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USP13 regulates ferroptosis in chicken follicle granulosa cells by deubiquitinating ATG7

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Abstract

The development and maturation of follicles are intricately linked to egg production and reproductive performance of chickens. Granulosa cells death directly affects the development and maturation of follicles, thereby impacting the reproductive performance of hens. Ferroptosis is a new type of cell death, it is

unknown how it affects the growth and development of chicken follicles. In this study, RNA-seq analysis revealed significant differences in the expression of ferroptosis-related genes between normal follicles and atretic follicles, suggesting a potential role for ferroptosis in follicle growth and development. In addition, we found that ubiquitin-specific protease 13 (**USP13**) was significantly upregulated in atrophic follicles. Overexpression of USP13 results in depletion of glutathione (**GSH**), peroxidation of lipids, accumulation of iron, and activation of ferroptosis in chicken granulosa cells. In contrast, USP13 knockdown significantly inhibited ferroptosis events. Mechanistically, USP13 prevents the degradation of autophagy related 7 (**ATG7**) by deubiquitinating it, thereby enhancing the stability of ATG7 protein and ultimately promoting ferroptosis. In conclusion, this study elucidates the crucial role of the USP13-ATG7 axis in regulating ferroptosis in chicken follicle granulosa cells, thereby presenting a novel avenue for molecular breeding research in chickens.

Key words: ATG7, deubiquitination, ferroptosis, granulosa cell, USP13

INTRODUCTION

The development of follicles in ovaries plays a crucial role in determining the egg-laying performance of chickens (Chen, et al., 2020). Follicle development is primarily regulated by the hypothalamic-pituitary-gonadal axis, which exerts a pivotal influence on hormone synthesis and oocyte maturation (Xie, et al., 2022). However, the majority of follicles fail to reach the preovulation stage throughout a chicken's lifespan and instead undergo a degenerative process known as follicular atresia (Ru, et al., 2024). Programmed cell death, including autophagy, apoptosis, and necrosis, has been reported to play a pivotal role in the regulation of follicular growth and development

(Chaudhary, et al., 2019; Bhardwaj, et al., 2022; Gallegos, et al., 2022). However, the precise role of ferroptosis as a recently discovered form of programmed cell death in chicken follicular growth and development remains elusive.

Ferroptosis is a form of cell death characterized by iron-dependent phospholipid peroxidation, which results in the accumulation of lipid reactive oxygen species and perturbs intracellular redox homeostasis, ultimately leading to cell death (Liang, et al., 2022). Recently, emerging evidence suggests a significant association between ferroptosis and female primary ovarian insufficiency (**POI**), infertility, and polycystic ovary syndrome (**PCOS**). Wang et al discovered that the deletion of BNC1 triggers POI by inducing oocyte ferroptosis (Wang et al, 2022). Lin et al. demonstrated a significant association between ferroptosis and PCOS, highlighting the potential of inhibiting ferroptosis as a therapeutic strategy for improving PCOS outcomes (Lin, et al., 2023). Zhang et al. found that iron overload is linked to female infertility, and regulating iron levels and ferroptosis shows promise for clinical interventions in treating this condition (Zhang, et al., 2024). These studies suggest that ferroptosis plays a crucial role in regulating reproductive function in female animals; however, its involvement in poultry follicular development remains unclear.

Cell homeostasis is regulated in large part by reversible post-translational changes of proteins called ubiquitination and deubiquitination (Han, et al., 2022). USP13, a crucial member of the deubiquitination family, exerts regulatory control over cellular activities by modulating target protein stability through the deubiquitination pathway (Esposito and Gutierrez, 2022). USP13 was found to have a regulatory relationship with several key genes involved in ferroptosis. USP13 facilitates Nrf2 expression through the deubiquitination pathway, thereby mitigating lipopolysaccharides-induced oxidative stress and inflammation by inducing Nrf2 (Wu, et al., 2023). Additionally,

USP13 binds to P53, impacting the expression of the P53 protein. P53 is a crucial regulator of ferroptosis, controlling iron accumulation, lipid peroxidation, glutathione peroxidase 4, and reactive oxygen species levels (Liu, et al., 2011). Li et al. also found that lncRNA FRMD6-AS1/miR-491-5p/USP13 axis can affect liver fibrosis through ferroptosis process (Li, et al., 2024). These studies suggest that USP13 may potentially play a regulatory role in the process of ferroptosis

In this study, we discovered that ferroptosis may play a role in the process of follicle development in chickens. Moreover, USP13 exerts regulatory control over the ferroptosis of follicle granulosa cells by deubiquitinating ATG7, thereby influencing the growth and development of follicles.

MATERIALS AND METHODS

Animals

The animal trials were conducted following the approved research protocol by the Animal Ethics Committee of Sichuan Animal Science Academy (approval no. 0235/2022). The experimental animals used in this study were the maternal S07 series of Dahen 799 broilers, which were provided by Sichuan Dahen Poultry Breeding Co., LTD. At 350 d of age, hens with consistent laying patterns and those that had ceased laying for more than 10 consecutive d were screened based on individual egg production records. Subsequently, a preliminary selection was made to identify 20 hens exhibiting potential cessation of laying, considering nest characteristics, as the experimental group. An equal number of hens with regular laying behavior were chosen as controls. The chickens were housed individually, following a 14-h light and 10-h dark photoperiod, and were given unrestricted access to commercial feed and water.

