



The miR-193a-3p regulated *PSEN1* gene suppresses the multi-chemoresistance of bladder cancer



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ABSTRACT

Chemoresistance prevents the curative cancer therapy, our understanding of which remains inadequate. Among the differentially expressed genes between the chemosensitive (5637) and chemoresistant (H-bc) bladder cancer cell lines, the expression level of the *PSEN1* gene (presenilin 1), a key component of the γ -secretase, is negatively correlated with chemoresistance. A small interfering RNA mediated repression of the *PSEN1* gene suppresses cell apoptosis and de-sensitizes 5637 cells, while overexpression of the presenilin 1 sensitizes H-bc cells to the drug-triggered cell death. As a direct target of microRNA-193a-3p that promotes the multi-chemoresistance of the bladder cancer cell, *PSEN1* acts as an important executor for the microRNA-193a-3p's positive impact on the multi-chemoresistance of bladder cancer, probably via its activating effect on DNA damage response pathway. In addition to the mechanistic insights, the key players in this microRNA-193a-3p/*PSEN1* axis are likely the diagnostic and/or therapeutic targets for an effective chemotherapy of bladder cancer.

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1. Introduction

Bladder cancer (BCa) is the world's second commonest type of urogenital cancer in men [1,2]. Together with the high recurring rate, BCa is highly refractory to drug therapy and regarded as one of the most costly and difficult types of cancer to be contained [3]. Although an eradication of the primary lesions by chemotherapy is achieved in about half of BCa patients, the disease often recurs, being refractory to the 2nd round of chemotherapy [4], irrespective of the previous drug

exposure [5]. As a hallmark of cancer, the multi-chemoresistance varies drastically among the cancer patients, the different cancer lesions or different regions of the same lesions within a single patient [6], presenting one of the biggest challenges to both the effective chemo-resistance prediction and the rational chemotherapy of cancer.

Despite years of intensive efforts, our knowledge of the multi-chemoresistance of cancer remains poor [7–10]. The mechanisms in discussion include an inefficient cellular drug uptake and accumulation, activation of the anti-oxidant glutathione system for detoxication, enhancement of DNA repair, and up-regulation of anti-apoptosis pathways [11–13]. The best characterized genes involved in the multi-chemoresistance of cancer are ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp), breast cancer resistance proteins (BCRP) and multidrug resistance associated proteins (MRPs) [14–16], which are often over-expressed in the multi-chemoresistant cancer cells [17].

In addition to the protein coding genes, the microRNA genes have a vital role in the multi-chemoresistance of cancer [18]. For instance, we showed in hepatocellular carcinoma (HCC) cells that the DNA methylation regulated miR-193a-3p dictates the 5-FU resistance via repressing SRSF2 expression [19]. We recently demonstrated the positive role of

Abbreviations: BCa, bladder cancer; MiR, microRNA; HCC, hepatocellular cancer; Pi, pirarubicin; Pa, paclitaxel; Ad, adriamycin; EH, epirubicin hydrochloride; Ci, cisplatin; UTR, untranslated region; *PSEN1*, presenilin 1; TERT, telomerase reverse transcriptase; ODC1, ornithine decarboxylase 1; CDKN1A, cyclin-dependent kinase inhibitor 1A (p21, Cip1); EDN1, endothelin 1; RelA, v-rel avian reticuloendotheliosis viral oncogene homolog A, p65

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miR-193a-3p in the multi-chemoresistance of BCa cells, which is executed via its suppression of five direct targets: SRSF2, HIC2, PLAU, LOXL4 and HOXC9 and in turn alter the activities of following five signaling pathways: DNA damage response, Notch, NF- κ B, Myc/Max and oxidative stress [20–22].

For the protein coding genes that contribute to the BCa's multi-chemoresistance, we performed a RNA-seq based-omic analysis for the differentially expressed genes in between a multi-chemoresistant (H-bc) and a multi-chemosensitive cell (5637) lines and found 9051 differentially expressed protein-coding genes (not shown). In the present study, we systematically investigated both role and mechanisms of the PSEN1 in the BCa multi-chemoresistance in both cultured cells and tumor xenograft/nude mouse system. The PSEN1 (presenilin) is a key component of a four protein entity, the γ -secretase complex that catalyzes the intramembrane cleavage of integral membrane proteins for the activation of Notch pathway [23]. The mutated *PSEN1* gene has been linked to the occurrence of the type III Alzheimer disease [24,25]. Relevant to its suppressing role in the BCa multi-chemoresistance, PSEN1 expression positively correlates with the activity of DNA damage response pathway. Furthermore, we showed that PSEN1 is a direct target of miR-193a-3p and relays in a significantly part the miR-193a-3p's effect on the multi-chemoresistance.

2. Material and methods

2.1. Cell lines and transfection

Cell lines — 5637 (ATCC NO. HTB-9) and H-bc (established by cancer research Institute of Kunming Medical College, 1986.) were purchased from the Chinese Academy of Sciences Committee on Culture Collection Cell Bank, Shanghai Institutes for Biological Sciences, Chinese Academy of Cell Resource Center. Both cell lines are cultured in RPMI1640 (Invitrogen, USA) + 10% fetal bovine serum (Invitrogen, USA) and 1% glutamine at 37 °C in 5% CO₂.

2.2. The mimic/antagomiR/siRNA/overexpression plasmid transfection

All the mimic, agomiR, antagomiR, siRNA and the scramble sequence control (NC) as well as the ribo FECT CP transfection kit were supplied by Guangzhou Ribobio, China). The mammalian expression constructs for PSEN1 (EX-G0389-M98-5) with GFP tag were supplied by Guangzhou Fulengen, China. Transfection of both ribonucleic acid reagents mentioned above and the reporter plasmids in a Cignal Finder Pathway Reporter package (Qiagen, US) was performed according to the manufacturer's instruction.

Chemically modified mimic oligonucleotides (agomir) were synthesized to regulate miR-193a-3p/5p expression in vivo. The 3' end of the oligonucleotides was conjugated to cholesterol, and all the bases were 2'-OMe modified. The agomir oligonucleotides were deprotected, desalted and purified by high-performance liquid chromatography.

