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Original Article

Immunohistochemical detection of p53 in endometrial carcinoma for the prospect of lymphatic permeation and nodal metastasis participating in the prognosis

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Immunohistochemical analysis of endometrial carcinoma was performed with the reagents including PCNA, Ki−67, p53, CD44v3, and CD44v6. Every reagent except for p53 reacted with every specimen including normal control endometria. Normal endometrium did not react with p53, and 9% of hyperplastic ones was positive for p53. p53 positivity of all was 40% and, furthermore, increased to 100% in cases with lymphatic permeation or nodal involvement (ly+/n+), though the positive rate remained in 19% in ones without lymphatic permeation or nodal involvement(ly−/n). This study indicated that p53 was the most useful immunohistochemical marker for the prediction of lymphatic involvement in endometrial carcinoma.

Key word : carcinoma of endometrium, p53, lymphatic permeation, nodal metastasis, prognosis

Introduction

It is said that the progress of histological atypism correlates to its poor prognosis because tumor gets the biological characters of lymphatic permeation and dessemination. There have been reported, on the other hand, many metastatic cases of well differentiated type of carcinomas. So the reliable assessment of both the lymphatic permeation and nodal involvement has been required for the definition of the treatment and the prognosis. It was often difficult to identify either the lymphatic permeation or the nodal metastasis with Magnetic resonance imaging (MRI) or Computed tomography (CT). Recent gene analyses reported that the cancerous progression accompanied the overexpression of the cellular proliferation markers (PCNA,Ki−67), the abortive suppressor gene markers (p53), and the aberrant cellular adhesion ligands (CD44v3, CD44v6).(1,2) The immunohistochemical analysis of these reagents was, therefore, studied in the resected specimens and the valuable immunohistochemical predictors for preoperative lymphatic involvement were decided.

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Materials and Methods

Specimens: Specimens were composed of 20 cases of normal endometrium, 22 of hyperplasia, and 22 of carcinoma consisted of 16 cases of ly−/n− and 6 cases of ly+/n+.

Reagemts: 1. Monoclnal mouse anti−proliferating cell nuclear antigen (DAKO−PCNA,PC10), provided by DAKO Co.(Denmark), was the polymerase delta accessory protein and positive in nucleus of G1/S/G2 phase. 2. Monoclonal mouse ani−nuclear antigen Ki−67 (MIB−1), provided by lmmunotech Co. (France), reacted with nuclei of G1−M phases. 3. Monoclonal mouse anti−p53 protein, provided by immunotech Co. (France). activated both p21 (the suppression protein in the progress through cyclin to G1/S via Rb) and apoptosis. It was, furthermore, accumulated in cells as variant forms in spite of the rapid turnover and disappearance of normal forms of p53. 4. Polyclonal

rabbit anti-CD44v3 and CD44v6 (CD44 variant containing an exon 7 and exon 10, respectively), provided by Zymed Co. (USA), were the homing cell adhesion molecules and connected with extracellular hyaluronate and, furthermore, their variants were reported to be positive in metastatic cases.

Staining procedure: Formalin-fixed, paraffin-embedded tissue sections were used with immunoperoxidase procedure. The heat treatment before preparation were performed in routinely emarciated sections.

The decision of staining positivity was unrelated to its number of stained cells and we judged positive for the specimen with any obviusly stained cells.

Results

PCNA was strongly positive in nuclei of normal endometrium, hyperplasia, and carcinoma. The number of positive cells was increased with the progress of their pathological lesions. The positive pattern of Ki-67 was similar to that of PCNA but the number of positive cells was fewer than that of PCNA. p53 reactants were also identified in nuclei. In normal endometrium p53 was negative. p53 was positive in both 9% of hyperplasia and 40% ones of carcinoma regardless of their histological grading. The rate of p53 positive cases in lymphatic factor-positive ones (ly+/n+) was higher than that in negative ones (ly-/n-) with a percentage of 100 to 19, respectively (Fig, 1) . There were diffuse intracytoplasmic positivity of CD44v3 and CD44v6 regardless of their pathognomonic lesions.

Fig.1. p53-significance in the immunohistochemical differentiation between malignancy and beni gnancy in endometrium

lesions/positivity	-/±	+/++	χ^2-independence
normal	10	0	
benign	22	0	*p=0.0001
non-atypical	14	0	#p=0.002
atypical	8	0	$p=0.03
malignant	2	5	*#$

*#$ χ^2-test for independence and its significant rate between two groups.

Discussion

p53 was regarded as one of suppressor gene products which inducted the activation of p21. This suppression mechanism was inducted by the activation of p21, which suppressed both cyclin and Rb as the inductors of cell proliferation cycle factors. The intracellulay turnover of normal p53 products was so rapid that we could not discolose any localization of p53 in normal cells. The rearrangement of p53 genes frequently occurred with the progression of malignancy. Because these variant products of p53 coule not be immediately degradated, they remained in nuclei for long time and we could easily demonstrate them immunohistochemically as an overexpression.(1) The p53 overexpresson was found in 10-28% of usual endometrioid adenocarcinoma of high grade atypism (G3).(1,2) In serous subtype of endometrial carcinoma, the most atypical carcinoma, the p53 overexpression rate increased to 80-86% of cases. (2) Our study revealed that the positive rate in all carcinoma, ly-/n-, and ly+/n+ were 40%, 19%, and 100%, respectively. The results desclosed that the increment of positivity depended on the lymphatic involvement and, that is, the immunohistochemical overexpression of p53 was the most predictable marker of the lymphatic involvement. We could also disclose the p53 overexpression in endometrial hyperplasia, though it was reported that even atypical hyperplasia could not reveal the p53 overexpression. (1,2)

In PCNA and Ki-67, the marker of cell proliferation, the positive cells increased in hyperplasia rather than that in normal endometrium. Carcinoma had more positive cells than those in hyperplasia but there was no difference between ly-/n- and ly+/n+.

Both CD44v3 and CD44v6 were thought as variants of standard forms of CD44 cell adhesion molecules. Either the loss of standard forms or the expression of these variant types was reported as the expression of invasion in malignancy, especially in urogenital tract carcinoma.(3) The intracytoplasm was deffusely stained but there was no staining difference among lesions.

Addendum

The abstract of this paper has been presented at the 38th. meeting of the Japanese Society of Clinical Cytology, Takamatu, Kagawa, May 29−30, 1997. (This abstract was published on Jpn Soc Clin Cytol 1997;36(Suppl.1):182.)

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子宮内膜腺癌予後因子（リンパ管侵襲とリンパ節転移）の予測としてのp53免疫組織学的検索

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子宮内膜線癌の臨床進行期分類は、治療方針や予後の決定に重要である。今日、CT、MRI、内視鏡等により、術前における癌進行度の把握はその正確さを向上してきている。しかしながら、従来より、その悪性度の判定がより簡便で安価なことが望まれている。今回、我々は、細胞増殖因子（PCNA,Ki−67)、癌抑制遺伝子（p53）、細胞接着因子（CD44）等の免疫組織学的検討により、腺癌のリンパ行性因子について検討した。細胞核内の p53 産物の過剰発現に関して、リンパ行性転移の認められない症例と認められた症例の頻度は、それぞれ、19%と100%であった。p53 過剰発現とリンパ行性進展は有意に正相関することがわかった。術前の組織標本による悪性度の判定に関しては、一般的組織細胞学的異型度のみでなく、高分化型腺癌においても p53 陽性腺癌はリンパ行性転移に注意しなければならない。

キーワード；子宮内膜腺癌、p53、リンパ管侵襲、リンパ節転移、予後

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