Granulosa Cells Isolation and Culture

After isolating the F1, F2, and F3 ovarian follicles from the hens, they were submerged in a PBS solution. Next, in accordance with methods outlined in prior descriptions (Hu, et al., 2024), granulosa cells were extracted from the membrane layer of the follicles above. The cell suspension was then seeded at a density of 1×10^6 cells per well into 24-well culture plates. The cells were grown in DMEM supplemented with 0.5% streptomycin and 10% fetal bovine serum at 37°C in a 5% CO_2 humidified environment. The cell culture media and supplements were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA), unless otherwise specified.

Plasmids, siRNA, and Cell Transfections

Chicken granulosa cells were seeded into 6-well plates and transfected with a pcDNA3.1 expression vector encoding either USP13 or USP13 siRNA in order to overexpress or silence USP13 in the cells. The siRNA sequence or overexpression plasmids targeting USP13 and were designed and synthesized by Shanghai Gemma Biological Company (GenePharma, Shanghai, China). Using Lipofectamine 3000 Transfection Reagent in accordance with the manufacturer's instructions, the siRNAs or plasmids were transfected into granulosa cells.

RNA Isolation and Real-Time PCR

Using a total RNA isolation kit (TaKaRa, Tokyo, Japan) and following the manufacturer's instructions, total RNA was isolated from follicles or granulosa cells. The TaKaRa PrimeScriptTM RT reagent Kit (TaKaRa) was employed for cDNA synthesis, while SYBR Premix Ex Taq II (TaKaRa) was utilized for real-time quantitative PCR (**qPCR**) analysis following the manufacturer's instructions. The oligonucleotide

primers used for qPCR analysis in this study were provided in Table 1.

Table 1.

The primers used in this study.

Genes	Forward primer (5′–3′)	Reverse primer
USP13	AGCATGTCGGAAAGCTGTGT	AATCCAACAGCCCC
ACSL4	CTCTCAGCCATTTTAGCAGCC	ATTCATGAAAATCT
COX2	TAGACCTCCCCCAACTAGCC	TGCCAATGGTTAGC
FTH1	GTCGTATCCACCGCATCTCT	GCCACATCATCCCG
GAPDH	GCAACCGTGTTGTGGACTTG	CTCCAACAAAGGG

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Western Blot Assay and Immunoprecipitation Assay

The yellow follicles and cells were subjected to protein extraction using cell lysis buffer (Beyotime, Beijing, China) supplemented with phosphatase inhibitor (Beyotime) and protease inhibitor (Beyotime). The Bradford test was used to quantify the amount of protein present (Bio-Rad, Hercules, CA). Protein weighing roughly 20 μg was electrophoresed on SDS-PAGE gels and then placed onto a PVDF membrane. With the use of a kaleidoscopic prestaining standard (Sigma-Aldrich), the molecular weight was found. The PVDF membrane was blocked with 5% milk and then incubated with the primary antibody at 4°C for a whole night. PVDF membranes were treated with enhanced chemiluminescence reagent (Millipore, Bedford, MA)

after an hour of incubation with HRP-labeled secondary antibodies the following day. The densitometric analysis of the bands was performed using ImageJ software. DF-1 (FuHeng, Shanghai, China) or granulosa cells were lysed with an IP lysis solution (Sigma-Aldrich) containing phosphatase inhibitors for the immunoprecipitation analysis. Total protein was obtained through immunoprecipitation using Flag, HA, and Myc-Tag antibodies in conjunction with protein A/G beads. After washing 3 times with IP lysis buffer, the immunocomplexes were analyzed using western blotting techniques.

Antibodies

The following primary antibodies were used: rabbit anti-USP13 (cat. no. sc-514416, Santa Cruz), Rabbit anti-ATG7 (cat. no. sc-514416, Santa Cruz), mouse anti-COX2 (cat. no. sc-514489, Santa Cruz), anti-ACSL4 (cat. no. SAB2100035, Sigma-Aldrich), rabbit anti-Ubiquitin (cat. no. ab134953, Abcam), mouse anti-Myc-Tag (cat. no. 05-724, Sigma), mouse anti-Flag-Tag (cat. no. F3165, Sigma-Aldrich), rabbit anti-HA-Tag (cat. no. ab9110, Abcam), mouse anti-GAPDH (cat. no. AB2302, Sigma-Aldrich). The following secondary antibodies were used: goat anti-mouse HRP (cat. no. sc2005, Santa Cruz), rabbit antimouse HRP (cat. no. ab6728, Abcam), goat anti-rabbit HRP (cat. no. AP510, Sigma-Aldrich).

Lipid Peroxidation, GSH, and Iron Assay

Cells were cultured in 12-well plates, and after specific treatment, cell lysates were obtained by centrifugation. The MDA (cat. no. ab118970, Abcam) lipid peroxidation detection kits were used by the manufacturer's instructions to measure lipid peroxidation product levels in the cell lysates. GSH concentration levels were quantified with a GSH assay kit (cat. no. CS0260; Sigma-Aldrich), and ferrous iron concentration

was determined using an iron assay kit (cat. no. ab83366, Abcam).

