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Marker Profile of Different Phases in the Transition of Normal Human Ovarian Epithelium to Ovarian Carcinomas

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To investigate whether early changes in the transformation of normal ovarian epithelial cells into tumor cells can be detected with monoclonal antibodies, a comparative immunohistochemical study was performed on normal human ovarian mesothelial cells, cystomas, cystadenomas, ovarian carcinomas, as well as granulosa cell tumor. Using monoclonal antibodies against different keratin subtypes, it was shown that mesothelial cells, ovarian cysts, cystadenomas, and carcinomas all reacted positively with broad-spectrum anti-keratin monoclonal antibodies (MAbs), as well as with MAbs to keratins 7, 8, 18, and 19. Keratins 4 and 13 were not found in mesothelial cells, but positive groups of cells were identified in several cystomas, adenomas, and carcinomas. While mesothelial cells did not react with the panepithelial marker BW495/36, invaginating metaplastic mesothelial cells, inclusion cysts, cystomas, adenomas, and carcinomas showed an increasing reactivity with BW495/36, with an increasing degree of malignancy. The reactivity of MAbs against ovarian carcinoma-associated antigens (OV-TL 3, OC 125, MOv 18, and OV-TL 10) was limited to weak staining reaction in some mesothelial cells but were found to be positive on more than 50% of the ovarian cystadenomas and more than 90% of the ovarian carcinomas. Thecal and granulosa cells of primordial, primary, and secondary follicles all reacted positively with antibodies to the broad-spectrum keratins OV-TL 12/5 and RCK 102, and to keratins 8 and 18, but not with keratins 4, 7, 13, and 19. These keratins decreased or disappeared in granulosa cells of mature follicles (Graafian follicles), whereas granulosa cell tumors did not react with anti-keratin antibodies. The reactivity of BW 495/36 was negative or limited to traces in some granulosa cells. Ovarian carcinoma-associated antigens were not expressed in granulosa cells or granulosa cell tumors. The data indicate that mesothelial cells undergoing metaplastic changes finally resulting in ovarian cystadenomas (and carcinomas) initiate the synthesis of a 200-kd glycoprotein recognized by MAb (BW 495/36), the production of ovarian carcinoma associated antigens, in addition to focal production of keratin 4 and/or 13, as seen in several samples. The granulosa cell tumors decrease or switch off their keratin production and remain negative for the 200-kd glycoprotein and the ovarian carcinoma-associated antigens. (Am J Pathol 1991, 138:455–463)

The normal ovarian surface is covered by a single layer of flat or cuboidal epithelial cells that histologically correspond to the mesothelial lining of the pleural, the pericardial, and the peritoneal cavities. The coelomic epithelium is thought to contribute, during embryogenesis, to the formation of the Müllerian duct from which the upper genital organs develop and, in later life, it can contribute to the formation of the majority of benign and malignant epithelial neoplasms of the ovary.[1] The formation of crypts, inclusion cysts, and papillary structures are considered steps in differentiation, proliferation, and possible hormone-mediated malignant transformation of entrapped ovarian epithelium.[2,3] To investigate whether early changes in the transformation of normal ovarian cells to tumor cells could be recognized with monoclonal antibodies, we carried out a comparative immunohistochemical study on normal human ovaries lined with mesothelial cells, cystomas, cystadenomas, ovarian carcinomas, as well as granulosa cells and granulosa cell tumors. The results indicate that the ovarian carcinomas derived from mesothelial cells have a phenotype that is distinct from

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the granulosa cell tumors, as marked by monoclonal antibodies to keratins, by BW495/36, and by monoclonal antibodies to ovarian carcinoma-associated antigens.

Material and Methods

Tissues

Normal and neoplastic human ovarian tissues were snap frozen immediately after surgery and stored at −80°C. For use in the indirect immunofluorescence and immunoperoxidase technique, 4- to 5-μm thick cryostat sections were air dried and used unfixed in all immunohistochemical assays. Parallel samples were processed for histologic examination (hematoxylin and eosin staining).