2.2.1. Chemotherapeutics

All the chemotherapeutic drugs used are of the clinic grade [26,27] (NCI Dictionary of Cancer Terms, <http://www.cancer.gov/dictionary>), pirarubicin (Pi, Wanle, Shenzhen), paclitaxel (Pa, Taiji, Sichuan), adriamycin (Ad, Pfizer, Jiangsu), epirubicin hydrochloride (EH, Haizheng, Zhejiang), and cisplatin (Ci, Haosen, Jiangsu). The chemo-resistance profiling (IC₅₀ measurements) was conducted as previously described [20].

2.3. Analysis of RNA

Total RNA was isolated using a TRIzol reagent (Tiagen Biotech Co., Ltd., Beijing, China). For mRNA analysis, the cDNA was made from total RNA by oligo-dT priming with a prime Script RT reagent kit (Tiagen Biotech Co., Ltd., Beijing, China) and the steady state mRNA

level expression of PSEN1 was measured by qRT-PCR with gene-specific fluorescent Taqman probe together with the β -actin using a differentially fluorescence-labeled probe (provided by Shing Gene, Shanghai, China) in the FTC-3000P (Funglyn Biotech Inc., Canada). Using the 2^{- $\Delta\Delta$} Ct method, the normalization with β -actin was performed before each's relative level between 5637 and H-bc was calculated (44). The primers and probes for complementary DNA synthesis and qRT-PCR analysis are:

hPSEN1F: 5'-TATGGCAGAAGGAGACCCG-3'
 hPSEN1R: 5'-CCATTCTCACTGAACCCG-3'
 hPSEN1 probe: 5'-CY5-CATTCTCTGCAACAGTGTCTTGTGACTCC-3'
 hPSEN2 F: 5'-CTTCACTGATGCTGCTGTCC-3'
 hPSEN2 R: 5'-GAAGTTCAGACAGTCAGCAAGA-3'
 hPSEN2 probe: 5'-CY5-ACCTACAATGTGGCCATGGACTACCC-3'
 hAPH1A F: 5'-TCGGTATCATCAGTGGTGTCTTC-3'
 hAPH1A R: 5'-AAAGGTATGGAGCAGGATAATGG-3'
 hAPH1A probe: 5'-CY5-CTTCTGACTTCAGCCITTTCTGACAGC-3'
 hCDKN1A F: 5'-CACTGTCTGTACCCTTGTGCC-3'
 hCDKN1A R: 5'-GGCTTCTCTTGGAGAAGATCA-3'
 hCDKN1A probe: 5'-ROX-CCCCAGGTGGACCTGGAGACTCTC-3'
 hEDN1 F: 5'-CTTCTGCCACCTGGACATCA-3'
 hEDN1 R: 5'-CATCTATTTTACGGTCTGTTC-3'
 hEDN1 probe: 5'-ROX-CGTTGTTCCTATGGACTTGAAGCC-3'
 hODC1 F: 5'-ATGATAGCAAAGCCATCGTA-3'
 hODC1 R: 5'-CCCAGACTCTGCACCAACTG-3'
 hODC probe: 5'-ROX-CTACCGGGACAGGATTGACTGTGC-3'
 hTERT F: 5'-GCTGCTCAGGCTTTCTTTTATG-3'
 hTERT R: 5'-ACCTCTGCTCCGACAGCTC-3'
 hTERT probe: 5'-ROX-CGGAAGAGTGTCTGGAGCAAGTTGC-3'
 hACTB F: 5'-GCCCATCTACGAGGGGTATG-3'
 hACTB R: 5'-GAGGTAGTCAGTCAGGTCCCG-3'
 hACTB probe: 5'-HEX-CCCCATGCCATCTGCGTC-3'

2.4. Analysis of protein

Cells lysate with 1 × SDS loading buffer (60 mM Tris-HCl, pH 6.8, 2% SDS, 20% glycerol, 0.25% bromophenol blue, 1.25% 2-mercaptoethanol) was heated at 100 °C for 10 min to facilitate the sample loading for the conventional Western analysis. The anti-PSEN1 (AJ1650a), anti- α -tubulin (AJ1034a) and anti-GAPDH (AM1020a) were provided by Wuxi Phama, Shanghai, China. The target proteins were then probed with anti-rabbit IgG peroxidase-conjugated antibody (LP1001b), or HRP goat anti-mouse IgG antibody (LP1002a) (all antibodies are from Abgent, San Diego, CA) followed by an enhanced chemiluminescence reaction (Pierce). The relative levels of proteins were quantified using densitometry with the Gel-Pro Analyzer (Media Cybernetics). The target bands over the GAPDH or α -tubulin band were densitometrically quantified and indicated under each band.

2.5. Analysis of cell

2.5.1. Apoptosis analysis

Every group of cells was harvested and diluted with PBS twice. Then 5 μ l of FITC-labeled enhanced-annexin V and 5 μ l of 20 μ g/ml of propidium iodide were added to 100 μ l cell. Upon incubation in the dark for 15 min at room temperature, samples were diluted with 400 μ l PBS. Flow cytometry was carried out on a FACSCalibur instrument. The result was analyzed by random software. The experiments were performed independently three times [28].

2.5.2. The luciferase reporter assay

A full length of the human PSEN1 3'-untranslated region (1112 bp) with the miR-193a-3p targeting sequence (WT) or mutant

target sequence (Mut) were cloned at the downstream of the firefly luciferase gene in pGL3 (Invitrogen) to construct pGL3-luc-PSEN1. All the constructs were confirmed by restriction digestion. The luciferase activity of 3'-UTR and pathway reporter assay was determined as previously described [20].

