# *Beta-Secretase 1 (BACE1)* Is Down-Regulated in Invasive Ductal Carcinoma of Breast

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## Abstract

***Background:*** The enzyme beta-secretase 1 (BACE1) and its antisense transcript (*BACE1-AS*) have been implicated in the pathogenesis of Alzheimer's disease. Moreover, several lines of evidence point to their contribution in tumorigenesis.

***Methods:*** In the present study, we evaluated expression of BACE1 mRNA (*BACE1)* and *BACE1-AS* in 54 breast cancer tissues and 54 adjacent non-cancerous tissues (ANCTs) from the same patients using quantitative real-time PCR.

***Results:*** *BACE1* was significantly down-regulated in tumoral tissues compared with ANCTs, while *BACE1-AS* expression was not significantly different between tumoral tissues and ANCTs. The Bayesian Multilevel model showed a significant difference in *BACE1* expression between stage 1 and 2 cancers after age-effect adjustments. *BACE1-AS* expression was significantly greater in ER-positive than in ER-negative samples (P=0.01). *BACE1* and *BACE1-AS* expression were not correlated with patient ages in any sample sets.

***Conclusions:*** Significant correlations were detected between expression of these genes in both tumoral tissues and ANCTs. The current study provides evidence for differential *BACE1* expression in breast tissues and suggests further assessment of the role of *BACE1* in the pathogenesis of cancer.

**Keywords:** BACE1, BACE1-AS, Breast Cancer, Lncrna.

## Introduction

The enzyme beta-secretase 1 (BACE1) is involved in the primary step of proteolytic cleavage of amyloid precursor protein (APP), which is completed by the subsequent function of gamma-secretase. Accumulation of the resultant amyloid beta (Abeta) peptide in neuritic plaque of Alzheimer's brain has underscored the role of BACE1 in the pathogenesis of this disorder and has been proposed as a therapeutic target in Alzheimer's disease (AD) (1). A long non-coding RNA (lncRNA) transcribed from the BACE1 gene (*BACE1)* antisense (AS) strand regulates its expression via a feed-forward loop. The observed up-regulation of *BACE1-AS* in human AD brains compared with matched controls provided further evidence for the participation of *BACE1* and *BACE1-AS* in the pathogenesis of AD (2). The significance of BACE1 in the pathogenesis of cancer has also been the focus of other researchers. The presence of APP in the endothelium during vessel synthesis implies its participation in angiogenesis. Moreover, BACE1 inhibitors have

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been shown to decrease endothelial cell proliferation and inhibit neoangiogenesis both *in vitro* and *in vivo* (3). Consistent with these studies, two independent studies have reported elevated APP levels in breast cancer cells (4, 5). Considering the regulatory role of *BACE1-AS* in *BACE1* expression and subsequent APP production (2) on one hand, and the detected high APP abundance in breast cancer cells (4) on the other, we hypothesized that *BACE1* and *BACE1-AS* expression in breast cancer tissues might differ from that in corresponding adjacent non-cancerous tissues (ANCTs). Further evidence for our hypothesis were provided by a growing number of studies reporting the role of lncRNAs in breast cancer (6-8) and the observed up-regulation of the lncRNA *BACE1-AS* by several cell stressors (9), which might be present in the cancer microenvironment as well. Consequently, we performed the present study to evaluate *BACE1* and *BACE1-AS* expression in invasive ductal carcinoma samples and their paired ANCTs.

## Materials and methods
### Patients
In the present study, we recruited 54 patients with invasive ductal carcinoma of breast who were hospitalized in Sina and Farmanieh hospitals (Tehran, Iran) for surgical removal of breast mass. The diagnosis of cancer was confirmed through histopathological examination. Hormone receptor status and other relevant information were extracted from medical records. All patients signed written informed-consent forms. The study protocol was approved by the local ethical committee of Shahid Beheshti University of Medical Sciences. Tumoral tissues and ANCTs were excised and transferred in liquid nitrogen to the Medical Genetics Laboratory for further assessments.

