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Original Article

TUBB3 as molecular marker for improved taxane efficacy of adjuvant chemotherapy for breast cancer

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Abstract: Objective: It has been reported that the expression levels of Tubulin β3 may help predict beneficial therapeutic responses for taxanes. However, few studies examined the exact benefits the receipts might have received, especially in the setting of breast cancer patients. *Methods:* Among the 97 breast cancer patients without overexpressing ER, PR, and Her2, 46 received a taxane therapeutic regimen regardless of TUBB3 expression profile (Control group); whereas another cohort of 51 breast cancer patients lacking ER, PR, and Her2 overexpression, TUBB3 expression was tested lower prior to deciding on choosing a taxane regimen (Personalized group). The PFS and OS of each group were statistically analyzed. Results: Those with low Tubulin 3 expression showed trends toward longer median PFS (7.6 *v* 6.7 months; $P = 0.037$) and OS (35.8 *v* 27.7 months; $P = 0.04$) compared to those without molecular testing. Conclusion: Molecular testing-based personalized medication should be applied universally to maximize the efficacy of tumor care.

Keywords: Breast cancer, adjuvant chemotherapy, docetaxel, personalized tumor care

Introduction

Breast cancer occurs with an incidence rate of 16.39 per 100,000 Chinese women [1], it is among the highest incidence of all cancers and among the most common cause of cancer death in China and much of the world, with an estimated mortality being 13.7% worldwide [2]. Well-established prognostic factors of breast cancer include tumor size, node status, occurrence of metastases, tumor grade, and hormone receptor expression, etc. Selection of appropriate initial therapy relies on the knowledge of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status. High tumor grade, expression of hormone receptors, and HER2-positivity provide bases for chemotherapy, endocrine manipulations, and anti-HER2 agents, respectively. Over 60% of breast cancer cases are present with ER/PR^+ [3], which render the patients highly responsive to anti-estrogen therapeutic strategies. However, over 20% of ER^+ breast cancer patients who have received hormonal adjuvant therapy will relapse; similarly, Her2-targeted therapies are also reported with rates of discordance ranging from 10% to 40%. All of the above cases may ultimately need chemotherapy instead. Moreover, the breast cancer cases lacking ER, PR, and Her2 overexpression-the so-called 'triple-negative' breast cancer (TNBC) cases-remain a major challenge. For breast cancer patients who are HER2-negative, regimens containing anthracyclines and taxanes are superior choices, it has even been suggested that a taxane followed by an anthracycline is a sequence option that can be incorporated into daily clinical practice [4].

However, not all of the breast cancer cases are suitable to apply regimens consisting of taxanes and anthracyclines, pharmacogenomics studies have indicated that, among other indications, only those with low expression levels of Tubulin β3 and high expression levels of TOPO IIα [5] are more likely to achieve ideal therapeutic efficacy.

The purpose of this study was to evaluate the association of TUBB3 expression level and the therapeutic efficacy of a taxane in the setting of Adjuvant Chemotherapy for a cohort of patients with stage I to III breast cancer.

Patients and methods

Patients

During the period August 2009 to May 2010, a total of 46 TNBC patients mostly from east regions of China, were enrolled in the first arm of a single-center study trying to determine the efficacy of taxanes as adjuvant regimen on Chinese TNBC patients (Control group).

From June 2010 to July 2011, a total of 51 TNBC patients whose tumors were tested with lower expression levels of TUBB3, were enrolled in the second arm of a single-center study trying to determine the impact of TUBB3 expression level on the efficacy of taxanes as adjuvant regimen on Chinese TNBC patients (Test group).

Selection of candidate molecular markers

Systematic review of the literature was carried out with databases such as PubMed/Medline, PharmGKB, "High Wire" etc., with search terms including "taxanes pharmacogenetics" "Personalized breast cancer" etc. After molecular marker TUBB3 had been chosen as indicator to predict taxanes efficacy, it was further checked in preliminary test before application to the patients in the Test group.

Chemotherapy and response evaluation

The chemotherapy regimens consisted of docetaxel (75 mg/m^2 of Taxotere; Sanofi Aventis, Paris, France) on day 1, and were repeated every 3 weeks. The clinical tumor response was assessed radiologically by computed tomography after every two courses of chemotherapy, according to the Response Evaluation Criteria in Solid Tumors (ver. 1.0) [6]. PFS was defined as the period from the start of chemotherapy to documentation of disease progression or death from any cause, whichever occurred first. If neither event had occurred at the time of the last record, the patient was censored at that time. OS was calculated from the start of chemotherapy to death from any cause. This study protocol was reviewed and approved by the Institutional Review Board of Huashan Hospital, Fudan University School of Medicine.

