Correlation between histological grading and growth-related factors in human endometrial adenocarcinomas

Y. Kuwashima

Department of Laboratory Medicine, Hanyu General Hospital - Hanyu, Saitama (Japan)

Summary

In order to clarify biological significance of histological grading of malignant tumors, several "growth-related factors" were examined and compared in human endometrial adenocarcinomas with different histological grades. Growth fraction (estimated by Ki-67 immunostain) and cytoplasmic bc1-2 and nuclear p53 overexpression were estimated immunohistochemically in 40 cases of carcinomas with different histological grades. When the grades were divided simply into 3 tiers, G1 (well); G2 (moderately); G3 (poorly differentiated), essentially no differences were found between histological grade and growth fraction. In addition, bc1-2 expression, which is known to negatively affect Ki-67 expression, had no correlation with histological grade. Only nuclear p53 overexpression, known to reflect gene alterations, tended to be more common in the higher grade groups. The results indicate that growth fraction does not correlate well with histological grade of human endometrial adenocarcinoma, as far as examined by Ki-67 and bc1-2 immunostains. The p53 gene status may have some significance in histological grade of human endometrial adenocarcinoma, although its role is not always clearly understood.

Introduction

Histological grading (differentiation status) has usually been given in pathological findings of malignant tumors from various organs [1]. However, its biological significance has not been well understood. A correlation between the tumor grade and its clinical aggressiveness has been documented in some organs [2, 3], but these reports have dealt with extreme subtypes of malignant tumors with a histogenesis which is not always clear [2, 3]. Common carcinomas are usually classified into the three grades, G1 (well); G2 (moderately); G3 (poorly differentiated) according to the morphological differentiation of tumor cells, especially in the structure of tumor cell nests [1]. Aggressiveness of the tumor is generally thought to correlate with its growth potential [4]. Here we examined the correlation between histological grade of common endometrial carcinomas and several growth-related factors which are applicable to histopathological examination. Our aim was to clarify whether histological grade of the tumor truly reflects its growth potential.

Materials and Methods

Tumors: 40 cases of endometrial adenocarcinoma of the uterus, resected surgically in the Saitama Cancer Center Hospital from 1990 to 1993 were examined [5]. None of the cases contained squamous cell carcinoma components and had received preoperative therapy. Histological grade of the tumor was expressed as G1, G2, G3 [6]. When foci of at least two histologic grades were intermingled in one case, the predominant grade was taken.

Immunohistochemistry: Representative sections(s) (1-3), usually from the central portion of the tumor, were examined. For immunostaining, the avidin-biotin (ABC) method was applied to formalin-fixed and paraffin-embedded tissue [7]. In staining for Ki-67 antigens and bc1-2 products, the sections were treated previously in a microwave for 10 min. The ABC method was performed using the Histofine SAB-PO-kit (Seikagaku Kogy, Tokyo, Japan). Counterstaining was carried out by hematoxylin for Ki-67 and by methyl green for other antigens. The primary antisera were used for the Ki-67 antigen (DAKO, CA, USA), bc1-2 product (DAKO) and p53 protein (Novocastra, U. K.). Ki-67 was reported to represent the growth fraction of the tumor [8-11]. The bc1-2 product has been reported to protect cells from apoptosis [12], this fact being confirmed in human endometrial adenocarcinoma in our previous report [13]. In addition, overexpression of bc1-2 inhibited growth of solid tumor cells in vitro [14] and greatly reduced growth fraction in human endometrial carcinoma in vivo [15].

The latter findings were described in our previous report [15]. The antiserum to p53 protein used was that against both the wild-type and mutant form of p53 gene products, and a positive reaction to this antisera suggests mutation of the p53 gene [16-18]. p53 protein has been known to inhibit cell growth [19].

Data evaluation: (a) For Ki-67 antigen, the growth fraction was assessed and expressed as percentages of at least 1,200 positive, randomly selected nuclei. (b) For bc1-2 product and p53 protein, classification was made only as negative (–), focally positive (+), or diffusely positive (++), irrespective of staining intensity. For bc1-2 product, strong staining of normal lymphocytes was used as an internal control.

Results

The results of the study are summarized in Table 1. As shown in the table, essentially no differences were found between histological grade and magnitude of growth fraction as estimated by Ki67 immunostaining. Expression status of bc1-2 products, which negatively affects growth fraction of human endometrial carcinoma (as demonstrated in our previous report [15]), also showed no essential differences among the three groups. A positive p53 signal was mainly detected in less differentiated groups, i.e. G2 and G3. Only one out of 20 G1 cases showed a focally positive signal for p53 protein.

