Lon Peptidase 2, Peroxisomal (LONP2) Contributes to Cervical Carcinogenesis via Oxidative Stress

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Background: Lon protease is responsible for degrading proteins injured by oxidation, and has 2 isoforms, located in mitochondria and peroxisomes. Recent research showed that Lon protease was upregulated in different types of human cancer, but the role of Lon peptidase 2, peroxisomal (LONP2) in cancer is not well understood. It is known, however, that in cancer biology, reduction-oxidation is one of the molecular mechanisms involved in tumorigenesis.

Material/Methods: Oncomine databases and tissue microarrays, initially using immunohistochemistry, were used to analyze LONP2 expression in cervical cancer. In order to uncover the biologic functions and mechanism(s) underlying LONP2 in cervical tumorigenesis, we downregulated the expression of LONP2 using 2 siRNAs transduced in HeLa and SiHa cells. CCK8 assays were performed to evaluate cell viability. Cell cycle and apoptosis assays were used to determine cell growth. Cell migration and invasion assays were used to study changes in cell migration and invasion capacity. Immunofluorescence and flow cytometry were performed to analyze the changes in ROS production.

Results: We found that the expression of LONP2 was significantly upregulated in cervical cancer, and there was a significant association with pathology type, pathology grade, and clinical stage, but not with age or lymph node metastasis. Moreover, we demonstrated that knocking down LONP2 in HeLa and SiHa cells reduced cell proliferation, cell cycle, apoptosis, migration, invasion, and oxidative stress levels.

Conclusions: Our findings suggest that LONP2 promotes cervical tumorigenesis via oxidative stress and may be a potential biomarker and therapeutic target in cervical cancer.

MeSH Keywords: Peroxisomes • Reactive Oxygen Species • Uterine Cervical Neoplasms

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Background

Cervical cancer is the fourth most frequent type of cancer found in women worldwide, accounting for 527 600 new cases and 265 700 deaths, more than for any other gynecologic tumor [1]. China has unfortunately shown a substantial increase in cervical cancer in contrast to developed countries, where the incidence trends have decreased [2]. Today, we know that carcinogenesis entails a multitude of factors and stages, and recent evidence shows that various oncogenes and tumor suppressor genes contribute to the biologic behavior of cervical cancer and its inherent clinical phenotypes [3]. However, the molecular mechanism(s) sub-serving tumorigenesis and its progression remain unknown.

Lon peptidase 2, peroxisomal (LONP2) is an evolutionarily conserved, ATP-dependent protease with chaperone-like activity present in prokaryote and eukaryote peroxisomes [4]. LONP2 plays an important role in the degradation of misfolded or damaged proteins [5], and controls protein quality to prevent the continual damage caused by the generation of reactive oxygen (ROS) [6], thus maintaining the peroxisomal protein homeostasis. This implies a vital mechanism that serves to avert the accumulation of oxidative damage. It should be noted that the chaperone-like activity of LONP2 may help to correct the normal folding and assembly of imported proteins during peroxisome biogenesis during periods of reduced metabolic needs [7]. Numerous recent studies showed that Lon protease is upregulated in different types of human cancers, including non-small-cell lung cancer [8], colon cancer [9], and bladder cancer [10], either at the RNA or protein level [11]. Moreover, knockdown of Lon can suppress the proliferation of tumor cells [12].

Peroxisomes play a key role in the maintenance of cellular oxidative balance and are recognized as potential regulators of oxidative stress-related signaling pathways [13]. This suggests that peroxisome dysfunction may be associated with the initiation and progression of many diseases, including diabetes, atherosclerosis, and cancer [14]. Interestingly, several studies indicated that LONP2 may act as a significant coordinator of metabolism-related ROS generation within peroxisomes [15–17]. Thus, in the present study, we hypothesized that the abnormal expression of LONP2 can lead to accumulation of oxidative damage and imbalance in peroxisomal protein homeostasis, resulting in tumorigenesis. We found that the expression of LONP2 was significantly upregulated in cervical cancer, and was significantly associated with pathology type, pathology grade, and clinical stage. We also demonstrated that LONP2 promoted proliferation, cell cycle kinetics, apoptosis, migration, invasion, and oxidative stress levels in these cancer cells. Our findings suggest that LONP2 promotes cervical cancer tumorigenesis via oxidative stress and may be a potential biomarker and therapeutic target in cervical cancer.

Material and Methods

Cervical cancer tissue microarray and immunohistochemistry (IHC)

A cervical cancer tissue microarray (CR2083, Xi'an Alena Biotechnology Ltd. Co.) containing squamous cell carcinomas (n=85), adenocarcinomas (n=7), adenosquamous carcinomas (n=2), and paracancerous normal tissues (n=10) were purchased for IHC. The tissue microarray was deparaffinized, microwave-heated in EDTA buffer (Aspen), and incubated in a 3% H_2O_2 solution; and non-specific binding was blocked using 5% BSA (Roche, USA). Then, samples were incubated overnight at 4°C with anti-LONP2 antibodies (1: 50). The corresponding secondary antibody was incubated for 50 min at 37°C. Slides were counterstained with hematoxylin (Aspen) and differentiated with 1% hydrochloric acid alcohol before dehydration and mounting.

Immunohistochemical stains were scored semi-quantitatively according to the intensity and percentage of positively staining cells. Briefly, the intensity was scored as 0 if negative; 1 if weak; 2 if moderate, and 3 if strongly stained. The percentage of positive cells was graded as 0 if <5% of cells were positively stained; 1 if 5–24% were positively stained; 2 if 25–49% were positively stained; 3 if 50–74% were positively stained; and 4 if ≥75% of cells were positively stained. We then calculated a staining index as intensity × positive rate (negative 0–1, weak 2–4, moderate 5–8, and strong 9–12) to calculate the final staining score.