### RNA extraction, cDNA synthesis, and expression analysis
RNA was extracted and cDNA synthesized using TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA) and a RevertAid First Strand cDNA Synthesis Kit (TaKaRa, Japan), respectively, according to company protocols. Relative *BACE1* and *BACE1-AS* expression were compared between tumoral tissues and ANCTs using TaqMan Fast Universal PCR Master Mix (Applied Biosystems) in a Rotor Gene 6000 Corbett Real-Time PCR System. Expression was normalized to the hypoxanthine-guanine phosphoribosyl transferase gene (*HPRT*). The primer sequences are shown in Table 1.

**Table 1.** Nucleotide sequences of primers and probes used in the study.

| Gene name | Primer and probe sequences | Product length |
|---|---|---|
| *HPRT1* | F: AGCCTAAGATGAGAGTTC | 88 |
| | R: CACAGAACTAGAACATTGATA | |
| | FAM -CATCTGGAGTCCTATTGACATCGC- TAMRA | |
| *BACE1* | F: CCAAGACGACTGTTACAA | 79 |
| | R: GAAGCCCTCCATGATAAC | |
| | FAM-TTGCCATCTCACAGTCATCCAC-TAMRA | |
| *BACE1-AS* | F: GACACTGTACCATCTCTTTTACCC | 113 |
| | R: CACCACCAACCTTCGTTTGC | |
| | FAM - AGTCCACTCACGGAGGAGGTCGCC -TAMRA | |

### Statistical methods
Statistics were analyzed using R software version 3.0.5 and SPSS 18 (Chicago, IL, USA). The Spearman correlation and Bayesian Multilevel model were used. Kruschke's Bayesian estimation was used to assess the significance of mean expression differences between tumoral tissues and ANCTs. A student-t prior distribution was assumed for parameters with 200,000 iteration and 5000 burn-outs. The 95% highest density interval (HDI) values were calculated based on Bayesian approach. The difference between relative mean gene expression values in patient

categories was evaluated using Tukey's honest significance test. CT values were corrected for the efficiencies of each primer set. The pairwise correlations between relative *BACE1* and *BACE1-AS* transcripts levels in each set of samples were calculated

using the regression model.

## Results

### *Patient demographic and clinical features*

Patient demographic and clinical features are shown in Table 2.

**Table 2.** Patient demographic and clinical features.

| Variables | Values |
|---|---|
| **Age (years) (mean± SD)** | $51.79 \pm 13.54$ (29-81) |
| **Menarche age (years) (mean± SD)** | $13 \pm 1.65$ (10-18) |
| **Menopause age (years) (mean± SD)** | $44.91 \pm 14.91$ (38-60) |
| **First pregnancy age (years) (mean± SD)** | $18.04 \pm 8.36$ (14-32) |
| **Breast feeding duration (months) (mean± SD)** | $41.62 \pm 34.1$ (3-120) |
| **Positive family history for other cancers (%)** | 17% |
| **Cancer stage (%)** | |
| I | 30.8 |
| II | 28.8 |
| III | 30.8 |
| IV | 9.6 |
| **Overall grade (%)** | |
| I | 17 |
| II | 49 |
| III | 34 |
| **Mitotic rate (%)** | |
| I | 45.2 |
| II | 42.9 |
| III | 11.9 |
| **Tumor size (%)** | |
| <2 cm | 32 |
| ≥2 cm, <5 cm | 66 |
| ≥5 cm | 2 |
| **Estrogen receptor (%)** | |
| Positive | 87.8 |
| Negative | 12.2 |
| **Progesterone receptor (%)** | |
| Positive | 77.1 |
| Negative | 22.9 |
| **Her2/neu expression (%)** | |
| Positive | 25 |
| Negative | 75 |
| **Ki67 expression (%)** | |
| Positive | 100 |
| Negative | 0 |

### *BACE1 and BACE1-AS expression in tumoral tissues and ANCTs*

*BACE1* expression was significantly less in tumoral tissues than in ANCTs; however, *BACE1-AS* expression was not significantly different in the two tissue types (Fig. 1).