Immunohistochemical Techniques

Unfixed, air-dried sections were incubated with optimally diluted monoclonal antibodies for 30 minutes at room temperature. After repeated washings in phosphate-buffered saline (PBS) containing 0.5% Tween-20 (Merck, Darmstadt, FRG), pH 7.4, sections were further incubated for 30 minutes at room temperature with peroxidase conjugated rabbit anti-mouse Ig (1:100, Dako, Glostrup, Denmark), washed in PBS-Tween, and peroxidase activity detected with 0.1% 3′-3-diaminobenzidine-HCl (Sigma Chemical Co., St. Louis, MO) and 0.01% H_2O_2 for 5 minutes. Sections were counterstained with hematoxylin and eosin according to standard procedures.

The indirect immunofluorescence assay on cryostat sections was performed as described previously.[4] The following antibodies were used in this study:

1) Mouse monoclonal antibodies against ovarian carcinoma: OC 125,[5] OV-TL 3,[4] OV-TL 10,[6] and MOv 18.[7]

2) Mouse monoclonal antibodies against intermediate filament proteins: broad-spectrum keratin antibodies: OV-TL 12/5 against keratins 5, 7, 14 and 19; RCK 102 against keratins 5 and 8;[8,9] keratin 7: OV-TL 12/30 and RCK 105;[8,9] keratin 18: RGE 53, RCK 106, CK18-2, 2C8;[10,8,9] keratin 4: 6B10;[11] keratin 8: CAM 5.2 and M20;[12] keratin 13: 1C7 and 2D7,[11] keratin 19: LP2K.[13]

3) Mouse monoclonal antibodies BW 431/31 against a carcinoembryonic antigen (CEA) epitope,[14] and BW 495/36 against a 200-kd glycoprotein in epithelial cell types.[15,16] The fluorescence intensity as well as the number of positive cells were arbitrarily scored as $^-$negative, $^{+/-}$weakly positive, $^+$positive, or $^{++}$strongly positive. The immunoperoxidase reaction was scored similarly.

Both the IPO and IFA technique have been applied to all normal and tumor cases.

Results

Immunocytochemical Studies on Mesothelial Cell Ovarian Cysts(adenomas) and Carcinomas

The immunohistochemical finding of the marker profile of ovarian mesothelium and ovarian tumors are summarized in Table 1 and depicted in Figures A to L. It is obvious that all stages of malignant transformation contain keratins, as concluded from their reaction with the broad-spectrum antibodies OV-TL 12/5 and RCK 102.

Table 1 shows also that mesothelial cells contain keratins 7, 8, 18 (Figure A), and 19. Keratin 4 and 13 nor the 200-kd glycoprotein defined by the panepithelial marker BW495/36 (Figure B) were expressed in these cells. The keratins expressed in mesothelial cells were also found in ovarian cysts (Figure D), cystadenomas as well as ovarian carcinomas (Figure G). Incidentally heterogeneity was noticed in the group of adenomas and/or carcinomas assayed with the four different monoclonal antibodies to keratin 18, and the two different MAbs against keratin 8 (Table 1). The strong fluorescence or peroxidase staining reaction as observed in mesothelial cells (Figure C) was reduced or negative in cysts (Figure F) and adenomas, but again strongly expressed in carcinomas. Keratins 4 and 13, being absent in mesothelial cells, became expressed in focal groups of carcinoma cells (Figure I) in about one half of the samples.

The monoclonal antibody BW 495/36 did not stain ovarian mesothelial cells (Figure B). Within the group of ovarian cysts both negative as well as positive cases were found (Figure E2 and E1) and even heterogeneous staining was observed within the epithelial lining of a cyst. All ovarian adenomas as well as all carcinomas (Figure H) were clearly positive. This means that BW 495/36 discriminates between mesothelial cells and ovarian adenomas c.q. carcinomas, while the group of ovarian cysts constitutes an intermediate, mixed group.

The markers OV-TL 3, OC 125, and MOv 18 were found at low expression levels in a minor number of samples of mesothelial cells, but the expression level increased in cysts and in adenomas.

They were strongly positive in more than 90% of the ovarian carcinomas (Figures J, K, and L), while OV-TL 10 reacted with 60% of the carcinomas.

