Expression of anaphase-promoting complex7 in normal tissues, fibroadenomas, and phyllodes tumors of the breast

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Directed by Professor Tai Seung Kim

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Abstract

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The proteolytic destruction of cyclins is a fundamental process during cell division. Cyclin B proteolysis is triggered by the anaphase-promoting complex (APC). However, the levels and patterns of APC expression in nonproliferating cells and tissue have yet to be determined, and it currently remains unknown as to whether the APC is expressed in differentiated cells from each organ. In addition, cell cycle dysregulation due to APC loss may contribute to cell transformation and human carcinogenesis. There have been only a few reports regarding APC expression in human cancer, particularly in stromal tumors, including phyllodes breast tumors.

In order to address these questions, this study investigates APC7 expression in a variety of normal tissues and cells, and in spindle cell breast tumors, and also includes a comparison between the proliferation antigen Ki-67 and S-phase fraction.

We selected a representative area in various normal tissues, 20 cases of fibroadenoma, 20 cases of juvenile fibroadenoma, 50 cases of benign phyllodes tumor, 20 cases of borderline phyllodes tumor, and 22 cases of malignant phyllodes tumor.

The following results were obtained.

1) Labile cells, such as the epithelial cells of the gastrointestinal tract, breast, and salivary gland, and the lymphoid cells of the lymphoreticular system, exhibited frequent expressions of APC7 and Ki-67. However, no or low-level APC7 and Ki-67 expression was noted in permanent and stable cells.
2) The average values of the APC7 and Ki-67 labeling indices increased in order from benign to malignant within the phyllodes tumor group, and the fibroadenomas and juvenile fibroadenomas exhibited lower levels of APC7 and Ki-67 expression than did the phyllodes tumors (26.67±13.60% versus 66.07±14.53% and 0.51±0.87% versus 4.29±7.42%, respectively, p=0.000).
3) A significant positive correlation was noted between the frequency of APC7 and mitotic count (R=0.32, p=0.002), cellularity (R=0.26, p=0.012), pleomorphism (R=0.34, p=0.001), and degree of stromal overgrowth (R=0.35, p=0.001) in the phyllodes tumor group. APC7 expression was not correlated with tumor size (R=-0.016, p=0.878).
4) The Ki-67 expression correlated positively with mitotic count (R=0.632, p=0.000), cellularity (R=0.29, p=0.005), pleomorphism (R=0.53, p=0.000), degree of stromal overgrowth (R=0.46, p=0.000) and tumor size (R=0.25.p=0.015) in phyllodes tumors.
5) The frequency of APC7 positive stromal cells correlated with Ki-67 expression in phyllodes tumors (R=0.21, p=0.048), and in all of the examined breast tumors (R=0.303, p=0.000).
6) The fibroadenoma group exhibited a lower S-phase fraction than did the phyllodes tumor group (4.19±2.15% versus 6.15±6.05%), but this was determined not to be statistically significant (p=0.163). APC7 expression did not correlate with the S-phase fraction or the G2M fraction. However, Ki-67 expression was

correlated positively with the S-phase fraction (R=0.375, p=0.001).

The above results indicate that APC7 and Ki-67 are closely related to cell proliferation. Also, phyllodes tumors, which are characterized by the monoclonal proliferation of stromal cells, can be differentiated from cellular fibroadenomas with increased mitotic figures mimicking phyllodes tumors by APC7 and Ki-67 immunochemistry. As APC7 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 tumors from fibroadenomas. In addition, considering APC7 loss in highly pleomorphic cells in cases of malignant phyllodes tumors, APC7 downregulation may be associated with sarcomatous transformation via chromosome instability, or accelerated oncogene signaling.

Key words: anaphase promoting complex, ki-67, phyllodes tumor, fibroadenoma

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I. Introduction

During the eukaryotic cell division cycle, cells initially replicate their DNA during S phase, package the DNA into sister chromatids during mitosis, then segregate the chromosome evenly into daughter cells.[1] Cyclin B, like several related mitotic cyclins, is a central regulatory element which triggers entry into mitosis, and cyclin B synthesis during interphase is essential for the activation of cyclin-dependent kinase 1 (cdk1), which initiates many early mitotic events.[2] Cyclin degradation at the end of mitosis results in the inactivation of cyclin-dependent kinases.[3] The inactivation of cdk1 during anaphase and telophase is crucial for the exit from mitosis and DNA replication in the subsequent cell cycle, due to its enabling of the formation of prereplicative complexes.[4] Also, partial cdk1 inactivation might be required for the separation of sister chromatids in anaphase in animal cells.[5]

Cyclin is destroyed via ubiquitin-dependent proteolysis. It is

targeted for degradation by the covalent attachment of a multiubiquitin chain. It can then be recognized and degraded by the 26S proteasome.[6,7] Cyclin B ubiquitination reactions are mediated by activation of anaphase-promoting complex (APC),in conjunction with several APC activator proteins, including Cdc20, Cdh1, universal ubiquitin-activating enzyme E1, and one of two ubiquitin-conjugating E2 enzymes (UbcH5 and Ubc H10).[8-10] APC is a cell cycle-regulated ubiquitin ligase, which assembles multiubiquitin chains on regulatory proteins, including securin and cyclin B.[2] APC has a high molecular mass complex, and is composed of at least 11 subunits, but is only fully active as a ubiquitin ligase after it has bound to Cdc20 or Cdh1, resulting in distinct assemblies, such as APC^{cdc20} or APC^{Cdh1}.[2] In mitosis, APC is activated by binding to Cdc20, and then Cdc20 is degraded during metaphase, then replaced by Cdh 1, which maintains the APC in an active state until the end of the subsequent G1 phase. Cyclin B degradation begins in metaphase and continues, while the sister chromatids separate.[11]

APC^{Cdc20} promotes the separation of sister chromatids at the metaphase-to-anaphase transition.[8] The activation of APC, in association with Cdc20 during metaphase, promotes the proteolysis of securin liberating separin, after all sister chromatids have achieved bi-orientation, and all of their kinetochores are under tension. Then, separase activates, and dissolves the cohesion between the sister chromatids.[12]

The APC's mitotic functions and regulation are now understood in some detail. Larger surprises, however, may originate from other cell cycle research, which may reveal unexpected roles for APC in nonproliferating cells and tissue.

The cells of the body are divided into three groups, predicated on their proliferative capacity and relationship to the cell cycle.

Continuously dividing cells (labile cells) follow the cell cycle from one mitosis to the next, and continue to proliferate throughout life. Quiescent cells (stable cells) normally demonstrate a low level of replication; however, these cells can undergo rapid division in response to a variety of stimuli. They are considered to be in G0 phase, but can also be stimulated into G1 phase. Nondividing cells (permanent cells) have exited the cell cycle and cannot undergo mitotic division in postnatal life. In vertebrates, APC^{Cdh1} is present and active in some quiescent cultured cells,[13] and is expressed in a variety of tissues predominantly composed of postmitotic cells, including the brain, in which it can be detected in the nuclei of differentiated neurons.[14] However, APC expression in the nonproliferating cells and tissue has yet to be characterized, and it is unknown as to whether the APC is expressed in differentiated cells from each organ.

Considering APC's prominent role in cell cycle regulation, it is tempting to speculate that its dysregulation triggered a primary perturbation of cell cycle progression, and contributed to cell transformation as well as human carcinogenesis. Complete inhibition of APC activity results in cell death. However, a quantitative or qualitative disruption of some APC subunits might be sufficient to induce a significant dysregulation of the key regulators of the cell cycle, thus contributing to malignant transformations.[15] Premature separation of sister chromatids leads to either the loss or gain of chromosomes in the daughter cells (aneuploidy), a prevalent form of genetic instability associated with human cancer.[16,17] After the carcinomatous transformation, in which alterations of the anaphase-promoting complex genes occur, APC expression may be decreased in the cancer cells. There have been only a few reports regarding APC expression in human cancer. For example, the APC7 expression rate in

breast carcinoma was found to increase with decreasing mitotic activity, both in cases with a low Ki-67 labeling index and in the diploid group. Therefore, the loss of APC7 expression may be related to the progression of breast cancer.[18]

However, the proliferative activities of stromal cells, rather than those of epithelial cells, appear to be essential in phyllodes tumor formation.