Cell culture and reagents

HeLa and SiHa cell lines obtained from our laboratory were cultured in Dulbecco's modified Eagle's medium (DMEM) (Hangzhou Gino Biopharmaceutical Technology Co., Ltd) containing 10% fetal bovine serum (FBS) (Hangzhou Tianhang Biopharmaceutical Technology Co., Ltd) and 1% penicillin-streptomycin at 37°C in a humidified atmosphere with 5% CO_2 in compressed air.

Rabbit polyclonal anti-LONP2 antibody was purchased from Novus (NBP1-81294); rabbit monoclonal anti-cyclin D1 antibody was purchased from CST (#2978); rabbit polyclonal β-Actin antibody was purchased from Servicebio (GB11001); and mouse anti-rabbit secondary antibodies were obtained from Santa Cruz Biotechnology (sc-2357).

siRNA transfection

Four sequences of interfering RNAs for LONP2 (si-LONP2) and interfering negative control RNA (si-NC) were constructed by Beijing Ou Lingge Biological Technology Co., Ltd. HeLa and

SiHa cells were transiently transfected with siRNAs of LONP2 for knockdown of LONP2 using Hiperfect Transfection Reagent (QIAGEN) according to the manufacturer's protocol. The cells were harvested 48 h after transfection for analysis of knockdown efficiency. After validation, the working siRNAs were chosen to perform further experiments.

Real-time quantitative PCR (RT-PCR)

Total RNA isolated from HeLa and SiHa cells was extracted with Trizol (Invitrogen), and RNAs were quantified using a NanoDrop Spectrophotometer (Shanghai Sunny Hengping Scientific Instrument Co., Ltd.). The cDNA was synthesized in a reverse-transcription reaction by using a Retroscript Kit (Takara, Japan). Expression of the LONP2 gene was detected using RT-qPCR Primer Assays and RT-SYBR Green Mastermixes (Takara) in an ABI 7500 Sequence Detection System (Applied Biosystems) according to the manufacturer's protocol. GAPDH was used as an endogenous control to normalize the differences in the amount of total RNA in each sample. RT-qPCR was performed in triplicate, and the analysis was carried out using the $2^{(-\Delta\Delta CT)}$ threshold cycle method.

Western immunoblotting analysis

Cells were lysed in RIPA buffer containing protease inhibitor cocktail of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF). Total protein was quantified with the bicinchoninic acid (BCA) protein assay kit. Equal amounts of protein samples were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto a polyvinylidene fluoride (PVDF) membrane. Following incubation with Tris-buffered saline-Tween-20 (TBST) containing 5% non-fat milk at room temperature for 1 h, the membrane was probed overnight with anti-LONP2 (1: 250)/cyclin D1 (1: 1000) antibodies at 4°C. Then, the membrane was incubated with mouse anti-rabbit secondary antibody (1: 5000). β-Actin was used as a loading control. The blots were visualized using chemiluminescence ECL reagent (ASPEN).

Cell viability analysis

Cell viability was performed by using a CCK-8 kit (Beyotime) to assess the effect of LONP2 on the viability of HeLa and SiHa cells at different time-points. After transfection for 48 h, HeLa and SiHa cells were detached and separately plated into 96-well plates at a density of 2×10^3 cells/well per 100 μl of DMEM and incubated. We then changed the medium and added fresh medium containing 10% CCK8 and incubated for 1 h at 37°C. OD absorbance at 450 nm was measured with a microplate reader (Perkin Elmer).

Cell cycle and apoptosis analysis

Cell cycle and apoptosis analysis were carried out to detect the effect of LONP2 on HeLa and SiHa cells. For cell cycle studies, cells were harvested 48 h after transfection, resuspended with 100 μl PBS, and centrifuged. Then, cells were fixed in cold ethanol and incubated overnight at 4°C. After washing twice with PBS, we incubated the cells with 500 μl of RNase/PI (Tianjin Sungene Biotech Co., Ltd.) for 20 min at room temperature away from light, and then used FACSCalibur (BD) for analysis.

For the apoptosis assay, at 48 h after transfection, cells were harvested, centrifuged, and washed with PBS. Then, cells were resuspended with 300 μl binding buffer, stained with 5 μl Annexin V-FITC (Tianjin Sungene Biotech Co., Ltd), and incubated for 15 min in the dark. We then added 5 μl PI, and the cells were incubated for 5 min in the dark and assessed with FACSCalibur (BD).

Transwell migration and invasion assays

Cell migration and invasion assays were performed in a 24-well plate with 8-μm pore size chamber inserts (BD Biosciences). Transfected cells (1×10^5) in serum-free medium were seeded into 200 μl upper chambers pre-coated with or without Matrigel (1 mg/ml, BD Biosciences) for the migration and invasion assays, respectively. The lower compartment was filled with 500 μl of DMEM containing 10% FBS as a nutritional attractant. After 48 h of incubation at 37°C, cells at the top surface that had not migrated or invaded to the lower surface of the insert were manually removed by wiping with a cotton swab. Cells that had migrated or invaded to the bottom surface of the chambers were fixed in paraformaldehyde for 10 min, stained with crystal violet, photographed over 3 randomly chosen fields, and counted under a light microscope (Olympus).