Although nearly all cystadenomas were stained positively with OV-TL 3, OC 125, and MOv 18, OV-TL 3 scored strongly positive with 2 of 12 samples of cystadenomas, OC 125 with 6 of 12, and MOv 18 with 6 of 11 samples. OV-TL 10, which reacted with only 60% of ovarian carcinomas, displayed also less reactivity with the benign tumors (3 of 10). The staining pattern of anti-CEA (BW431/31) was not discriminative between the groups

Table 1. *Marker Profile of Ovarian Mesothelium and Ovarian Tumors*

MAb	Antigen	Mesothelium	Cystoma Simplex	Cystadenomas	Carcinomas
RGE 53	Keratin 18	+/++ 6/6	+−/++ 11/11	+/++ 8/9 −− 1/9	+/++ 60/60
RCK 106		++ 6/6	++ 8/8	++ 9/9	++ 60/60
CK18-2		+ 4/4	++ 9/9	nd	nd
2C8		+−/+ 4/4	+H 9/9	+ 7/9 −− 2/9	nd
OV-TL 12/5	Keratins 5/7/14/19	++ 6/6	++ 12/12	++ 12/12	++ 60/60
OV-TL 12/30	Keratin	++ 6/6	++ 12/12	++ 12/12	+/++ 60/60
RCK 105	7	++ 6/6	++ 5/5	++ 4/4	++ 10/10
CAM 5.2	Keratin 8	++ 4/4	+/++ 3/3	+/++ 4/4	+/++ 1/1 −/++ 6/6
M 20		++ 4/4	++ 3/3	++ 4/4	+/++ 7/7
RCK 102	Keratins 5/8	++ 6/6	++ 9/9	++ 9/9	++ 60/60
LP2K	Keratin 19	+/++ 6/6	+−/+ 6/8 −− 2/8	+−/+ 4/8 −− 4/8	++ 8/10 +− 2/10
6B10	Keratin 4	−− 6/6	−− 7/8 ++ 1/8	−− 6/9 +H 3/9 −− 4/9	++H 4/9 +− 1/9
2D7	Keratin 13	−− 6/6	−− 5/8 +− 3/8	−− 8/9 +H 1/9	+H 4/10 −− 6/10
IC7		−− 6/6	−− 6/8 +− 2/8	−− 8/9 +H 1/9	+H 4/9 −− 5/9
BW 495/36	Panepith.	−− 6/6	−− 4/12 +− 3/12 + 5/12	+/++ 12/12	+/++ 44/44
OV-TL 3	Ovar. carc. OA3	+− 6/6	+ 5/11 +− 2/11 −− 4/11	+/++ 2/12 +−/+ 7/12 +− 3/12	++ 68/74 +− 6/74
OC 125	CA 125	−− 5/6 +− 1/6	+/++ 8/12 +− 1/12 −− 3/12	+/++ 6/12 +− 3/12 −− 3/12	++ 68/79 +− 5/79 −− 6/79
MOv 18	Glycoprot. 38-40 kDa	−− 5/5 +− 1/6	+−/+ 5/9 −− 4/9	+/++ 6/11 +−/+ 4/11 −− 1/11	++ 45/51 +− 4/51 −− 2/51
OV-TL 10	OA10	+− 4/6 −− 2/6	+/++ 5/10 −− 5/10	+ 3/10 −− 7/10	++ 30/50
BW 431/31	CEA	−− 4/6 +− 2/6	+/++ 4/12 +− 1/12 −− 7/12	+ 5/11 +− 1/11 −− 5/11	++ 17/54 +− 12/54 −− 25/54

Immunoreactivity: −− negative; +− weakly or sporadically positive; + moderately positive; ++ strongly positive; H, heterogen positive. No differences were observed in the results of IPO as well as IFA technique applied to all normal and tumor cases.

of ovarian cystomas, adenomas, and carcinomas because of its variable staining pattern.

Immunocytochemical Studies on Granulosa Cells and Granulosa Cell Tumors

Table 2 summarizes the marker profile of granulosa cells and granulosa cell tumors, which are depicted in Figures M to P. Several keratins were found in primary (Figure M) and growing follicles (Figure N) of the ovaries, while the expression of keratins in mature Graafian follicles was usually reduced to traces (Figure O and P) or undetectable. The thecal cells of the primordial follicle, as well as the granulosa cells up to the stage beyond the secondary follicles reacted with MAbs to keratin 8, 18, and with two broad spectrum antibodies OV-TL 12/5 and RCK 102. Keratins 7 and 19 were not observed in granulosa cells, except for traces in a single sample. Keratins 4 and 13 also were absent in granulosa cells. No or sporadic reactivity of BW 495/36, and of the markers OV-TL 3, OC 125, MOv 18, and OV-TL 10 was found in sporadic granulosa cells.