Analysis of ATG7 Deubiquitylation

To conduct the in vivo Beclin1 ubiquitylation assay, cells were transfected with the designated plasmids and exposed to 20 μM MG132 for 12 h. Subsequently, the samples were harvested and subjected to lysis using NETN buffer (Sigma) supplemented with 0.1% SDS, 20 μM MG132, and a cocktail of protease inhibitors. The lysates were incubated with an anti-ATG7 antibody for 3 h and protein A/G agarose beads for an additional 8 h at 4 °C. The precipitated proteins were liberated from the beads through a boiling procedure lasting 10 mins in the SDS-PAGE loading buffer. Subsequently, these proteins were subjected to immunoblotting (IB) using the anti-HA antibody.

RNA Seq

To prevent contamination of the genomic DNA, total RNA was extracted from F1 to F3 follicles using a RNeasy Mini Kit (Sigma) with on-column DNase digestion (RNase-Free DNase Set, Sigma). The transcriptome data analysis, sequencing, and cDNA library construction were performed by Personalbio Biotechnology Co., Ltd. (Shanghai, China).

Cell Viability Assay

Cell death was assessed by staining with propidium iodide (**PI**) (cat. no. S6874, Selleck) according to the manufacturer's protocol, followed by fluorescence microscopy observation (ThermoFisher, Waltham, MA).

Statistical Analysis

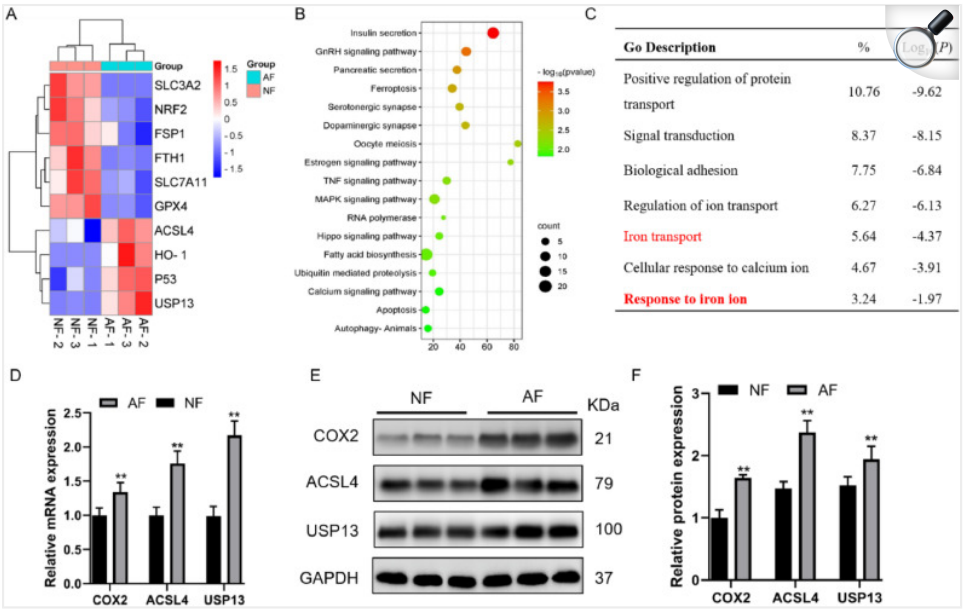
The experimental results were presented as the mean ± standard deviation (**SD**) of the mean. GraphPad Prism 7 software was used to perform statistical analysis using the unpaired Student's *t*-test or 1-way analysis of variance. Disparities were deemed statistically significant when they met the $P < 0.05$ threshold of significance. *P*-values were represented using the following notation: $*P < 0.05$, $**P < 0.01$.

RESULTS

Ferroptosis Impacts the Development of Chicken Follicles

RNA-Seq was used to quantify gene expression levels in normal and atrophic follicles, revealing significant differential expression of numerous ferroptosis-associated genes between the 2 experimental groups (Figure 1A). Next, we performed Kyoto encyclopedia of genes and genomes (**KEGG**) pathway analysis and gene ontology (**GO**) enrichment analysis using the RNA-seq data. It's interesting to note that the ferroptosis pathway, which influences cell death, has been proposed as important for chicken follicle development (Figure 1B). GO enrichment analysis revealed the involvement of iron transport and response to iron ion in follicular development (Figure 1C). Subsequently, qPCR and western blot analysis revealed that the expression of USP13 was significantly up-regulated in atrophic follicles, which was consistent with the expression of ferroptosis marker genes cytochrome c oxidase subunit II (**COX2**) and acyl-CoA synthetase long chain family member 4 (**ACSL4**) (Figures 1D, 1E). This suggests that ferroptosis is involved in follicle development, and USP13 may affect follicle development through ferroptosis pathway.

Figure 1.



