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Expression Profiles and Functional Roles of BRCC3 and NLRP3 in Malignant Transformation of Endometriosis

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#These authors contributed equally to this work as the co-first authors

Abstract

Objective: The mechanisms underlying the progression from endometriosis to Endometriosis-Associated Ovarian

the progression of endometriosis to EAOC.

Method: The clinical tissue samples of endometriosis and EAOC endometrium were collected with normal endometrium served as controls. Immuno-histochemical staining was performed to determine the expression patterns

transwell assays.

Results:
samples compared to the normal endometrium. NLRP3 expression was also positively correlated with FIGO stage,

Conclusion:

[illegible]

BRCC3 has been reported to be an upstream regulator of NLRP3 [13], we therefore evaluated the clinical significance of BRCC3 and its association with NLRP3 in these tissue samples. We found that BRCC3 protein level was significantly increased in EAOC and endometriosis samples compared to that of normal endometrium (Figure 3A and B). In addition, mRNA expression of BRCC3 was also elevated in EAOC and endometriosis samples. Particularly, the protein and mRNA levels of BRCC3 in EAOC samples were dramatically higher than those in the endometriosis samples (Figure 3C). Furthermore, correlation analysis showed that BRCC3 was positively related to NLRP3 in EAOC tissues (Figure 3D).

[illegible]

To explore the functional role of BRCC3 in endometriosis malignant transformation, the endometriosis cell line CRL-7566 was transfected with BRCC3 overexpression plasmids or specific siRNA, respectively (Figure 4A). By conducting cell proliferation assay, we found that overexpression of BRCC3 in CRL-7566 cells notably increased cell proliferation (Figure 4B), while knockdown of BRCC3 significantly decreased cell proliferation (Figure 4B). In addition, BRCC3 overexpression significantly inhibited apoptosis whereas inhibition of BRCC3 promoted apoptosis in CRL-7566 cells (Figure 4C and E). Transwell assays revealed that BRCC3 dramatically enhanced the migration and invasion, while knockdown of BRCC3 suppressed such effects in endometriosis cells (Figure 4D, F and G). Furthermore, western blot analysis showed that overexpression of BRCC3 significantly increased the expression levels of NLRP3, MMP2/9, caspase-1, and IL-1β, whereas depletion of BRCC3 suppressed their protein expression

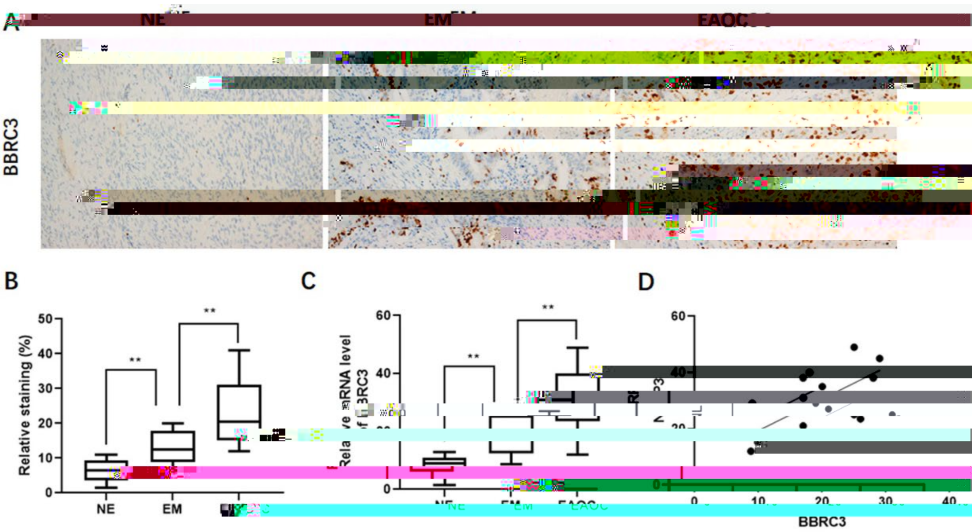

Figure 3: IHC analysis for BRCC3 expression in patients with EAOC and endometriosis