2.6. In vivo study

The xenograft model on nude mice was generated and analyzed as previously described [20]. Expressions of PSEN1 protein were measured using immunohistochemical analysis on 5-mm slices of formalin fixed paraffin-embedded tumor xenografts in nude mice. To avoid inter-treatment bias, the tissue slides from different groups were made on a single slide and subject to the same immuno-staining simultaneously. Antigens were retrieved by pretreating dewaxed sections in a microwave oven at 750 W for 5 min in a citrate buffer (pH 6) processed with the Super sensitive link-labeled detection system (Biogenex, Menarini, Florence, Italy). The enzymatic activities were developed using 3-amino-9-ethylcarbazole (Dako, Milan, Italy) as a chromogenic substrate. Following counter staining with Mayer hematoxylin (Invitrogen), slides were mounted in aqueous mounting medium (glycergel, Dako). Pictures were taken using LEICA DM 4000B microscope, while the relative level of each protein was calculated using LEICA software, and the percentage of the mock over the chemotherapeutic treated tumors was calculated and plotted.

2.7. Statistical analysis

Data are presented as means, and error bars indicate the S.D. or S.E. All statistical analyses were performed with Excel (Microsoft, Redmond, WA) or Prism (GraphPad Software Inc., La Jolla, CA). Two-tailed Student's t-test, a one-way analysis of variance or Mann-Whitney U test was used to calculate statistical significance. A P value of <0.05 was considered to be significant.

3. Results

3.1. PSEN1 is a negative regulator of the multi-chemoresistance of BCa

Among 9051 differentially expressed genes between a multi-chemosensitive (5637) versus a resistant BCa cell line (H-bc) by the RNA-seq based omic analysis, the *PSEN1* gene (Fig. 1A) was selected for further study. Its level was higher in 5637 than H-bc cells at both protein (4.09:1, Fig. 1A and C) and mRNA levels (RNA-seq based omic: 2.25:1, and qRT-PCR analysis: 1.92:1, Fig. 1A and B).

To demonstrate its role in the BCa chemoresistance, we suppressed the PSEN1 expression in 5637 cells by siRNA (Fig. 1D) and found that the cell death triggered by each of the five drugs (paclitaxel, Pa; adriamycin, Ad; cisplatin, Ci; pirarubicin, Pi; epirubicin hydrochloride, EH) were suppressed in the PSEN1 siRNA transfected 5637 cells (Fig. 1E). Conversely, an over-expressed GFP-tagged PSEN1 protein (by Western analysis, Fig. 1F and an immunofluorescence analysis,

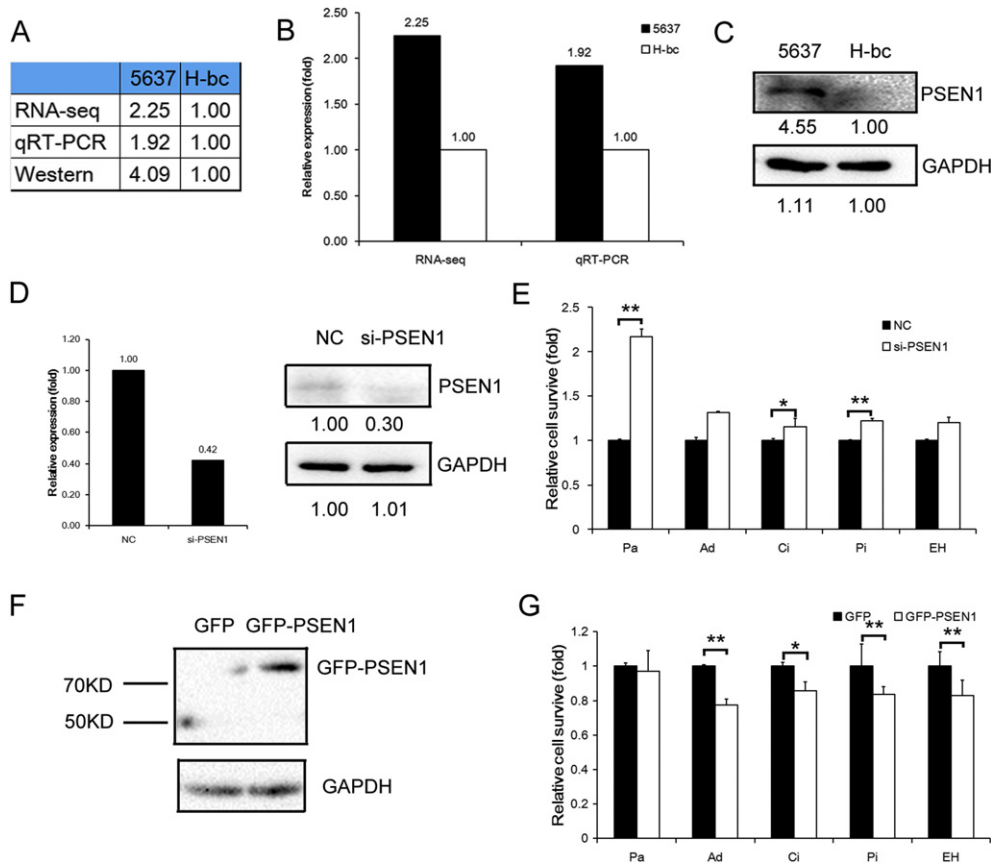


Fig. 1. The PSEN1 expresses in a negative correlation with chemoresistance in BCa cells. A, the PSEN1 level in 5637 versus H-bc cells, at mRNA level (by the miR-seq and qRT-PCR analyses) and the protein level (by Western analysis) shown in both Table and plots (B, RNA level; C, protein level). D, the level of the PSEN1 mRNA (by qRT-PCR) and protein (by Western analysis) in the siRNA transfected versus the NC transfected 5637 cells. E, the relative cell survival in the 5637 cells transfected with the PSEN1 over the NC siRNAs, assayed at 72 h post-treatment of the IC₅₀ dosed drugs. F, the PSEN1 protein level in the EGFP-PSEN1 transfected versus the GFP construct transfected H-bc cells, measured by Western analysis. G, the relative cell survival of the H-bc cells transfected by an EGFP-PSEN1 over the GFP expression vectors, assayed at 72 h post-treatment of the IC₅₀ dosed drugs. (*, P < 0.05; **, P < 0.01).

Supplementary Fig. 1) sensitized H-bc cells to Ad, Ci, Pi, or EH, but not Pa (Fig. 1G).