ROS analysis

HeLa and SiHa cells were seeded on glass coverslips in 6-well plates and grown to approximately 50% confluence. After washing, were added DHE (Sigma, USA) to the cells and incubated them for 30 min at room temperature in the dark. Then, we stained the cells with DAPI (Aspen) for 10 min at room temperature. Finally, the slides were mounted with anti-fluorescent quenching tablets (Aspen). The samples were observed and photographed at 400×magnification under confocal microscopy (NIKON, Japan).

We also used flow cytometry to detect intracellular ROS. First, transfected cells were harvested after 48 h, centrifuged, and washed. Second, cells were fixed with DCFH-DA (Beyotime) (1: 1000, final concentration, 10 μM). Finally, cells were assessed with BD Arialll (BD) (the excitation wavelength was

488 nm, and emission wavelength was 525 nm). Notably, we used 200 µM H_2O_2 (Sinopharm Chemical Reagent Co., Ltd.) as the oxidant, which enhanced the si-LONP2 reduced production of ROS to reverse the phenotype.

Statistical analysis

All experiments were carried out at least 3 times and results are reported as means ± standard error bars. Correlations between clinicopathologic and immunohistochemical variables were calculated according to a χ^2 test or a Person χ^2 test. The t test was applied to evaluate the differences between groups. SPSS 24.0 was used to conduct statistical analyses and $P<0.05$ was considered to be statistically significant.

Results

LONP2 is significantly upregulated in cervical cancer

In an effort to determine the expression profile of LONP2 in cervical cancer, we analyzed LONP2 mRNA expression in 2 independent microarray datasets from the publicly accessible database Oncomine, and we found significantly upregulated LONP2 expression in tumor tissue (Figure 1A, 1B).

Furthermore, to confirm the correlation between LONP2 expression level and clinical significance in cervical cancer compared with normal tissues, we performed IHC analysis using a cervical cancer tissue microarray (consisting of 85 squamous cell carcinomas, 7 adenocarcinomas, 2 adenosquamous carcinomas, and 10 paracancerous normal tissues). LONP2 expression in tumorous tissues was classified as high (IHC staining index ≥6) or low (IHC staining index <6) in 94 cervical cancer patients, and we confirmed that the expression level of LONP2 was significantly higher in cervical cancer than in adjacent normal tissues (Figure 1C–1E). To further explore the role of LONP2 in cervical cancer progression, we also investigated the correlation between LONP2 expression and the clinicopathologic parameters of 94 cervical cancer tissue samples, and found that the level of LONP2 expression was significantly associated with pathology type, pathology grade, and clinical stage. However, there was no significant association with age or lymph node metastasis (Table 1). It should be noted that from each patient we removed 2 tissue samples from different areas to control for differences induced by regional tissue selection.

Reduced expression of LONP2 inhibits cervical cancer cell proliferation, cell cycle, and apoptosis

To investigate whether LONP2 affected the growth of cervical cancer, we tested cellular viability after transient transfection of si-LONP2 or si-NC in HeLa and SiHa cells. As demonstrated by CCK-8 assay, cells transfected with si-LONP2 showed significantly reduced proliferation compared to transfections with si-NC in both HeLa (Figure 2A) and SiHa (Figure 2B) cells ($P<0.05$). We also investigated the expression of cyclin D1, because it is a crucial proliferative hallmark of numerous cancers, and demonstrated that downregulation of LONP2 decreased the expression of cyclin D1 (Figure 2C, 2D). To further investigate how LONP2 promoted cellular viability, we next analyzed the effects of LONP2 on cell cycle kinetics and apoptosis by flow cytometry. We found that the number of cells in the G0/G1 phase in si-LONP2 groups was higher than in the si-NC groups ($P<0.05$) (Figure 2E, 2F), revealing that downregulated LONP2 significantly arrested the cell cycle in G0/G1. Moreover, the apoptosis assay showed that the percentage of early apoptotic cells was increased among cells transfected with si-LONP2 ($P<0.05$) (Figure 2G, 2H). These findings consistently suggest that downregulated LONP2 negatively regulates cellular proliferation, cell cycle, and apoptosis of cervical cancer cells.

Reduced expression of LONP2 suppresses cervical cancer cell migration and invasion

To assess whether LONP2 affected migration and invasion of cervical cancer cells, we performed transwell assays, and showed that downregulated expression of LONP2 significantly inhibited cellular migration compared with the control group in both HeLa and SiHa cells ($P<0.05$) (Figure 3A, 3B). Conversely, the transwell invasion assay showed that LONP2 depletion significantly decreased invasive ability in both cell lines ($P<0.05$) (Figure 3C, 3D).