Phyllodes tumors 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 phyllodes tumor, but this still varies considerably. Several grading systems have been proposed, with three subgroups,[19,20] but none has been universally applied, since the prediction of this sort of behavior remains difficult in individual cases. In addition, benign phyllodes tumors may be difficult to distinguish from fibroadenomas in which stromal cellularity and mitotic count are increased.

Therefore, some ancillary indicators should be verified for the differentiation of phyllodes tumors from fibroadenomas, and the prediction of clinical outcomes. DNA content and the expression of proliferative antigen, Ki-67, were considered in a limited number of phyllodes tumors, and their prognostic value remains an open problem.[21-24]

The present study focused in the quantitaton of APC7 in normal labile cells, stable cells, and permanent cells, using a mouse fibroblast cell line (3T3 L1), and a variety of normal tissues, and analyzed APC7 expression in spindle cell breast tumors by immunohistochemistry and western blotting. Also, I compared APC7 expression with pathologic parameters, DNA index, S-phase fraction,

and G2M fraction, as well as the expression of the proliferative antigen, Ki-67. The goals of this study were to analyze the histologic features and expression of APC7 and Ki-67, as well as the status of DNA ploidy in spindle cell breast tumors, in order to assess the usefulness of these markers in distinguishing between them. In addition, I expect to determine the characteristics of APC7, according to cellular differentiation and proliferation characteristics, by correlating the APC7 expression patterns in normal tissue and breast tumors with the Ki-67 proliferation index, and DNA contents.

II. Materials and Methods

1. Materials

The study was performed with paraffin tissue blocks which contained a variety of normal tissues and breast tumor tissues from 132 patients. The breast tumor cases consisted of 20 fibroadenomas, 20 juvenile fibroadenomas, 50 benign phyllodes tumors, 20 borderline phyllodes tumors, and 22 malignant phyllodes tumors from the Ajou University Hospital (Suwon, Korea) and the Severance Hospital (Seoul, Korea), between January 1993 and December 2003.

2. Methods

A. Production of polyclonal antibodies against APC7

Polyclonal antibodies against mouse APC7 were raised in a New Zealand White (NZW) rabbit via immunization with recombinant APC7 protein. In brief, recombinant mouse APC7 were produced in *E.coli* using a pET32 expression vector system (Novagen, Madison, WI, USA), and the 6x histidine-tagged APC7 proteins were then purified via Ni-NTA affinity chromatography (Qiagen, Hilden, Germany). A NZW 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 (10,000g, 10 min). APC7-specific antibodies were then further purified by binding the serum to APC7-coupled nitrocellulose, and eluting this with 100mM/l glycine-HCL buffer (pH 2.5).

B. Adipocyte differentiation from 3T3 L1 cells (mouse fibroblast)

3T3-L1 fibroblasts were purchased from the American Type Culture

Collection (ATCC, Rockville, MD, USA). Adipocyte differentiation was induced according to the methods of Liu *et al.*[25] with modifications. 3T3-L1 cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gemini, Calabasas, CA USA), and supplemented with 10% fetal bovine serum (Gemini, Calabasas, CA, USA) at 37℃, to confluency. Adipocyte differentiation was induced by supplementation of the media with a combination of 1mg/L insulin (Eli Lilly, Indianapolis, IN, USA), 120mg/L 3-isobutyl-1-methylxanthine (IBMX; Sigma, St. Louis, MO, USA), and 0.1mg/L dexamethasone (DEX; Sigma, St. Louis, MO, USA) for 48 hours, followed by treatment with insulin alone for an additional 48 hours. The media was then replaced with fresh culture medium (DMEM supplemented with 10% FBS) after 2 days, and then every three days thereafter.

C. Construction of a tissue microarray using normal tissue

Two representative areas were taken from a paraffin tissue which contained a variety of normal tissue types. A total of 87 core tissue biopsies (diameter, 1.0 mm) were taken and arrayed into a new recipient paraffin block.

D. Pathologic examination of breast tumor

The paraffin blocks were retrieved and 4-um slides routinely prepared, and stained with hematoxylin and eosin. The main features favoring phyllodes tumor over fibroadenoma are a greater amount of cellular stroma, the formation of leaf-like structures, and infiltrative border. In the differentiation of fibroadenoma from juvenile fibroadenoma, I considered the patient's age, tumor size, and stromal cellularity. All of the slides from the phyllodes tumors 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. The phyllodes tumors were graded as benign, borderline, or malignant, according to the criteria developed by Moffat *et al.*[19] and Gary *et al.*,[26] after which they were modified. A diagnosis of benign phyllodes tumor was made in cases in which there was low cellularity, mild stromal overgrowth, mild nuclear pleomorphism, a rounded margin, and a mitotic count of two or less per 10 high-power fields. Malignant phyllodes tumors 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 phyllodes tumor. Borderline phyllodes tumors were diagnosed in cases in which the criteria for a benign or malignant tumor could not be fulfilled.

E.Immunohistochemistry and evaluation

Five-micrometer sections of the above tissue array block, and 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. Sections were treated with protein-blocking solution, and primary antibodies such as anti-mouse APC7 antibody or human Ki-67

antibody (DAKO, Copenhagen, Denmark) were applied overnight at a 1:50 or 1:100 dilution. After several rinses in phosphate-buffered saline, the sections were incubated in biotinylated secondary antibody. The bound antibodies were detected by a streptavidin-biotin method, with a Cap-Plus detection kit (Zymed Laboratories Inc, San Francisco, CA, USA). The slides were rinsed in phosphate-buffered saline, exposed to diaminobenzidine, and counterstained with Mayer hematoxylin.

For normal tissue, APC7 positive cells were assessed according to their proportion. The staining pattern was graded from 0 to 3, with 0 representing no staining; 1 when <33% of nuclei were stained; 2 when 33-67% of nuclei were stained; and 3 when >67% of nuclei were stained. And Ki-67 positive cells were expressed as a percentage. For the tumor tissues, stromal nuclear APC7 and Ki-67 staining were recorded as the numbers of APC7-positive nuclei and Ki-67 positive nuclei, divided by the total number of stromal nuclei of at least 10 fields, then expressed as a percentage.

F. Western blotting for APC7

Protein extracts were prepared by solubilizing cells in RIPA buffer (150 mmol/NaCl, 1% NP40, 0.5% deoxycholate, 0.1% sodium dodecylsulfate, 50mmol/l Tris.Cl, pH 7.5, protease inhibitor) and differential centrifugation (10,000g for 10 min). Of the protein fractions obtained, 30ug was resolved by 12% SDS-PAGE, and then the separated proteins were electrotransferred onto Immobilon membranes (Millipore, Bedford, MA, USA). After preblocking these membranes with 5% skimmed milk, they were treated with anti-mouse APC7 antibodies as a primary antibody, and horseradish peroxidase-conjugated anti-rabbit antibodies were used as a secondary antibody. Immunoreactive bands were developed with an enhanced

chemiluminescence system (Amersham Pharmacia Biotech, Uppsala, Sweden).

G. Flow cytometry

Three or four 40-um sections were cut from paraffin blocks of 11 fibroadenomas, 9 juvenile fibroadenomas, 30 benign phyllodes tumors, 17 borderline phyllodes tumors, and 20 malignant phyllodes tumors. Then, each section was rehydrated in a descending ethanol series, and washed in phosphate-buffered saline. The sections were then placed in 10mmol/l citrate solution (pH 6.0), and incubated for 2 hours at 80℃. After cooling, 1mg/ml pepsin in 0.1 N HCl was added to the sections, and they were digested for 30 minutes. The resulting suspension (2×10^6 nuclei) was filtered through 50u-mesh, and further suspended in 500 ul of 1% bovine serum albumin solution. DNAs were stained with a Cycle TEST PLUS DNA Reagent Kit™ (Becton Dickinson, Ontario, Canada). The stained cells were analyzed with a FACscan (Becton Dickinson, San Jose, CA, USA), and the fraction of aneuploid cells was calculated with Cell Fit software (Becton Dickinson).

