliferation and invasiveness (25). Our model may explain a mechanism for abnormal primary ciliogenesis not only in endometrial carcinoma but also in other malignant tumors.

One advantage of the approaches used in this study was the availability of patient specimens with clinical information rather than having to rely on experimental systems such as the use of cultured cells. A variety of endometrioid carcinoma

Table II. Characteristics of patients with endometrioid carcinoma.

Endometrioid carcinoma histology	Grade 1 ^a (n=17)	Grade 3 ^b (n=8)
Mean age, (age range)	51 (44-71)	58.5 (47-76)
Surgical Stage, n		
I , II	17	3
III , IV	0	5
Recurrence, n	0	3
Death, n	0	3

^aGrade 1, exhibiting <5% solid non-glandular, non-squamous growth;

^bGrade 3; exhibiting >50% solid non-glandular tumor.

specimens was employed in this study. Some specimens of Grade 3 endometrioid carcinoma were taken from advanced cancers at the time of diagnosis, and some were recurrent cancers which led to patient death. In other words, the distribution of cilia and the expression of p62/OFD1 could be examined in tissues of cancers that are considered to be highly metastatic and have proliferative potential. If we had used cell lines for this study, ciliogenesis and autophagy could not have been examined in the different states of endometrial carcinoma cells. One of the weaknesses was the limited use of paraffine-fixed specimens and our inability to perform additional studies such as functional analysis. Another limitation was the relatively small sample size of endometrioid carcinoma Grade 3. Considering the above two limitations, our observations in this study must be confirmed in future by using a large number of patient samples.

From the findings of this study, we hypothesized that autophagy is severely blocked in highly malignant endometrial cancer, leading to the abnormal accumulation of OFD1 and the inhibition of ciliogenesis. Therefore, the restoration of autophagy may induce proper expression of OFD1 around the ciliary basal body and generate primary cilia on endometrioid cancer cells. We were not able to discover the triggers of autophagy inhibition nor any correlation between OFD1 and ciliogenesis in this study. These issues