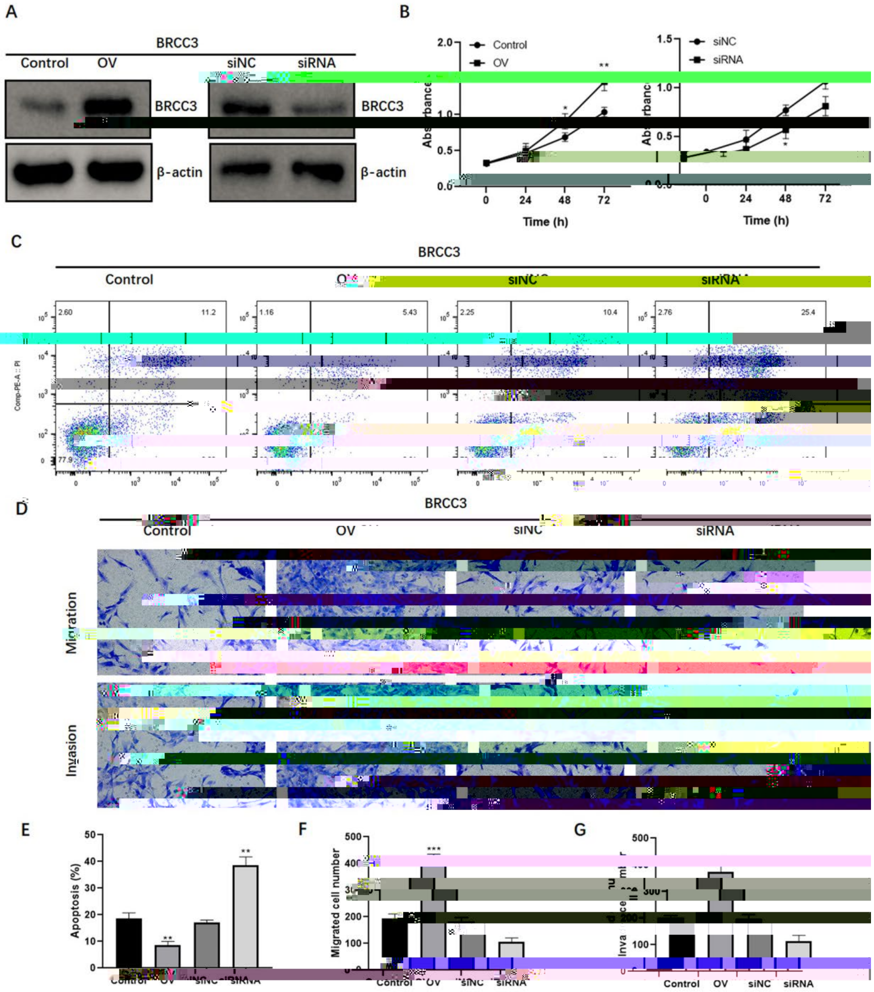

Figure 4: BRCC3 promotes malignant transformation of endometriosis

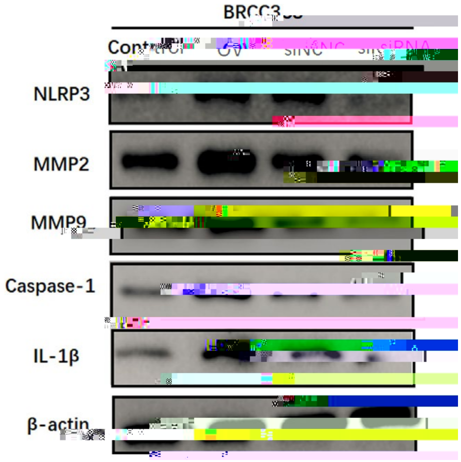

Figure 5: Effects of BRCC3 on NLRP3 expression in endometriosis cells

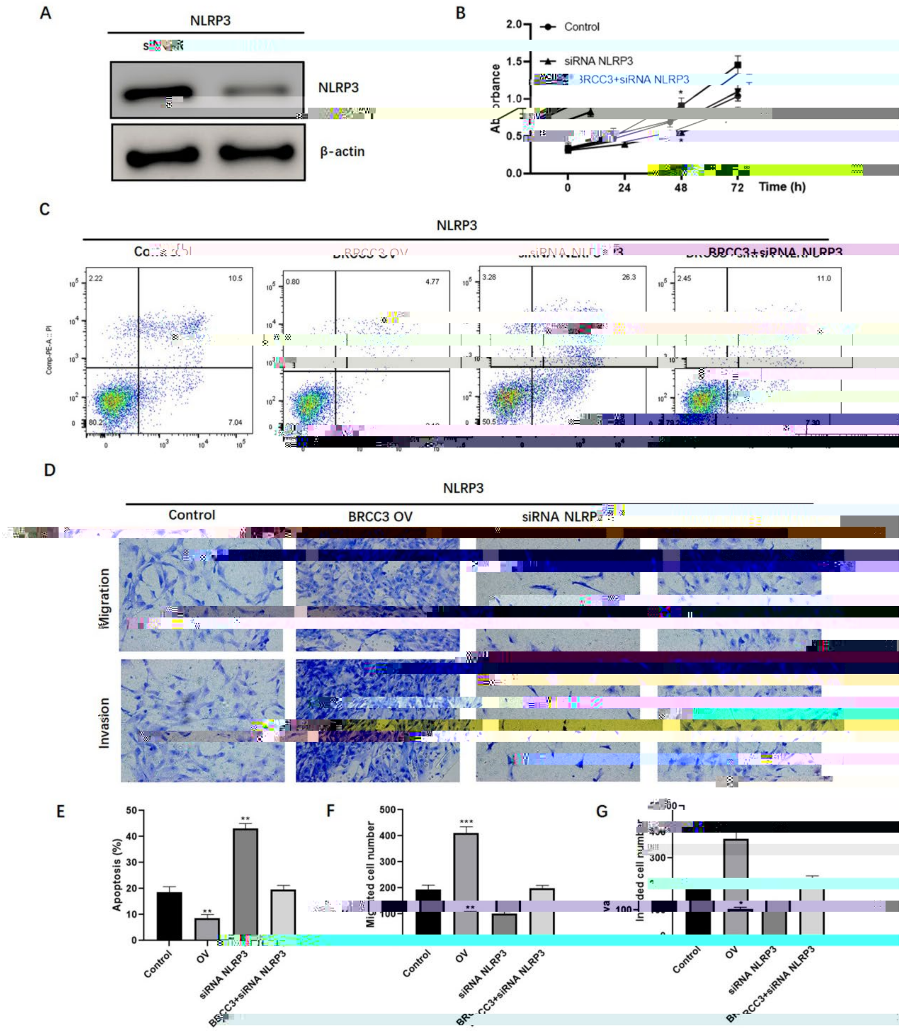

Figure 6: BRCC3 modulates cell proliferation, apoptosis, migration, and invasion of endometriosis cells through NLRP3

(A)

transformation via NLRP3. To validate such hypothesis, we firstly knocked down of NLRP3 by siRNA (Figure 6A). Consequently, depletion of NLRP3 significantly decreased cell proliferation, and reversed BRCC3-induced overexpression of CRL-7566 cells (Figure 6B). Moreover, overexpression of BRCC3 could inhibit apoptosis (Figure 6C and E), and enhance migration (Figure 6D and F) and invasion (Figure 6D and G) of CRL-7566 cells; these effects were abrogated by NLRP3 knockdown (Figure 6C-G). These results suggest that BRCC3 promotes the malignant phenotype of endometriosis cells through NLRP3.

[illegible]

Although endometriosis is usually considered benign, its biological behaviors exhibit some common features with ovarian malignancies, such as cell proliferation, apoptosis, migration, and invasion [14]. It has been widely acknowledged that endometriosis may increase the risk of epithelial ovarian cancer. However, it remains unknown about the pathological mechanism of endometriosis malignant transformation to EAOC [15]. This study evaluated the expression and functional role of NLRP3 inflammasome in endometriosis. Our findings showed that activation of NLRP3 inflammasome and its upstream BRCC3 occurred and induced malignant transformation of endometriosis cells.

Endometriosis and ovarian cancer share abnormal immunological and apoptotic features in the peripheral blood and peritoneal fluids [14, 16]. Several genes are found to be associated with tumor development in endometriotic tissues from patients with endometriosis [14, 17-19]. Inflammatory responses play critical roles for tumorigenesis and are also capable of disturbing the tumor responses to therapy. It has been found that inflammasome-related genes (e.g., NLRP3, IL-1, TLR1, TNF) are differentially expressed