H. Statistical analysis

Differences in APC7 expression and Ki-67 proliferation indices between fibroadenomas and phyllodes tumors were assessed using independent *t*-tests, or analysis of variance with Bonferroni's correction. I also compared pathologic parameters and flow cytometric parameters with the APC7, or Ki-67 labeling index with Pearson product moment correlation (size, mitotic count, cellularity, pleomorphism, stromal overgrowth, S-phase fraction, G2M fraction), or by independent *t*-tests (status of tumor margin).

III. Results

1. Morphological analysis of cultured 3T3 L1 cells and differentiated adipocytes

As shown in figure 1, the 3T3 L1 fibroblasts were usually spindle-shaped, often stellate (spiderlike), and revealed long cytoplasmic extensions. Being proliferated to the point of confluency, these cells became polyhedral-shaped, but lipid droplets were not detected. The differentiated adipocytes then became closely packed, with an accumulation of lipids which formed many unilocular or multilocular spherical cells.

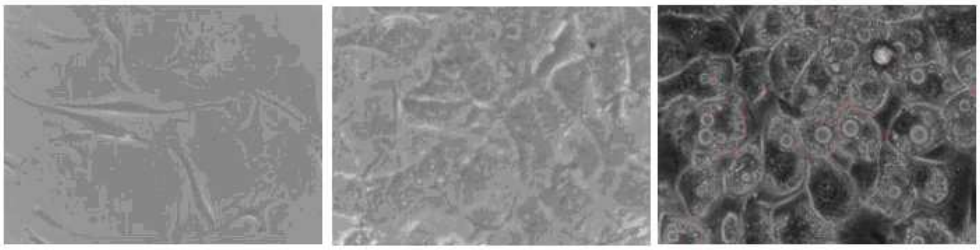

Fig. 1. Morphological features of cultured 3T3 L1 cells.

2. Clinicopathologic characteristics

The patients were aged 22-49 (mean 33.8 years) in fibroadenoma cases, 2-29 (mean 15.8 years) in juvenile fibroadenoma cases, 12-52 (mean 35.8 years) in benign phyllodes tumor cases, 26-60 (mean 41.6 years) in borderline phyllodes tumor cases, and 19-57 (mean 38.8 years) in malignant phyllodes tumor cases.

The size of the tumors ranged from 1.3 to 4.5cm (mean 2.69±0.89cm) in cases of fibroadenoma, from 2.0 to 5.4cm (mean 5.40±2.81cm) in cases of juvenile fibroadenoma, from 1.5 to 12.0cm (mean 4.18±2.23cm) in cases of benign phyllodes tumors, 1.5 to 15.0cm

(mean 7.37±4.57cm) in cases of borderline phyllodes tumors, and from 2.0 to 12.0cm (mean 6.0±3.38cm) in cases of malignant phyllodes tumors.

All of the fibroadenomas were well circumscribed with pushing growth margins, and exhibited no hypercellularity or nuclear pleomorphism (Fig. 2A). The number of mitotic figures per 10 high-power fields was less than 2. Some juvenile fibroadenomas exhibited slightly increased hypercellularity and epithelial proliferation (Fig. 2B). The leaf-like structures, nuclear pleomorphism, or increased mitotic figures were not found. The phyllodes tumors exhibited increased stromal cellularity and definite leaf-like structures. Most of the benign phyllodes tumors exhibited a mild degree of stromal cellularity, nuclear pleomorphism and stromal overgrowth, and rounded pushing margins (Fig. 2C, D). The mitotic count was less than 2, except in 2 cases, which revealed 2.5 mitotic figures per 10 high-power fields. The malignant phyllodes tumors exhibited a severe degree of stromal cellularity, nuclear pleomorphism, stromal overgrowth, and infiltrative margin (Fig. 2E,F). In malignant phyllodes tumors exhibiting severe nuclear pleomorphism, highly atypical stromal cells constituted a minor component. The mitotic counts were over 5 except in 2 cases. The borderline phyllodes tumors exhibited variable degrees of pathologic parameters.

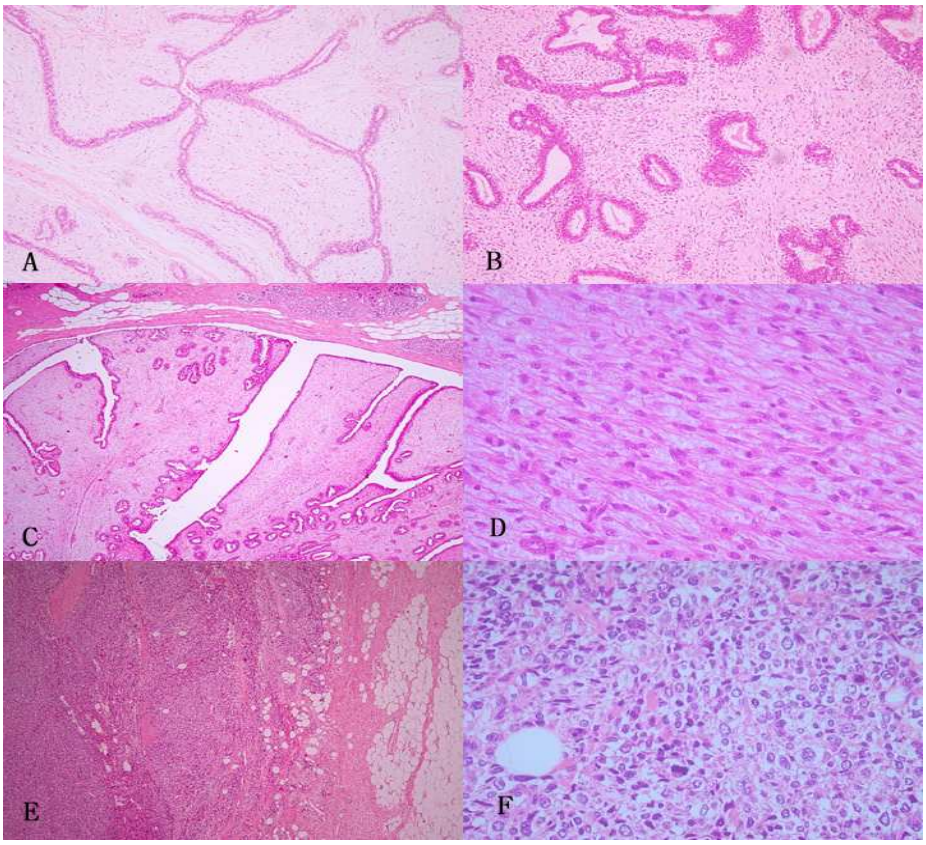

Fig. 2. Pathologic features of fibroadenoma (A), juvenile fibroadenoma (B), benign phyllodes tumor (C, D), and malignant phyllodes tumor (E, F). A. Fibroadenoma shows myxoid stroma with low stromal cellularity (H&E, x100). B. Juvenile fibroadenoma shows epithelial proliferation and higher stromal cellularity than fibroadenoma (H&E, x100). C. Benign phyllodes tumor shows leaf-like pattern and well-defined interface with the surrounding normal tissue (H&E, x40). D. Increased number of stromal cells shows monomorphic nuclei and rare mitosis (H&E, x400). E. Malignant phyllodes tumor shows infiltrative margin and markedly increased stromal cellularity (H&E, x40). F. The stromal cells exhibit pleomorphic nuclei and frequent mitosis (H&E, x400).

3. Immunohistochemistry for APC7 and Ki-67 in normal tissue and breast tumor

Immunohistochemical studies on the paraffin-embedded sections of the various normal tissues revealed that the APC7 and Ki-67 antibodies recognized antigens which were located in the nucleus (Fig. 3).

Table 1 lists the APC7 and Ki-67 expressions of a variety of normal tissues or cells. Labile cells, such as epithelial cells of the gastrointestinal tract, breast, salivary gland, and lymphoid cells of the lymphoreticular system, exhibited pronounced APC7 and Ki-67 expression. By way of contrast, no or low degrees of APC7 and Ki-67 expression were noted in the permanent and the stable cells.