Reduced expression of LONP2 decreases ROS production

We evaluated the relationships among LONP2, tumorigenesis, and the influence of ROS using immunofluorescence assays with DHE and confocal microscopy to determine intracellular ROS production, and found that ROS was dramatically decreased in si-LONP2 groups, and the H_2O_2 treatment increased ROS induced intracellular oxidative stress level, in both HeLa (Figure 4A, 4B) and SiHa (Supplementary Figure 1) cells ($P<0.05$). We also used flow cytometry to corroborate the production of ROS, loading the cells with DCHF-DA probes, and noted a marked reduction in intracellular ROS production in the si-LONP2 groups, and an obvious increase in H_2O_2 treatment groups ($P<0.05$). (Figure 4C–4F). Collectively, these results clearly demonstrated that reduced expression of LONP2 decreased ROS production, and oxidant H_2O_2 could enhance the si-LONP2-reduced production of ROS, reversing the phenotype.
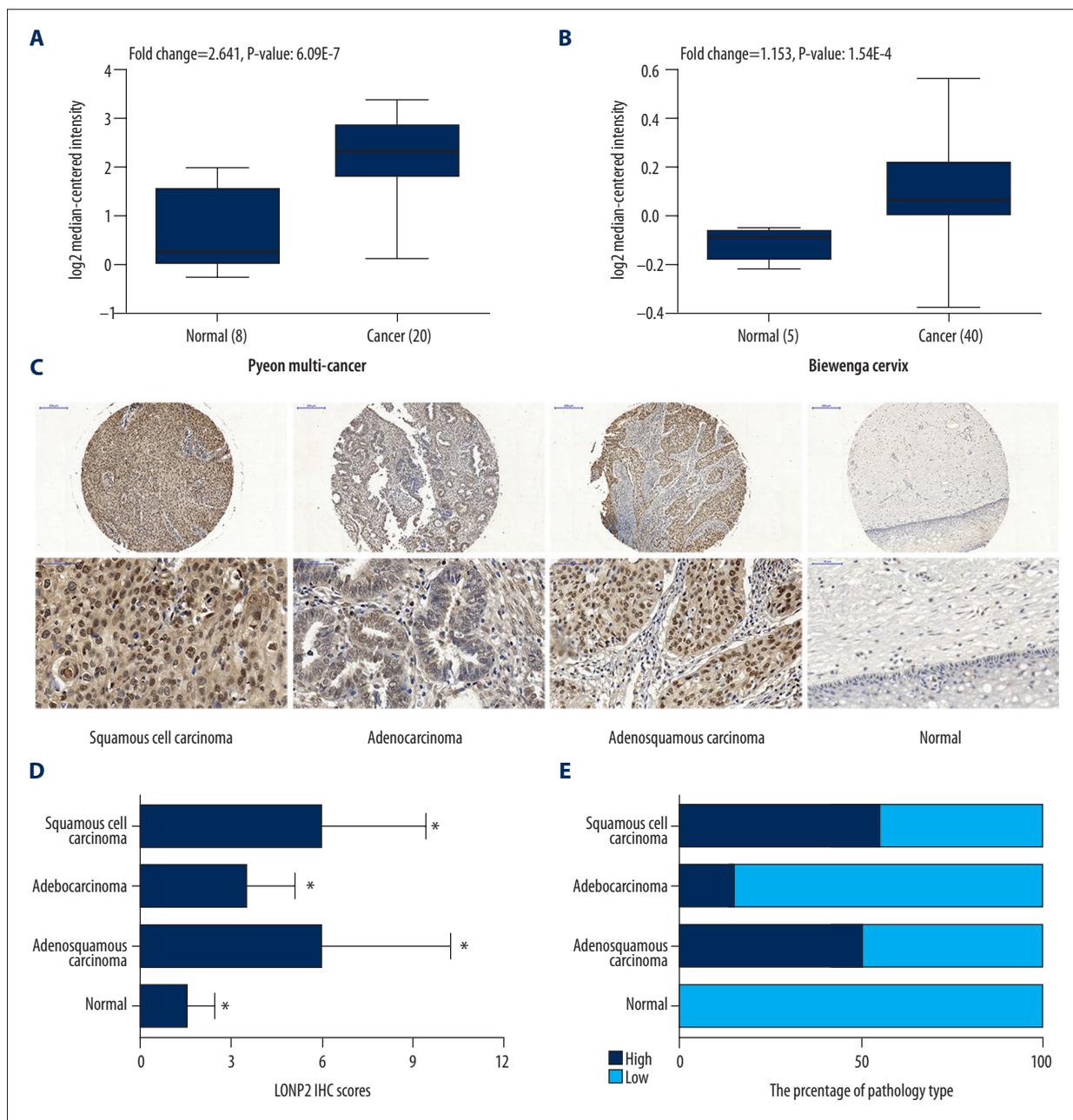
Figure 1. LONP2 is upregulated in cervical cancer tissues. Validation of LONP2 gene upregulation in cervical cancer using Oncomine datasets (**A, B**). Representative photomicrographs of LONP2 IHC staining (200×) of carcinomas and normal tissues (**C**). Semi-quantitative results of LONP2 expression displayed as means ±SD, * *P*<0.05 (**D**). Distributions of LONP2 expression in squamous cell carcinomas, adenocarcinomas, adenosquamous carcinomas, and paracancerous normal tissues (**E**).

Discussion

Recently, studies examining the role of Lon in the peroxisome have revealed LONP2 to be a protease that mimics the biologic function of the mitochondrial isoform, LONP1. Sequence comparisons of the mitochondrial and peroxisomal isoforms of Lon revealed a high degree of overlap within the functional domains, suggesting that LONP2 possesses a function similar to that of LONP1 [18]. There is now growing evidence that LONP1 plays a very active role in progression of several chronic diseases involving tumorigenesis. For example, higher LONP1 expression was observed in gastric cancer relative to noncancerous tissues, and LONP1 is now identified as a key regulator of metabolic reprogramming in gastric cancer [19]. Thus, we herein speculate that LONP2 is also involved in cervical carcinogenesis, potentially serving as a diagnostic biomarker for

Table 1. Correlations between LONP2 expression and clinicopathologic characteristics in 94 cervical cancer patients*.