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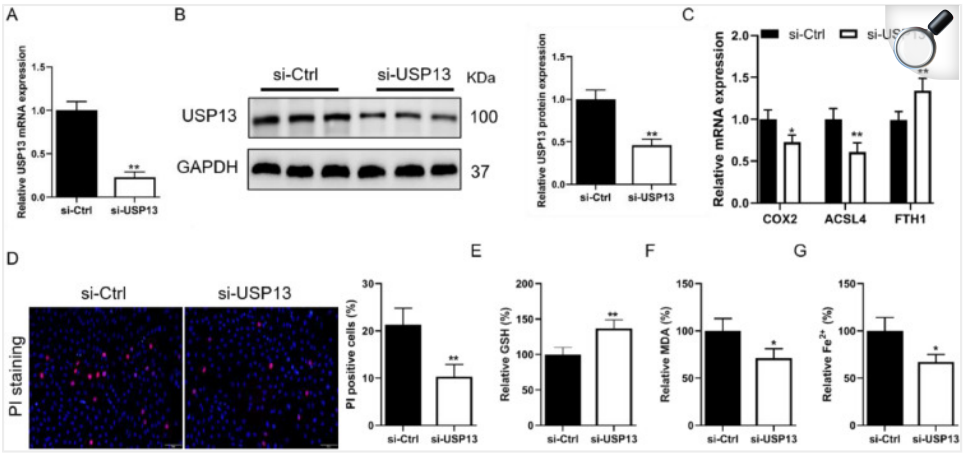
Ferroptosis is involved in the growth and development of chicken follicles. (A) Grading clustering and heatmap analysis were performed to compare the expression of ferroptosis-related genes in normal or atrophic follicles. (B) Enrichment analysis of signaling pathways associated with differentially expressed genes in normal or atrophic follicles. (C) Functional annotation was analyzed in relation to differentially expressed genes observed in normal or atrophic follicles. (D) PCR analysis was performed to assess the mRNA expression levels of COX2, ACSL4, and USP13 in normal or atrophic follicles. (E, F) Western blot analysis was performed to assess the protein expression levels of COX2, ACSL4, and USP13 in normal or atrophic follicles. Data are means ± SD. n = 3 hens each group. *$P < 0.05$; **$P < 0.01$.

Effect of USP13 on Ferroptosis of Follicle Granulosa Cells in chicken

To investigate the effect of USP13 on ferroptosis in chicken granule cells, we employed USP13 siRNA transfection to effectively suppress the expression of USP13. The results showed that transfecting cells with USP13 siRNA significantly inhibited the mRNA and protein expression levels of USP13 compared to the control (Figures 2A, 2B). qCPR analysis revealed a significant decrease in the expression of ferroptosis marker genes COX2 and ACSL4, while the expression of ferritin heavy chain 1 (**FTH1**) was significantly increased in USP13 silenced cells (Figure 1C). PI staining revealed that the downregulation of USP13 markedly augmented the live cells (Figure 1D). The key processes of ferroptosis encompass the accumulation of Fe^{2+}, elevation in lipid peroxidation, and depletion of glutathione (**GSH**). Elisa assay revealed a decrease in GSH levels, accompanied by an increase in MDA and Fe^{2+} concentrations in USP13 silenced cells (Figures 2E-2G). Consequently, we enhanced the expression of USP13 by transfecting an overexpression vector for USP13 (Figures 3A, 3B). The results showed that the overexpression of USP13 significantly induced ferroptosis in granulosa cells (Figures 3C-3G). These data suggest that USP13 exerts a positive regulatory effect on ferroptosis in chicken follicle granulosa cells.

Figure 2.



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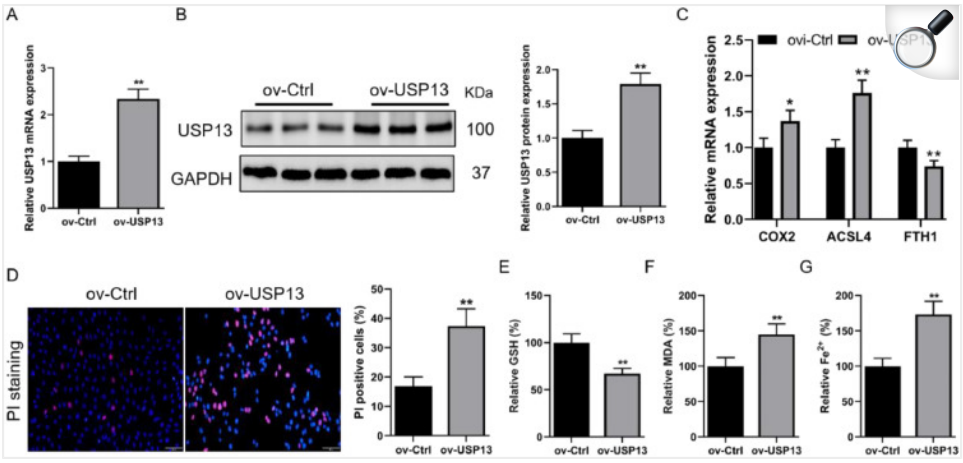
Effect of USP13 knockdown on ferroptosis in chicken granulosa cells. (A) The mRNA expression levels of USP13 were assessed using PCR analysis in control or USP13 silenced cells. (B) The protein expression levels of USP13 were assessed using western blot analysis in control or USP13 silenced cells. (C) PCR analysis was performed to assess the mRNA expression levels of COX2, ACSL4, and FTH1 in control or USP13 silenced cells. (D) PI staining was used to detect cell activity in control or USP13 silenced cells, Scale bar=100 μm. (E-G) The concentrations of GSH, MDA, and Fe^{2+} in control cells or USP13 silenced cells were assessed using the Elisa assay. Data are means ± SD. n = 3 independent cell cultures. *$P < 0.05$; **$P < 0.01$.

Figure 3.