Although a limited number (n = 2) of granulosa cell tumors were available, none of them showed significant reactivity with the monoclonal antibodies used. Slight staining was observed on ovarian stroma in some samples with OV-TL 3.

Discussion

The ovarian surface epithelium or mesothelium is the site of origin of the largest group of ovarian tumors.[16] The

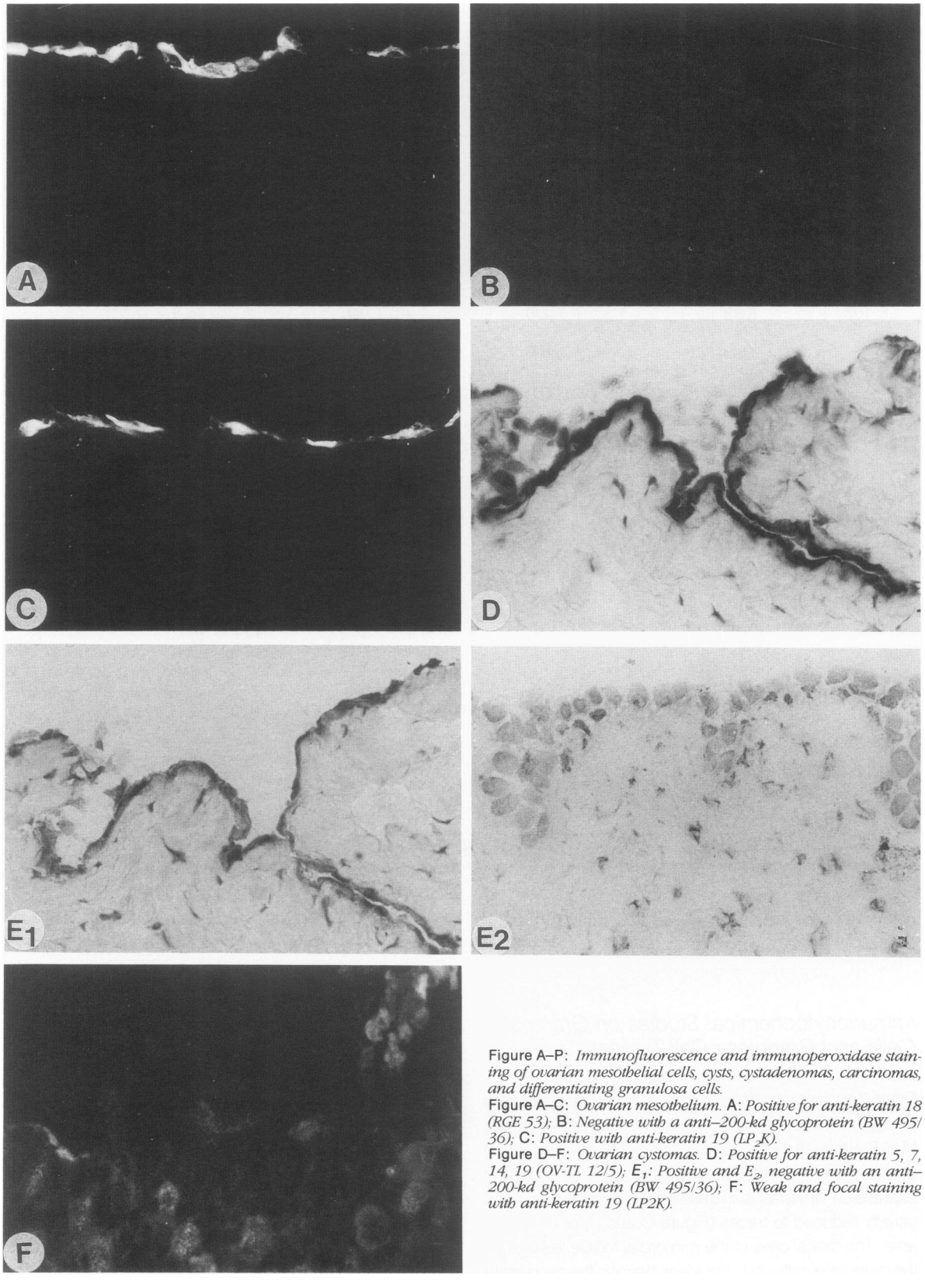

Figure A–P: *Immunofluorescence and immunoperoxidase staining of ovarian mesothelial cells, cysts, cystadenomas, carcinomas, and differentiating granulosa cells.*
Figure A–C: *Ovarian mesothelium.* A: *Positive for anti-keratin 18 (RGE 53);* B: *Negative with a anti–200-kd glycoprotein (BW 495/36);* C: *Positive with anti-keratin 19 (LP_2K).*
Figure D–F: *Ovarian cystomas.* D: *Positive for anti-keratin 5, 7, 14, 19 (OV-TL 12/5);* E_1: *Positive and* E_2, *negative with an anti–200-kd glycoprotein (BW 495/36);* F: *Weak and focal staining with anti-keratin 19 (LP2K).*