3.2. The PSEN1 is a direct target of miR-193a-3p in BCa cells

MiR-193a-3p has been noted for its role in the multi-chemoresistance in both HCC [19] and BCa cells [20–22]. The miR-193a-3p level is significantly lower in 5637 than in H-bc, while its target genes SRSF2, HIC2 and PLAU levels are lower in H-bc than 5637 cells [20], a same expression pattern of the PSEN1 gene in the context of the multi-chemoresistance of BCa (Fig. 1A).

Incidentally, miR-193a-3p is one of 56 miRs that are bioinformatically capable by the TargetScan software (<http://www.targetscan.org/>) of repressing the PSEN1 gene expression at the post-transcriptional level. To check the PSEN1 status as one of the miR-193a-3p's targets, we determined the PSEN1 level in the miR-193a-3p mimic transfected 5637 and the antagomiR transfected H-bc cells versus the scramble sequence control (NC) transfected. Following the changes of the miR-193a-3p level (up to roughly 4000 folds by mimic and 0.4% by antagomiR relative to the NC, Fig. 2A), the miR-193a-3p mimic transfection brought down the PSEN1 mRNA to 52% (Fig. 2B) and the protein to 88% (Fig. 2C) of the NC transfected 5637 cells. As expected, a miR-193a-3p antagomiR

transfection raised the mRNA level of PSEN1 by roughly 5 folds (Fig. 2B) and the protein level by 147% of the NC level in H-bc cells (Fig. 2C).

To conclude whether the PSEN1 is a direct target of miR-193a-3p, we put the full length wild type or mutant 3'-untranslated region (UTR) mega to create pGL3-PSEN1 UTR WT or the PGL3-PSEN1 UTR Mut (Fig. 2D). Both constructs and pGL3-control were transfected into 5637 and H-bc cells, respectively, to determine whether the differentially expressed miR-193a-3p in the context of the chemoresistance BCa cells is really functional. pGL3-PSEN1-UTR WT but none of other two reporter constructs gave a significantly higher luciferase activity in 5637 than in H-bc cells (Fig. 2E, upper panel). Furthermore, the luciferase activity of pGL3-PSEN1-UTR WT but not other two was brought down in the mimic transfected 5637 cells and raised in the antagomiR transfected H-bc cells (Fig. 2E, middle and lower panels).

Following the changes of the PSEN1 protein level in both the miR-193a-3p mimic and PSEN1 siRNA transfected 5637 cells (Fig. 3A), the cell death triggered by all five drugs was reduced (Fig. 3B). Conversely, a transfection of the GFP-tagged PSEN1 expression construct raised the PSEN1 protein level (Fig. 3C), sensitized H-bc cells to Ad, Pi, or EH (Fig. 3D). In line with its negative effect on chemoresistance (Fig. 1D and F), a siRNA mediated PSEN1 repression reduced the apoptotic cells from 2.35% to 1.36%, an effect that was not seen in the mimic transfected