Revised manuscript accepted for publication July 3, 1999.

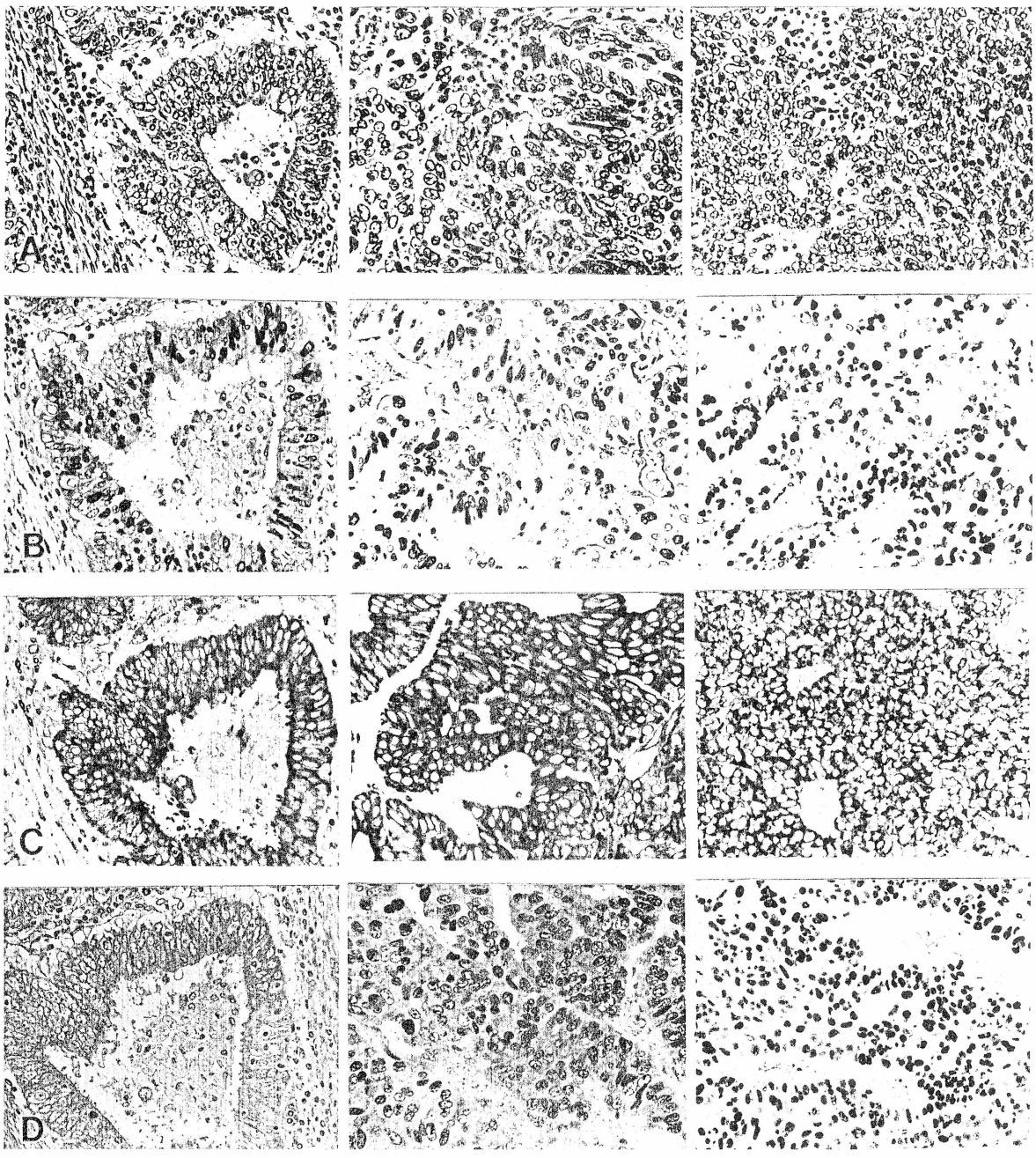

Figure 1. — Representative staining patterns for Ki-67, bc1-2 product and p53 protein. A; HE, B; Ki-67, C; bc1-2; D; p53. Left; G1, middle; G2, and right side; G3 (X170).

