

lncRNA APOC1P1-3 promoting anoikis-resistance of breast cancer cells

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Research

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Abstract

Background

Anoikis resistance plays a critical role in the tumor metastasis by allowing survival of cancer cells in the systemic circulation. We previously showed that long non-coding RNAs APOC1P1-3 (lncRNA APOC1P1-3) inhibits breast cancer cell apoptosis. However, its role in the anoikis resistance remains unclear.

Methods

We induced anoikis resistance in two breast cancer cell lines (MCF-7 and MDA-MB-231) under anchorage-independent culture condition and studied the effects of lncRNA APOC1P1-3 on the apoptosis. The Dual-Luciferase activity assay were used to whether miRNA-188-3P can specifically bind to lncRNA APOC1P1-3. We further explored the role of APOC1P1-3 in the lung metastasis by injecting MDA-MB-231 and MDA-MB-231-APOC1P1-3-knock-down cells in the female BALB/c nude mice.

Results

We found that it suppressed early apoptosis of these cells by gain or loss of their function, respectively. We further explored its mechanism related to anoikis resistance, and found this molecule promoted the resistance via activating Caspase 3, 8, 9 and PARP. Moreover, it was specifically binding to the target miRNA-188-3p to block its inhibition of Bcl-2 (an anti-apoptosis protein). These findings suggest that lncRNA APOC1P1-3 plays an important role in the development of breast cancer metastasis via anoikis resistance.

Conclusions

This study demonstrates that lncRNA APOC1P1-3 can promote the anoikis resistance of breast cancer cells and specifically bind to miRNA-188-3p acting as a “sponge” to block the inhibition of anti-apoptotic protein Bcl-2.

Background

Metastasis, the major cause of death in cancer patients, is a complicated process involving detachment of cancer cells from the original site, survival in the circulatory and lymphatic systems, and colonization in distant target organs [1]. Anoikis resistance is a prerequisite for metastasis, facilitating secondary tumor formation in distal organs [2]. Anoikis is a particular type of apoptosis of epithelial and endothelial cells as losing attachment from the appropriate extracellular matrix (ECM) or neighboring cells. It is a physiological process for organ development and tissue homeostasis [3]. Anoikis resistance plays a vital role in the tumor survival via circulating tumor cells in the blood [4]. It involves in many intracellular

molecules, such as growth factor receptors, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) [5].

lncRNAs are a set of key regulatory RNAs with transcripts longer than 200 nucleotides. Increasing evidence indicates that they involve in almost all physiological and pathological processes in the human body, including cell proliferation, apoptosis, stem cell maintenance, and cancer metastasis [6, 7, 8, 9]. They directly regulate the metastasis both *in vitro* and *in vivo*. lncRNA HOTAIR aberrantly expressed in many tumors (liver, breast, colon and pancreatic cancers), contributing to the metastasis [10, 11]. lncRNA MALAT1 was discovered first in non-small cell lung cancer patients with high metastasis and poor prognosis [12]. It was upregulated in prostate, colorectal, and bladder cancers [13, 14, 15]. lncRNAs were also involved in various mechanisms, including sponge of miRNA, epithelial-mesenchymal transition (EMT) and epigenetic gene regulations [16]. However, their mechanisms in the anoikis resistance of breast cancer remains unclear. In our previous studies, we sought out long intergenic non-coding RNA APOC1P1-3 from breast cancer tissues and cell lines, and found that it was upregulated in most cases, and correlated with tumor size and hypomethylation in its promoter region. Moreover, it promoted cell proliferation and inhibited apoptosis via α -tubulin acetylation [17]. In the present study, we further explored its role in the anoikis resistance of breast cancer.

Methods

Human breast cancer tissues and cell lines

A total of 24 freshly resected breast cancer samples and paired paracancerous tissues were collected from Fudan University Shanghai Cancer Center. Our present research has been approved by Basic Medical Ethics Committee of the Fudan University (2017-F001). The patient signed the informed consent. Specimens were snap-frozen in liquid nitrogen immediately after resection and kept in -80°C .

The breast cancer MCF-7 and MDA-MB-231 cell lines were obtained from the cell bank of the Shanghai Institute of Biological Sciences, Chinese Academy of Sciences. The cells were cultured in DMEM/F12 medium with 10% (v/v) fetal bovine serum (Gibco, USA), 100 units penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin, at 37°C with 5% CO_2 .

Cytoplasmic and nuclear separation

The content of RNA in cytoplasm and nucleus was studied with PARIS™ Kit (ThermoFisher AM1921). When the confluence rate was $>90\%$ in the 60 mm dish, the cells were collected. Cytoplasm and nuclear were separated by centrifugation following the manual of kit. The cytoplasm supernatant and nucleus precipitate were transferred to different tubes, equal volume of 2 \times Lysis Binding Buffer and mixed thoroughly with a pipette. The obtained RNA was stored at -80°C with an appropriate amount of DNAase to prevent degradation.

RNA-Chip and bioinformatics analysis

The expression of lncRNA was screened by pairwise random variance in the cancer and control tissue samples. The specific criteria were: lncRNA expression was 1.5 times higher in cancer tissue, and the $P \leq 0.05$. The relevant hierarchical clustering analysis was assisted by Kang Cheng Biotechnology Co, Ltd., using Cluster Treeview software (Stanford).

Real-time quantitative PCR

RT-PCR was used to verify the expression of lncRNA APOC1P1-3. Total RNA from cells and tissues was extracted by TRIzol from Invitrogen. Reverse transcription was performed with TOYOBO's ReverTra Ace qPCR RT Kit. Real-time PCR was performed with Bio-RAD iTaq™ Universal SYBR® Green Supermix. The primer sequences are described in Table S4.

