*Original Article*

# High Expression of p300 in Human Breast Cancer Correlates with Tumor Recurrence and Predicts Adverse Prognosis

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## ABSTRACT

**Objective:** Transcriptional coactivator p300 has been shown to play a variety of roles in the transcription process and mutation of p300 has been found in certain types of human cancers. However, the expression dynamics of p300 in breast cancer (BC) and its effect on BC patients' prognosis are poorly understood.

**Methods:** In the present study, the methods of tissue microarray and immunohistochemistry (IHC) were used to investigate the protein expression of p300 in BCs. Receiver operating characteristic (ROC) curve analysis, Spearman's rank correlation, Kaplan-Meier plots and Cox proportional hazards regression model were utilized to analyze the data.

**Results:** Based on the ROC curve analysis, the cutoff value for p300 high expression was defined when the H score for p300 was more than 105. High expression of p300 could be observed in 105/193 (54.4%) of BCs, in 6/25 (24.0%) of non-malignant breast tissues, respectively ($P$=0.004). Further correlation analysis showed that high expression of p300 was positively correlated with higher histological grade, advanced clinical stage and tumor recurrence ($P$<0.05). In univariate survival analysis, a significant association between high expression of p300 and shortened patients' survival and poor progression-free survival was found ($P$<0.05). Importantly, p300 expression was evaluated as an independent prognostic factor in multivariate analysis ($P$<0.05).

**Conclusion:** Our findings provide a basis for the concept that high expression of p300 in BC may be important in the acquisition of a recurrence phenotype, suggesting that p300 high expression, as examined by IHC, is an independent biomarker for poor prognosis of patients with BC.

**Key words:** Breast cancer; p300; Tumor recurrence; Prognosis

## INTRODUCTION

Breast cancer (BC) is the most common malignancy among women, and accounted for approximately 1.15 million new cases and 411,000 deaths worldwide in 2002[1]. Particularly in the last two decades, incidence and mortality rate of BC have climbed sharply in China[2]. The survival rate for BC patients has increased dramatically due to earlier detection and new treatment protocols in the last decade[3]. Presently, various clinicopathologic factors, such as lymph node status, histological grade, tumor size, vascular invasion, hormone receptor status and HER2 expression are utilized to predict BC prognosis and provide accurate treatment[4]. However, these factors are insufficient and approximately 20% to 30% of BC patients will die from BC within five years of primary diagnosis[5].

p300, a member of the histone acetyltransferase family of transcriptional coactivator, has been found to play a critical role in the transcription process and catalyzes histone acetylation through its histone acetyltransferase activity[6, 7]. Transcriptional coactivator p300 has been indicated to regulate different cellular processes including differentiation, cell-cycle regulation, proliferation, apoptosis and DNA damage response[8]. A role for p300 in tumor suppression has been proposed by the evidence that disturbance of p300 function by viral oncoproteins is essential for the transformation of rodent primary cells[9, 10]. However, several studies revealed that transcriptional coactivator p300 is a positive regulator of cancer progression and related to tumorigenesis of various human cancers[11-13]. It has been suggested that the cooperation between CtBP1 and p300 appears to be central in discriminating nuclear receptor repression versus stimulation of genes at early times after hormone exposure in breast cancer cells[14]. Stronger p300 expression was observed in malignant epithelia compared to normal mammary glands in animal models and human breast carcinoma[15]. In addition, Marije et al suggested that

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p300 is a cofactor highly correlated with p53 accumulation and HIF-1a levels in invasive breast cancer[16].

Up to date, the clinicopathologic/prognostic significance of p300 in BCs is poorly understood. In this study, immunohistochemistry (IHC) was utilized to examine the distribution and frequency of p300 expression in a well-characterized breast cancers prepared by tissue microarray. In order to avoid predetermined cutpoint, receiver operating characteristic (ROC) curve analysis was applied to define the cutoff score for separating p300 highly expressed tumors from p300 low expressed tumors. Subsequently, the clinicopathologic/prognostic significance of p300 expression in BCs was analyzed.