Fig. 1 illustrates the representative staining pattern of Ki-67, bc1-2 product and p53 protein in each of the three groups.

Discussion

Our present results clearly show that histological grade of the tumor did not reflect its growth fraction. Growth fraction (percentages of tumor cells in the cell proliferation cycle of the total tumor cells examined) has been thought to represent one of the major factors in tumor growth [20, 21]. Thus, it is speculated that histological grade of the tumor is not closely related to the "growth" of the tumor. Because "growth" of the tumor is thought to be one of the most important factors determining its aggressiveness [4], histological grading may be considered less important in the clinical outcome of the tumor.

We previously have shown apparent differences in growth fraction between undifferentiated and well differentiated ovarian cancer [22]. However, undifferentiated carci-

nomas are rare and extreme variants of ovarian cancer, and their histogenesis is still not well understood [23].

Thus so far in "common types" of carcinomas, histological grading may have only little value with respect to the growth, aggressiveness and clinical outcome of the tumor, at least in endometrial cancer, although importance of histologic grading has been reported in the prognosis of ovarian epithelial carcinoma [24].

A high frequency of p53 immunopositivity in higher grade groups (less differentiated cases) should be mentioned, although its significance may not be conclusive because p53 protein functions are extremely diverse [19]. p53 has been reported to be related to cancer-suppression in general, being involved in the control of cell growth and apoptosis [19].

Further analysis is necessary to clarify the correlation between histological grade of the tumor and p53 gene status.

Table 1. — *Summary of the present results*[a].

Case no.	Grade[b]	Growth fraction (%)[b]	bcl-2[b]	p53[b]
1	G 1	38	+	–
2	G 1	26	–	–
3	G 1	20	++	–
4	G 1	41	++	–
5	G 1	53	–	–
6	G 1	25	++	–
7	G 1	49	–	–
8	G 1	47	++	–
9	G 1	32	+	–
10	G 1	10	++	–
11	G 1	26	++	+
12	G 1	23	++	–
13	G 1	26	–	–
14	G 1	22	–	–
15	G 1	32	–	–
16	G 1	35	++	–
17	G 1	30	++	–
18	G 1	34	++	–
19	G 1	27	–	–
20	G 1	32	–	–
		(average 31%)	(12/20)[c]	(1/20)[c]
21	G 2	25	+	–
22	G 2	19	–	–
23	G 2	26	–	+
24	G 2	35	–	–
25	G 2	14	–	+
26	G 2	35	++	++
27	G 2	33	–	–
28	G 2	20	–	–
29	G 2	28	–	–
30	G 2	34	+	+
31	G 2	35	++	–
32	G 2	26	++	++
33	G 2	20	–	–
		(average 27%)	(5/13)[c]	(5/13)[c]
34	G 3	46	–	–
35	G 3	25	–	++
36	G 3	42	++	+
37	G 3	30	–	–
38	G 3	28	+	–
39	G 3	32	++	++
40	G 3	48	–	–
		(average 36%)	(3/7)[c]	(3/7)[c]

[a] The results were extracted from our previous study [5] dealing with macroscopic tumor appearance.
[b] Evaluated as in the materials and methods section.
[c] Proportion of positive cases in each group.

Acknowledgement

The authors thank Dr. H. Matsudaira for reading the manuscript.

