Available online at www.sciencedirect.com
ScienceDirect
Pathology – Research and Practice 202 (2006) 705–712
PATHOLOGY RESEARCH AND PRACTICE
www.elsevier.de/prp

ORIGINAL ARTICLE

Correlation of p53 and MIB-1 expression with both the systemic recurrence and survival in cases of phyllodes tumors of the breast

Kan Yonemori[a,*], Tadashi Hasegawa[b], Chikako Shimizu[a], Taro Shibata[c], Koji Matsumoto[a], Tsutomu Kouno[a], Masashi Ando[a], Noriyuki Katsumata[a], Yasuhiro Fujiwara[a]

[a]*Breast and Medical Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*
[b]*Department of Surgical Pathology, Sapporo Medical University School of Medicine, South 1, West 16, Chuo-ku, Sapporo 060-8543, Japan*
[c]*Statistics and Cancer Control Division, Research Center for Cancer Prevention and Screening, National Cancer Center, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan*

Received 21 April 2006; accepted 24 May 2006

Abstract

Phyllodes tumors are rare primary tumors of the breast. The study aimed at evaluating the immunohistochemical features of phyllodes tumors of the breast that may be useful for predicting the clinical outcome. We examined the immunohistochemical expression of the epidermal growth factor receptor (EGFR), HER2/neu, CD117/c-kit, p53, and MIB-1, and analyzed correlations between the immunohistochemical findings and the clinical outcome. The study included 41 patients with phyllodes tumor (20 benign, 5 borderline, and 16 malignant). Systemic recurrence occurred in 9 patients. The 2-year survival rate was 84%, and the 2-year recurrence-free survival rate was 77%. Six patients developed systemic recurrence within the first year after surgery. None of the phyllodes tumors was positive for HER2/neu or CD117/c-kit. Positive staining for p53 was seen in 10 phyllodes tumors (24%), and the median MIB-1 index was 10%. Both p53 expression and the MIB-1 index, but not the expression status of EGFR, were significantly correlated with the recurrence-free and overall survival. p53 expression status and MIB-1 index may be significant prognostic factors in patients with phyllodes tumors, and careful postoperative follow-up may be important in those cases showing positive expression of p53 and/or MIB-1 index.

Keywords: Phyllodes tumor; p53; MIB-1; Systemic recurrence; Survival

Introduction

Phyllodes tumors of the breast are rare, accounting for less than 1% of all breast tumors. [16]. Phyllodes tumors occur predominantly in middle-aged women, and the average tumor size is 4–5 cm. Histopathologically, these tumors are distinguished from true sarcomas by the presence of epithelial elements within the cellular connective tissue stroma. At present, phyllodes tumors are classified into benign, borderline, and malignant subtypes based on a combination of histological features, stromal cellular atypia, mitotic activity, stromal overgrowth, and tumor margins [15]. Approximately 50% of phyllodes tumors are benign, while the

*Corresponding author. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.
E-mail address: kyonemor@ncc.go.jp (K. Yonemori).

0344-0338/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2006.05.006

incidence of the malignant subtype has been reported to range from 26% to 35% [16,17]. While local recurrence can occur in all phyllodes tumors, systemic recurrence may also develop in cases of borderline or malignant phyllodes tumors [8].

Several studies have been carried out in which different pathologists have evaluated the same histological slides, resulting in a discordance of up to 25% in the final histopathological typing [5,9]. It remains difficult to predict the clinical outcome of the patients based solely on the histological features, and no effective treatment strategies have been developed for systemic involvement in these cases. Previous studies have been conducted to investigate the usefulness of immunohistochemical analyses of the tumors for various tumor markers to predict the clinical outcome. Immunohistochemical detection of p53 expression, commonly used as an identification for tumor-suppressor gene mutation, has been correlated with tumor grade [6,13,19]. Several studies on MIB-1 immunostaining, cell proliferation, have also shown a correlation between MIB-1 positivity and the histological grade [6,11,12,24]. Furthermore, several studies have also investigated the expression of other tumor markers in phyllodes tumors, including actin, epidermal growth factor receptor (EGFR), HER2/neu, BM28/cdc, CD34, CD117/c-kit, platelet-derived growth factor, and vascular endothelial growth factor [4,7,18,19,21–23], but these previously conducted studies did not add substantially to the information already provided by standard histopathological analysis.