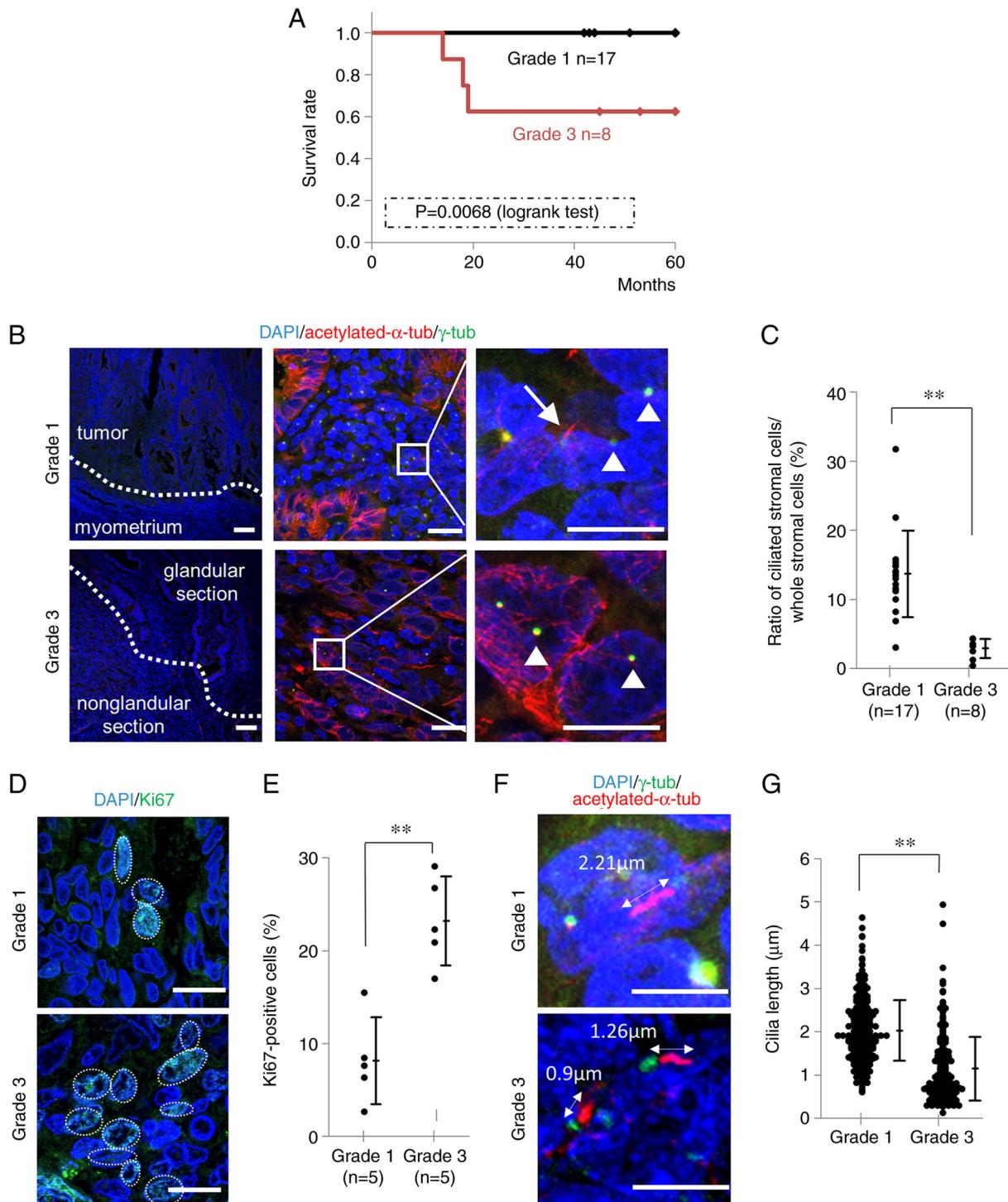


Figure 2. State of primary cilia in endometrioid carcinoma grades 1 and 3. (A) Kaplan-Meier survival curve for Grade 1 and Grade 3 of endometrioid carcinoma. (B) Primary cilia on endometrioid carcinoma grades 1 and 3 stained with acetylated- α -tubulin antibody for ciliary axonemes (stained red), γ -tubulin antibody for centrosomes (stained green) and Hoechst for nuclei (stained blue). In the right panels, the structure with a single axoneme extending from the centrosome is a primary cilium (indicated using arrows). Scale bars: Left panels, 100 μ m; center panels, 20 μ m; right panels, 10 μ m. (C) Ratio of ciliated stromal cells per whole stromal cells in endometrioid carcinoma grades 1 and 3. (D) Endometrioid carcinoma grade 1 and 3 stained with Ki67 antibody (stained green) and Hoechst (stained blue). The dotted line indicates Ki67-positive cells (scale bar, 20 μ m). (E) Ratio of Ki67-positive cells per whole cells in endometrioid carcinoma grade 1 and 3. (F) Primary cilium length on stromal cells of endometrioid carcinoma grades 1 and 3. The double-headed arrow indicates the length of the axoneme (scale bar, 5 μ m). (G) Dot plot of primary cilium lengths in endometrioid carcinoma grade 1 (n=372) and grade 3 (n=225). The center bars represent averages and the error bars represent standard deviations. **P<0.01. OS, overall survival; tub, tubulin.

must be addressed in the future. In addition, we intend to further examine whether the restoration of primary cilia on endometrioid carcinoma cells might lead to the suppression of tumor growth.

In conclusion, the present study found an increase in the number of primary cilia from the proliferative to the secretory phase of the normal menstrual cycle. We also found a decrease in the number of primary cilia as well as an excessive