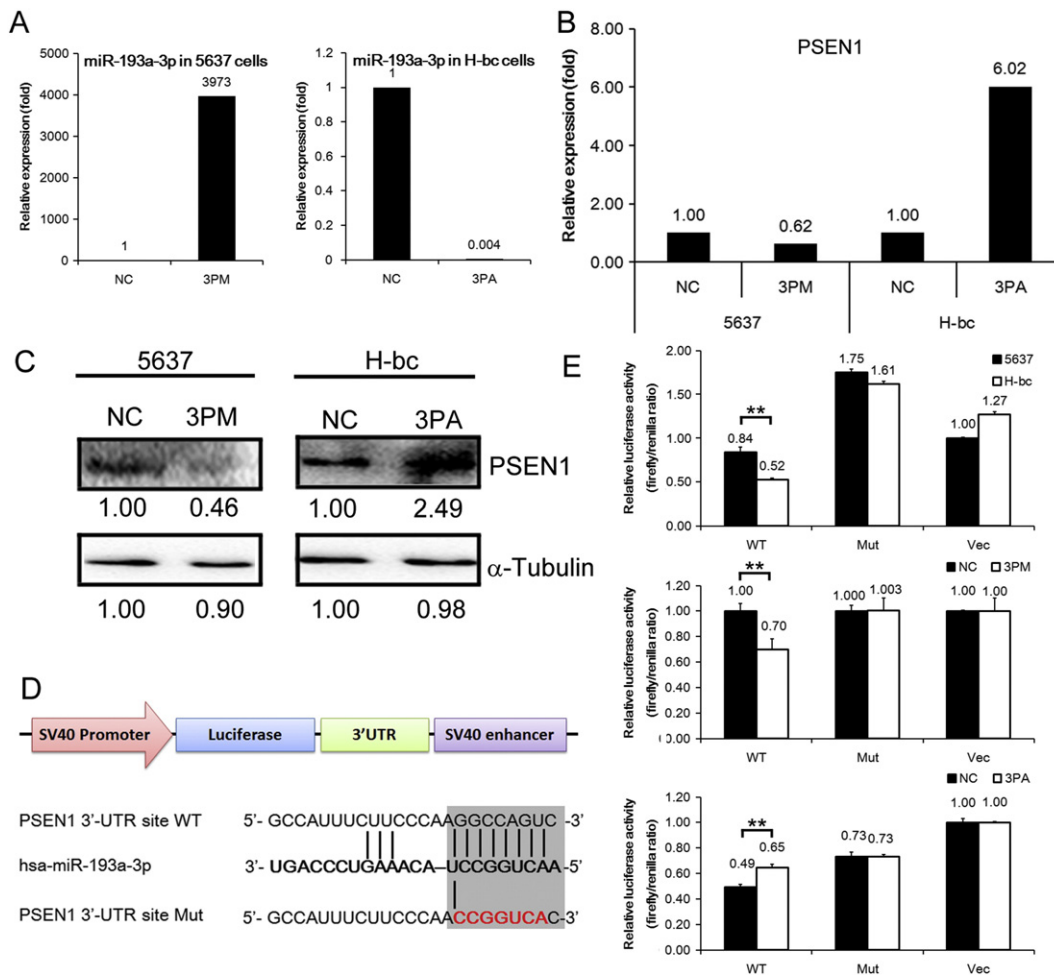


Fig. 2. The PSEN1 mRNA is a direct target of miR-193a-3p in BCa cells. The level of miR-193a-3p (A, by qRT-PCR), the PSEN1 mRNA (B, by qRT-PCR) and protein (C, by Western analysis) in the miR-193a-3p mimic (3PM) transfected 5637 cells and the miR-193a-3p antagomiR (3PA) transfected H-bc cells versus the negative control (NC). D, the miR-193a-3p target sequences (the wild-type in shadow and mutant in red) in the 3'-UTR region of PSEN1 gene. E, the relative luciferase activity (fold) of the reporter with wild-type (WT) or mutant (Mut) PSEN1-UTR or without UTR (Vec) were determined in the miR-193a-3p mimic (3PM) in 5637 or antagomiR (3PA) in H-bc versus Mock (NC) transfected BCa cells. The Renilla luciferase activity of the co-transfected reporter construct was used to control the transfection efficacy. A representative result from three independent experiments shown. Error bars represent s.e.m. *P < 0.05; **P < 0.01; by Student's t-test.

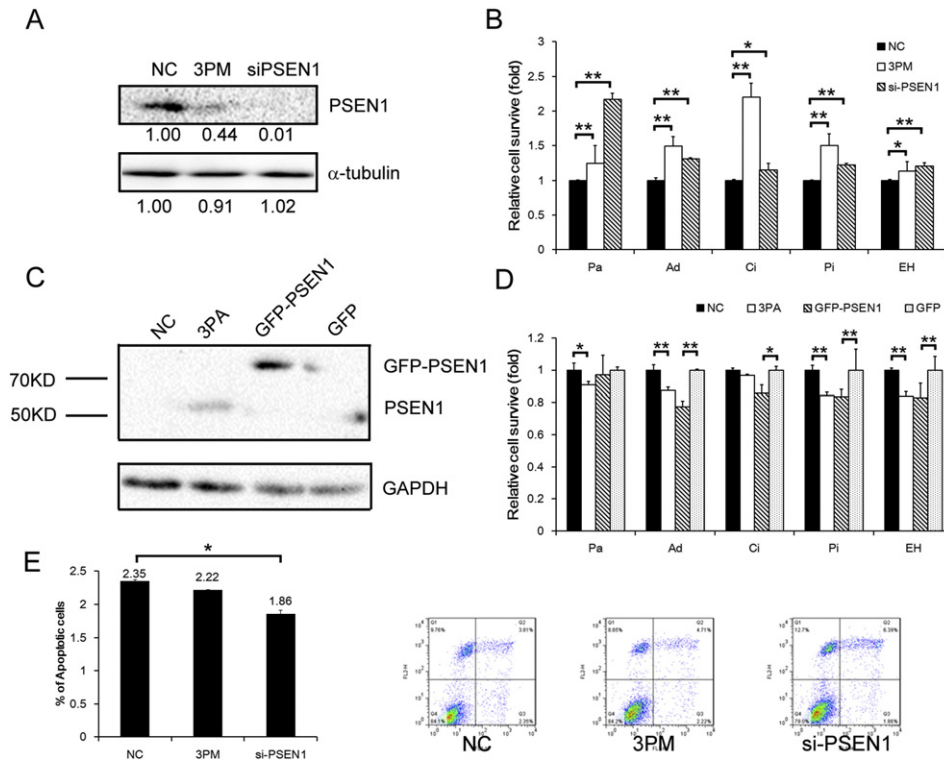


Fig. 3. PSEN1's effects on the drug-triggered cell death in BCa cells is partially reproduced by a forced alteration of miR-193a-3p level in cells. A, the PSEN1 protein level (Western analysis) in the 3PM or siRNA transfected versus the NC transfected 5637 cells. B, the relative cell survival of the 5637 cells transfected with miR-193a-3p mimic (3PM) over the NC transfected 5637 cells, 72 h after a treatment of the IC₅₀ dosed drugs. C, the PSEN1 protein level (Western analysis) in the 3PA or GFP tagged overexpression construct transfected versus the NC transfected H-bc cells. D, the relative cell survival of the H-bc cells transfected with miR-193a-3p antagonist (3PA) over the NC transfected H-bc cells, assayed at 72 h post-treatment of the IC₅₀ dosed drugs. E, the effects of the forced reversal of both miR-193a-3p and PSEN1 levels on the apoptosis of 5637 cells by FACS analysis in plot and in the original. NB, *, P value < 0.05; **, P value < 0.01.

5637 cells (Fig. 3E), the interpretation remains to be found. Nevertheless, the PSEN1 gene seems to have a negative role opposite to the miR-193a-3p's positive effect on the multi-chemoresistance of BCa cells. Getting all these observations together, PSEN1 executes to a great extent the miR-193a-3p's effect on the BCa chemoresistance to all five drugs according to the results in the transfected 5637 cells (Figs. 1E and 3B) or the following three drugs: Ad, Pi and EH according to the results in the transfected H-bc cells (Figs. 1G and 3D).

