# ABSTRACT

**Purpose:** To evaluate whether changes in genomic expression that occur beginning with breast cancer (BC) diagnosis and through to tumor resection after neo-adjuvant chemotherapy (NCT) unveil biomarkers that can help in the prediction of therapeutic response and survival.

**Materials and Methods:** We determined gene expression profiles in tumor samples from 39 BC patients who showed pathologic complete response (pCR) or therapeutic failure (no pCR) after NCT (cyclophosphamide-doxorubicin/epirubicin). On the basis of unsupervised classification analysis of microarray data and interactome studies, we selected the genes *NUSAP1*, *KIAA0101*, *MME*, and *DST* for analyses of NCT response, expression in BC histologic subtypes, and presence of tumor-infiltrating lymphocytes. Finally, correlation analyzes between *NUSAP1* and *KIAA0101* (the most discriminating genes) against disease-free survival (DFS) and overall survival (OS) were performed.

**Results:** A signature of 43 genes discriminated pCR from non-pCR patients (fold change = ±3, false discovery rate (FDR) *P* value < .0298). Patients achieving pCR showed downregulation of *NUSAP1* and *KIAA0101* in tumor tissues and increased DFS and OS, while overexpression of these genes correlated with poor therapeutic response and OS. These genes are known to be involved in regulation of mitotic division.

**Conclusions:** Downregulation of *NUSAP1* and *KIAA0101* after NCT has a significant effect on tumor response to chemotherapy and patient survival.

**Keywords:** disease-free survival, *KIAA0101*, *NUSAP1*, neo-adjuvant chemotherapy treatment, neo-adjuvant treatment, overall survival, pathologic complete response, pathologic response, survival.

# Introduction

Therapeutic response and prognosis in breast cancer (BC) are affected by such factors as patient age [1], clinical stage [2], tumor histopathology, and molecular subtypes [3]. Gene expression profiles and genomic signatures performed prior to therapy can provide additional information on tumor biology, and algorithms have been developed to predict risk of relapse and survival and to define the best treatment options [4-6]. A program of genomic testing may allow for the identification of low-risk tumors associated with a favorable prognosis and as such would facilitate therapeutic decision-making for aggressive tumors that have a poor response to conventional therapies. In addition, genomic signatures can identify gene expression patterns related to chemotherapy resistance, immune system response, and tumor invasion [7-10].

Comparisons of gene expression analyses of biopsy specimens taken before and after neo-adjuvant chemotherapy treatment (NCT), may be useful to define tumor molecular adaptations to a specific chemotherapeutic agent or regime [7-10]. The pathologic complete response (pCR) in BC is defined as the absence of all invasive tumor tissue after completion of NCT cycles [11]. The achievement of pCR after NCT correlates with patient survival [12]. Alternative treatment regimens may improve survival when pCR is not achieved [13]. Comparisons of the changing patterns of gene signatures in response to chemotherapy may enable predictions of clinical response and prognosis, and sometimes, to recognize new response biomarkers of specific pathways related to treatment resistance and recurrence.

There is no genomic signature to define therapeutic alternatives in patients with incomplete pathologic response (non-pCR). Therefore, the identification of gene expression profiles in tumor tissue after NCT that are associated with a good or a bad pathological response or with survival could facilitate the identification of patients who could benefit from second-line adjuvant treatment or improve clinical follow-up, as has been shown in some studies assessing pathologic response [14]. Review of the biochemical pathways in which these genes are involved could also provide potential therapeutic targets or identify markers for high-risk patients who require closer follow-up.

The aim of this work was to analyze changes in genomic expression in primary BC tumors in patients undergoing NCT and to identify genes associated with prognosis in nonresponding patients that could guide new pharmaceutical interventions for a second line of treatment. Our studies that showed downregulation of *NUSAP1* and *KIAA0101* and overexpression of *MME* and *DST* in tumor biopsies of patients correlated with pCR after NCT and significantly correlated with both disease-free survival (DFS) and overall survival (OS). *NUSAP1* is involved in cell proliferation and migration and *KIAA0101* participates in cell cycle control and apoptosis [15, 16]. Overexpression of these genes have each been correlated with tumor progression and metastasis [17-19]. Downregulation of *MME* is associated with tumor recurrence and metastasis [20]. Underexpression of *DST*, which produces a cytoskeletal protein, promotes breast cancer progression independently of tumor hormonal status [21].

# Materials and Methods

**Patient population.** Patients with BC were recruited, engaged in informed consent, and enrolled in the study in the Centro de Cáncer de Mama (Breast Cancer Center) of the Hospital San José in Monterrey, Mexico. The Institutional Review Board of the School of Medicine of Tecnologico de Monterrey (CONBIOETICA 19 CEI 011-2016-10-17) authorized the research protocol with the number: P000088-Altru-Pro-CI-CR002. In accordance with the Declaration of Helsinki, informed written consent was obtained from all patients participating in this study. Tissue samples were collected from 54 patients with clinical and/or radiologic diagnosis of BC (tumor size > 2 cm and palpable lymph nodes) from July 2011 to October 2014.

**Neo-adjuvant chemotherapeutic regimens.** Regimens were established according to the clinical stage and the immunohistochemistry of the breast tumors by medical oncologists. They consisted of 4 cycles every 3 weeks of either intravenous cyclophosphamide (500–1500 mg/m2) and doxorubicin (≥40 mg/m2) or intravenous cyclophosphamide (500–1500 mg/m2) and epirubicin (≥60 mg/m2). After receiving either of these regimens, patients received 12 week