Immunohistochemical staining

Tumor samples collected from every participating patient were fixed in 10% buffered formalin and then processed and embedded in paraffin. Serial 4-micron sections were cut and placed on positive charged slides, and were prepared and first stained with hematoxylin and eosin (H&E). The H&E stained histological slides were reviewed by a certified pathologist. Slides were deparaffinized in xylene and hydrated through graded concentrations of ethanol and finally distilled water. Antigen retrieval was carried out at this stage with 10 mM citrate buffer, pH 6.0, in a pressure cooker. Primary antibodies for Tuj1 (sc-58888; used at 1:500 dilutions) was purchased from Santa Cruz Biotechnology, Inc.

Statistical analyses

SPSS 17.0 software (Chicago, IL, USA) was utilized to perform the data analysis. Clinical outcomes were expressed as progression-free survival (PFS), and overall survival (OS), PFS and OS curves were constructed with the Kaplan-Meier method. Factors with significant influence on survival in univariate analysis were further analyzed by multivariate Cox regression analysis. A significance level of $P < 0.05$ was used.

Results

TUBB3 expression and clinicopathological parameters

In the test group with known lower TUBB3 expression, the response rate (CR + PR) to chemotherapy was 41.4%, which was not significantly different from that seen in Control group (37.1% vs. 41.4%), suggesting that the TUBB3 expression did not significantly impact the overall response rate.

Molecular detection of TUBB3 expression

In order to confirm that the TUBB3 expression could be used to predict the therapeutic efficacy of taxanes, in the Test Group, all of the breast cancer patients were tested to ascertain that the TUBB3 expression level was low. A representative low expression figure was shown in **Figure 1A**, whereas a higher expression example was shown in **Figure 1B**.

The effect of TUBB3 expression-based personalized taxane application on breast patients

Although the TUBB3 expression did not impact the overall response rate between the Test

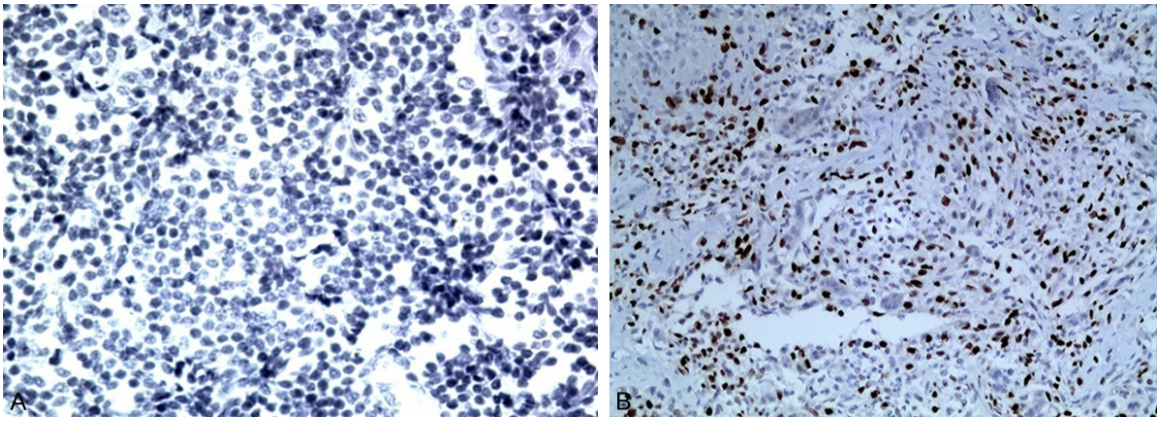

Figure 1. TUBB3 expression. A. Low level of TUBB3 expression; B. High expression level of TUBB3.