## MATERIALS AND METHODS

### Patients and Tissue Specimens

In the current study, the paraffin-embedded pathologic specimens from 193 patients with BC were collected from the archives of Department of Pathology, Sun Yat-Sen University Cancer Center, Guangzhou, China, between September 1997 and October 2004. The cases selected were based on distinctive pathologic diagnosis of BC, undergoing primary and curative resection for tumor without preoperative anticancer treatment, availability of resection tissue and follow-up data. The mean age of these patients was 48.1 years. Average follow-up time was 50.57 months (median, 47.97 months; range, 3.73 to 94.77 months). Additional 25 non-malignant breast specimens were obtained from reduction mammoplasties.

Patients whose cause of death remained unknown were excluded from our study. Clinicopathologic characteristics for these patients including age, tumor size, histological grade, clinical stage and relapse were detailed in Table 1. Histological grade was based on the criteria proposed by Elston and Ellis[17]. Tumor stage was defined according to American Joint Committee on Cancer/International Union Against Cancer tumor-node-metastasis (TNM) classification system[18]. Institute Research Medical Ethics Committee of Sun Yat-Sen University granted approval for this study.

### Tissue Microarray (TMA) Construction

Tissue microarray was constructed as the method described in our previous study[19]. Briefly, formalin-fixed, paraffin-embedded tissue blocks and the corresponding H&E-stained slides were overlaid for TMA sampling. The slides were reviewed by a senior pathologist (M-Y. C.) to determine and mark out representative tumor areas. Triplicates of 1.0 mm diameter cylinders were punched from representative tumor areas of individual donor tissue block and re-embedded into a recipient paraffin block at defined position, using a tissue arraying instrument (Beecher Instruments, Silver Spring, MD, USA).

### Immunohistochemistry (IHC)

The TMA slides were dried overnight at 37°C, deparaffinized in xylene, rehydrated through graded alcohol, immersed in 3% hydrogen peroxide for 20 minutes to block endogenous peroxidase activity, and antigen-retrieved by pressure cooking for 3 minutes in ethylenediamine tetraacetic acid (EDTA) buffer (pH=8.0). Then the slides were preincubated with 10% normal goat serum at room temperature for 30 minutes to reduce nonspecific reaction. Subsequently, the slides were incubated with mouse monoclonal anti-p300 (Abcam, Cambridge, MA, USA) at a concentration of 3ng/ml for 2 hours at room temperature. The slides were sequentially incubated with a secondary antibody (Envision; Dako, Glostrup, Denmark) for 1 hour at room temperature, and stained with 3,3-diaminobenzidine (DAB). Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. A negative control was obtained by replacing the primary antibody with a normal murine IgG. Known immunostaining positive slides were used as positive controls.

### IHC Evaluation

Immunoreactivity for p300 protein was evaluated in semi-quantitative method as described previously[20]. Each TMA spot was assigned an intensity score from 0–3 (I0, I1–3) and proportion of tumor cells for that intensity over the total number of tumor cells was recorded as 5% increments from a range of 0–100 (P0, P1–3). A final H score (range 0–300) was achieved by adding the sum of scores obtained for each intensity and proportion of area stained (H score = I1XP1+ I2XP2+I3XP3).

### Selection of Cutoff Score

ROC curve analysis was utilized to determine cutoff value for separating tumors with p300 high expression from tumors with p300 low expression by using the 0,1-criterion[21]. At the p300 H score, the sensitivity and specificity for each outcome under study was plotted, thus generating various ROC curves (Figure 1). The score was selected as the cutoff value, which was closest to the point with both maximum sensitivity and specificity. Tumors designated as "low expression" for p300 were those with scores below or equal to the cutoff value, while "high expression" tumors were those with scores above the value. In order to perform ROC curve analysis, the clinicopathologic features were dichotomized: tumor size (≤3.2 cm, or >3.2 cm), histological grade (Grade I+Grade II or Grade III), lymph node metastasis (N0+N1 or N3), clinical stage (I+II or III), relapse (absence or presence) and survival status (death due to BC or censored).

### Statistical Analysis

Statistical analysis was performed by using the SPSS statistical software package (standard version 13.0; SPSS, Chicago, IL, USA). ROC curve analysis was applied to determine the cutoff score for high expression of p300. The correlation between p300 expression and clinicopathological features of BC patients was evaluated by $\chi^2$-test. Univariate and multivariate survival analyses were performed using the Cox proportional hazards regression model. Survival curves were obtained with the Kaplan-Meier method. Predictive accuracy was quantified using the Harrell concordance index. Differences were considered significant if the *P*-value from a two-tailed test was $<0.05$.