The Bayesian t test results comparing *BACE1* and *BACE1-AS* relative expression in tumoral tissues and ANCTs are shown in Table 3.
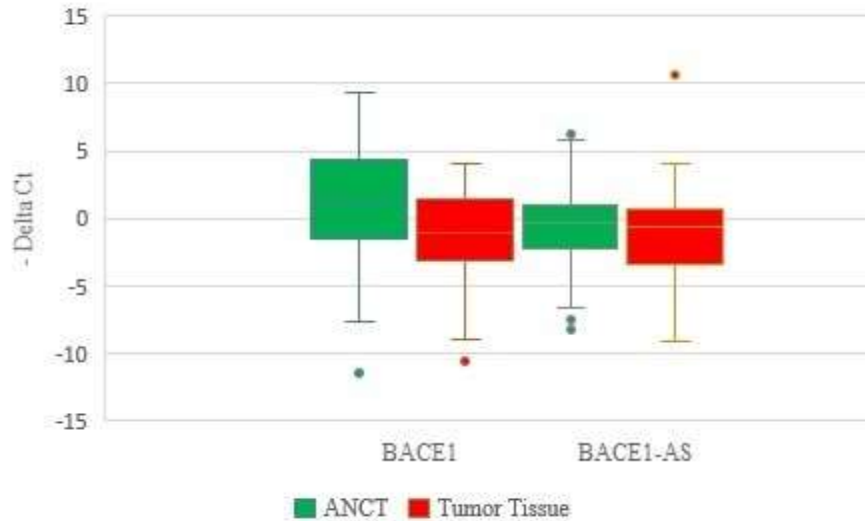
**Fig. 1.** Relative *BACE1* and *BACE1-AS* expression in tumoral tissues and ANCTs.

**Table 3.** Bayesian t test to compare relative gene expression between two paired groups (a: Tumor-ANCTs, b: computed from frequentist method, c: 95% highest density interval (HDI)

| Gene | Posterior mean | | Relative Expression difference[a] | SD | Effect Size | P value[b] | 95% HDI[c] |
|------|------------------|--------|------------------|------|-------------|------------|------------|
| | **Tumoral tissues** | **ANCTs** | | | | | |
| *BACE1* | -0.44±0.68 | 1.02±0.67 | -1.637 | 0.48 | -0.487 | 0.001 | [-2.58, -0.71] |
| *BACE1-AS* | 0.42±0.43 | 0.63±0.5 | -0.3562 | 0.51 | 0.094 | 0.499 | [-1.37, 0.62] |

### Association between expression levels and patients' clinical data

We assessed associations between expression levels and cancer stages using the Bayesian Multilevel model after age-effect adjustments and found higher *BACE1* expression in stage 1 compared with stage 2 cancers in a way that expression in stage 2 cancers were 1.81 unit higher than stage 2 cancers (Table 4); however, *BACE1-AS* expression was not significantly different between the various stages (Table 5).

**Table 4.** Bayesian Multilevel results of association between *BACE1* expression and stage with age-effect adjustments (Stage 1= Reference group).

| *BACE1* expression | Estimate | SE | P value | 95% Credible Interval |
|--------------------|----------|------|---------|------------------------|
| Stage2 | 1.81 | 1.25 | 0.035 | [0.65, 4.28] |
| Stage 3 | 0.38 | 1.28 | 0.141 | [-2.14, 2.96] |
| Stage 4 | -0.29 | 1.77 | 0.8 | [-3.68, 3.24] |
| Age | -0.03 | 0.04 | 0.272 | [-0.11, 0.04] |

**Table 5.** Bayesian Multilevel results of association between *BACE1-AS* expression and stage with age-effect adjustments (Stage 1= Reference group).

| *BACE1-AS* expression | Estimate | SE | P value | 95% Credible Interval |
|-----------------------|----------|------|---------|------------------------|
| Stage 2 | 0.46 | 1.47 | 0.347 | [-2.43, 3.34] |
| Stage 3 | 0.33 | 1.42 | 0.543 | [-2.43, 3.17] |
| Stage 4 | -2.14 | 2.1 | 0.482 | [-6.28, 2.04] |
| Age | -0.06 | 0.05 | 0.248 | [-0.15, 0.03] |

We also assessed correlations between expression levels in each sample set and patients' ages. *BACE1* and *BACE1-AS* expression were not correlated with patients' ages in any sample sets (Table 6).