Characteristics	LONP2 staining		P value
	High expression (IHC staining index ≥6)	Low expression (IHC staining index <6)	
Age (years)			0.948**
≤45	57	55	
>45	40	36	
Pathology type			0.015***
Squamous cell carcinoma	93	77	
Adenocarcinoma	2	12	
Adenosquamous carcinoma	2	2	
Grade			0.00[a]
–	9	11	
1	18	17	
2	64	37	
3	6	26	
FIGO Stage			0.034[a]
I	75	71	
II	11	13	
III	11	3	
IV	0	4	
Lymph node metastasis			0.065[b]
Negative	89	89	
Positive	8	2	

* Two tissue samples were taken from different areas of each patient; ** χ^2 test; *** Pearson χ^2 test.

cervical cancer, as well as a sign of cervical cancer progression and a therapeutic target for the treatment of cervical cancer. To confirm our hypothesis, we first demonstrated that LONP2 expression was significantly increased in cervical cancer tissues relative to that in adjacent normal tissues, and that it was highly correlated with several clinicopathologic indicators such as pathology type, pathology grade, and clinical stage, whereas age and lymph node metastasis showed no association. These results strongly suggest that LONP2 is a cancer-related gene involved in cervical cancer.

A growing number of studies have focussed on the function of LONP1, and there is a clear role of LONP1 in tumorigenesis. In colorectal and melanoma cells, knockdown of LONP1 inhibits *in vivo* tumor growth and metastasis, whereas overexpression promotes tumorigenesis [11]. Downregulating the Lon gene can inhibit the proliferation of MCF7 breast cancer cells, and

induces apoptosis and enhances sensitivity of MCF7 cells to UV light, cisplatin, and heat stress [20]. Regarding malignant glioma cells, Lon downregulation also leads to impaired cell viability under normoxic conditions [21]. LONP2 and LONP1 demonstrate similarly conserved structural domains, and the ability of oxidative damage generated by one organelle to affect the whole cell also emphasizes a similarity in function [18]. In order to investigate the role of LONP2 in cervical cancer progression, we downregulated LONP2 expression in HeLa and SiHa cells using siRNA, and found that attenuated LONP2 reduced the cellular proliferation of HeLa and SiHa cells, suppressed cell cycle kinetics and apoptosis, and inhibited cellular migration and invasion.