3.3. PSEN1 positively regulates the DNA damage response pathway in the context of BCa multi-chemoresistance

We recently showed that miR-193a-3p regulates the multi-chemoresistance via repressing three of its downstream targets: SRSF2, PLAU and HIC2 and in turn altering the activities of following five signaling pathways: DNA damage response, Notch, NF- κ B, Myc/Max and oxidative stress [20]. Therefore, we determined which of these five pathways are also affected by the forced changes of PSEN1 level in both 5637 and H-bc cells. As shown in Fig. 4A, when the PSEN1 level was repressed by either miR-193a-3p mimic or PSEN1 siRNA transfection (Fig. 3A), activities of the DNA damage response and Myc/Max pathways were affected. For instance, the activity of the DNA damage response pathway was reduced by PSEN1 siRNA to 85% of the level in the NC transfected control, in contrast to 17% of the NC level by the miR-193a-3p mimic. For the Myc/Max pathway, the PSEN1 siRNA led to a greater repression than the miR-193a-3p mimic (Fig. 4A). We further assessed the changes of the mRNA levels of the following genes in 5637 cells transfected by the PSEN1 siRNA in comparison with the NC and miR-193a-3p mimic: the downstream genes of the Myc/Max pathway (TERT and ODC1) and of the DNA damage response pathway (CDKN1A and EDN1) as well as three members of γ -secretase: PSEN1/2 and APH1A by qRT-PCR analysis. Along with the reduction of

the PSEN1 mRNA by miR-193a-3p mimic or PSEN1 siRNA, one of the downstream targets of the Myc/Max pathway, ODC1 mRNA level was reduced as expected. However, the PSEN1 siRNA raised the mRNAs of the DNA damage response pathway targets rather than repressed by the mimic in 5637 cells. One plausible explanation for this conflicting observation is that the repression of these two downstream genes by miR-193a-3p is not achieved via its repression of PSEN1 expression at the post-transcriptional level (Fig. 4B). Intriguingly, miR-193a-3p mimic transfection not only brought down the PSEN1 level, but also PSEN2 and APH1A levels (Fig. 4B). A PSEN1 independent mechanism may account for the seeming conflicting results in the context of BCa multi-drug chemoresistance (Figs. 1–3).

We further compared the activities of these five pathways in the H-bc cells transfected with a GFP-PSEN1 expression construct or a GFP alone construct and (the expression of GFP-PSEN1 gene in the transfected H-bc cells was shown in panel, Fig. 3C) and showed that among the two pathways suggested by the tests in 5637 cells (Fig. 4A), only the activity of the DNA damage response pathway was raised drastically by both miR-193a-3p antagonist and GFP-PSEN1 (Fig. 4C). Therefore, the PSEN1's role to relay the miR-193a-3p's effect on the BCa chemoresistance is principally accomplished via its effect on the DNA damage response pathway.

3.4. PSEN1 expression was reduced in the miR-193a-3p agomiR injected 5637 tumor xenografts and raised in the antagonist-injected H-bc tumor xenografts

Recently, it has been shown that miR-193a-3p promotes the Pa chemoresistance of BCa in tumor xenografts of nude mice via its repression of three of its targets in tumor tissues: SRSF2, PLAU and HIC2 [20]. In the present study, we semi-quantified by an immuno-histological analysis of the levels of PSEN1 protein (Fig. 5) in the same set of the

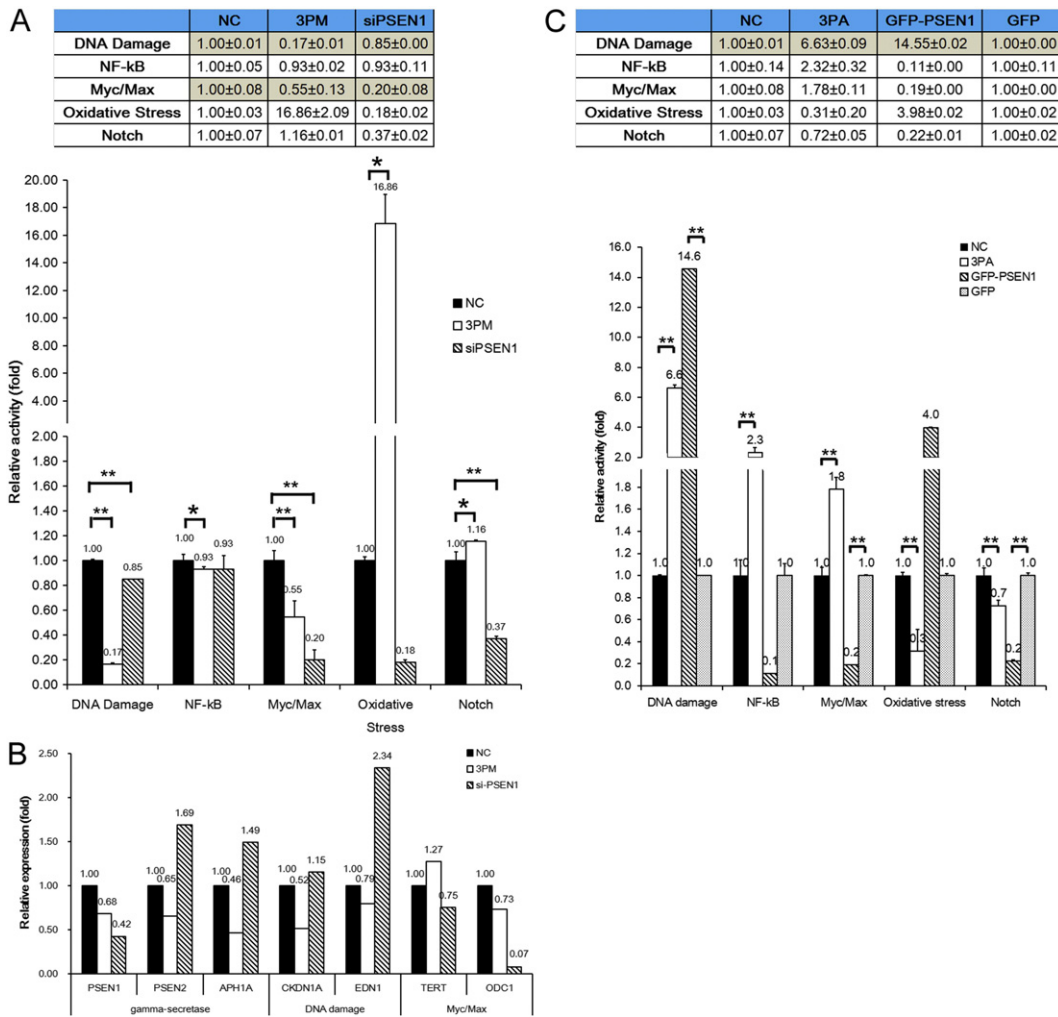


Fig. 4. A forced altering of the PSEN1 level changes the activities of signaling pathways assayed by using a Qiagen™ pathway reporter system. A, The relative activities (mean ± S.D.) of five pathways in the PSEN1 siRNA or miR-193a-3p mimic transfected versus the NC transfected 5637 cells. B, the mRNA levels (qRT-PCR analysis) of the effective genes of the DNA damage and Myc/Max pathways' and the components of the γ -secretase complex in the miR-193a-3p mimic or PSEN1 siRNA transfected 5637 cells. C, the relative activities (mean ± S.D.) of five pathways in the EGFP-PSEN1 vector or miR-193s-3p antagmiR (3PA) transfected versus the NC transfected H-bc cells. NB, *, P value < 0.05.