**Table 6.** Spearman correlation between gene expression in each sample set and patients' ages.

|  |  | *BACE1* | *BACE1-AS* |
|---|---|---|---|
| Group | Tumoral tissues | -0.252 | 0.059 |
|  | ANCTs | -0.164 | 0.107 |

We also evaluated associations between relative gene expression and clinicopathological data (Table 7). *BACE1-AS* expression was significantly greater in estrogen receptor (ER)-positive than in ER-negative samples (P=0.01). No significant gene expression differences were found between patient subgroups.
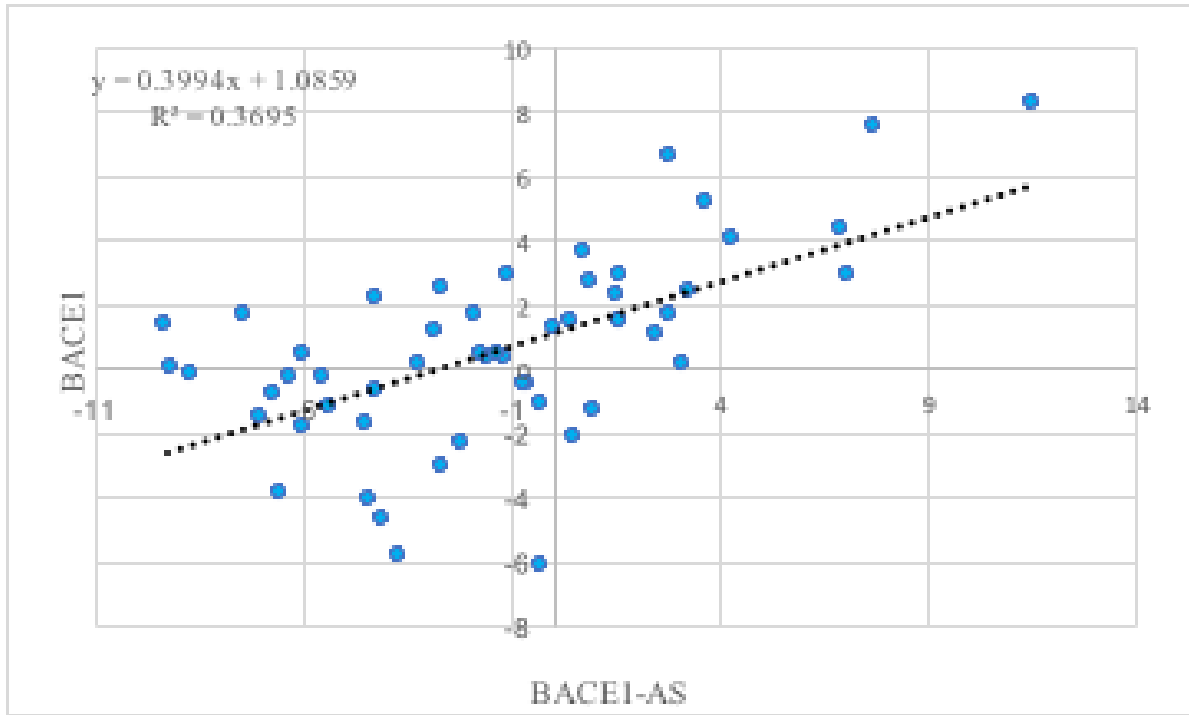
**Table 7.** Association between *BACE1* and *BACE1-AS* transcript levels and tumor characteristics. Mean ± standard deviation values of Efficiency ^CT reference gene-Efficiency ^CT target gene are presented.