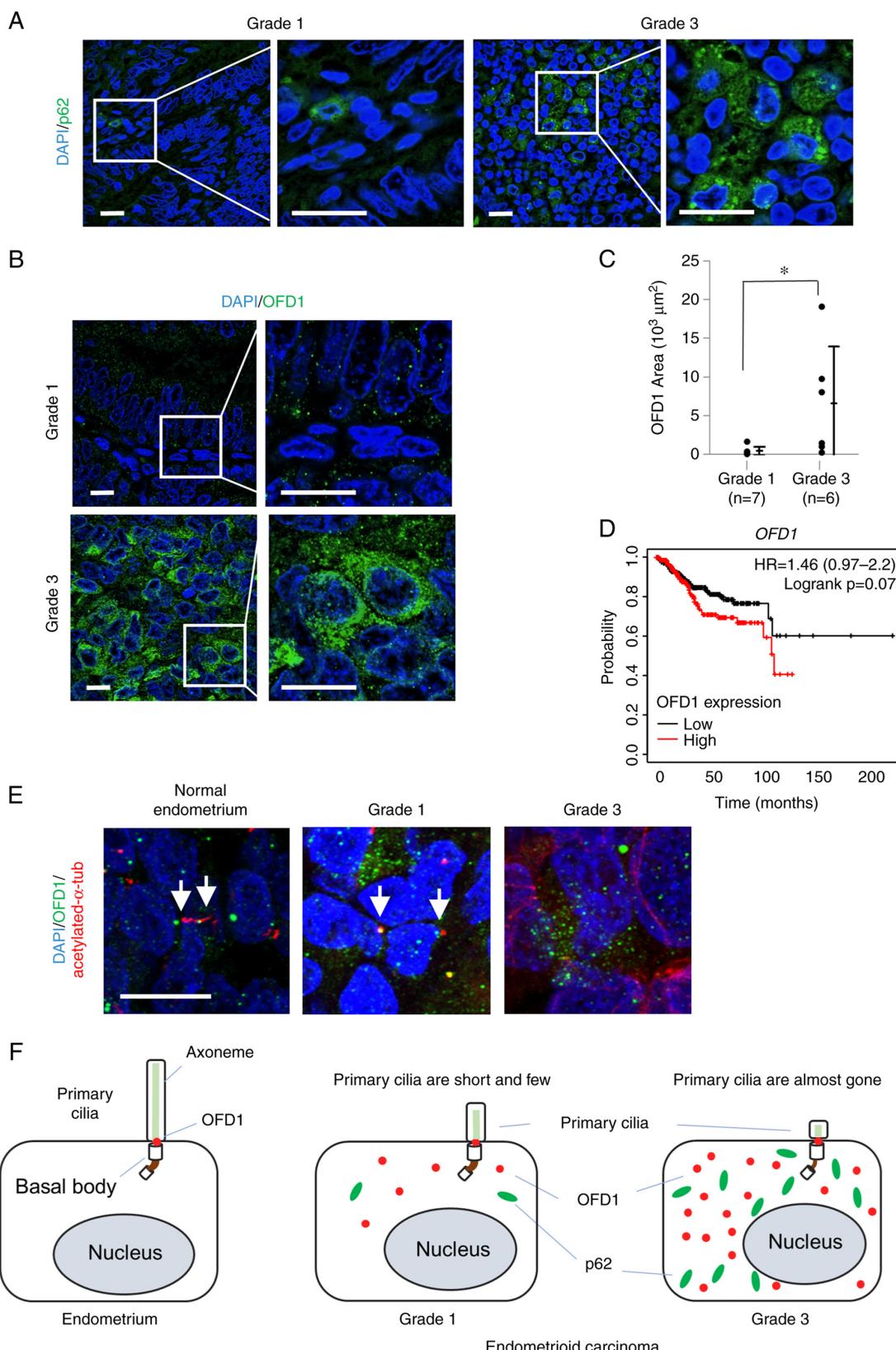


Figure 3. Association between primary cilia and autophagy in endometrioid carcinoma Grade 1 and Grade 3. (A) Expression of p62 in endometrioid carcinoma Grade 1 and Grade 3 stained with p62 antibody (green) and Hoechst (blue) (scale bars, 20 μm). (B) Expression of OFD1 in Grade 1 and Grade 3 tissues stained with OFD1 antibody (green) and Hoechst (blue) (scale bars, 20 μm). (C) Dot plot of OFD1 intensity in endometrioid carcinoma Grade 1 and Grade 3. The OFD1 intensity in three fields of view for each sample was measured. Error bars represent standard deviations. * $P<0.05$ (D) Survival curves for endometrial cancer patients (n=543) with different expression of OFD1 mRNA. The Kaplan Meier plotter was used. Patients with an OFD1 expression above the median are indicated in the red line, and patients with OFD1 expression below the median are shown in the black line. (E) OFD1 expression (stained green) and primary cilia staining with acetylated- α -tubulin antibody (stained red) in normal endometrial tissue at the secretory phase, and tissue of endometrioid carcinoma Grade 1 and Grade 3. The axoneme of a primary cilium is indicated by an arrow (scale bar, 10 μm). (F) A working model for the relationship between primary cilia and autophagy in normal endometrial stromal cells, and in cells of endometrioid carcinoma Grade 1 and Grade 3. HR, hazard ratio; OFD1, oral-facial-digital syndrome 1 protein.

accumulation of the ciliary protein OFD1 induced by the impairment of autophagy in highly malignant endometrial cancers. Since the prognosis of endometrial carcinoma patients with high expression of OFD1 is poor, the extent of OFD1 expression may be used as an index to determine prognosis in endometrial carcinoma patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Authors' contributions

RK was involved in sample collection, investigation, methodology, analysis, writing of the original draft and funding acquisition. EH, FO, CYN and SG performed the experiments. SO, SM and RN performed experiments and writing. HI provided analysis and interpretation of data. YK was involved in the conceptualization, writing and review of the manuscript and supervision of the research. MSO was involved in the conceptualization, writing, review and editing of the manuscript as well as supervision. All authors approved the final version of the manuscript. RK and YK confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

This study was approved by the Medical Research Ethics Review Committee of the Nagoya City University Graduate School of Medicine. Written consent was obtained from each patient after being given an explanation of the purpose of the research.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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