Oxidative stress has been shown to underlie various hallmarks of cancer [22], and maintenance of reduction-oxidation homeostasis is critical for carcinogenesis [23]. The peroxisome is one
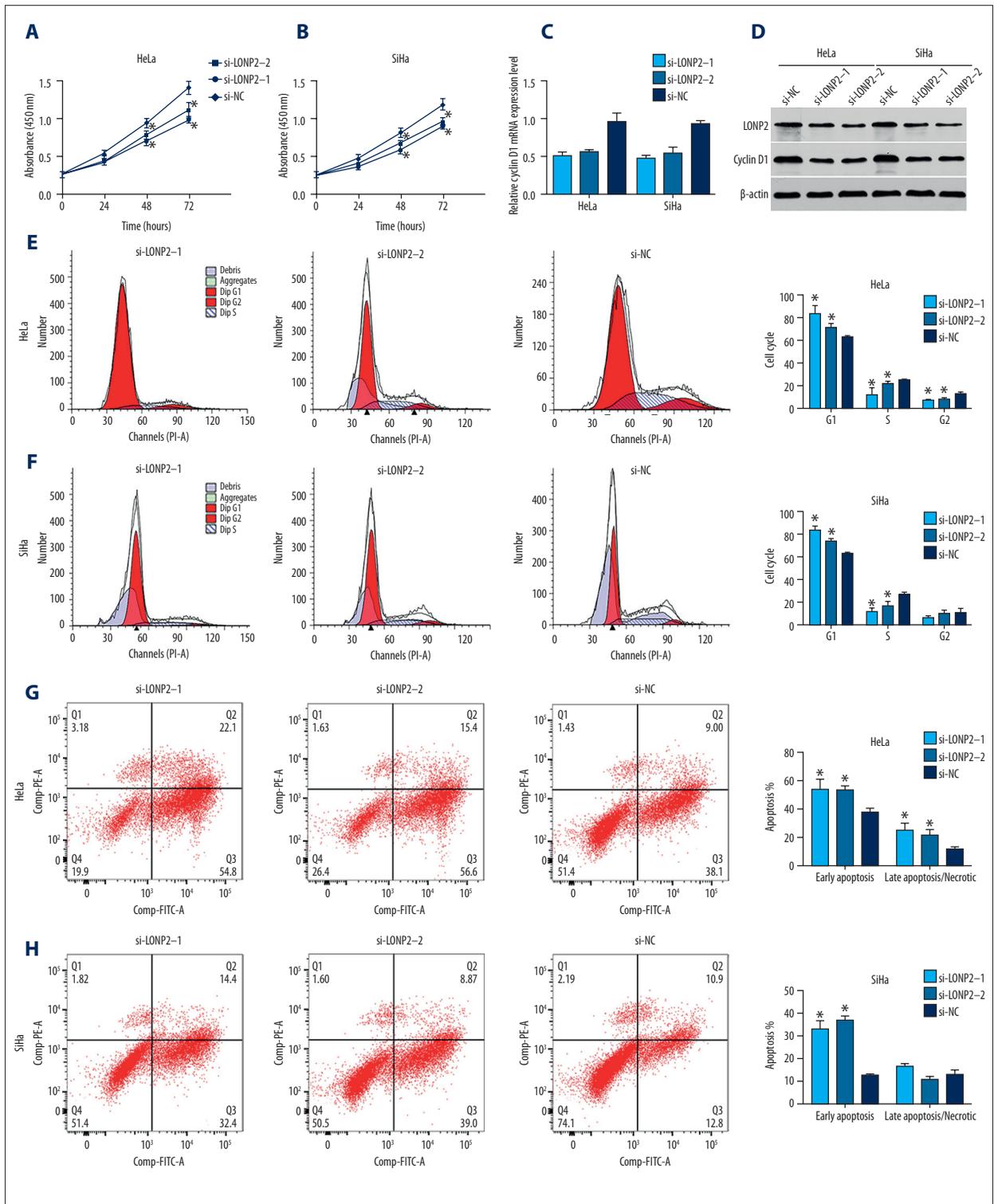
Figure 2. Downregulation of LONP2 suppresses cervical cancer proliferation, cell cycle, and apoptosis. HeLa (**A**) and SiHa (**B**) cell proliferation was measured using CCK-8 assay after transfecting 24 h, 48 h, or 72 h. RT-PCR (**C**) and Western immunoblot (**D**) were used to evaluate cellular proliferation related protein (Cyclin D1) expression. Representative results from cell cycle assays of HeLa (**E**) and SiHa (**F**) cells transfected with si-LONP2-1, si-LONP2-2, or si-NC. Representative results of cellular apoptosis assay with HeLa (**G**) and SiHa (**H**) cells transfected with si-LONP2-1, si-LONP2-2, or si-NC. The data are presented as means ±SD, * *P*<0.05.
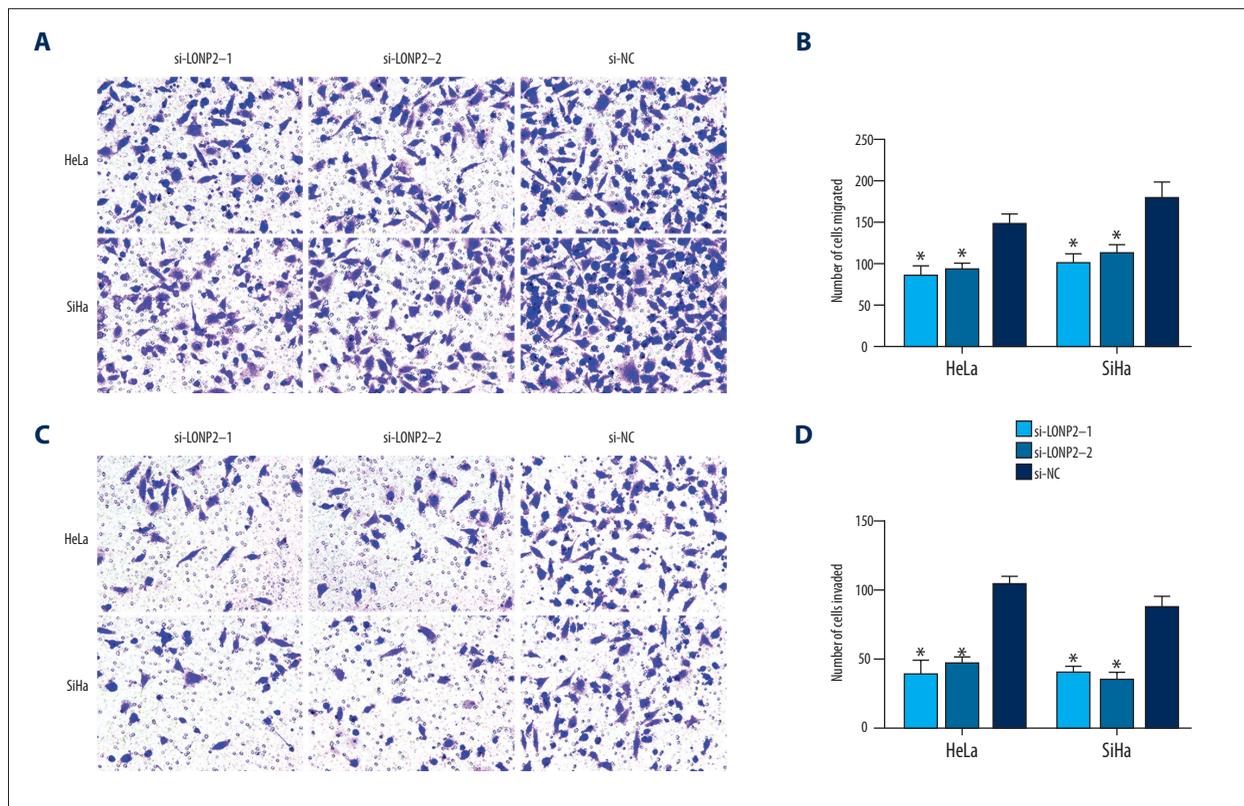
Figure 3. Downregulation of LONP2 suppresses migration and invasion of cervical cancer cells. Representative images (200×) and quantification of transwell migration (**A, B**) and invasion (**C, D**) assays of HeLa and SiHa cells transfected with si-LONP2-1, si-LONP2-2, or si-NC. The data are presented as means ±SD, * *P*<0.05.

of the organelles that generates intracellular ROS, while others are mitochondria and endoplasmic reticulum. The growth suppression of cervical cancer cells might be partially attributed to a dysfunction in peroxisomes, whereby increased expression of metabolic enzymes is associated with cancer [24] because of a lack of LONP2 protease and chaperone activity. This may ultimately result in the pernicious accumulation of misfolded, unassembled, and/or oxidatively damaged proteins [18]. For example, downregulation of LONP1 in bladder cancer cells significantly inhibited proliferation due to decreased ROS production [10]. Interestingly, peroxisome-mediated ROS production is thought to exert a profound effect on mitochondrial integrity [24], and induction of intraperoxisomal ROS production resulted in increased mitochondrial fragmentation [25]. These studies reflect the interactions between the 2 organelles, suggesting that peroxisomal dysfunction is a precursor of mitochondrial dysfunction. Our findings show for the first time that the expression levels of LONP2 are upregulated in cervical cancer, downregulation of LONP2 leads to decreased cellular ROS production, and oxidant H_2O_2 enhances the si-LONP2-reduced production of ROS, reversing the phenotype and revealing an essential role for Lon protease in the tumorigenesis of cervical cancer via in oxidative stress pathways. However, it must be noted that the ROS that we detected in