Anoikis model of breast cancer cells

Anoikis model was established by poly-2-hydroxyethyl methacrylate (polyHEMA) (Sigma company) culture [35]. PolyHEMA was dissolved in ethanol (final concentration of 120mg/ml) in a 65°C water bath. The PolyHEMA solution (1:10 in 95% ethanol) was sterilized under a 0.22 µm filter and pipetted into a 6-well culture plate (1ml/well). The plate was incubated overnight at room temperature, washed three times with PBS, and incubated with DMEM/F12 medium prior to use. 1×10^6 cells were seeded into the PolyHEMA-coated wells for 12, 24, 48 or 72h in a humidified (37°C, 5% CO₂) incubator.

Annexin V-FITC/PI double staining for apoptosis

MDA-MB-231a and MCF-7a cells cultured for 48 h were collected, digested with 0.25% EDTA-free trypsin and pipetted to achieve a uniform single cell suspension. Cell concentration was adjusted to L-15/DMEM medium containing 10% FBS, 10% double antibiotics to 1×10^6 cells/ml. The Annexin V-FITC/PI double staining was performed with the Annexin V-FITC Apoptosis Detection Kit (Dojindo).

Dual-Luciferase report gene vectors

The pmirGLO Dual-Luciferase miRNA Target Expression Vector and Dual-Luciferase® Reporter Assay were purchased from Promega. Using bioinformatics to analyze and predict the binding site of miRNA-188-3p to APOC1P1-3, we designed primers and cloned the target fragment. The fragment was inserted into the pmirGLO Dual-Luciferase miRNA Target Expression Vector by molecular cloning, and the recombinant reporter plasmid and the target microRNA mimic were co-transfected into the target cells. The luciferase was extracted and its activity was tested. The mutated fragment was ligated into the dual luciferase reporter gene vector, and the target microRNA mimetic was co-transfected with the target cell for fluorescence detection. The DNA purification kit-Glue recovery kit and Rapid site-directed mutagenesis kit were purchased from TIANGEN. Endonuclease XhoI-Sall and T4 ligase were from NBE.

In vivo metastasis studies

We firstly designed a lentivirus for knock-down human APOC1P1-3 (5' GAAAGACCCTGGAGGACTA 3') from GeneChem Corporation (Shanghai, China). We seeded 2×10^5 MDA-MB-231 cells in 6-well plates and carried out the transfection with Lv-sh-APOC1P1-3 and Lv-sh-Control 24 h. At 72 h, we extracted total RNA from the cells and evaluated the inhibitory effects (about 60%) with RT-PCR (Supplementary materials). Then, we established the APOC1P1-3 knock-down and control stable MDA-MB-231 cell lines with transfected Lv-sh-APOC1P1-3, Lv-sh-Control and the Puromycin (2.5 μ g/ml).

All animal experiments were performed in accordance with the guidelines and approved by the Institutional Animal Care and Use Committee at Fudan University (20160225-108). Six-week-old female BALB/c nude mice were used (8 mice/group). MDA-MB-231 and MDA-MB-231 with APOC1P1-3 knock-down cells (2×10^6 cells/group) were injected into the tail vein. After 7 weeks, the mice were sacrificed and lung metastases were quantified and imaged from Hematoxylin and Eosin (HE) stained slides.

Statistical analyses

All values are reported as mean \pm SE and analyzed with the SPSS 17.0 (Chicago, IL, USA). For comparisons, one-way analyses of variance, Fisher's exact tests, χ^2 -tests, and two-tailed student's t-tests were performed. $P \leq 0.05$ was considered to be statistically significant. The diagrams were completed with Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

Characterization of lncRNA APOC1P1-3

Previously, we measured and compared lncRNA APOC1P1-3 expression in breast cancer and its adjacent tissues via microarray chip. In order to identify its protein-noncoding property, we checked the Open Reading Frame (ORF) of APOC1P1-3 by ORF finder (<http://www.ncbi.nlm.nih.gov/orffinder/>), and found that it has 8 ORFs and none is more than 200nt, suggesting no protein coding potential (Table S1). CSF is the mutation rate of the codon. Codons are relatively conservative in the coding region, but prone to mutations in the non-coding region. Due to the problem of sequence conservation, Michael [18] proposed a new value to introduce the evolution model PhyloCSF. We also checked the PhyloCSF of APOC1P1-3 on UCSC (<http://genome.ucsc.edu/>). All values in the exon of APOC1P1-3 are less than 0, indicating no conservative type.

Correlation between lncRNA-APOC1P1-3 and breast cancer

We measured the expression of lncRNA APOC1P1-3 in 3 malignant (T47D, MCF-7, MDA-MB-231) and a normal (MCF-10A) breast cell lines and in breast cancer tissues, by real-time qPCR (Figure. 1A-1C). The expressions in malignant cells upregulated, especially in the MDA-MB-231 cells (increased by 1.5 fold). In addition, lncRNA APOC1P1-3 was found both in cytoplasm and nucleus in MDA-MB-231 cells (Figure. 1B). To evaluate the role of lncRNA APOC1P1-3 in survival, we used the cBioPortal database in TCGA for related bioinformatics analysis. The survival rate is much higher in patients with low expression (blue

line) than with high expression (red line) (Figure. 1D; n=816, P<0.01). This may be related to distant metastasis of the tumor. We further explored the role of APOC1P1-3 in the lung metastasis by injecting MDA-MB-231 and MDA-MB-231-APOC1P1-3-knock-down cells into the tail vein, and found that the lung metastases burden in nude mice of APOC1P1-3-knock-down cells significantly decreased (Figure. 1Ea-b).