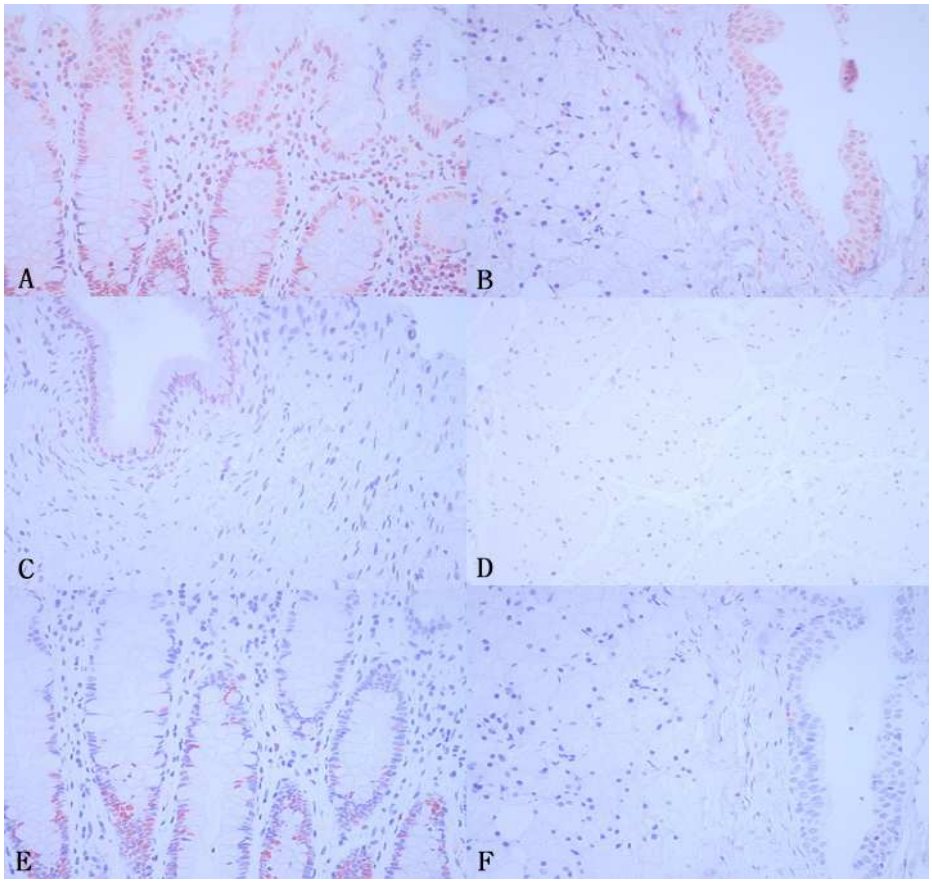

Fig. 3. Expression of APC7 (A-D) and Ki-67 (E-F) in various normal tissues or cells by immunohistochemistry. A. The colonic mucosa showing frequent APC7 expression in the crypt epithelia and lymphoid cells in the lamina propria (x400). B. The parotid gland exhibiting frequent APC7 expression in the ductal epithelia, but not in the acinic cells (x400). C. The uterine endocervix exhibiting frequent APC7 expression in epithelia, but not in the stromal cells (x400). D. The smooth muscle from uterus evidencing no APC7 expression (x400). E. The colonic mucosa showing frequent Ki-67 expression in the crypt epithelia (x400). F. The parotid gland exhibiting rare Ki-67 expression in both ductal epithelia and acinic cells (x400).

Table 1. Expression of APC7 & Ki-67 in various human normal tissues

Tissue	Cell	APC7 (grade)	Ki-67 (%)
Brain	Neuron	1	0
	Glia	1	0
Spleen	Lymphocyte	3	12
Lymph node	Lymphocyte	3	36
Skin	Epidermis	2	9
Breast	Duct	3	1
	Acinus	3	1
Salivary	Duct	3	1
	Acinus	1	0
Thyroid	Follicle	3	4
Pancreas	Acinus	1	0
	Islet cell	1	0
Heart	Myocardium	0	0
Lung	Pneumocyte	1	0
Liver	Hepatocyte	1	0
Kidney	Glomerulus	0	0
	Tubule	1	0
Ureter	Urothelium	2	0
Epididymis	Epithelium	1	0
Ovary	Stromal cell	2	0.5
Uterus	Cervix, epithelium	2	2
	Endocervix, epithelium	3	0.5
	Endometrial stromal cell	3	17
	Endometrial gland	2	19
	Myometrium	1	0
F. tube	Epithelium	1	0
Esophagus	Epithelium	2	7
Stomach	Epithelium	2	17
Jejunum	Epithelium	3	48
Ileum	Epithelium	3	52
Colon	Epithelium	3	44
Rectum	Epithelium	3	45
Skeletal M		0	0
Thymus	Lymphocyte	1	14
Pituitary		1	0
Parathyroid		1	0
Adrenal	Cortex	3	0
Bladder	Epithelium	2	0
Appendix	Epithelium	3	7
Adipocyte		0	0

Immunohistochemical data from tissue microarray. The APC7 staining pattern was graded from 0 to 3, with 0 representing no staining; 1 when <33% of nuclei were stained; 2 when 33-67% of nuclei were stained; and 3 when >67% of nuclei were stained. Ki-67 positive cells were expressed as a percentage.

Immunohistochemical staining revealed the nuclear localization of APC7 and Ki-67 proteins in all breast tumor groups in the epithelial and stromal cells. Immunostaining for APC7 revealed diffuse patterns, irrespective of area, and these tended to be unexpressed in the highly pleomorphic cells. Most of the mitotic stromal cells were found to express Ki-67. By way of contrast, APC7 expression was not determined to be related to mitosis.

The percentages of positive stromal cells expressing APC7 in the cases of fibroadenoma and juvenile fibroadenoma ranged from 7 to 64% (mean, 27.90±14.88%) and from 8 to 55% (mean, 25.45±12.44%), respectively. The percentages of positive stromal cells expressing APC7 in the cases of phyllodes tumors ranged from 36 to 87% (mean, 62.06±14.06%) in the benign, from 33 to 95% (mean, 67.30±15.42%) in the borderline, and from 45 to 92% (mean, 74.05±11.48%) in the malignant tumors. The average value of APC7 increased in order from benign to malignant within the phyllodes tumor group, and the fibroadenomas and juvenile fibroadenomas exhibited lower APC7 expression than did the cases of phyllodes tumors (26.67±13.60% versus 66.07±14.53%).

The percentages of positive stromal cells expressing Ki-67 in cases

of fibroadenoma and juvenile fibroadenoma ranged from 0.0 to 1.0% (mean, 0.14±0.21%), and from 0.1 to 3.1% (mean, 0.88±1.11%), respectively. The percentages of positive stromal cells expressing Ki-67 in the cases of phyllodes tumors ranged from 0.1 to 13.0% (mean, 1.77±2.35%) in the benign, from 0.1 to 15.0% (mean, 2.87±3.84%) in the borderline, and from 0.2 to 45.0% (mean, 11.34±11.97%) in the malignant tumors. The average value of the Ki-67 labeling index increased in order from fibroadenoma to malignant phyllodes tumor, and the cases of fibroadenomas and juvenile fibroadenomas exhibited lower Ki-67 labeling indices than did the phyllodes tumors (0.51±0.87% versus 4.29±7.42%). Table 2 summarizes the percentages of expressed APC7 and Ki-67 protein in the stromal cells of all tumor groups. Fig. 4 shows APC7 and Ki-67 immunostaining in the fibroadenoma and phyllodes tumor.

Table. 2 Labeling index of APC7 and Ki-67 in breast tumors

		APC7 (%)	Ki-67 (%)
Fibroadenoma	Conventional	27.90 ± 14.88	0.14 ± 0.21
	Juvenile	25.45 ± 12.44	0.88 ± 1.11
	Subtotal	26.67 ± 13.60	0.51 ± 0.87
Phyllodes tumor	Benign	62.06 ± 14.06	1.77 ± 2.35
	Borderline	67.30 ± 15.42	2.87 ± 3.84
	Malignant	74.05 ± 11.48	11.34 ± 11.97
	Subtotal	66.07 ± 14.53	4.29 ± 7.42

Stromal nuclear APC7 and Ki-67 staining were recorded as the numbers of APC7-positive nuclei and Ki-67 positive nuclei, divided by the total number of stromal nuclei of at least 10 fields, then expressed as a percentage.