| | *BACE1* | P value | *BACE1-AS* | P value |
|---|---|---|---|---|
| **Age** | | | | |
| <55 years old vs. ≥55 years old | 3.06 (1.42) vs. 91.45 (288.38) | 0.35 | 327.39 (1.54) vs. 489.87 (2.15) | 0.78 |
| **ER Status** | | | | |
| ER (+) vs. ER (-) | 2.39 (1.26) vs. 484.36 (1.17) | 0.71 | 55.67 (282.95) vs. 1.47 (3.61) | 0.01 |
| **PR Status** | | | | |
| PR (+) vs. PR (-) | 571 (2.98) vs. 7.699 (2.45) | 0.08 | 63.68 (304.84) vs. 809.02 (2.66) | 0.09 |
| **HER2 Status** | | | | |
| HER2 (+) vs. HER2 (-) | 6.81 (2.36) vs. 666.5 (3.04) | 0.12 | 1.73 (1.97) vs. 312.08 (1.49) | 0.47 |
| **Tumor Grade** | | | | |
| Grade 1 vs. 2 | 336.89 (419.44) vs. 10.6 (37.95) | 0.9 | 56.93 (45.06) vs. 1.4 (1.84) | 0.95 |
| Grade 1 vs. 3 | 336.89 (419.44) vs. 6.42 (2.05) | 0.47 | 56.93 (45.06) vs. 1.27 (3.14) | 0.28 |
| Grade 2 vs. 3 | 10.6 (37.95) vs. 6.42 (2.05) | 0.24 | 1.4 (1.84) vs. 1.27 (3.14) | 0.09 |

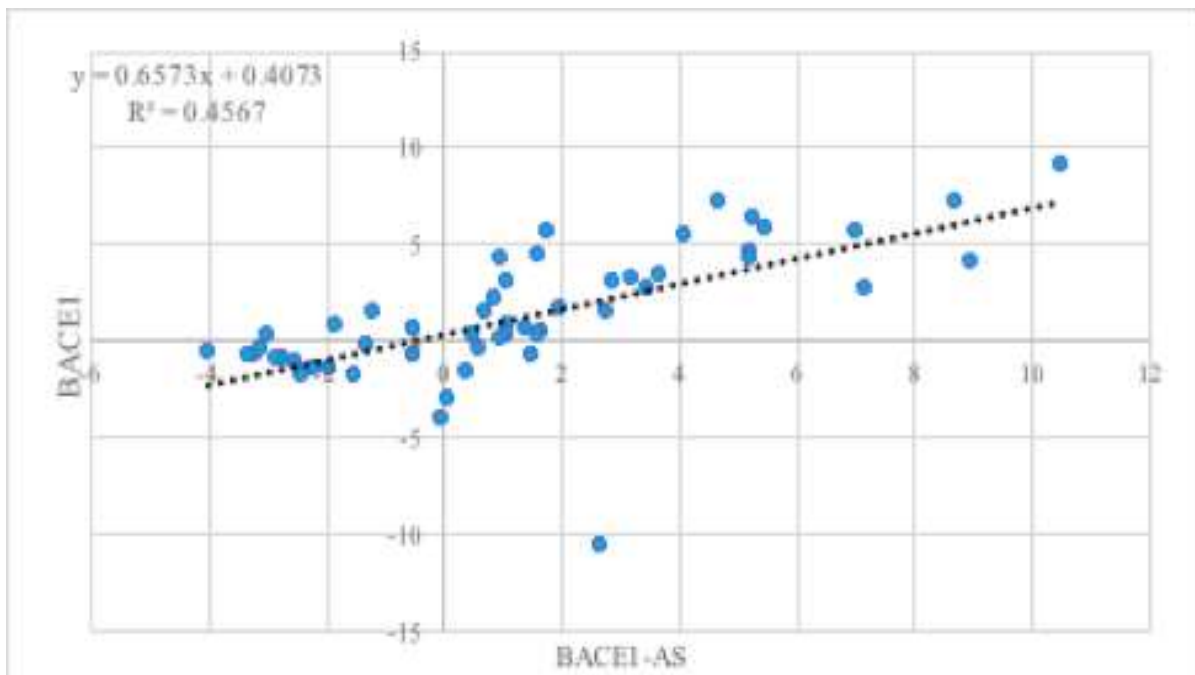**Correlation between *BACE1* and *BACE1-AS* expression in tumors and ANCTs**

Significant correlation was found between *RACE1* and *BACE1-AS* expression in both tumors and ANCTs (Figs. 2A and 2B).

