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Tissue microarray analysis of connexin expression and its prognostic significance in human breast cancer

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Abstract

Breast cancer accounts for approximately 15% of all cancer deaths. Currently, axillary nodal status is the most reliable prognostic indicator for breast cancer. Tumor size and histological grade are used to stage breast cancer. Estrogen receptor/progesterone receptor (ER/PR) and HER-2/neu status are useful in predicting patient survival and relapse. Ki67, an indicator of proliferative activity, also correlates well with prognosis. Connexin proteins form gap junction channels, permitting intercellular exchange of ions and small molecules. Reduced connexin protein levels and impaired gap junctional intercellular communication are associated with tumor phenotypes. This study investigated the prognostic value of connexin proteins as breast cancer markers. Tissue microarrays, containing 438 cases of invasive breast carcinoma, were stained with Cx26, Cx32, and Cx43 antibodies. The degree of connexin immunoreactivity was determined and then correlated with patient outcome, tumor grade, tumor size, lymph node status, and immunohistochemical markers, such as p53, ER/PR status, Ki67 and c-erbB-2 expression. Cx26, Cx32, or Cx43 did not correlate well with tumor grade, tumor size, p53 or c-erbB-2 status. There was an inverse correlation between Cx32 and lymph node status ($P < 0.05$) and a positive correlation between Cx43 and PR status ($P < 0.01$). Cx32 and Cx43 correlated positively with ER status ($P < 0.01$). Cx43 correlated negatively with Ki67 expression ($P < 0.01$). Cx26, Cx32, and Cx43 did not correlate with patient outcome. Based on our observations in this study, connexin proteins do not appear to be reliable indicators of breast cancer prognosis.

Keywords: Breast cancer; Connexin; Gap junctions; Tissue microarrays

1. Introduction

Breast cancer is one of the most common malignancies and accounts for more than 15% of all female cancer deaths [1]. Current prognostic indicators exist including axillary nodal status, histological grade, and tumor size [2]. More recently, estrogen receptor (ER) and progesterone receptor (PR) status [3,4], along with the proliferative marker, Ki67 have been used as prognostic breast cancer indicators [2]. Histopathological (nodal status, histological tumor grade, tumor size) and immuno-

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histochemical (ER/PR status, Ki67, HER-2/neu) breast cancer markers are important in predicting patient survival and relapse [5,6]. Furthermore, many treatment decisions rely upon breast cancer markers [2]. Current breast cancer treatments include hormone therapy, radiotherapy, chemotherapy, and surgical tumor removal. All these treatments have significant physiological and psychological side effects. Furthermore, they do not ensure a cure. The search for improved treatments and a more complete understanding of cancer behavior inspires the discovery of new molecular markers.

Connexin proteins form gap junction channels which are able to participate in the exchange of possible growth regulatory signals [7,8]. Impaired gap junctional intercellular communication (GJIC) has been associated with tumor phenotype [9–11]. Additionally, connexin proteins have been reported to have functions independent of GJIC. Specific to breast cancer, Cx43 and Cx26 were transfected into cultured human breast cancer cells (MDA-MB-435), resulting in improved differentiation and growth regulation capacity [12]. Qin et al. [13] also transfected human breast cancer cells (MDA-MB-231) with Cx43. The result was suppressed tumor growth independent of GJIC.

Few studies have observed the presence and distribution of connexin proteins in nontumorigenic and breast tumor tissues [14,15]. These studies have been compromised by a relatively small sample size. For example, Jamieson et al. [14] examined the immunocytochemical expression of Cx26 and Cx43 in 11 benign breast lesions, 1 mucoid and 1 lobular carcinoma, and 27 invasive carcinomas of no special histological type. Comparatively, this present study investigated Cx26, Cx32, and Cx43 expression in over 400 human breast tissue samples.

The purpose of this study was to determine if connexin proteins are reliable markers for breast cancer behavior. After scoring tissue sections for the abundance of connexin proteins, statistical analysis was performed by correlating connexin protein levels with patient survival, tumor grade, tumor size, lymph node status and previously established immunohistochemical markers such as p53, ER/PR status, Ki67, and c-erbB-2 expression. If connexin proteins are found to have a protective role in breast cancer progression then this may lead to new therapeutic methods and a more complete understanding of breast tumor behavior.

2. Materials and methods

2.1. Tissue source and preparation

Tissue previously obtained from 438 patients with invasive breast cancer at Vancouver General Hospital between 1974 and 1995, which had been was formalin fixed and paraffin embedded, was used for tissue microarray (TMA) construction. The tissue blocks were sectioned and stained with hemotoxylin and eosin (H&E), and then graded according to the Nottingham modification of the Scarth, Bloom Richardson method [16] prior to TMA construction. Then representative areas of invasive carcinomas were marked on both the slide and matching paraffin tissue block for TMA construction.

2.2. TMA construction

TMAs were prepared at the Genetic Pathology Evaluation Center in Vancouver General Hospital, British Columbia. Representative areas of breast carcinomas were selected and marked on the H&E slide. The corresponding tissue block, used to create the H&E slide, was identified and primed for TMA construction. A tissue arraying instrument (Beecher Instruments, Silver Springs, MD) was used to create the TMAs as described by Parker et al. [17]. Briefly, the instrument was used to create holes in the recipient block with defined array coordinates. A hollow needle was used to transfer the tissue cores into the recipient block. Two 0.6-mm diameter tissue cores were taken from each breast cancer case. Three composite high-density TMA blocks were designed, and serial 4-μm sections were cut with a Leica microtome and transferred to adhesive-coated slides.

2.3. Immunohistochemistry protocol

Citrate buffer (pH 6.0) was prepared by mixing 18 ml of 0.1 M citrate acid with 82 ml of 0.1 M sodium citrate and bringing the volume to 1 l with distilled water (dH_2O). The citrate buffer was preheated to 60–90 °C while the slides were deparaffinized and rehydrated by the following procedure: xylene incubation three times for 5 min, 100% absolute alcohol incubation twice for 3 min, 95% ethanol incubation for 3 min, 80% ethanol incubation for 3 min, followed by a 2 min rinse with ddH_2O. The slides were immediately placed in the heated citrate buffer for 30 min for antigen retrieval. The citrate buffer was then cooled to room temperature for 20 min. The slides were removed and then rinsed with phosphate buffered saline (PBS; pH 7.0) three times for 5 min. In order to block endogenous peroxide activity, the slides were incubated with 3% (v/v) hydrogen peroxide for 30 min, followed by three times 5 min rinses with PBS.