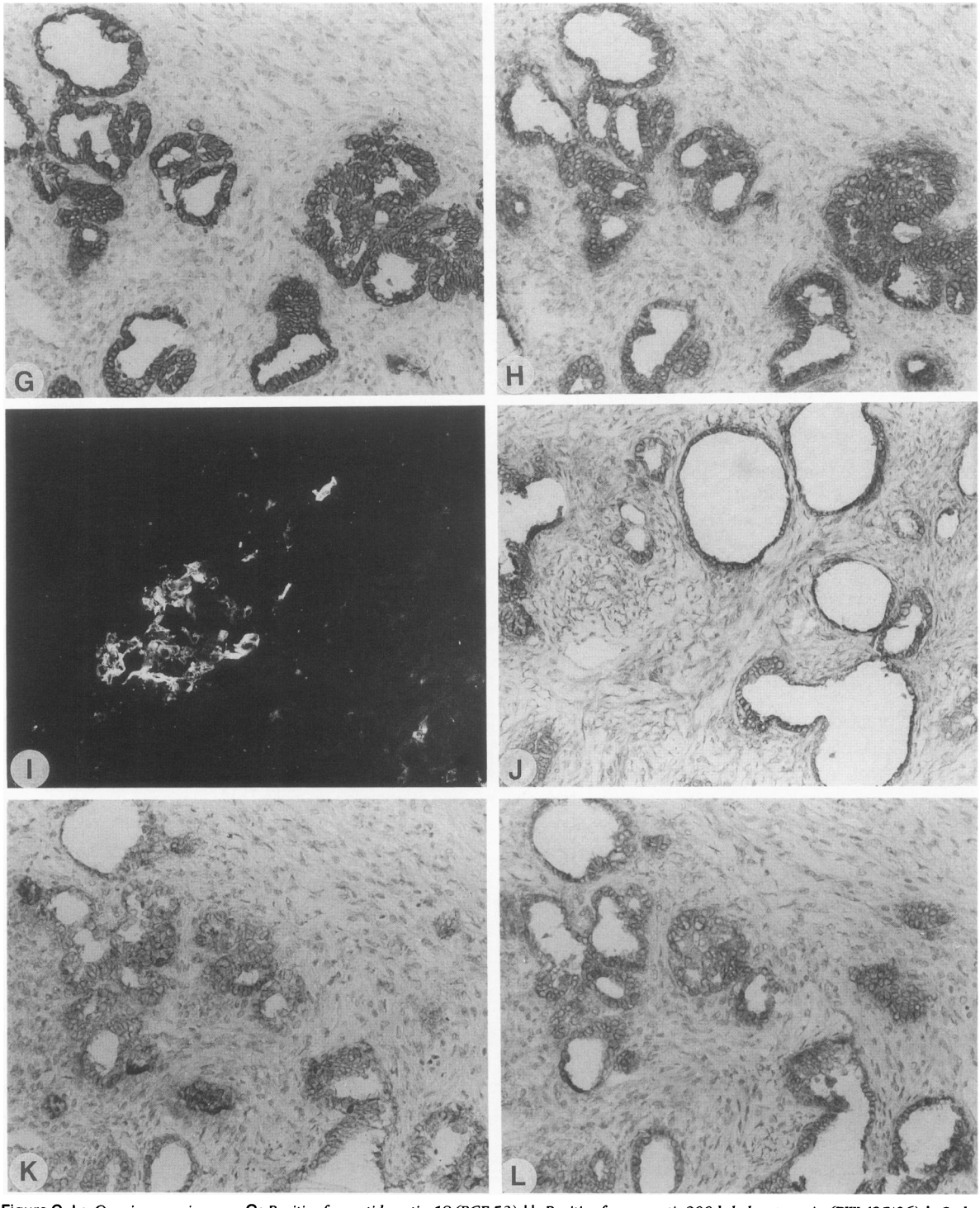

Figure G–L: *Ovarian carcinomas.* **G:** *Positive for anti-keratin 18 (RGE 53);* **H:** *Positive for an anti–200-kd glycoprotein (BW 495/36);* **I:** *Only groups of cells are positive stained with anti-keratin 4 (6B10);* **J:** *Positive for anti-ovarian carcinoma antigen (OV-TL 3);* **K:** *Positive for anti-ovarian carcinoma antigen (OC 125);* **L:** *Positive for anti-ovarian carcinoma antigen (MOv 18).*

formation of crypts, inclusion cysts, cystadenomas, and papillary structures are considered to represent steps in differentiation, proliferation, and possible malignant transformation of entrapped ovarian germinal epithelium, thought to be mediated through hormones.[2]

To investigate whether early changes in the transformation of normal ovarian epithelial cells into tumor cells can be detected with monoclonal antibodies, we compared the marker profile of normal ovaries, ovarian cystomas, adenomas, carcinomas, as well as granulosa

Table 2. *Marker Profile of Granulosa Cells and Granulosa Cell Tumors*

MAb	Antigen	Primor. foll (Gran. cells) n = 6	Primair foll (Gran. cells) n =6	Sec. foll (Gran. cells) n = 6	Graafian foll n = 2	Gran. cell tumor n = 2
RGE 53	Keratin	+/++	+	+	+−/+	−−
RCK 106	18	++	++	++	+− 1/1	−−
CK18-2		+/++ 4/4	+/++ 4/4	+/++ 4/4	−− 1/1	−−