**A**



**B**

**Fig. 2.** Correlation between *BACE1* and *BACE1-AS* expression in tumors (A) and ANCTs (B).

## Discussion

In the present study we evaluated *BACE1* and *BACE1-AS* expression in invasive ductal carcinoma of breast and found significantly less *BACE1* in tumors than in ANCTs. BACE1 catalyzes the primary step of APP production (1), which has been shown to be elevated in some human malignancies and to enhance cell proliferation (10). In breast cancer cell lines, *APP* expression was up-regulated by dihydrotestosterone and its expression was associated with cell proliferation. In addition, APP protein abundance has been suggested as a prognostic factor in ER-positive breast cancer patients (4). In addition, increased APP levels have been shown to increase tumorigenicity and invasiveness of aggressive breast cancer cells (5). However, BACE1 activity and abundance have not yet been assessed in breast cancer samples. Several lines of evidences have highlighted the role of BACE1 in the pathogenesis of other cancer types. BACE1 inhibitors have been suggested as putative therapeutic agents in some human malignancies as they suppressed the growth and angiogenesis of human glioblastoma and lung adenocarcinoma tumors in a xenograft animal model (3). BACE1 is also involved in the photolytic cleavage of the insulin receptor (IR). The soluble IR fragment (IRsol) generated by this enzymatic action is increased in the plasma of hepatic cancer patients (11). Our study showed different *BACE1* expression patterns in breast cancer tissues than were seen in other malignancy types. A possible explanation for this discrepancy is the presence of a tissue-specific function for BACE1. On the other hand, as previous studies have shown up-regulation of APP in breast cancer tissues, we hypothesize that the observed down-regulation of *BACE1* in our study might be compensated by activation of or over-expression of gamma-secretase to accomplish the final step of APP production. Simultaneous analysis of BACE, gamma-secretase, and APP levels in a larger cohort of breast cancer patients might help clarify the role of these proteins in the pathogenesis of breast cancer. The similar level of *BACE1-AS* in tumoral tissues and ANCTs, despite the down-regulation of *BACE1* in tumoral tissues as revealed by our study, might imply a defect in regulation of *BACE1-AS*

expression or in the feed-forward loop between these two transcripts in tumoral tissues. The latter possibility is also reflected in the observed stronger correlation between these two transcripts in ANCTs than in tumoral tissues, as demonstrated by $R^2$ values. Future studies are needed to assess the interaction between these two transcripts in cancerous and normal tissues.

The Bayesian Multilevel model results showed a significant difference in *BACE1* expression between stages 1 and 2 cancers after age-effect adjustments. Such a finding might imply a putative stage-specific signature for this lncRNA or its involvement in certain stages of cancer development, which should be assessed in larger patient cohorts.

Previous animal studies have shown age-dependent differential expression of *BACE* splice variants in brain tissues (12). Further evidence for age-dependent regulation of BACE1 in brain tissue included the observed down-regulation of miR-186 as a negative regulator of BACE1 in aged brains (13); however, we found no correlations between *BACE1* or *BACE1-AS* transcript levels in breast tissues and patient age. Such inconsistencies might be due to the presence of tissue-specific regulatory mechanisms for *BACE1* expression.

Finally, we found greater *BACE1-AS* expression in ER-positive than in ER-negative samples. Previous studies have shown an association between BACE1 activity and brain estrogen reduction both in female AD patients and animal models (14). Moreover, estrogen treatment significantly decreased BACE1 protein levels in an ER-dependent manner (15). The observed association between *BACE1-AS* expression and ER status suggest an extra level of complexity in estrogen-regulated BACE production.

In conclusion, we demonstrated down-regulation of *BACE1* in invasive breast cancer samples in association with some patient clinicopathologies. Future studies are needed to elaborate the role of BACE1 in breast cancer pathogenesis.

## Acknowledgment

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