Available online at www.sciencedirect.com
ScienceDirect
Annals of Diagnostic Pathology 13 (2009) 89–95
Annals of DIAGNOSTIC PATHOLOGY

Expression of immunoreactivity and genetic mutation in eosinophilic and ciliated metaplastic changes of endometrial glandular and stromal breakdown: cytodiagnostic implications

Keiko Shimizu, CT[a,d], Yoshiaki Norimatsu, PhD[b], Tadao K. Kobayashi, PhD[c,*], Masami Sakurai, MD, PhD[e], Seiko Ogura, CT[a], Akihiko Yoshizawa, MD[f], Tomoyuki Miyamoto, CT[g], Yasuyuki Miyake, CT[g], Yatsuki Aratake, PhD[d,g], Takuya Sakaguchi, PhD[d,g], Eiji Ohno, PhD[d,g]

[a]*Department of Pathology, Saiseikai Noe Hospital, Imperial Gift Foundation Inc, Osaka 536-0002, Japan*
[b]*Department of Medical Technology, Faculty of Health Sciences, Ehime Prefectural University of Health Sciences, Ehime 791-2101, Japan*
[c]*Department of Pathology, Saiseikai Shiga Hospital, Imperial Gift Foundation Inc, Ritto, Shiga 520-3046, Japan*
[d]*Department of Medical Life Science, College of Life Science, Kurashiki University of Science and the Arts, Kurashiki, Okayama 712-8505, Japan*
[e]*Japan Institute of Pathology Inc, Osaka 530-0014, Japan*
[f]*Department of Pathology, Kyoto University Hospital, Kyoto 606-8507, Japan*
[g]*Department of Cytopathology, Graduate School of Science and Industrial Technology, Kurashiki University of Science and the Arts, Kurashiki, Okayama 712-8505, Japan*

Abstract Various metaplastic changes may be present in endometrium, in which also cellular atypias may often be observed. Particularly, eosinophilic and ciliated changes (ECCs) occur in both nonneoplastic and neoplastic endometrium. This may cause confusion in the cytodiagnosis. This study was enterprised to investigate the possible help of immunocytochemical and cytogenetic study in the diagnostic and biologic assessment of ECC cells. In immunocytochemistry for p53 protein, Ki-67, and cyclin A, the material consists of 40 cases of cytologic smears examined by direct sampling of the endometrial cavity comprising 30 cases of ECC in endometrial glandular and stromal breakdown (EGBD) and 10 cases of well-differentiated adenocarcinoma (G1). After cytodiagnosis, immunostaining for p53 protein, Ki-67, and cyclin A was performed on multiple wet-fixed slides from each single case to evaluate the immunoreactivity, intensity of nuclear staining, and nuclear labeling index (N-LI). The intensity of nuclear staining was scored as negative (0), weak (1), moderate (2), or strong (3), and the N-LI was scored as less than 10% (0), from 10% to 25% (1), from 26% to 50% (2), or more than 50% (3), and the final score was calculated by adding both partial scores. A statistical significance test was performed by using Mann-Whitney *U* test, and the result was judged as significant when the *P* value was less than .05. For genetic mutation analysis of *p53*, the material comprised 6 cases of EGBD in which a high score was measured with immunocytochemistry for p53 protein, and the presence of ECC was confirmed on the hematoxylin and eosin. The ECC cells on paraffin-embedded specimens were captured using laser capture microdissection technology. Mutations in *p53* gene (exons 5-8) were examined using DNA sequencing analysis. In immunocytochemistry for p53 protein, Ki-67, and cyclin A, the proportions of immunoreacticve cells for p53 were statistically higher in ECC compared with those of G1 ($P < .05$). The proportions of the immunoreactive cells for Ki-67 and cyclin A were statistically higher in G1 compared with those of ECC ($P < .05$). (2) In genetic mutation analysis of *p53*, DNA sequencing of *p53* in 6 cases revealed no mutations. The percentage of immunoreactive cells for p53 protein were higher in ECC than in G1, but the

* Corresponding author: Tel.: +81 77 552 1221; fax: +81 77 552 3942.
E-mail address: tkkobaya@msn.com (T.K. Kobayashi).

1092-9134/$ – see front matter
doi:10.1016/j.anndiagpath.2008.12.007

mutation point was not confirmed in genetic mutation analysis. The differential expression of these biologic parameters in ECC cells could be considered of possible relevance to the cytopathologic diagnosis in the future.