tumor tissues in the mice that were subjected to an injection of Pa or PBS. The intratumor injection of either miR-193a-3p's agomiR (into 5637)/antagomiR (into H-bc tumor) indeed led to the expected changes of the PSEN1 protein in the tumor tissues (Fig. 5). This observation further supports the notion that PSEN1 gene has a role in the miR-193a-3p's promoting effect in the Pa-chemoresistance of the BCa cells.

4. Discussion

Despite years of intensive research efforts, the questions such as how many genes contribute to the multi-chemoresistance of cancer and by what mechanisms remain elusive. Our previous works have shown that the miR-193a-3p is a potent promoter of the multi-chemoresistance in both HCC [19] and BCa via repressing the expression of its three downstream targets: SRSF2, PLAU and HIC2 [20]. For the new protein-coding genes instrumental to the multi-chemoresistance of BCa, we performed a comparative RNA-seq omic analysis of a multi-chemosensitive (5637) and resistant (H-bc) cell lines and identified a panel of genes that are differentially expressed, including the PSEN1 gene in this paper that exhibits a negative correlation with the chemoresistance (Fig. 1). Both role and mechanisms of the PSEN1 gene in the context of BCa chemoresistance were systematically addressed in both cultured cells and tumor xenografts in nude mice. A forced reversion of the PSEN1 level in 5637 cells suppressed the drug-triggered

cell death by each of the five drugs that are also affected by miR-193a-3p in a same way (Figs. 1 and 3). We then showed that PSEN1 gene is another direct target of miR-193a-3p and acts as a key executor of the former's promoting effects on the BCa chemoresistance (Figs. 2–5)

PSEN1 encodes the catalytic subunit of γ -secretase, a full function of which is required for an activation of the Notch pathway [29]. It has been reported that PSEN1 elevates the expression of the multidrug resistance gene, ABC11/MRP1 through its positive effect on Notch signaling [30], and the inhibitor of γ -secretase will significantly enhance the anti-tumor activity of docetaxel and relieved its resistance in breast cancer by several different mechanisms [31]. The PSEN1's involvement in the BCa multi-chemoresistance seems more complicated. Although a siRNA mediated repression of the PSEN1 expression desensitized 5637 cells to the drug-triggered cell death (Fig. 3A) and reduced the activity of the Notch pathway (Fig. 4A), which is opposite to the positive effect of the miR-193a-3p mimic on the Notch pathway (Fig. 4A). The functional γ -secretase is a four component complex consisting of Presenilin (PSEN1/2), Nicastrin, Aph-1, and PSENEN (Pen-2) [29]. It is intriguing to observe that while the transfection of PSEN1 siRNA only suppressed the PSEN1 mRNA level, miR-193a-3p mimic also repressed the expression of both PSEN2 and Aph-1 genes (Fig. 4B). Since both PSEN2 and Aph-1 genes do not have the target sequence of miR-193a-3p at the 3'-UTR region analyzed by Targetscan [www.targetscan.org] and miRDB [http://mirdb.org/miRDB/] as well as both 5'-UTR and

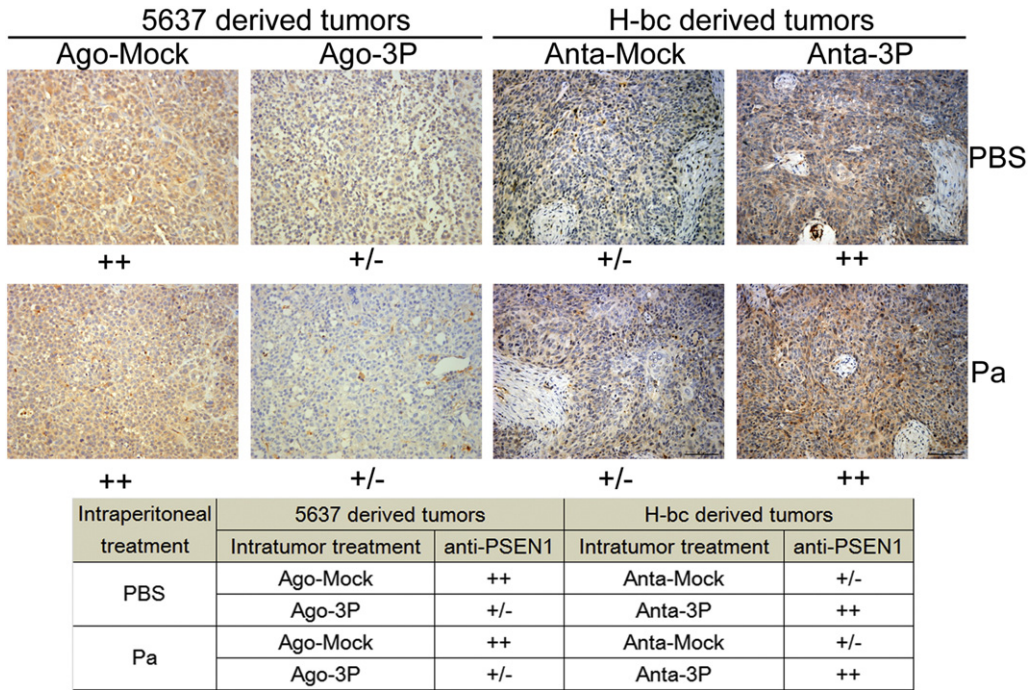


Fig. 5. The PSEN1 level in the miR-193a-3p agomiR injected 5637 and the miR-193a-3p antagomiR injected H-bc tumor xenograft versus the NC injected tumor xenografts determined by an immunohistochemical staining. The 5637 and H-bc tumor tissues from each group were fixed on one slide and immunostained for indicated antibody, respectively. Levels of PSEN1 proteins in each group were determined by immunostaining and summarized in the table.

coding regions by RNA22 v2 [https://cm.jefferson.edu/rna22v2/], the miR-193a-3p's effect on BCa chemoresistance observed is probably achieved by the mechanisms involving other targets of miR-193a-3p. In addition, the classic connection of the PSEN1 with the Notch pathway [29], seems to have a little role in the BCa chemoresistance in the present study (Fig. 6).