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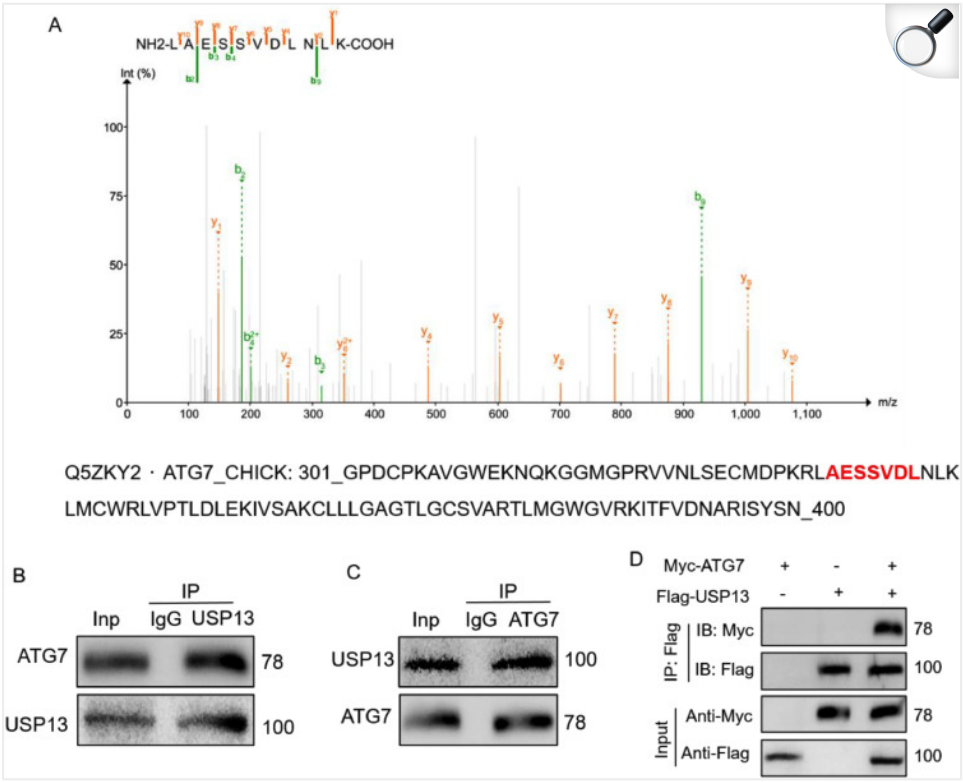
Effect of USP13 overexpression on ferroptosis in chicken granulosa cells. (A) The mRNA expression levels of USP13 were assessed using PCR analysis in control or USP13 overexpression cells. (B) The protein expression levels of USP13 were assessed using western blot analysis in control or USP13 overexpression cells. (C) PCR analysis was performed to assess the mRNA expression levels of COX2, ACSL4, and FTH1 in control or USP13 overexpression cells. (D) PI staining was used to detect cell activity in control or USP13 overexpression cells, Scale bar=100 μm. (E-G) The concentrations of GSH, MDA, and Fe^{2+} in control cells or USP13 overexpression cells were assessed using the Elisa assay. Data are means ± SD. n = 3 independent cell cultures. *$P < 0.05$; **$P < 0.01$.

USP13 Interacts With ATG7 in the Granulosa Cells of Chicken

To investigate the molecular mechanisms of USP13-mediated

ferroptosis, we employed immunoprecipitation coupled with mass spectrometry (**IP/MS**) to analyze the protein interactome associated with USP13. ATG7 was identified as a potential interacting protein of USP13 through IP/MS analysis (Figure 4A). The IP/MS results were validated by co-IP analysis, which demonstrated that USP13 precipitated ATG7 in granulosa cells, while control IgG did not (Figure 4B). It was verified by reverse co-IP that ATG7 strongly precipitated USP13 in granulosa cells (Figure 4C). Additionally, we used epitope-tagged proteins in DF-1 cells for a Co-IP test. We also performed a Co-IP test with epitope-tagged proteins in DF-1 cells. As predicted, the Myc- and Flag-labeled ATG7 and USP13 showed effectively co-precipitation in DF-1 cells (Figure 4D). These findings show that USP13 and ATG7 interact in chicken granulosa cells.

Figure 4.