our study were total ROS in the cell, not just in the peroxisomal and mitochondrial fractions, suggesting a great need for further study in this area. In addition, investigations into the interactions among LONP1 and LONP2, mitochondria, and peroxisomes are certainly also required in further studies, as these might contribute to novel findings regarding the connection between reduction-oxidation and tumorigenesis. Notably, human papillomaviruses (HPV) is the major etiological factor in the development of cervical cancer [26], in which HPV integration is one of the key events. Recent research has shown that HPV integration can begin with DNA damage or double-strand breaks induced by oxidative stress of HPV protein. Our results combined with those of other studies suggest that LONP2 leads to cervical cancer by oxidative stress, and we can speculate that LONP2 may play a role in HPV integration, which deserves further exploration [27].

Our study has several limitations. First, as the tissue samples we used were purchased from a tissue microarray, not directly collected from the patients, they could only be used for IHC semi-quantitative method to detect LONP expression, rather than more accurate methods such as Western blot or RT-PCR. Secondly, our research mainly tried to validate and prove that LONP2 is involved in driving malignancy via oxidative stress,
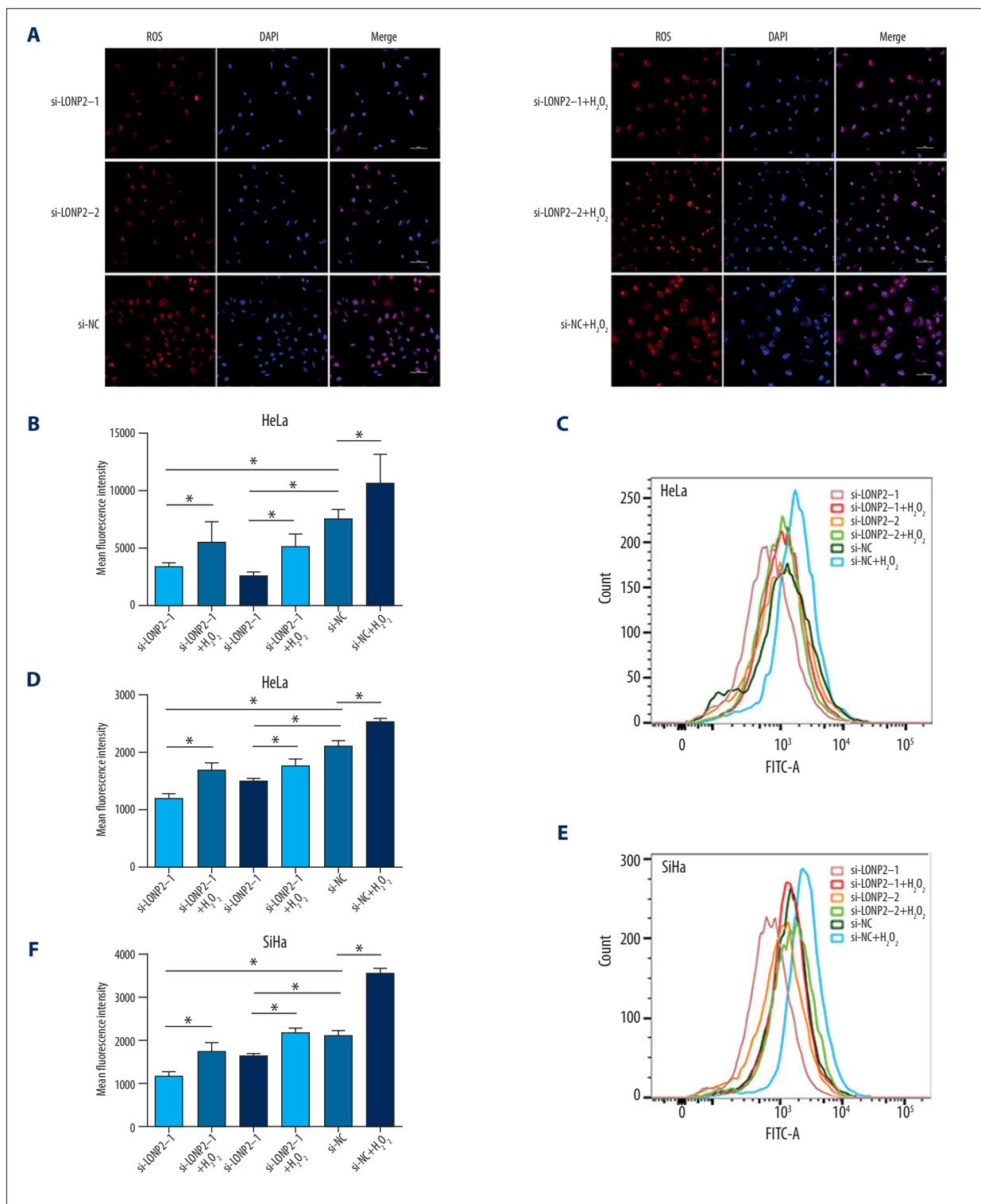
Figure 4. Downregulation of LONP2 decreases ROS production. ROS levels in cells were measured by using the fluorescence probe DHE/DCFH-DA. Representative images (400×) of HeLa (**A, B**) cell transfected with si-LONP2-1, si-LONP2-2 or si-NC, and the H_2O_2 treatment, taken with confocal microscopy and their quantification are shown. Immunofluorescence staining was performed using DHE (ROS, red fluorescence) and DAPI (nucleus, blue fluorescence). The results of flow cytometry are also shown for HeLa (**C, D**) and SiHa (**E, F**) cells transfected with si-LONP2-1, si-LONP2-2 or si-NC, and the H2O2 treatment. The probe used was DCFH-DA. The data are presented as means ±SD, * $P<0.05$.