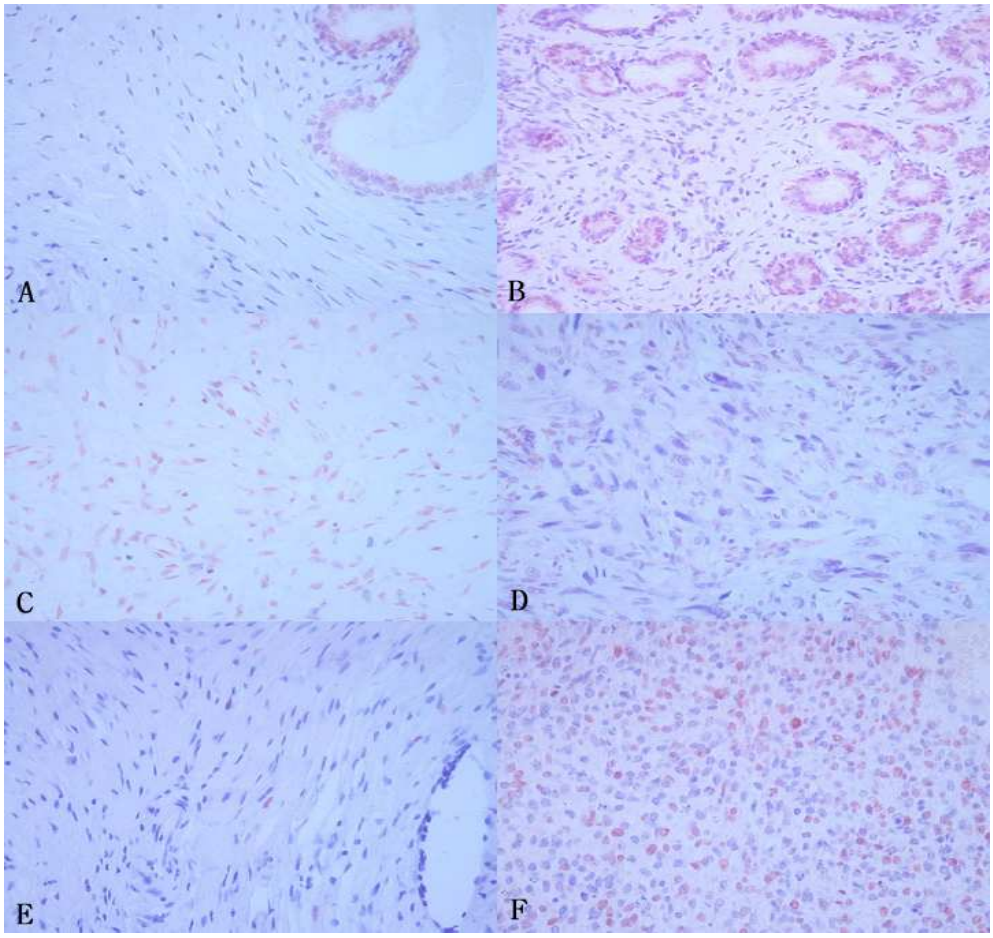

Fig. 4. Expression of immunohistochemical markers, APC7 (A-D) and Ki-67 (E-F), in breast tumors. A. Fibroadenoma with few stromal nuclei staining in low stromal cellularity; B. Juvenile fibroadenoma with low frequency of APC7 expression in relatively increased stromal cellularity; C. Benign or borderline phyllodes tumor with ample stromal nuclei staining. D. Malignant phyllodes tumor showing extensive stromal APC7 expression. The most atypical stromal nuclei are not stained. E. Benign phyllodes tumor showing a few scattered stained nuclei. F. Malignant phyllodes tumor showing frequent Ki-67 positive stromal cells.

4. Western blotting analysis of 3T3 L1 cell extract and breast tumors with APC7 antibody

Fig. 5 reveals the western blotting results for APC7 in the cultured cells and the representative breast tumor. The normal growing fibroblasts exhibited relatively high levels of expression, whereas the differentiated adipocytes exhibited no expression.

In analyzing western blotting from human breast tumors, I attempted to minimize epithelial components and choose tumors with dense stromal cellularity by examining hematoxylin-eosin stained slides, as the epithelial cells in the breast tumors expressed a relatively high amount of APC7, and the amount of APC7 was correlated with stromal cellularity. The APC7 expression levels were high in the phyllodes tumors, irrespective of subtype, and low in the cases of juvenile fibroadenoma. These data also revealed that APC7 band intensity was proportional to the APC7 labeling index, according to the results of immunohistochemistry.

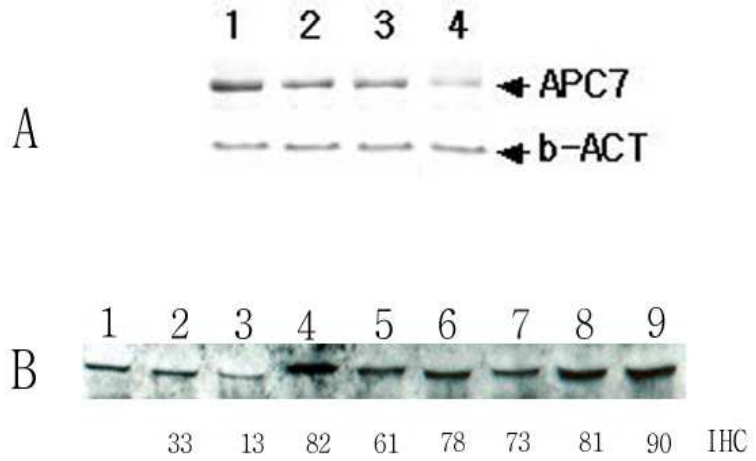

Fig. 5. Representative western blotting analysis of APC7 in 3T3 L1 cell extract (A) and breast tumor extract (B). A. Western blot of cell extracts confirmed the presence of immunoreactive APC7 in normal growing fibroblasts, but not in differentiated adipocytes. Lane 1: normal 3T3 L1 cell extract; 2, serum-free 3T3 L1 cell extract; 3, confluent 3T3 L1 cell extract; 4 differentiated adipocyte extract. B. The expression levels of APC7 were higher in the phyllodes tumors, irrespective of subtype, than in fibroadenomas. Lane 1: normal breast; 2-3, juvenile fibroadenoma; 4-5 benign phyllodes tumor; 6-7, borderline phyllodes tumor; 8-9, malignant phyllodes tumor. IHC: Labeling index of APC7 by immunohistochemistry.

5. Flow cytometry analysis

Interpretable DNA histograms were obtained in 87 cases; most of these were diploid, 3 cases were aneuploid, and 3 cases were tetraploid (Table 3). The fibroadenomas and juvenile fibroadenomas exhibited lower S-phase fractions than did the phyllodes tumors (4.19±2.15% versus 6.15±6.05%).

Two cases exhibiting aneuploidy also evidenced relatively lower levels of APC7 expression (33 and 51%, respectively).

Table 3. DNA ploidy analysis in fibroadenomas & phyllodes tumors

	FA(%)	Juvenile FA(%)	Benign PT(%)	Borderline PT(%)	Malignant PT(%)
Diploidy	11(100)	9(100)	30(100)	15(88.2)	16(80.0)
Tetraploidy	0(0)	0(0)	0(0)	1(5.9)	2(10.0)
Aneuploidy	0(0)	0(0)	0(0)	1(5.9)	2(10.0)
% S in diploid	3.49	5.05	4.14	4.63	10.95
% G2M	2.26	2.15	2.12	2.07	3.56
% S+G2M	5.75	7.20	6.26	6.70	14.52

FA: fibroadenoma, PT: phyllodes tumor

6. Statistical analysis

APC7 and Ki-67 expressions were found to be more frequent in the phyllodes tumor group than in the fibroadenoma group, by independent *t* test (p=0.000, respectively).

When analyzing APC7 expression in the five groups by the Bonferroni method (Fig. 6A), significant differences between the benign phyllodes tumors and the malignant phyllodes tumors existed (p=0.009). There were no significant differences between the fibroadenoma and juvenile fibroadenoma cases; benign phyllodes tumors and borderline phyllodes tumors; borderline phyllodes tumors and malignant phyllodes tumors.

When analyzing Ki-67 expression by the same method (Fig. 6B), significant differences between benign or borderline phyllodes tumors and malignant phyllodes tumors were detected (p=0.000). I detected no significant differences between fibroadenoma and juvenile fibroadenoma; benign phyllodes tumors and borderline phyllodes tumors.