The capacity of anoikis resistance of breast cancer cells

Anoikis resistance model was established by culturing the cells on a low-adherent culture plate with polyHEMA [19]. Both MDA-MB-231 and MCF-7 cells survived under adherent and anchorage-independent conditions for 72 h by forming micro-tissues (Figure. 2A-2B), indicating the anoikis resistance. MCF-7a aggregated in large clusters with more cells were dead than MDA-MB-231a, which is similar to a previous report [20]. MDA-MB-231a cells formed many small irregular clumps, which may be responsible for detachment from primary tumor tissues.

Under both adherent culture and ultralow attachment culture, we collected cells with or without underwent anoikis resistance for 48h. Aggregation occurred in all cells (MCF-7a, Figure. 2C and MDA-MB-231a, Figure. 2D). The aggregation was much more in the anoikis group than in the normal group (n=7, **P<0.01).

We compared the number of apoptotic cells under adherent culture and low-adherent culture condition Fluorescence-activated cell sorting (FACS). After 48h culture, the numbers of apoptotic cells were significantly fewer in MCF-7a and MDA-MB-231a (Figure. 2E-F) than in MCF-7 and MDA-MB-231 groups (n= 3, **P<0.01), indicating an effective induction of anoikis resistance.

LncRNA APOC1P1-3 suppressing apoptosis via apoptosis-related proteins

In the gain/loss functionality studies, the expression of lncRNA APOC1P1-3 efficiently up-regulated by transfection with pcDNA3.1-sense in MCF-7 cells and down-regulated by transfection with siRNA-196 in MDA-MB-231 cells (Figure. 3A-B). The percentage of early apoptosis (FACS) was significantly decreased in MCF-7a cells (Figure. 3C) (n=3, *P<0.05), and increased in MDA-MB-231a cells (Figure. 3D) (n=3, *P<0.05). The results support that the lncRNA APOC1P1-3 could promote anoikis resistance.

To probe the mechanisms of anoikis resistance, we measured apoptosis related proteins (Western Blot) from MCF-7a transfected with siRNA-196, as well as MDA-MB-231a transfected with pcDNA3.1-sense (Figure. 3E-F) (n=3, **P<0.0). The expressions of activated-caspase 3, 8, 9, and activated-PARP upregulated in MDA-MB-231a cells which were knockdown with siRNA-196. In contrast, the expressions decreased in MCF-7a cells which were overexpressed with pcDNA3.1-sense. These results indicate that upregulation of lncRNA APOC1P1-3 could influence anoikis resistance via caspase-dependent pathway.

Regulation of anoikis resistance via binding to miRNA-188-3p

We used RNA microarrays (provided by Kangcheng Company) to screen related target microRNAs of lncRNA-APOC1P1-3 in breast cancer tissues, and predicted microRNAs in multiple databases like PITA,

MIRDB4.0 and Miranda by matching between sequences (Table. S2). There are 84 microRNAs that may bind to APOC1P1-3. With Venn diagram to compare the predicted results of databases and chip, miRNA-188-3p most likely binds to lncRNA APOC1P1-3 (Figure. 4A). The microarray results suggest that the binding site is at 222 bp-248 bp of APOC1P1-3 (Figure. 4B).

The pmirGLO-APOC1P1-3'UTR and the pmirGLO-APOC1P1-Mut vectors were separately constructed with the Dual-Luciferase Report Gene System. We used the BiBiServ-RNAhybrid database to perform thermodynamic analysis of the predicted binding sites before making a point mutation. The mfe value of the predicted site is -28.5kcal/mol (Figure. S1), the highest absolute value among all 11 predicted sites, indicating a good thermodynamic effect. Group 4 mfe random mutation was -25.4kcal/mol (Table. S3), indicating a better thermodynamic effect.

By transfected miRNA 188-3p mimics, we found that the expression of miRNA-188-3P was significantly higher than that in the control group (Figure. 4C). The pmirGLO-APOC1P1-3'UTR plasmid was bound to the co-transfected miRNA-188-3P mimics. Since the transcription of the luciferase reporter gene was blocked, the fluorescence activity was significantly lowered. After mutation (Figure. S2), miRNA-188-3P mimics could no longer bind to it, and the luciferase reporter gene was transcribed normally (Figure. 4D). The fluorescence activity was not significantly different from the control, suggesting that miRNA-188-3P can specifically bind to lncRNA-APOC1P1-3.

The cross-talking between miRNA-188-3p and lncRNA-APOC1P1-3

We further explored the effect of miRNA-188-3p on anoikis resistance. miRNA-188-3p was first overexpressed in MDA-MB-231a cells by transfection with miRNA-188-3p mimics. Early apoptosis was highly increased after the overexpression (Figure. 4E), suggesting an inhibition of anoikis resistance. After co-overexpression of lncRNA APC1P1-3 and miRNA-188-3p, there was no significant difference in early apoptosis between Sense + Mimics and the controls (Figure. 4F, revealing that miRNA-188-3p can reverse the effect of lncRNA-APOC1P1-3 in anoikis resistance.

Moreover, we studied the mutual regulation between lncRNA APOC1P1-3 and miRNA-188-3p. Firstly, we overexpressed APOC1P1-3 in MCF-7a cells by transfection with pcDNA3.1-sense, and evaluated the expression of miRNA-188-3p, and vice versa. Neither overexpression of miRNA-188-3p nor lncRNA-APOC1P1-3 caused a change (Figure. 4G-H). Thus, although lncRNA APOC1P1-3 binds to miRNA-188-3p, the two molecules do not have any mutual impact.

lncRNA-APOC1P1-3 synergizes with miRNA-188-3p to affect Bcl-2

To study the effect of lncRNA APOC1P1-3 and miRNA-188-3p on the anti-apoptotic protein Bcl-2, we examined the Bcl-2 expression in 4 groups (sense+/mimics-, sense-/mimics+, sense+/mimics+, sense-/mimics-) (Figure. 5A-C). Overexpression of lncRNA APC1P1-3 (sense+/mimics-), significantly increased Bcl-2 (n=3, **P <0.01), while overexpression of miRNA-188-3p (sense-/mimics+) decreased it (n=3, **P <0.01). Interestingly, overexpression of both lncRNA APOC1P1-3 and miRNA-188-3p

(sense+/mimics+) did not alter Bcl-2 expression of (n=3, **P>0.01). We obtained the same result with Western Blot (Figure. 5D-E). Thus, APOC1P1-3 may enhance the anoikis resistance by blocking the inhibition of miRNA-188-3p against the Bcl-2.