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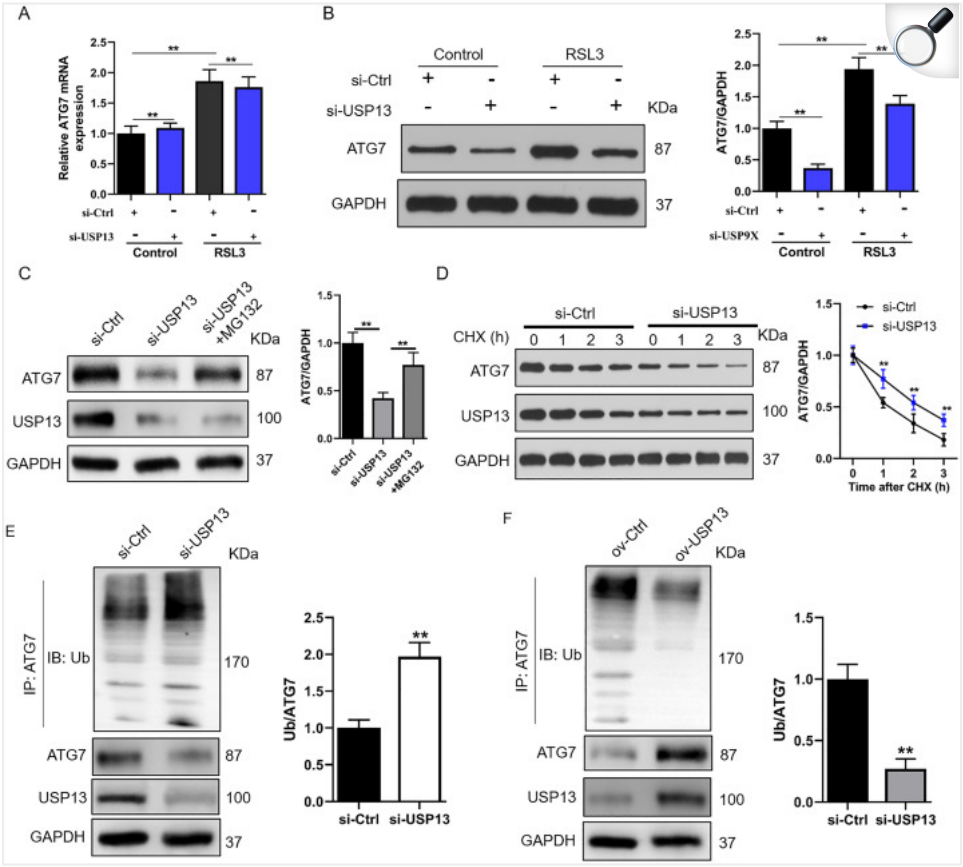
USP13 interacts with ATG7 in chicken. (A) ATG7 was identified as an interacting protein by using IP/MS analysis to detect which proteins bind USP13. (B) Anti-USP13 or anti-IgG immunoprecipitation was used to measure endogenous protein binding in the lysate of granulosa cells, and anti-ATG7 immunoblotting was then performed. (C) Anti-ATG7 or anti-IgG was used in immunoprecipitation to verify endogenous protein interactions in lysates of granulosa cells, and anti-USP13 was used in immunoblotting. (D) After transfecting DF-1 cells with Flag-tagged USP13 and Myc-tagged ATG7, the lysate was submitted to immunoprecipitation using anti-Flag antibody and immunoblotting using anti-Myc (ATG7) and anti-Flag (USP13) antibodies. Data are means ± SD. n = 3 independent cell cultures. $*P < 0.05$; $**P < 0.01$.

USP13 Suppresses the Ubiquitination and Degradation of ATG7

Subsequently, we investigated the impact of USP13 knockdown on ATG7 expression. Regardless of the presence of RSL3, USP13 knockdown significantly reduced ATG7 protein levels without affecting ATG7 mRNA levels (Figures 5A, 5B). This suggests that USP13 potentially modulates the expression of ATG7 via post-transcriptional modifications. Subsequently, we found that the protease inhibitor MG132 reversed the decline in ATG7 protein levels caused by USP13 knockdown (Figure 5C). Next, we employed the protein synthesis inhibitor cycloheximide to impede the process of protein synthesis and observed a diminished half-life of ATG7 protein in cells exhibiting reduced USP13 knockdown as compared to the control group (Figure 5D). Next, we investigated the possibility that USP13 could regulate the ubiquitination and degradation of ATG7. Knockdown of USP13 effectively enhances ATG7 ubiquitination

and reduces ATG7 protein levels compared to the control group (Figure 5E). In contrast, overexpression of USP13 significantly reduced the ubiquitination of ATG7 and increased the levels of ATG7 protein (Figure 5F). These data suggest that USP13 deubiquitinates ATG7, thereby enhancing the stability of the ATG7 protein in chicken granulosa cells.

Figure 5.



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USP13 inhibits ATG7 ubiquitination and degradation in granulosa cells. (A) qPCR was used to assess the expression of ATG7 mRNA in control and USP13 silenced cells treated with RSL3 (2 μM, 24 h). (B) Western blot analysis of ATG7 protein expression was performed in control and USP13 silenced cells treated with RSL3 (2 μM, 24 h). (C) Western