Keywords: Endometrial cytologic examination; Eosinophilic and ciliated metaplasia; p53; Ki-67; Cyclin A; Immunocytochemistry; DNA sequencing

1. Introduction

Endometrial tissue is a sensitive target for steroid sex hormones and is able to modify its structural characteristics with promptness and versatility. Furthermore, it is known that various metaplastic changes occur in endometrial mucosa and may cause deep changes in its cytomorphological appearance, to the point of simulating malignancy [1-7]. Because nuclear enlargement is involved, eosinophilic and ciliated changes (ECCs) of the endometrium are often thought as atypical. Therefore, ECC may undermine the precision of endometrial cytodiagnosis. This tendency is especially recognized in cases of endometrial glandular and stromal breakdown (EGBD) [8]. We reported that abnormal cell clumps often observed in cytologic smears with dysfunctional uterine bleeding due to an anovulatory cycle usually contain EGBD, and they were composed of metaplastic cells [9]. The nuclear enlargement of ECC cells made the differentiation of EGBD and well-differentiated adenocarcinoma (G1) difficult. In 1999, Quddus et al [10] described the immunohistochemical overexpression of p53 in tissue sections on ECC cells. They unexpectedly observed the presence of weak and heterogenous p53 immunoreactivity in ECC cells.

We report our experience in evaluating the immunocytochemical expression of p53 protein, Ki-67, and cyclin A and genetic mutation analysis of *p53* in cytologic smears obtained by direct endometrial sampling. The aim of the current study is that of investigating in this way the possible biologic meaning of such changes in ECC cells with its reflexes on the cytopathologic diagnosis.

2. Material and methods

2.1. Immunocytochemistry for p53 protein, Ki-67, and cyclin A

The material consists of 40 cases of cytologic smears examined by direct sampling of the endometrial cavity on which histopathologic diagnoses were obtained by endometrial curettage for the period between January 2004 and December 2007. Tissue samples were routinely formalin fixed, paraffin embedded, and processed for staining with hematoxylin and eosin. There were 30 cases of ECC (EGBD) (patients aged 44-60; median age, 50.5 years) and 10 cases of G1 (patients aged 29-76; median age, 54.2 years). The endometrial cytologic samples used in the study were prepared as follows. Material was collected using the Endocyte (Laboratoire CCD, Paris, France) and then the cellular components were spread on a glass slide and smeared conventionally. Cytologic smears were immediately fixed in 95% alcohol and subsequently stained by the standard Papanicolaou method. After cytodiagnosis, smears were transferred to 3 glass slides by using a previously described method for creation of multiple slides from a single smear preparation [12,13].

For immunostaining, the smears were hydrated through descending alcohols and phosphate buffered solution. Endogenous peroxidase activity was then blocked by immersing the slides in 3% hydrogen peroxide in methyl alcohol for 10 minutes at 7°C. Heat-induced antigen retrieval was performed using a pressure cooker for 10 minutes in 0.01 mol/L of citrate buffer, pH 6.0. Nonspecific staining was eliminated by incubating the smears with normal goat serum for 10 minutes at room temperature. Monoclonal antibodies were applied as follows: against p53 (clone DO-7, dilution 1:50, Novocastra, Newcastle, United Kingdom) for 60 minutes at room temperature, against Ki-67 (clone MIB-1, dilution 1:25, Dako, Glostrup, Denmark) for 60 minutes at room temperature, and against cyclin A (clone 6E6, dilution 1:50, Novocastra) for 60 minutes at room temperature, then all the slides were washed thoroughly and incubated with Simple Stain Max-Po (Multi) (mouse and rabbit /houseradish peroxidase) reagent (Nichirei, Tokyo, Japan) for 30 minutes at room temperature, and visualized by demonstration of conjugated peroxidase with diaminobenzidine as the substrate. Smears were counterstained with hematoxylin. Staining results were evaluated by 2 independent observers (KS and SO). For the evaluation of immunoreactivity, the intensity of nuclear staining and the nuclear labeling index (N-LI) were taken into consideration. At least 150 cells in randomly selected fields were counted. The intensity of nuclear staining was scored as negative (0), weak (1), moderate (2), or strong (3). The N-LI was scored as less than 10% (0), from 10% to 25% (1), from 26% to 50% (2), or more than 50% (3). The final score was calculated of the addition of both partial scores (Table 1). For the evaluation of results, a statistical significance test was performed by using

Table 1
Immunoreactivity scoring system

Score	%	Intensity
0	<10	Negative
1	10-25	Weak
2	26-50	Moderate
3	>50	Strong

The final score was calculated by adding both partial scores.