Discussion

Increasing reports indicate the importance of lncRNAs in the breast cancer metastasis [21, 22, 23], however, the relation between specific lncRNAs and anoikis resistance remains undetermined. In the current studies, we investigated the role of lncRNA APOC1P1-3 in anoikis resistance and its regulatory mechanisms. We found that it suppressed early apoptosis of breast cancer cells (MCF-7 and MDA-MB-231) in the anoikis resistance model by activating Caspase 3, 8, 9 and PARP (Figure. 6). The miRNA-188-3p is the potential target molecule (bioinformatics analysis and Dual-Luciferase report system). The lncRNA APOC1P1-3 suppresses miRNA-188-3p through competitively binding to alter the Bcl-2 expression.

lncRNAs play a crucial role in cancer development. Since anoikis resistance is critical for cancer metastases, lncRNAs could regulate the anoikis resistance to promote metastasis [24]. Indeed, Seitz et al. reported that lncRNA LINC00958 significantly reduced anoikis resistance in FL3 cell after knock-down [25]. We previously found that lncRNA APOC1P1-3 aberrantly overexpressed in breast cancer, and correlated with the tumor size and hypomethylation in its promoter. It decreased α -tubulin acetylation and apoptosis. In this study, we further established that it could promote anoikis resistance by regulating the early apoptosis.

Many proteins are involved in the apoptosis, and cause anoikis resistance. We investigated the relationship between lncRNA APOC1P1-3 and apoptotic proteins, and found that caspase 3, 8, 9 and PARP increased as the lncRNA APOC1P1-3 decreased. Whereas, activated caspase and PARP proteins decreased as the lncRNA APOC1P1-3 increased (Figure. 3E-F). All these results suggest that lncRNA APOC1P1-3 could promote anoikis resistance via caspase-dependent pathway. Indeed, activated-caspase proteins could promote apoptosis of cancer cells [26].

The lncRNAs could interplay with proteins, DNA, and RNA to regulate biological processes [27]. There is a cross-regulation between lncRNAs and microRNA [28, 29]. lncRNAs interact with microRNA through different pathways. For example, microRNA can influence the stability of lncRNAs. lncRNAs can act as miRNA decoys or sponges to repress target mRNAs [30]. lncRNAs can also compete with miRNAs by directly binding to the shared target mRNAs. In the current study, we isolated the lncRNA APOC1P1-3 from cytoplasm and explored its interaction with specific miRNAs. We found that miRNA-188-3p is most likely binding to lncRNA APOC1P1-3 (Figure. 4A) at 222–248 bp of APOC1P1-3 (Figure. 4B-D).

The miRNA-188-3p is a novel independent prognostic factor in colorectal cancer patients because it promotes cancer cell migration [31]. In addition, it negatively regulates NCAPG2 via activating NF- κ B to block NCAPG2-mediated hepatocarcinoma proliferation and metastasis [32]. The miRNA-188-3p is also involved in sevoflurane-induced cognitive dysfunction via MDM2 proto-oncogene [33]. Furthermore, it

negatively regulates TMED3 to inhibit the proliferation, migration and invasion of breast tumor [34]. These studies indicate that miRNA-188-3p has different functions in diverse pathological conditions. In our study, miRNA-188-3p promotes the early apoptosis of MDA-MB-231a cells, suggesting an inhibition of anoikis resistance. It specifically binds to lncRNA APOC1P1-3, and regulate the expression of Bcl-2 (Fig. 6) to promote the anoikis resistance.

Conclusion

In summary, our study demonstrates that lncRNA APOC1P1-3 can promote the anoikis resistance of breast cancer cells and specifically bind to miRNA-188-3p acting as a “sponge” to block the inhibition of anti-apoptotic protein Bcl-2.

Abbreviations

lncRNA APOC1P1-3

long non-coding RNAs APOC1P1-3;

ECM

extracellular matrix;

ORF

Open Reading Frame;

polyHEMA

poly-2-hydroxyethyl methacrylate;

FACS

Fluorescence-activated cell sorting.

Declarations

Ethics approval and consent to participate

All studies involving breast cancer samples in our present research have been approved by Ethics Committee of Fudan University. All animal experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee at Fudan University.

Consent for publication

All Authors have approved the manuscript and agree with submission to Journal of Experimental and Clinical Cancer Research.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Ping Zhou and Xingjun Qin conceived the project and supervised all studies. Qi Lu performed all experiments, analyzed and interpreted all the data. Li Wang constructed the manuscript and Jerry Yu refined and modified the article. Ping Zhu, Luying Li, Xue Wang and Youping Jin collected clinical sample and information. Xiuling Zhi and Xin Li support the experimental techniques. All authors participated in preparing the manuscript and approved the submitted and published version.