Each fibroadenoma or juvenile fibroadenoma case exhibited significant differences with each phyllodes tumor with regard to APC7 expression. However, I detected no significant differences between each fibroadenoma or juvenile fibroadenoma and the benign or borderline phyllodes tumors with regard to Ki-67 expression.

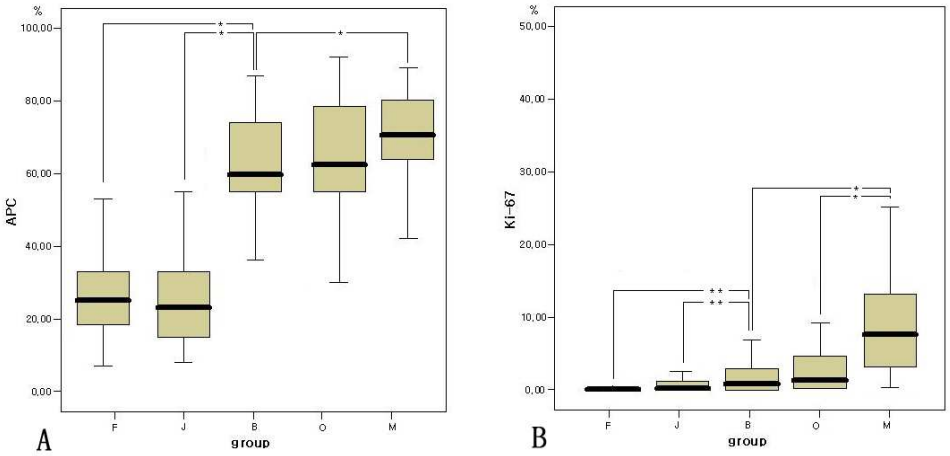

Fig. 6. Analysis of multiple groups according to APC7 (A) and Ki-67 (B) in breast tumors.
F: fibroadenoma, J: juvenile fibroadenoma, B: benign phyllodes tumor, O: borderline phyllodes tumor, M: malignant phyllodes tumor
*: statistical differences between each group, $p<0.01$
**: no statistical differences between each group, $p>0.01$

A significant positive correlation was noted between the frequency of APC7 and mitotic count ($R=0.32$, $p=0.002$), cellularity ($R=0.26$, $p=0.012$), pleomorphism ($R=0.34$, $p=0.001$) and degree of stromal overgrowth ($R=0.35$, $p=0.001$) in the phyllodes tumor group. APC7

expression was not determined to correlate with tumor size (R=-0.016, p=0.878) (Table 4). Also, Ki-67 expression correlated positively with mitotic count (R=0.632, p=0.000), cellularity (R=0.29, p=0.005), pleomorphism (R=0.53, p=0.000), degree of stromal overgrowth (R=0.46, p=0.000) and tumor size (R=0.25.p=0.015) in the cases of phyllodes tumors. In addition, APC7 and Ki-67 expressions were more frequent in the phyllodes tumors which exhibited infiltrative margins than those which exhibited pushing margins, according to the results of independent *t* tests (p=0.020, p=0.003, respectively).

The frequency of APC7 positive stromal cells correlated with Ki-67 in the cases of phyllodes tumors (R=0.21, p=0.048) and in all of the examined breast tumors (R=0.30, p=0.000).

Table 4. Pearson correlation coefficient of pathologic parameters according to APC7 & Ki-67 labeling index

	APC7 (n=92)		Ki-67 (n=92)	
Pathologic parameters	R	P-value	R	P-value
Size	-0.02	0.878	0.25	0.015
Mitosis	0.32	0.002	0.63	0.000
Cellualrity	0.26	0.012	0.29	0.005
Pleomorphism	0.34	0.001	0.55	0.000
Stromal overgrowth	0.46	0.001	0.46	0.000

Although the mean percentage of the S-phase fraction in the phyllodes tumor group was higher than that of the fibroadenoma group (6.15±6.05% versus 4.19±2.15%), the differences between the two groups were not found to be statistically significant (p=0.163).

When analyzing the percentages of S-phase fraction in the five groups by the Bonferroni method, I determined there to be a significant difference between the malignant phyllodes tumors and

other tumors.

APC7 expression did not correlate with the S-phase or G2M fractions. By way of contrast, Ki-67 expression correlated positively with the S-phase fraction (R=0.375, p=0.001) (Table 5).

Table 5. Pearson correlation coefficient of flow cytometric parameters according to APC7 & Ki-67 labeling index

Flow cytometric parameters	APC7 (n=81) R	APC7 (n=81) P-value	Ki-67 (n=81) R	Ki-67 (n=81) P-value
%S in diploid case	0.086	0.457	0.375	0.001
%G2M in diploid case	-0.047	0.680	0.042	0.716
%SG2M in diploid case	0.053	0.642	0.471	0.001

IV. Discussion

The cell-cycle functions of the APC, as well as its activator proteins, have been analyzed in proliferating animal cells. The APC core subunits APC2, CDC27, and APC7 could be detected, albeit in low amounts, in the stomach, kidney, and liver, whereas the highest amounts were discovered in extracts from the thymus, spleen, ovary, lung, and brain.[14] However, each organ is composed of various cell types, and whether the APC is expressed in the differentiated cells from each organ remains unknown. In addition, considering the mitotic functions of APC in cell proliferation, it was an unexpected finding that actively proliferating gastric epithelial cells exhibited low amounts of APC, and lung tissue predominantly composed of stable cells exhibited the highest amounts of APC. Interestingly, the neuron, which is considered a permanent cell, expressed only a small amount of APC7. The brain is the prototype of a nonproliferative tissue in mammals. However, the long-established dogma that no new neurons are generated in the brain has been determined to be incorrect, as neurogenesis does appear to occur in some areas of the adult brain. Neuronal stem cells have been identified in two areas of adult rodent brains, the olfactory bulb and the dentate gyrus of the hippocampus.[27-29]

In order to address these questions, I analyzed APC expression in a variety of normal human tissues via immunohistochemistry. As no significant differences were determined to exist with regard to APC expression among its subunits, APC2, CDC27, and APC7,[14] and as there were nonspecific cross-reactivity between APC3, APC6, and APC7, [30] I selected APC7.

In the present study, an immunohistochemical study using a normal tissue array determined that the epithelial and lymphoid cells exhibited frequent APC7 expression and the neural or glial cells of

the brain evidenced infrequent APC7 expression. The APC7 antibody exhibited nuclear staining, whereas little or no staining was observed in the cytoplasm. Consistent with the previous localization of CDC27 and CDC16,[31] APC7 resides predominantly in the nuclei of proliferating cells.

APC activity is closely related to its activator proteins, such as Cdc20 or Cdh1. APC which is fully activated at a late stage in mitosis, remains active through most of G1, and is rapidly inactivated at the G1/S phase transition in an APC^{Cdh1}-dependent manner.[32] Also, Cdc20 must be resynthesized during the S and G2 phases when APC^{Cdh1} is inactive and Cdc20 transcription is upregulated.[12,32-34] APC^{Cdc20} is sufficient to allow for cell proliferation, whereas the G1-specific function of APC^{Cdh1} is to keep cdk inactive. In yeast, Cdc20 accumulates during G2M as a result of the transcriptional activation of the Cdc20 gene.[12] However, considering that there was no significant relationship between APC7 expression and the percentage of S-phase or G2M growth fractions, as well as the detection of APC7, even in stable or permanent cells such as neurons, the immunochemistry and western blotting analysis of APC7 was not related to its activity.