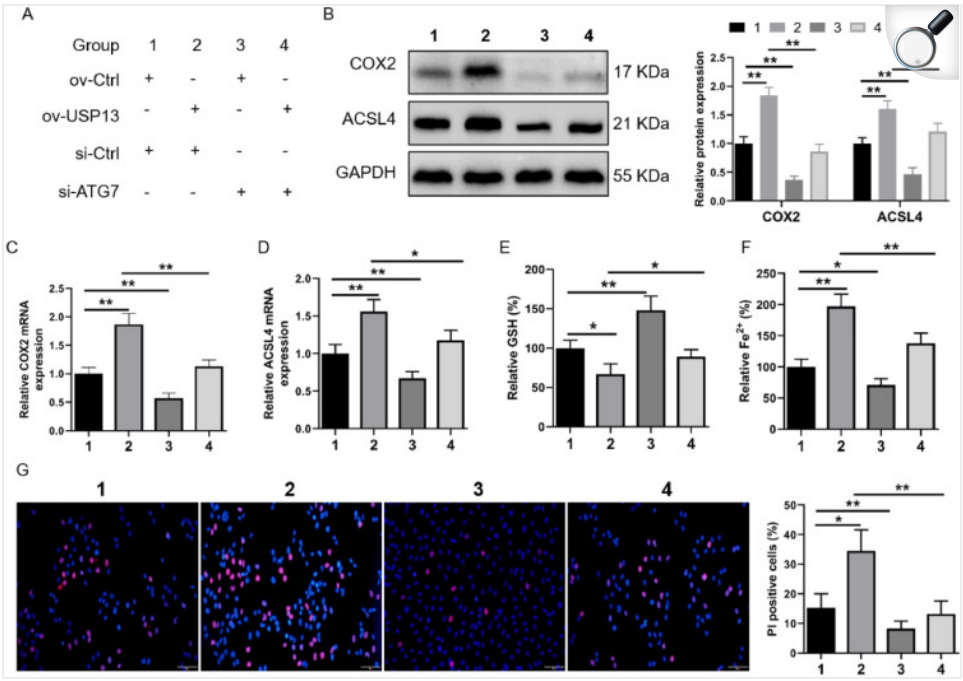
blot analysis was performed to examine the expression of ATG7 protein in control and USP13 silenced cells, with or without treatment of RSL3. (D) The protein levels of ATG7 in control and USP13 silenced cells were quantified using western blotting, with specific antibodies against ATG7 and USP13, in the absence or presence of cycloheximide (CHX, 10 μg/mL) for a specified duration. (E) The lysates from cells transfected with control or USP13 siRNA, and treated with MG132 before collection, were immunoprecipitated and detected using the specified antibodies. (F) The lysates from cells transfected with a control or USP13 overexpression plasmid, and treated with MG132 before collection, were immunoprecipitated and detected using the specified antibodies. Data are means ± SD. n = 3 independent cell cultures. *$P < 0.05$; **$P < 0.01$.

USP13 Mediates ATG7 to Regulate Ferroptosis in Chicken Follicle Granulosa Cells

We further investigated whether USP13 could regulate the ferroptosis process by mediating ATG7. An overexpression vector of USP13 and a siRNA vector targeting ATG7 were co-transfected to establish the experimental group, as depicted in Figure 6A. Western blotting revealed that USP13 enhances the expression levels of COX2 and ACSL4 mRNA and proteins, while knockdown of ATG7 significantly reduces their expression (Figures 6B-6D). Additionally, we found that ATG7 knockdown could restore the reduced GSH content resulting from USP13 overexpression (Figure 6E). The overexpression of USP13 significantly enhanced intracellular Fe^{2+} levels, while co-transfection with ATG7 siRNA markedly decreased the content of Fe^{2+} (Figure 6F). PI staining also provided evidence that overexpression of USP13 led to a reduction in cell viability, while co-transfection of ATG7 siRNA significantly enhanced cell

viability (Figure 6G). These results suggest that USP13 regulates ferroptosis in chicken granulosa cells by mediating ATG7, and deficiency of ATG7 can block USP13-induced ferroptosis.

Figure 6.



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USP13 regulates feroptosis in chicken follicle granulosa cells by mediating ATG7. (A) Detailed information on the experimental group in this section of the study. (B-D) qPCR and western blot analysis were performed to assess ACSL4 and COX2 mRNA and protein expression in USP13 overexpression or control cells transfected with either control siRNA or ATG7 siRNA. (E, F) Elisa assay detected the concentrations of GSH and Fe^{2+} in USP13 overexpression or control cells transfected with either control siRNA or ATG7 siRNA. (G) PI staining was used to detect cell activity after transfected with either control siRNA or ATG7 siRNA in USP13 overexpression or control cells, Scale bar=100 μm. Data are means ± SD. n = 3 independent cell cultures. *$P < 0.05$; **$P < 0.01$.

DISCUSSION

Follicle development and maturation play a crucial role in enhancing the laying performance of hens. Repressing follicle degeneration and augmenting the count of follicles progressing to the dominant stage bear significant economic implications in enhancing chicken egg production performance. Apoptosis and autophagy of follicular granulosa cells play a pivotal role in the process of ovarian follicular atresia in animals (Meng, et al., 2018). In this study, we have discovered that ferroptosis, a recently identified form of programmed cell death, exerts a significant influence on follicular growth and development in chickens. Therefore, elucidating and comprehending the molecular regulatory mechanism underlying ferroptosis holds immense significance in terms of suppressing granulosa cell death and enhancing laying performance in hens.

Mounting evidence substantiates the pivotal role of USP13 in governing cellular senescence (He, et al., 2023), cell cycle regulation (Esposito, et al., 2020), lung fibrosis (Liu, et al., 2023), and cancer metastasis (Guo, et al., 2023). Lee et al. discovered that USP13 functions as a deubiquitinase for p62, thereby promoting autophagy and facilitating the release of Nrf2, consequently activating the cellular antioxidant capacity (Lee, et al., 2023). Wu et al. also found that USP13 can induce Nrf2 expression by deubiquitinating NRF2, thereby reducing oxidative stress and inflammation induced by lipopolysaccharides (Wu et al, 2023). Nrf2 is a key gene in the ferroptosis pathway, which inhibits the process of ferroptosis by reducing lipid peroxidation and accumulation of iron ions (Dodson, et al., 2019). This suggests that USP13 may play an important role in the metabolism or homeostasis of iron ions by targeting proteins involved in the ferroptosis pathway. In this study, we found that USP13 inhibits GSH and promotes lipid peroxidation and iron accumulation to induce ferroptosis in chicken granule cells. This finding can also provide a theoretical

basis for improving the utilization rate of follicles in chicken molecular breeding. Further investigation into the mechanism of USP13 in regulating ferroptosis in granulosa cells of chickens will not only enhance our comprehension of the physiological processes underlying the reproductive system of laying hens, but also foster technological innovation and optimization of management strategies for breeding programs, thereby augmenting production efficiency and economic benefits associated with laying hen farming.