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Figures

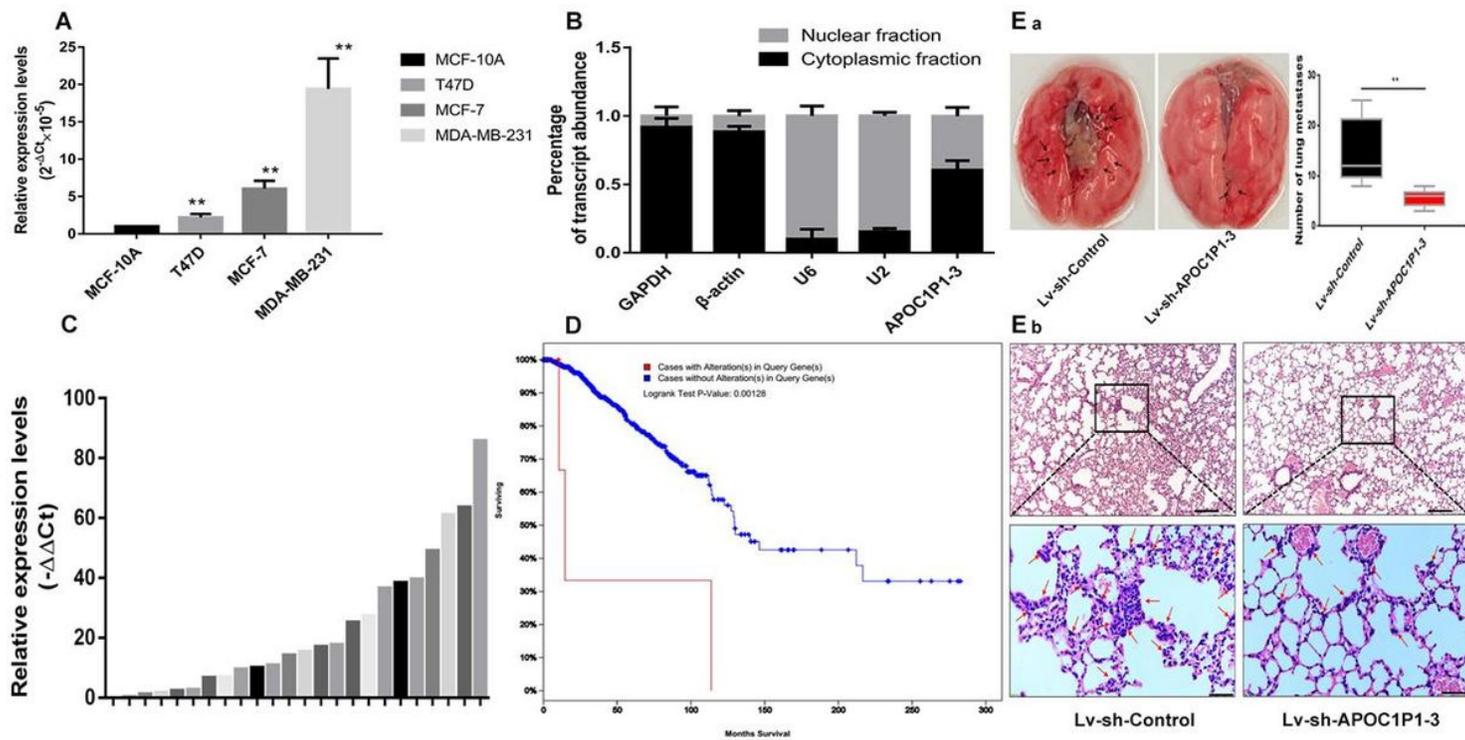


Figure 1

Correlation between lncRNA-APOC1P1-3 and breast cancer. a: The expression in breast cancer cells (T47D, MCF-7, MDA-MB-231) and normal mammary epithelial cell (MACF-10A) (RT-PCR). n=3, relative to the control group**P<0.01. b: In cytoplasmic nuclear separation of MDA-MB-231 cells, APOC1P1-3 expressed in different cell compartments (qRT-PCR). U6 RNA and U2 RNA were used for nuclear gene expression; and GAPDH RNA and β -catin RNA were used for cytoplasmic gene expression. c: The expression of lncRNA-APOC1P1-3 in 24 fresh breast cancer tissues (RT-PCR). d: The overall survival rate of breast cancer patients. Red line, high expression of APOC1P1-3 (110 months), blue line, low expression of APOC1P1-3 (300 months) (n=816, P<0.01). e: APOC1P1-3 promotes lung metastasis. (a) The number of lung metastases after tail vein injection with MDA-MB-231 cells infected with APOC1P1-3-knock-down or control vector. (b) Representative Hematoxylin and Eosin stain (H&E) of lung metastases after the tail vein injection. Scale bars: two pictures above is 200 μ m, two pictures below is 50 μ m. **, P < 0.01.