References

[1] Rosai J.: "Ackerman's Surgical Pathology". 1996, Mosby, St. Louis.
[2] Silva E. G., Tornos C., Bailey M. A., Morris M.: "Undifferentiated carcinoma of the ovary". *Arch. Pathol. Lab. Med.*, 1991, *155*, 377.
[3] Aldinger K. A., Samaan N. A., Ibanez M., Hill C. S. Jr.: "Anaplastic carcinoma of the thyroid: a review of 84 cases of spindle and giant cell carcinoma of the thyroid". *Cancer*, 1978, *41*, 2267-2275.
[4] Sell S., Wisecarver J. L.: "Neoplasia". In: "Anderson's Pathology". 10th ed., 1996, 513. Editor Damjanov I. and Linder J., Mosbyn, St. Louis.
[5] Kuwashima Y., Uehara T., Kishi K., Shiromizu K., Matsuzawa M., Takayama S.: "Proliferative and apoptotic status in endometrial adenocarcinoma". *Int. J. Gynecol. Pathol.*, 1995, *14*, 45.
[6] Scully R. E., Bonfiglio T. A., Kurman R. J. *et al.*: "Histological Typing of Female Genital Tract Tumors". 1994, 2nd ed. Springer-Verlag.
[7] Hsu S. M., Raine L., Fanger H.: "Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures". *J. Histochem. Cytochem.*, 1981, *29*, 577.
[8] Gerdes J., Schwab U., Lemke H., Stein H.: "Production of a monoclonal antibody reactive with a human antigen associated with cell proliferation". *Int. J. Cancer*, 1983, *31*, 13.
[9] Gerdes J., Lemke H., Baisch H., Wacker H.-H., Schwab U., Stain H.: "Cell cycle analysis of a cell proliferation associated human nuclear antigen defined by monoclonal antibody Ki-67". *J. Imunol.*, 1984, *133*, 1710.
[10] Simony J., Pujol J.-L., Radel M., Ursule E., Michel F.-B., Pujol H.: "In situ evaluation of growth fraction determined by monoclonal antibody Ki-67 and ploidy in surgically resected non-small cell lung cancers". *Cancer Res.*, 1990, *50*, 4382.
[11] Schluter C., Duchrow M., Wohlenberg C., Becker M. H. G., Key G., Flad H.-D., Gerdes J.: "The cell proliferation-associated antigen of antibody Ki-67: a very large ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle maintaining proteins". *J. Cell. Biol.*, 1993, *123*, 513.
[12] Korsmeyer S. J.: "Bcl-2: an antidote to programmed cell death". *Cancer Surv.*, 1992, *15*, 105.
[13] Kuwashima Y., Kobayashi Y., Kawarai A., Uehara T., Kurosumi M., Tanuma J., Shiromizu K.: "Expression of bcl-2 and apoptotic DNA fragmentation in human endometrial adenocarcinoma calls". *Anticancer Res.*, 1996, *16*, 3221.
[14] Pietenpol J. A., Papadopoulos N., Markowitz S., Willson J. K. V., Kinzler K. W., Vogelstein B.: "Paradoxical inhibition of solid tumor cell growth by bcl-2". *Cancer Res.*, 1994, *54*, 3714.
[15] Kuwashima Y., Kobayashi Y., Kurosumi M., Tanuma J., Shiromizu K., Kishi K.: "Inverse correlation between bcl-2 expression and cell growth fraction in human endometrial adenocarcinoma tissue". *Anticancer Res.*, 1997, *17*, 3773.

[16] Lane D. P., Benchimol S.: "p53: oncogene or anti-oncogene?". *Gene. Develop.*, 1990, *4*, 1.
[17] Bass I. O., Muldder J.-W.R., Offerhaus J. A., Vogelstein B., Hamilton S. R.: "An evaluation of six antibodies for immunohistochemistry of mutant p53 gene product in archival colorectal neoplasms". *J. Pathol.*, 1994, *172*, 5.
[18] Umekita Y., Kobatashi K., Saheki T., Yoshida H.: "Nuclear accumulation of p53 protein correlates with mutations in the p53 gene on archival paraffin-embedded tissue of human breast cancer". *Jpn. J. Cancer Res.*, 1994, *85*, 825.
[19] Harris C. C.: "p53 tumor suppressor gene: from basic research laboratory to the clinic - an abridged historical perspective". *Carcinogenesis*, 1996, *17*, 1187.
[20] Steel G. G.: "Growth Kinetics of Tumors". 1977, Clarendon Press, Oxford.
[21] Denekamp J.: "Cell Kinetics and Cancer Therapy". 1982, Charles C. Thomas Publisher, Illinois.
[22] Kuwashima Y., Uehara T., Kurosumi M., Shiromizu K., Matsuzawa M., Kishi K.: "Cell dynamics of undifferentiated carcinomas of the ovary. Immunohistochemical estimation of their growth fraction and apoptotic status". *Eur. J. Gynaecol. Oncol.*, 1995, *16*, 268.
[23] Scully R.: "Tumors of the ovary and maldeveloped gonads". 1979, Armed Forces Institute of Pathology, Washington D. C.
[24] Sorbe B., Frankendal B., Veress B.: "Importance of histologic grading in the prognosis of epithelial ovarian carcinoma". *Obst. Gynecol.*, 1982, *59*, 576.

Address reprint requests to:
Y. KUWASHIMA
Department of Laboratory Medicine
Hanyu General Hospital
551 Kamiiwase, Hanyu
Saitama 348 - 8505 (Japan)