but proving this in transformed cell lines is of limited biological relevance. Thirdly, the relevant literature is currently limited to phenomenological studies and few specific molecular mechanisms; therefore, we plan to perform cell and animal studies to investigate the role of LONP2 in cervical cancer. Large and well-designed studies investigating topics such as whether LONP2 can activate the MAPK/Erk1/2 signaling to regulate oxidative stress contributing to cervical carcinogenesis, as well as clinical research trials, are needed to elucidate the pathogenesis of cervical cancer.

Conclusions

In this study, we explored the relationship between LONP2 expression and cervical cancer. We demonstrated that LONP2 plays a critical role in the progression of cervical cancer by upregulating the oxidative stress, and that it could be a useful biomarker. Nevertheless, further basic studies are required to elucidate the molecular mechanisms by which LONP2 promotes tumorigenicity in cervical cancer; and clinical research trials are needed as well.

Acknowledgment

Supplementary Figure
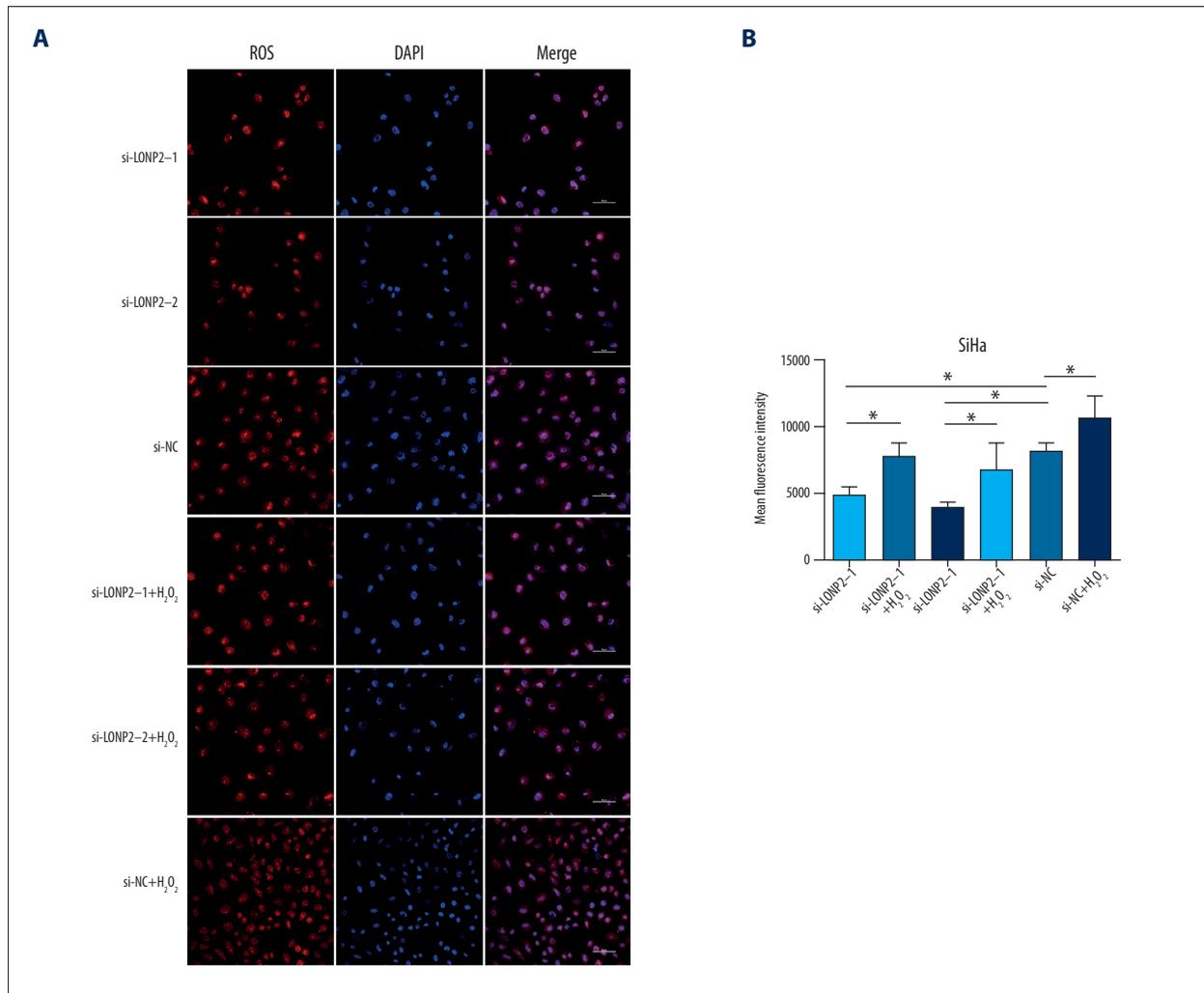
Supplementary Figure 1. Downregulation of LONP2 decreases ROS production. ROS levels in cells were measured by using the fluorescence probe DHE. Representative images (400×) of SiHa cell transfected with si-LONP2-1, si-LONP2-2 or si-NC, and the H_2O_2 treatment (**A**), taken with confocal microscopy and their quantification are shown (**B**).

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