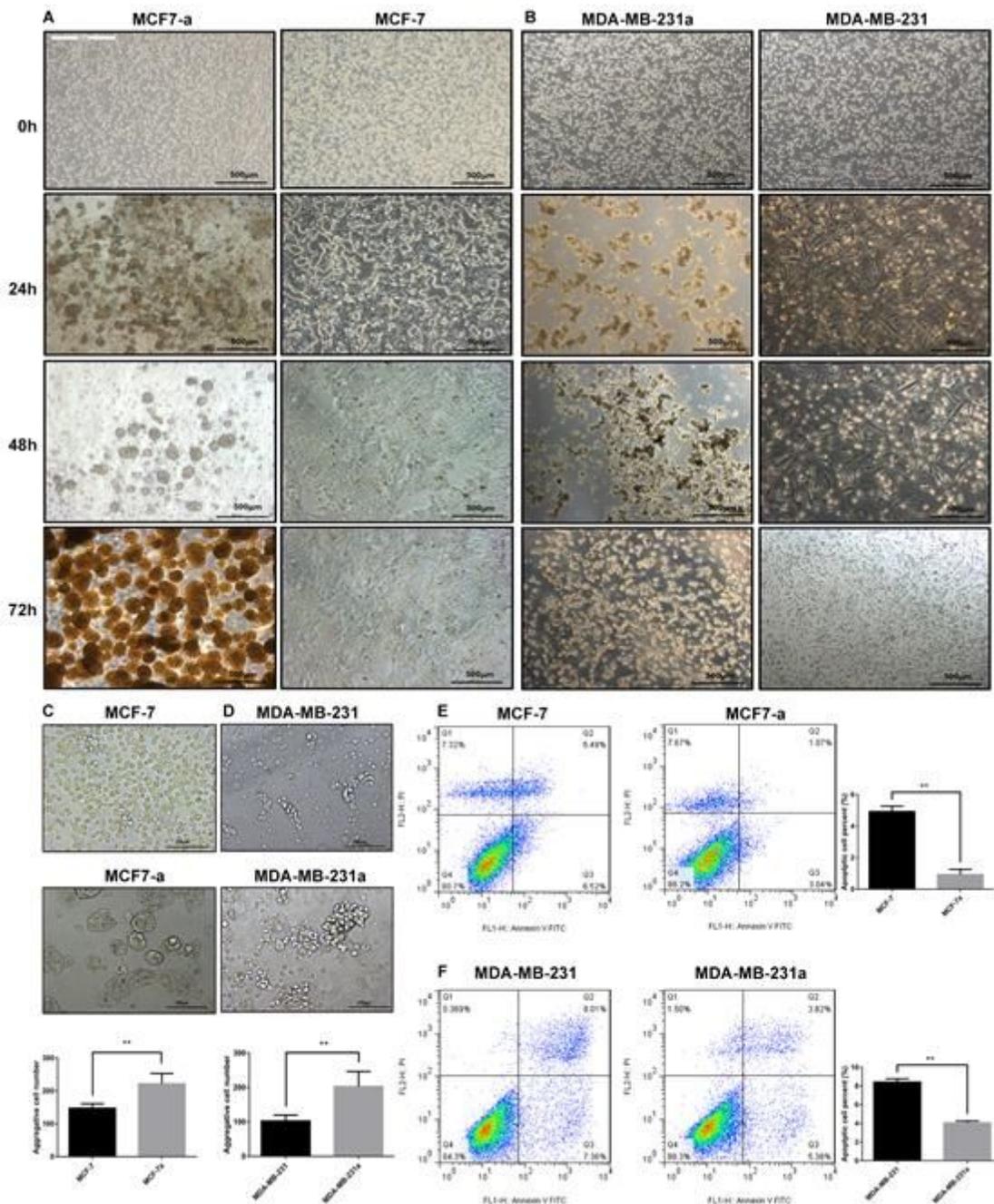


Figure 2

Morphological changes, aggregation rate and apoptotic changes of anoikis resistant cells. a&b: MCF-7a/MDA-MB-231a cells were cultured in anoikis model for 24, 48 and 72h. MCF-7/MDA-MB-231 cells were cultured in adherent state as normal control. As the induction time of MCF-7a/MDA-MB-231a increases in the anoikis model, the suspended single breast cancer cells begin to aggregate gradually. c&d: Microscopic morphology of MCF-7a/MDA-MB-231a and MCF-7/MDA-MB-231 cells 12h culture in anoikis or normal groups. The aggregation rate in MCF-7a/MDA-MB-231a is significantly more than MCF-7/MDA-MB-231 groups (n=7, **P<0.01). e&f: The effect of anoikis resistant on MCF-7a/MDA-MB-231a cells (Annexin V-FITC/PI double staining). Early apoptosis was significantly reduced in the MCF-7a/MDA-MB-231a group than in the MCF-7/MDA-MB-231 group (n=3, **P<0.01).