The epithelial cells of the gastrointestinal tract and the ductal epithelium of the breast or salivary gland revealed the highest APC7 expressions. Of the epithelial cells, the parenchymal cells of the liver, kidney, or lung, stable cells which proliferate under some circumstances, revealed lower APC7 expression. The amounts of APC7 were found to be relatively low in most of the stromal cells, as shown in figure 3C. However, some stromal cells of the ovary and endometrium revealed frequent APC7 expression. These cells are influenced by endogenous hormones, and are thought to exhibit proliferative activity. I determined that APC7 was expressed in a

variety of mammalian tissues, and that its levels were different according to the cell type. In addition, APC7 was clearly correlated with the Ki-67 labeling index. These above results, together with the prior observation that APC loss during embryogenesis is lethal before the embryonic period,[35] suggest APC's key role in the promotion of cellular proliferation. However, the significance of APC in quiescent cells remains poorly understood. Although I was unable to demonstrate that APC7 exists in quiescent cells or permanent cells, including neurons, as a nonfunctional remnant which has been left over from the mitotic past of the cells, I have considered this possibility. With regard to cellular differentiation, APC7 was barely detectable in differentiated cells, such as adipocytes or smooth muscle cells. Of the epithelial cells, the surface epithelial cells located on the basal layer are thought to be relatively undifferentiated, as opposed to the superficial layer, which exhibited larger amounts of APC7. The APC7 levels decreased when cultured fibroblasts became differentiated adipocytes. These results suggest that the amount of APC7 is related to cellular differentiation. However, the observation that the immunoblot analysis of cell lysates prepared from hippocampal neurons differentiated in culture exhibited a constant level of the APC core subunit CDC27[14] militates against this possibility. Therefore, I suggest that the role of APC in cellular differentiation is related to the type of tissue or cell. Why, then, did APC7 disappear in the differentiated adipocytes from the fibroblasts? The answer to this question remains unknown.

Uncontrolled APC activity may be involved in tumorigenesis. As most knowledge regarding APC regulation was published in recent years, little remains known regarding the relevance of APC in cancer development. The APC substrate, securin, is crucial to the

separation of sister chromatids, and is also involved in tumorigenesis.[36] The deletion of securin, as well as the blockage of proteolysis of securin by APC^{Cdc20} both result in genomic instability.[37,38] It has recently been demonstrated that several heterozygotic mutations of the APC subunit gene can be detected in colon cancer cells.[15] It will be of interest to determine whether APC is overexpressed or down-regulated in the stromal cells which are related to tumorigenesis, such as fibroepithelial tumor of the breast.

Fibroepithelial tumor of the breast comprises a heterogenous group of biphasic lesions which combine an epithelial component and a quantitatively predominant mesenchymal component. They are classified into two categories: fibroadenomas and phyllodes tumors.

Fibroadenomas consist of duct epithelial components and stromal cell components, and the latter constitute their main component. Phyllodes tumors comprise a group of circumscribed biphasic tumors, characterized by a double layer of epithelial components arranged in clefts, and surrounded by an overgrowing hypercellular mesenchymal component, which is typically organized into leaf-like structures. Local recurrence occurs in both benign and malignant tumors. The frequency of local recurrence and metastasis correlate with the tumor grade, but vary considerably from one series to another.

The division of phyllodes tumors into benign, borderline, and malignant is essentially arbitrary, as these lie along a histologic continuum, rather than falling into discrete histological categories. This continuum is illustrated by the fact that, in different large series, the proportion of borderline phyllodes tumors differed significantly, ranging from 11.2 to 28.2%.[21,26,39-41] In this study, differentiating between benign phyllodes tumors and borderline phyllodes tumors proved difficult. For example, a few cases

diagnosed as benign phyllodes tumors would be considered borderline if at least one pathologic parameter was present to more than a mild degree. The definition of the degree of pathologic parameters into mild or moderate categories was somewhat subjective.

The average in the published data suggests a 21% rate of local recurrence overall, with 17%, 25% and 27% rates of benign, borderline, and malignant phyllodes tumors, respectively, and a 10% overall rate of metastases, with 0%, 4% and 22% rates of benign, borderline, and malignant phyllodes tumors, respectively.[19] This division is important as malignant phyllodes tumors exhibit a higher potential to metastasize. Additional factors which have been studied to assist in the differentiation of different categories of phyllodes tumors include p53, CD34, bcl-2, Ki-67, Factor XIIIa, microvessel density and DNA ploidy.[20,26,40,42-44,45]

In this study, Ki-67 exhibited significant differences between malignant phyllodes tumors and benign or borderline phyllodes tumors (p=0.000, respectively), but not between benign phyllodes tumors and borderline phyllodes tumors. These results are consistent with the results of previous reports.[20,40] APC7 revealed significant differences between benign phyllodes tumors and malignant phyllodes tumors (p=0.009), but not between borderline phyllodes tumors and benign or malignant phyllodes tumors. These results indicate that Ki-67 immunohistochemistry is superior to APC7 with regard to the differentiation between phyllodes tumors, and also show that the analysis of the cell cycle proteins, Ki-67 and APC7 in benign and malignant phyllodes tumors may also prove useful. As there were no significant differences in APC7 expression between phyllodes tumors, benign or borderline phyllodes tumors may exhibit similar potential to proliferate and form malignant tumors. In addition, sarcomatous transformations from benign phyllodes tumors may be associated with

the accumulation of various genetic alterations, including APC7 loss or other tumor suppressor genes. This hypothesis is bolstered by the fact that highly atypical stromal cells in malignant phyllodes tumors revealed no expression of APC7 in this study and some high-grade breast carcinoma showed negative APC7 expression, especially in aneuploid breast carcinoma cases.[30] In addition, a few aneuploidy cases exhibited relatively lower APC7 labeling indices. This is analogous to the progression from the normal colonic epithelium to adenoma, ultimately leading to carcinoma.

There is also a histological overlap between benign phyllodes tumors and fibroadenomas, particularly the so-called phyllodal variants, which exhibit morphological resemblances to benign phyllodes tumors. In many cases, the differential diagnosis of fibroadenoma from phyllodes tumor poses no difficulty, but benign phyllodes tumors may be difficult to distinguish from fibroadenomas. The main characteristic features include more cellular stroma, and the formation of leaf-like processes. However, the degree of hypercellularity required to qualify a phyllodes tumor at its lower limit is problematic to define. Also, some fibroadenoma and juvenile fibroadenoma exhibit highly cellular stroma, rendering it difficult to differentiate from phyllodes tumors on the basis of the stromal cell mitotic counts. Leaf-like processes may be found in intracanalicular fibroadenomas. Recently, stereotactic or ultrasonographically (US) guided breast biopsy with large-core needles has become a widely accepted alternative to needle-guided surgical biopsy with regard to the diagnosis of pathologic breast lesions, particularly benign lesions. In such cases, the differentiation between fibroadenoma and phyllodes tumors becomes more problematic, as the leaf-like structures in the phyllodes tumors, as well as the borders between the tumors and normal breast

tissue are obscure. There is a broad consensus that the discrimination between phyllodes tumors and fibroadenoma is quite important, as the malignant potential of phyllodes tumors is higher than that associated with fibroadenomas.[46] Therefore, a detailed characterization of the stromal cells of phyllodes tumors or fibroadenomas appears to be essential in the clarification of its biological characteristics. In the previous report, the analysis of the clonality of phyllodes tumors and fibroadenomas showed that both epithelial and stromal cells are polyclonal in fibroadenomas. It also showed that, in phyllodes tumors, epithelial cells are polyclonal but stromal cells are monoclonal.[47] The epithelial components are capable of undergoing somatic mutations. In turn, this may result in monoclonal proliferation, which eventually progresses to a phyllodes tumor.[46] Therefore, evaluation of the proliferating activity of the stromal cells of fibroepithelial tumors can prove helpful.

In this study, the Ki-67 labeling index did not prove helpful in the differentiation of benign phyllodes tumors from the fibroadenoma group. I found juvenile fibroadenomas with higher Ki-67 indices than some of the benign phyllodes tumors. This result is consistent with the results of previous studies.[21,48] In addition, lower Ki-67 labeling indices in both fibroadenoma groups (mean, 0.51±0.87%) and benign phyllodes tumors (mean, 1.77±2.35%) may not be helpful in small biopsied specimens. Therefore, new objective diagnostic factors, which are useful in the discrimination of fibroadenomas from benign phyllodes tumors with low Ki-67 labeling indices may be needed. The APC7 labeling index was higher than that of Ki-67, and proved useful in the differentiation of benign phyllodes tumors from fibroadenoma or juvenile fibroadenoma cases. Monoclonal antibody Ki-67 is a reliable and simple means for the accurate assessment of the

growth fraction, and G1, S, G2 phases in the cell cycle.[49] Also, tumor cells in the G0 phase can not be detected by this. If almost all of the stromal cells of benign phyllodes tumors are in G0 phase, the Ki-67 proliferation index will be as low as in a fibroadenoma, and the differentiation between two neoplasms showing different biologic behavior is difficult when using Ki-67 immunohistochemistry, as was seen in our data. By way of contrast, immunohistochemistry with APC7 detects even stromal cells in the G0 phase, which retain proliferative potential in benign phyllodes tumors.