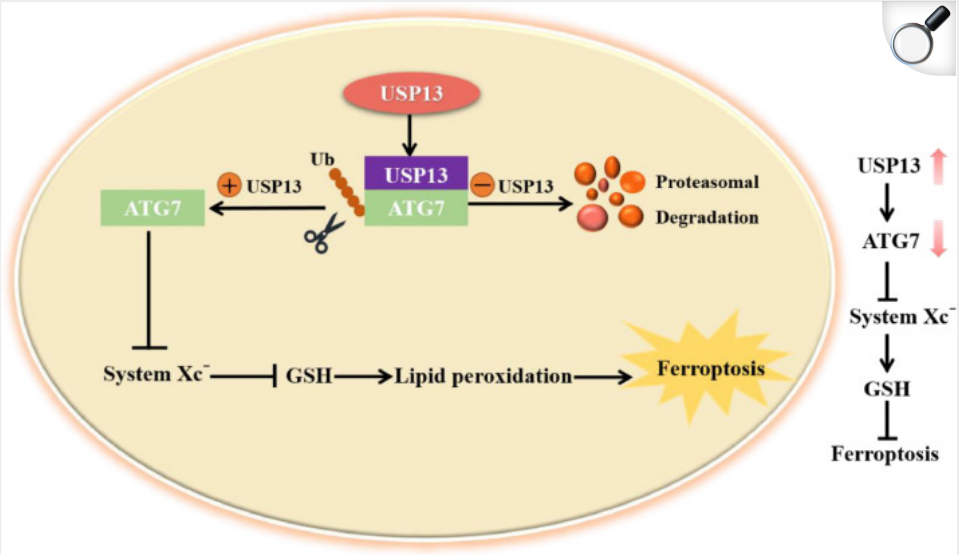
ATG7, a key member of autophagy, regulates the formation of autophagy vesicles and the assembly of autophagosomes, thus playing a crucial role in autophagy (Zhou, et al., 2022). In recent years, numerous studies have found that autophagy can act as an upstream regulatory mechanism of ferroptosis and play a crucial role in determining sensitivity to ferroptosis (Liu, et al., 2020). Yan et al. discovered that the deficiency of ATG7 hinders MI-2-induced ferroptosis, thereby attenuating the endothelial cell response to inflammatory stimuli (Yan, et al., 2023). Moreover, ATG7 facilitates the degradation of ferritin through autophagy activation, thereby enhancing intracellular Fe^{2+} accumulation and triggering ferroptosis (Yang, et al., 2019). These studies provide evidence for the crucial involvement of ATG7 in the ferroptosis pathway. Our Co-IP results showed that USP13 regulates the ferroptosis process in chicken granulosa cells by interacting with ATG7. We subsequently investigated the mRNA and protein expression levels of ATG7 in USP13-deficient chicken granulosa cells. The results showed that the downregulation of USP13 significantly reduced ATG7 protein levels, but not ATG7 mRNA levels. This suggests that post-translational modifications may play an important role in regulating the stability of ATG7. We further found that USP13 plays an important role in the stability of ATG7 protein by deubiquitinating ATG7. Deletion of ATG7 also mitigates USP13-induced ferroptosis in follicular granulosa cells. ATG7 has also been found to play a key role in follicle

development and maturation through the activation of autophagy (Ommati, et al., 2020). Therefore, USP13 may play an important role in the growth and development of chicken follicles by regulating the stability of ATG7 protein. Ferroptosis of granulosa cells may result in the shedding of the granulosa layer, thereby impacting the substantial reduction in sex steroid and antioxidant levels. These alterations are directly associated with poultry reproductive health and egg production capacity. A comprehensive comprehension of ferroptosis in follicular granulosa cells and its regulatory mechanism holds immense significance for comprehending poultry reproductive health and enhancing egg production rates.

CONCLUSION

In summary, ferroptosis is closely related to follicle growth and development. USP13 promotes the expression of ATG7 by deubiquitinating it, thereby activating iron-dependent lipid peroxidation levels and inducing ferroptosis in chicken follicular granulosa cells (Figure 7). Given the involvement of diverse physiological processes in ferroptosis, gaining a comprehensive understanding of the role of the USP13-ATG7 axis in chicken follicle growth and development can provide valuable insights into molecular-assisted breeding strategies and serve as a foundation for enhancing poultry yield.

Figure 7.



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Role of USP13 as a model in the growth and development of chicken follicles. USP13 suppresses System Xc^- activity by deubiquitinating ATG7 and facilitates ferroptosis in chicken follicle granulosa cells.

DISCLOSURES

The authors declare no conflicts of interest.

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