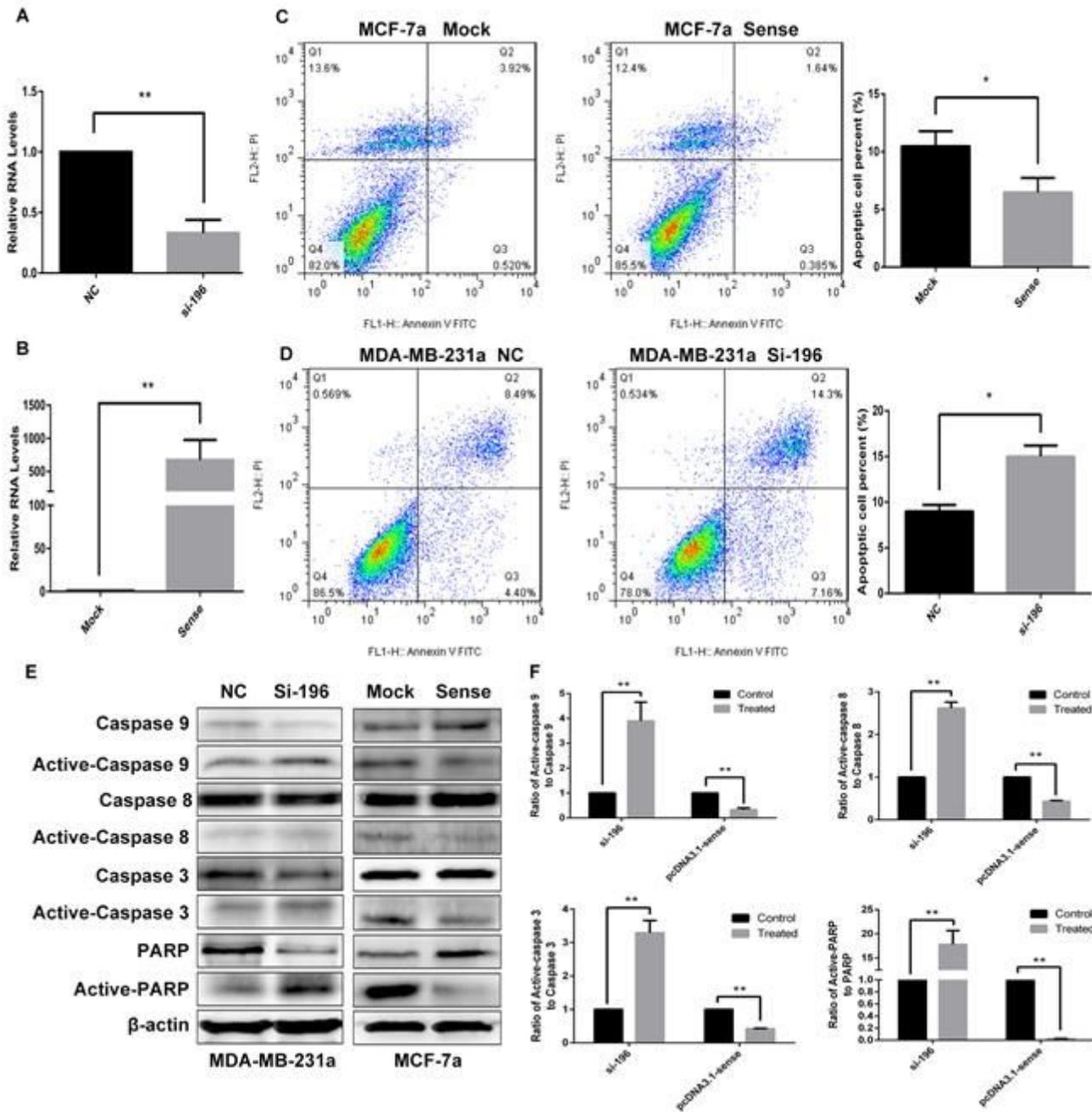


Figure 3

Effects of lncRNA-APOC1P1-3 on anoikis resistant. a&b: The expression of APOC1P1-3 was inhibited in MDA-MB-231 cells transfected with siRNA-196, but increased in MCF-7 cells transfected with pcDNA3.1-sense (qRT-PCR) ($n=3$, $**P<0.01$). c&d: Early apoptosis was more in MDA-MB-231a cells (low expression of APOC1P1-3) than in MCF-7a cells (high expression). e&f: Apoptosis-related protein (Western Blot). Similarly, in MDA-MB-231a cells active-Caspase 3, 8,9 and active-PARP expressions and the shear rate of apoptotic proteins increased, but decreased in MCF-7a cells.