There are reports for a positive regulatory loop between γ -secretase and p53 (the master transcriptional factor for the DNA damage response pathway), the former is capable to upregulate both level and activity of p53 [32], at the same time to be transcriptionally repressed by p53 [33–35]. The PSEN1 transcription is also under the control of Ets1/2 transcription factors, removal of which from its promoter

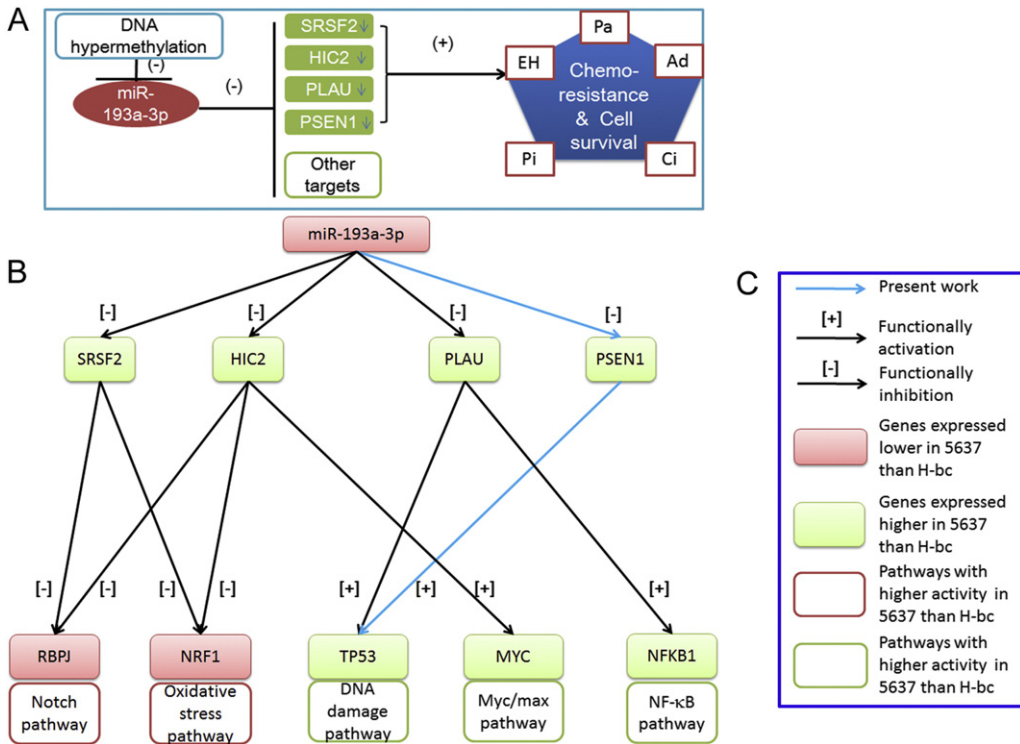


Fig. 6. The working model for the miR-193a-3p's role in regulation of the chemoresistance of BCa. A, expression of SRSF2, HIC2, PLAU (CDDis paper) and PSEN1 (here) genes under the negative regulation of the by DNA methylation regulated miR-193a-3p, positively correlate with the chemoresistance to four indicated chemotherapeutics. B, the miR-193a-3p's effect on the signaling pathways via its repression of SRSF2, HIC2 and PLAU (previous work) and PSEN1 (this work). C, figure legend.

chromatin caused both the disruption of its direct physical interaction with p53 and a transcriptional silencing of the PSEN1 gene [36]. The PSEN1 mutations have been suggested capable to induce the p53-dependent apoptosis, which is repressed by γ -secretase inhibitor [37, 38]. Indeed, a siRNA mediated repression of the PSEN1 gene suppressed both cell apoptosis (Fig. 3) and the activity of the DNA damage response pathway (Fig. 4). The functional links between the PSEN1 gene and the Myc/Max pathway may chiefly involved p53 as a critical mediator within the ARF–Mdm2–p53 regulatory loop [39–41]. It is clear from this study that among the five pathways controlled by miR-193-3p centered axis in BCa multi-chemoresistance, the DNA damage response pathway is only one that is negatively regulated by PSEN1 (Fig. 4A and C, and summarized in Fig. 6). Further studies are needed for the detailed mechanisms whereby PSEN1 regulates the BCa multi-chemoresistance. It is also worthwhile exploring whether the observations described in this report can be translated into the clinical practice for better chemotherapy of BCa.

5. Conclusion

We have previously shown the DNA methylation analysis of the promoter region of the following 11 genes: SALL3, CFTR, ABCC6, HPR1, RASSF1A, MT1A, RUNX3, ITGA4, BCL2, ALX4, MYOD1, DRM, CDH13, BMP3B, CCNA1, RPRM, MINT1, and BRCA1, in urine sediments confirmed the existing diagnosis of 121 among 132 bladder cancer cases (sensitivity, 91.7%) with 87% accuracy [42]. In this study, we demonstrated that PSEN1 is a direct target of miR-193a-3p, and negatively regulates the multi-chemoresistance of BCa by activation of the DNA damage response pathway. Therefore, the DNA methylation state of the miR-193a gene and expression state of both PSEN1 gene the DNA damage response pathway associated genes in both cancer tissues and urine sediments should be promising diagnostic targets for the guided anti-bladder cancer chemotherapy.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbdis.2014.12.014>.

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