Human Pathology (2009) **40**, 98–107

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Expression of anaphase-promoting complex7 in fibroadenomas and phyllodes tumors of breast

Yup Kang PhD[a], Jang Hee Kim MD[b], Tae Hui Lee MD[b], Tai Seung Kim MD, PhD[c], Woo Hee Jung MD, PhD[c], Hyun Cheol Chung MD, PhD[d], Byeong Woo Park MD, PhD[e], Seung Soo Sheen MD, PhD[f], Jae Ho Han MD, PhD[b,*]

[a]*Institute for Medical Science, Ajou University School of Medicine, Suwon 443-721, Korea*
[b]*Deparment of Pathology, Ajou University School of Medicine, Suwon 443-721, Korea*
[c]*Department of Pathology, Yonsei University College of Medicine, Seoul 120-752, Korea*
[d]*Cancer Metastasis Research Center, Yonsei Cancer Center, Yonsei University College of Medicine, Seoul 120-752, Korea*
[e]*Department of Surgery, Yonsei University College of Medicine, Seoul 120-752, Korea*
[f]*Department of Internal Medicine, Ajou University School of Medicine, Suwon 443-721, Korea*

Received 31 December 2007; revised 7 April 2008; accepted 11 April 2008

Keywords:
Anaphase-promoting complex;
Ki-67;
Phyllodes tumor;
Fibroadenoma

Summary The proteolytic destruction of cyclin B is an important event during cell division. Cyclin B proteolysis is triggered by the anaphase-promoting complex. Therefore, cell cycle dysregulation due to anaphase-promoting complex loss contributes to cell transformation and human carcinogenesis. This study investigates anaphase-promoting complex7 expression in spindle cell breast tumors and also includes a comparison between the proliferation antigen Ki-67 and S-phase fraction. The average values of the anaphase-promoting complex7 and Ki-67 labeling indices increased in order from benign to malignant within the phyllodes tumor group, and the fibroadenoma and juvenile fibroadenoma exhibited lower levels of anaphase-promoting complex7 and Ki-67 expression than did the phyllodes tumor. The frequency of anaphase-promoting complex7–positive stromal cells correlated with Ki-67 expression in phyllodes tumor and in all of the examined breast tumors. The above results indicate that anaphase-promoting complex7 and Ki-67 are closely related to cell proliferation. In addition, phyllodes tumor can be differentiated from juvenile fibroadenoma with increased mitotic figures mimicking phyllodes tumor by anaphase-promoting complex7 and Ki-67 immunochemistry. Because anaphase-promoting complex7 is expressed at higher levels than is Ki-67, it may overcome the limitations of the Ki-67 labeling index with regard to the differentiation of benign phyllodes tumor from fibroadenoma.

1. Introduction

The proteolytic destruction of cyclin B is an important event in the cell cycle and degradation of its results in inactivation of cyclin-dependent kinase. Cyclin B is destroyed by ubiquitin-dependent proteolysis, which is triggered by the anaphase-promoting complex (APC) [1,2]. Because of APC's prominent role in cell cycle regulation, dysregulation of APC triggered a primary obstacle of cell cycle progression and contributed to cell transformation and human carcinogenesis. A quantitative or qualitative disruption of some APC subunits might be

* Corresponding author.
E-mail address: hanpathol@yahoo.co.kr (J. H. Han).

0046-8177/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2008.04.023

sufficient to induce a significant dysregulation of the key regulators of the cell cycle, thus contributing to malignant transformations [3]. After the carcinomatous transformation, in which alterations of the APC genes occur, APC expression may be decreased in the cancer cells. There have been only a few reports regarding APC expression in human cancer [3,4].

However, the proliferative activities of phyllodes tumor (PT) and fibroadenoma (FA) stromal cells, rather than those of epithelial cells, appear to be essential in tumor formation. PTs are usually benign, but recurrences are not uncommon, and a relatively small number of patients eventually develop hematogenous metastases. The frequency with which local recurrence and metastases occur correlates strongly with the grade of the PT, but this still varies considerably. Several grading systems have been proposed, with 3 subgroups [5,6], but none has been universally applied because the prediction of this sort of behavior remains difficult in individual cases. In addition, benign PTs may be difficult to distinguish from FAs in which stromal cellularity and mitotic count are increased. Therefore, some ancillary indicators should be verified for the differentiation of PTs from FAs and the prediction of clinical outcomes. DNA content and the expression of proliferative antigens (Ki-67) were considered in a limited number of PTs, and their prognostic value remains an open problem [7-10].