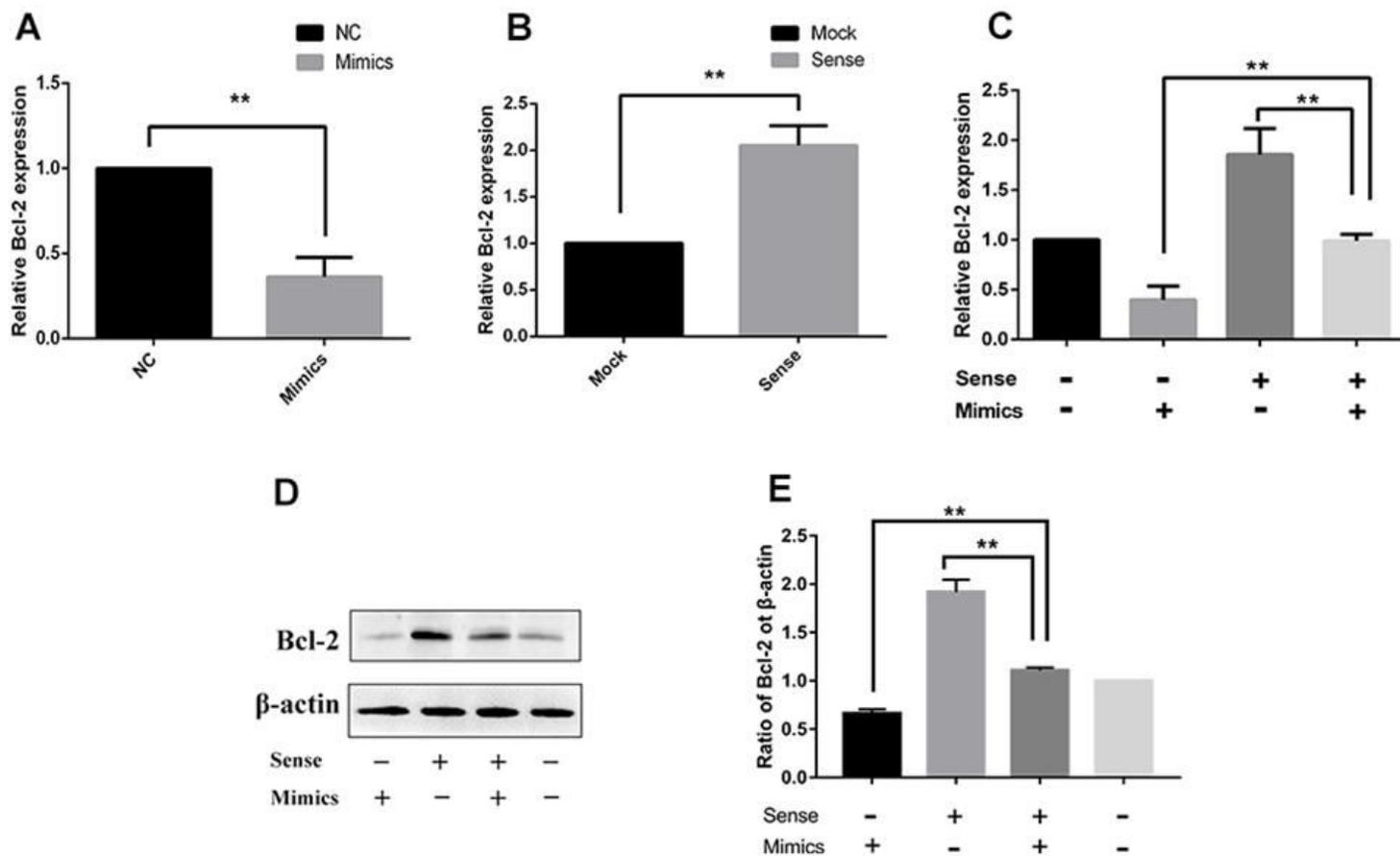


Figure 5

Effects of LncRNA-APC1P1-3 and miRNA-188-3p on the expression of Bcl-2. a&b: Overexpression of miRNA-188-3p decreases, but overexpression of LncRNA-APC1P1-3 increases the expression of Bcl-2. c: Overexpression of LncRNA-APC1P1-3 followed by up-regulation of miRNA-188-3p abolished the LncRNA-APC1P1-3 effect on Bcl-2. d&e: In four groups (sense+/mimics-, sense-/mimics+, sense+/mimics+, sense-/mimics-) simultaneous transfection of sense and mimics reversed the expression of Bcl-2 when separately transfected with sense/mimics.

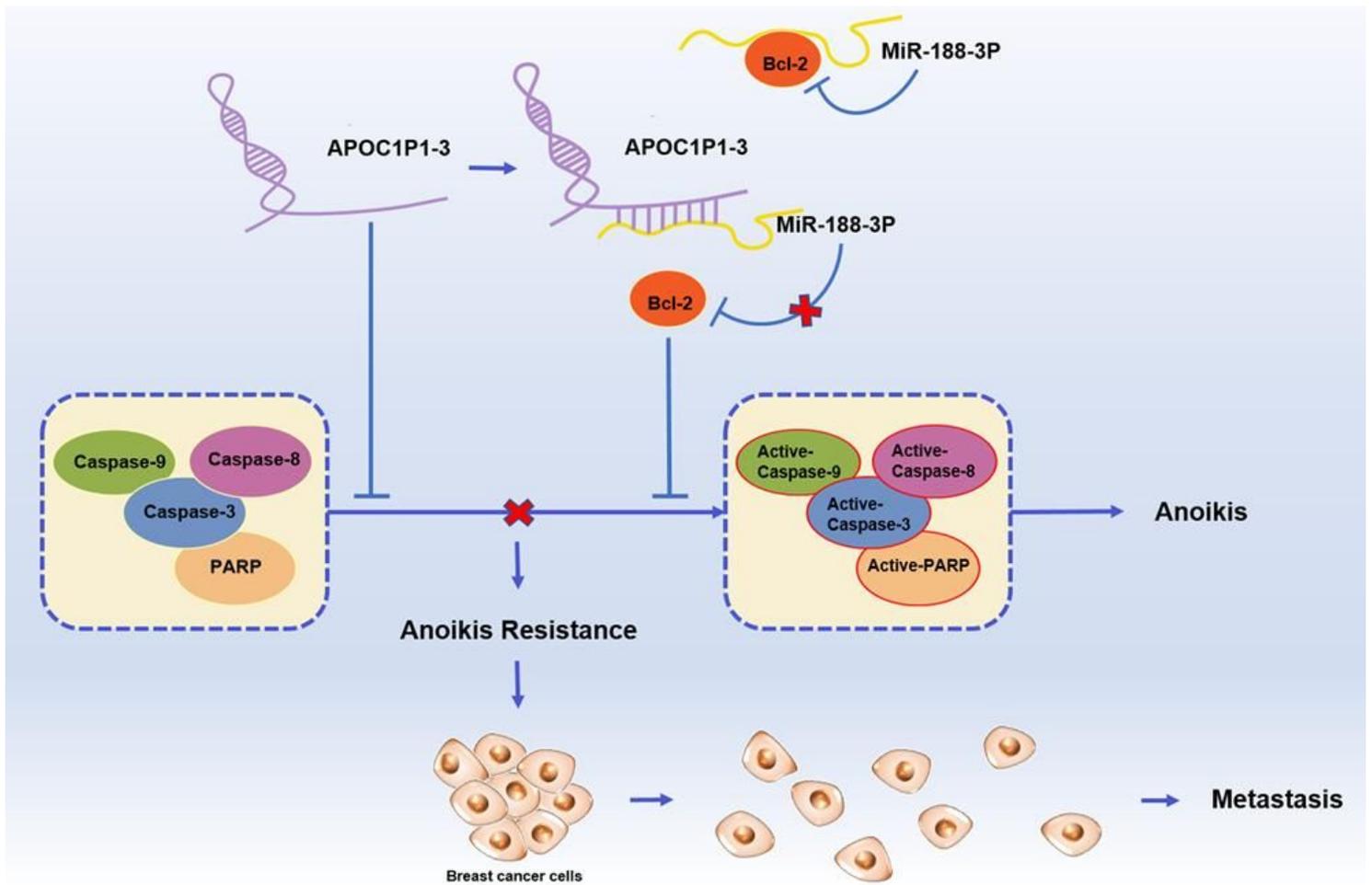


Figure 6

The molecular mechanism of lncRNA-APC1P1-3 in anoikis resistance. Two pathways, lncRNA APC1P1-3 activates Caspase 3, 8, 9 and PARP, and specifically binds to miRNA-188-3p to block its inhibition of Bcl-2.

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