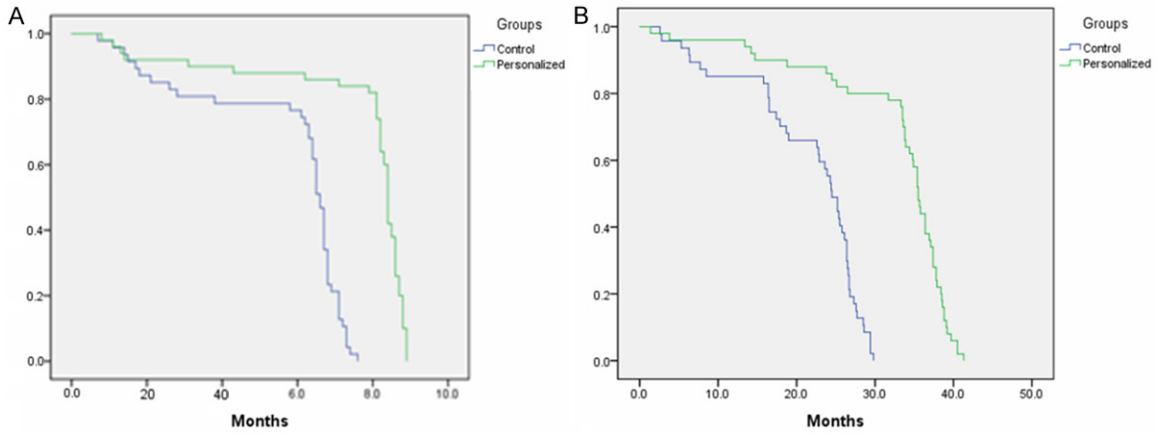

Figure 2. A. Proportional PFS (Y axis) relative to survival months (X axis), showing the difference in median PFS between control group (without molecular testing) and the personalized group (application of chemotherapy regimens was based on molecular testing) was significant (P = 0.037). B. Proportional OS (Y axis) relative to survival months (X axis), showing the difference in median OS between control group (without molecular testing) and the personalized group (application of chemotherapy regimens was based on molecular testing) was significant (P = 0.03).

Group and the Control group, the PFS and OS from the groups differed significantly. As shown in **Figure 2A** and **2B**.

Among the 97 patients treated with anthracycline+ taxanes, those with low Tubulin 3 expression showed trends toward longer median PFS (7.6 *v* 6.7 months; *P* = 0.037) and OS (35.8 *v* 27.7 months; *P* = 0.04) compared to those without molecular testing.

Correlation between and TUBB3 expression and clinical features

The expression of TUBB3 in different clinical features were compared and summarized. It showed that the difference of TUBB3 was only significant between some parts of clinical features. Correlations were observed between TUBB3 expression and TNM stage (P < 0.05). No correlation was observed between TUBB3 expression and gender, age, histology, differentiation of tumor, and chemotherapy status or performance status.

Discussion

Chemoresistance is one of the challenges breast cancer therapy has to overcome. Some of the difference in response to specific therapies can be attributed to somatic tumor characteristics, such as degree of estrogen receptor expression and HER2 status [7-10]. Pharmacogenomics refers to the study of the influence of a patient's genetic makeup or genetic expression status, on their response to drug therapy,

including toxicity and efficacy. Traditionally, clinical and histopathologic factors alone have been used to guide choice of therapy. These factors include tumor stage, tumor size, nodal status, and intra-tumoral characteristics such as grade, expression of estrogen and progesterone receptors, and HER2 status. These factors may be prognostic, indicating the aggressiveness of a tumor and likelihood of relapse without systemic therapy, predictive of response to specific treatments, or both.

In recent years, advances in technology such as the sequencing of the human genome, development of high-throughput DNA analysis, and popularization of the idea of "personalized medicine" have led to a significant interest in how differences in genetic makeup may be used to predict treatment safety and efficacy. Technologic advances have allowed the rapid assessment of gene expression and function. This includes assessment of both tumor (somatic) and host (germline) genetic variation. The goal of pharmacogenomic studies is to identify genetic alterations such as SNPs that considerably affect the function or expression of proteins involved in the pharmacokinetics or pharmacodynamics of therapeutic drugs. The ultimate goal of selecting a particular drug for a patient based on their genetic makeup is to improve efficacy and safety. To date, numerous studies have been conducted not only focusing on drug targets but also on cell cycle control and apoptosis, DNA damage and repair, and drug metabolism and transport.

Anthracyclines inhibit TOP2A protein activity, a key enzyme in DNA replication and RNA transcription [11]. It has been found that breast cancer patients with overexpressed the TOP2A protein [12] or had *TOP2A* gene amplification/deletion [13] in the tumor tissue would benefit more treatment with TOP2A inhibitors.

Disclosure of conflict of interest

None.

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