The aim of the present study was to conduct an immunohistochemical analysis to determine the expression status of EGFR, HER2/neu, CD117/c-kit, p53, and MIB-1 in phyllodes tumors. We assessed the correlation between the results of the immunohistochemical analysis and the clinical outcome in an attempt to identify factors predictive of the prognosis in cases of phyllodes tumors of the breast.

Patients and methods

The study group consisted of all patients with phyllodes tumor of the breast diagnosed at the National Cancer Center Hospital, Tokyo, between 1994 and 2004. The histological sections were re-reviewed by a single pathologist (T.H.) for diagnosis. Patient history and follow-up data were obtained by a review of the medical records. Recurrence-free survival (RFS) time was measured from the time of surgery until the appearance of systemic recurrence or until the last day of follow-up without evidence of systemic recurrence, and the overall survival time (OS) was measured from the time of surgery until the last day of follow-up or death, whichever came earlier.

Immunohistochemical analysis of tissue samples

Immunohistochemical staining of the tissue sections obtained from formalin-fixed, paraffin-embedded blocks was performed for EGFR, HER2/neu, CD117/c-kit, p53, and MIB-1 using the labeled streptavidin–biotin method. The antibodies used for the immunohistochemical staining were as follows: EGFR (EGFR pharmDx Kit, 2-18C9, DakoCytomation, Glostrup, Denmark), HER2/neu (CB11, BioGenex, San Ramon, USA), CD117/c-kit (A4502, DakoCytomation, Glostrup, Denmark), p53 (DO7, DakoCytomation, Glostrup, Denmark), and MIB-1 (Immunotech, Marseille, France). The anti-EGFR monoclonal antibody, clone 2-18C9, which binds to an epitope located near the ligand-binding domain on the extracellular domain of EGFR, has been shown to be specific for EGFR and not to cross-react with HER2 or other receptors of the HER family [20].

The immunohistochemical analysis of the primary tumor in all patients was conducted by the same investigator (T.H.), blinded to the clinical status of the patients. The intensity of the immunochistochemical staining for p53, EGFR, HER2/neu, and CD117/c-kit was also similarly scored as 0, negative; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. Negative controls, in which the primary antibody was omitted, were also included in each run. As for positive controls, invasive breast cancers showing strong staining (3+) were used as the positive controls for EGFR and HER2/neu staining, and tissue mast cells showing strong staining (3+) were used as the internal positive controls for CD117/c-kit staining. The proportion of positive cells was categorized as sporadic (positive cells $<10\%$); focal ($11\% <$ positive cells $<50\%$); and diffuse (positive cells $\geqslant 50\%$). The immunohistochemical scores of 2+ and 3+ with focal to diffuse distribution were considered to represent positive expression of the respective markers.

The MIB-1 index was defined as the percentage of nuclei showing positive staining calculated after counting 1,000 neoplastic cells per slide. The cut-off point for the value of the MIB-1 index ($>11.2\%$ vs. $\leqslant 11.2\%$) was defined based on the results of a previous study [13].

Statistical analysis

The Kaplan–Meier method was used to describe the distribution of the RFS and the median OS. The prognostic factors of primary tumor size ($\leqslant 10$ cm vs >10 cm) and histological types (benign vs. borderline vs. malignant) were analyzed statistically. The relationships between the expression of the biomarkers (EGFR, p53, and MIB-1 index) and the clinical outcomes of the patients were compared with the log-rank test, and the