The present study focuses in the quantititaton of APC7 and analyzes APC7 expression in spindle cell breast tumors by immunohistochemistry and Western blotting. In addition, we compare APC7 expression with pathologic parameters of PTs, DNA index, S-phase fraction, and G2M fraction using flow cytometry, as well as the expression of proliferative antigen Ki-67 by immunohistochemistry.

2. Materials and methods

2.1. Tissue samples

The study was performed with paraffin tissue blocks, which contained breast tumor tissues from 132 patients. The breast tumor cases consisted of 20 FAs, 20 juvenile FAs, 50 benign PTs, 20 borderline PTs, and 22 malignant PTs from the Ajou University Hospital (Suwon, Korea) and the Severance Hospital (Seoul, Korea) between January 1993 and December 2005.

2.2. Production of polyclonal antibodies against APC7

Polyclonal antibodies against mouse APC7 were raised in a New Zealand White rabbit via immunization with recombinant APC7 protein. In brief, recombinant mouse APC were produced in *Escherichia coli* using a pET32 expression vector system (Novagen, Madison, WI), and the 6× histidine-tagged APC7 proteins were then purified via Ni-NTA affinity chromatography (Qiagen, Hilden, Germany). A New Zealand White rabbit was then immunized with the purified APC7 protein and was further boosted twice every 3 weeks. Blood was collected from the rabbit's auricular artery, and the serum was then prepared via clotting and differential centrifugal separation (10 000 × *g*, 10 minutes). APC7-specific antibodies were then further purified by binding the serum to APC7-coupled nitrocellulose and eluting this with 100 mM/L glycine-HCL buffer (pH 2.5).

2.3. Pathologic examination of breast tumor

The paraffin blocks were retrieved, and 4-μm slides were prepared and stained with hematoxylin and eosin (H&E). The main features favoring PT over FA are a greater amount of cellular stroma, the formation of leaf-like structures, and infiltrative border. In the differentiation of FA from juvenile FA, we considered the patient's age, tumor size, and stromal cellularity. All of the slides from the PTs were reviewed with regard to the following histologic parameters: (1) stromal cellularity; (2) nuclear pleomorphism; (3) stromal overgrowth; (4) mitotic count; and (5) tumor margin, whether infiltrative or rounded. Stromal cellularity, nuclear pleomorphism, and stromal overgrowth were graded as mild/low, moderate, or severe; and mitotic count was assessed as the number of mitotic figures per 10 high-power fields. We assessed all H&E sections in detail to find the most mitotically active areas. The PTs were graded as benign, borderline, or malignant according to the criteria developed by Moffat et al [5] and Tse et al [11], after which they were modified. A diagnosis of benign PT was made in cases in which there was low cellularity, mild stromal overgrowth, mild nuclear pleomorphism, a rounded margin, and a mitotic count of 2 or less per 10 high-power fields. Malignant PTs were diagnosed in cases in which there was severe cellularity, severe nuclear pleomorphism, severe stromal overgrowth, an infiltrative margin, and a mitotic count of at least 5 per 10 high-power fields. At least 3 parameters were necessary for the diagnosis of a benign or malignant PT. Borderline PTs were diagnosed in cases in which the criteria for a benign or malignant tumor could not be fulfilled.

2.4. Immunohistochemistry and evaluation

Five-micrometer sections of 132 representative breast tumor blocks were cut, placed on charged poly-L-lysine–coated slides, and then used for immunohistochemical analysis.

The section containing the highest graded area was selected for immunohistochemical analysis. Sections were deparaffinized in zylene and rehydrated in graded alcohols and water. Endogenous peroxidase activity was blocked via treatment with 3% hydrogen peroxide for 10 minutes.