in endometriosis and EAOC, and correlated with poor progression-free survival, suggesting a vital role of inflammasome in EAOC carcinogenesis [20]. NLRP3 is a complex protein involved in the inflammatory responses. Our study found that caspase-1, NLRP3, and IL-1β were significantly activated in EAOC and endometriosis compared to normal endometrium. NLRP3 expression was also correlated with clinical parameters in patients with EAOC, such as FIGO stage and differentiation.

Increasing evidence has revealed that BRCC3, an upstream regulator of NLRP3, promotes inflammasome activation by deubiquitinating NLRP3 [13, 21-23]. Our data suggested that BRCC3 levels were upregulated in EAOC and endometriosis, positively correlated with NLRP3. Deregulation of BRCC3 is associated with tumor progression [24, 25]. To unravel the functional role of BRCC3 in endometriosis cells, we performed in vitro assays with manipulating BRCC3 in the endometriosis cell line. Data showed that BRCC3 overexpression induced cell overgrowth, inhibited apoptosis, and enhanced migration and invasion of endometriosis cells. Conversely, knockdown of BRCC3 suppressed such effects in endometriosis cells. Furthermore, we found that BRCC3 activated NLRP3 expression, and NLRP3 knockdown could abrogate the effects of BRCC3 on endometriosis cells, suggesting that RCC3-NLRP3 axis is critical for inflammasome activation and malignant transformation in endometriosis.

[illegible]

In conclusion, the present study demonstrates that BRCC3 could regulate NLPR3 expression, and promote cell proliferation, migration, and invasion, and inhibit apoptosis rate. The RCC3-NLRP3 axis might be a key target to prevent the malignant conversion of endometriosis to EAOC.

[illegible]

Endometriosis-Associated Ovarian Cancer, EAOC; Nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3, NLRP3; caspase-1 recruitment domain, ASC; interleukin-1β, IL-1β; Immunohistochemistry, IHC; BRCA1/BRCA2 Containing Complex Subunit 3, BRCC3

[illegible]

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genetic evidence that endometriosis is a precursor of ovarian cancer

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