A few fibroadenomas and juvenile fibroadenomas evidenced frequent APC7 expression, of more than 50%, although the Ki-67 labeling index was either 0.1 or 0.2 in all of those cases. In addition, we detected a few cases which exhibited low APC7 and Ki-67 in benign phyllodes tumors, in which the percentages were similar to those of the fibroadenoma group. In such cases, other pathological parameters proved insufficient for the diagnosis of phyllodes tumor, with the exception of the presence of prominent leaf-like structures. Therefore, the possibility of fibroadenoma rather than phyllodes tumor may be considered.

In conclusion, APC7 may circumvent the limitations of the Ki-67 labeling index, as it tends to be expressed at a higher frequency than does Ki-67, and APC7 and Ki-67 appear to represent useful markers in the differentiation of phyllodes tumors from fibroadenomas. However, we did not determine whether Ki-67 or APC7 expression correlated with disease-free survival, as most of the patients have shown no evidence of recurrence. Larger studies of phyllodes tumors, with more recurrent cases, will have to be assessed in order to determine the prognostic significance of APC7 expression. In addition, as APC is closely related to cell cycle regulators, it is necessary to correlate cyclin or cyclin dependent

kinase with APC, in order to determine its role.

V. Conclusion

In the present study, the expression of APC7 and Ki-67 were compared in various normal tissues and fibroepithelial tumors of breast which were divided into 5 groups: 20 cases of fibroadenoma, 20 cases of juvenile fibroadenoma, 50 cases of benign phyllodes tumor, 20 cases of borderline phyllodes tumor, and 22 cases of malignant phyllodes tumor. The relationship between pathological parameters and the above markers were also analyzed. The following results were obtained.

1) Labile cells, such as the epithelial cells of the gastrointestinal tract, breast, and salivary gland, and the lymphoid cells of the lymphoreticular system, exhibited frequent expressions of APC7 and Ki-67. However, no or low-level APC7 and Ki-67 expression was noted in permanent and stable cells.
2) The average values of the APC7 and Ki-67 labeling indices increased in order from benign to malignant within the phyllodes tumor group, and the fibroadenomas and juvenile fibroadenomas exhibited lower levels of APC7 and Ki-67 expression than did the phyllodes tumors (26.67±13.60% versus 66.07±14.53% and 0.51±0.87% versus 4.29±7.42%, respectively, p=0.000).
3) A significant positive correlation was noted between the frequency of APC7 and mitotic count (R=0.32, p=0.002), cellularity (R=0.26, p=0.012), pleomorphism (R=0.34, p=0.001), and degree of stromal overgrowth (R=0.35, p=0.001) in the phyllodes tumor group. APC7 expression was not correlated with tumor size (R=-0.016, p=0.878).
4) The Ki-67 expression correlated positively with mitotic count (R=0.632, p=0.000), cellularity (R=0.29, p=0.005), pleomorphism (R=0.53, p=0.000), degree of stromal overgrowth (R=0.46, p=0.000) and tumor size (R=0.25.p=0.015) in phyllodes tumors.

5) The frequency of APC7 positive stromal cells correlated with Ki-67 expression in phyllodes tumors (R=0.21, p=0.048), and in all of the examined breast tumors (R=0.303, p=0.000).
6) The fibroadenoma group exhibited a lower S-phase fraction than did the phyllodes tumor group (4.19±2.15% versus 6.15±6.05%), but this was determined not to be statistically significant (p=0.163). APC7 expression did not correlate with the S-phase fraction or the G2M fraction. However, Ki-67 expression was correlated positively with the S-phase fraction (R=0.375, p=0.001).

The above results indicate that APC7 and Ki-67 are closely related to cell proliferation. Also, phyllodes tumors, which are characterized by the monoclonal proliferation of stromal cells, can be differentiated from cellular fibroadenoma with increased mitotic figures mimicking phyllodes tumors by APC7 and Ki-67 immunochemistry. As APC7 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 tumors from fibroadenoma. In addition, considering APC7 loss in highly pleomorphic cells in cases of malignant phyllodes tumors, APC7 downregulation may be associated with sarcomatous transformation via chromosome instability, or accelerated oncogene signaling.

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Abstract(in Korean)

정상 조직 및 유방의 섬유샘종, 엽상종양에서의 anaphase-promoting complex7의 발현

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한재호

세포주기에 따른 사이클린(Cyclins)의 단백질 가수 분해는 세포 분열을 위한 기본적인 과정이다. 사이클린 B 단백질 분해는 APC(Anaphase-promoting complex)에 의해 이루어진다. 그러나 증식능이 없는 세포와 조직에서 APC의 발현은 잘 알려지지 않았고, 각 장기의 분화된 세포에서 APC의 발현 여부도 알려지지 않았다. 게다가 APC 발현이상 또는 기능이상에 의한 세포주기의 조절이상은 세포의 형질전환 및 암을 유발할 수 있는데, 인간의 암, 특히 유방의 엽상종양과 같은 기질성 종양(stromal tumor)에서 APC 발현 이상에 관한 보고는 거의 없었다.

이러한 문제들을 다루기 위해서, 이 연구는 다양한 정상 조직 혹은 세포, 그리고 유방의 기질세포종양에서의 ACP7 발현에 대해 조사하고, 증식표식자 Ki-67, 합성기 분획(S-phase fraction)과 비교하였다.

다양한 정상 조직과 섬유샘종 20예, 소아섬유샘종 20예, 양성 엽상종양 50예, 경계성 엽상종양 20예, 악성 엽상종양 22예로부터 아래와 같은 결과를 얻었다.

1) 위장관, 유방 및 타액선의 상피세포, 림프망상계의 림프구와 같은 불안정 세포는 빈번한 APC7과 Ki-67의 발현을 보였다. 이와는 대조적으로 영구세포와 안정세포는 APC7과 Ki-67 발현이 낮거나 거의 없었다.
2) APC7과 Ki-67 표지지수의 평균치는 엽상종양군에서 양성에서 악성으

로 갈수록 증가했다. 그리고 섬유샘종과 소아섬유샘종의 APC7과 Ki-67의 발현은 엽상종양에서의 발현보다 낮았다.

3) 엽상종양군에서 APC7의 발현은 유사분열 수, 세포밀도, 다형성, 기질의 증식 정도와 관련이 있었으나 종양의 크기와는 관련이 없었다.
4) 엽상종양군에서 Ki-67의 발현은 유사분열 수, 세포밀도, 다형성, 기질의 증식 정도 그리고 종양의 크기와 관련이 있었다.
5) 엽상종양군과 모든 유방 종양에서 APC7 양성 간질세포의 빈도는 Ki-67과 상호 관련이 있었다.
6) 유세포 측정기에 의한 합성기 분획값은 엽상종양군이 섬유샘종군 보다 더 높았으며, 합성기 분획값은 Ki-67 지수와 관련이 있었다.

위의 결과는 APC7과 Ki-67이 세포증식과 밀접한 관련이 있음을 보여준다. 그러므로 간질세포의 증식을 특징으로 하는 엽상종양은 APC7과 Ki-67 면역조직화학 검사에 의해 그와 유사하게 유사분열 수와 세포밀도가 증가된 섬유샘종과 구별될 것이다. APC7이 Ki-67보다 높은 빈도수로 발현되기 때문에, 섬유샘종으로부터 양성 엽상종양을 구별해내는데 있어 Ki-67 표지지수의 단점을 보완할 수 있다. 게다가 악성 엽상종양에서 다형성이 심한 세포에서 APC7의 발현이 안 되는 소견은 APC7의 소실이 염색체 불안정이나 종양과 관련된 유전자의 변이를 통하여 악성 종양을 유발시키는데 중요함을 시사한다.

핵심되는 말: anaphase-promoting complex, Ki-67, 섬유샘종, 엽상종양