2C8		+−/+ 4/4	+−/+ 4/4	+−/+ 4/4	−− 1/1	−−
OV-TL 12/5	Keratins					
	5/7/14/19	+/++ 4/4	+/++ 4/4	+/++ 4/4	+−	−−
OV-TL 12/30	Keratin	−−/±	−−/±	−−	+−	−−
RCK 105	7	−−/±	−−/±	−−		−−
CAM 5.2	Keratin	+/++ 4/4	+/++ 4/4	+/++ 4/4	+−/+ 1/1	− 1/1
M20	8	+/++ 4/4	+/++ 4/4	+/++ 4/4	+−/+ 1/1	− 1/1
RCK 102	Keratins					
	5/8	++	++	+	+−	−−
LP2K	Keratin	−− 5/6	−− 5/6	−− 5/6	−−	nd
	19	+− 1/6	+− 1/6	+− 1/6		
6B10	Keratin					
	4	−−	−−	−−	−−	nd
2D7	Keratin	−−	−−	−−	−−	nd
1C7	13	−−	−−	−−	−−	nd
BW 495/36	Panepith. Ov. Carc.	+−	+−	+−	−/+−	−/+−
OV-TL 3	OA3	−−	−−	−−	+−	−−
OC 125	Ca125	−−	−−	−−	−−	−−
MOv 18	Glycoprot. 38-40 kDa	−−	−−	−−	+−	−−
OV-TL 10	80A10	−−	−−	−−	−−	−−
BW 431/31	CEA	−−	−−	−−	−−	−−

Immunoreactivity: − negative; + − weakly or sporadically positive; + moderately positive; + + strongly positive; H, heterogen positive. No differences were observed in the results of IPO as well as IFA technique applied to all normal and tumor cases.

cells and granulosa cell tumors. Using monoclonal antibodies to different keratin subtypes, ovarian mesothelial cells, cystomas, adenomas, and carcinomas displayed virtually identical keratin expression patterns, although within some ovarian carcinoma subtypes heterogeneous reactivities were observed. Positive staining was seen in all cases with the broad-spectrum monoclonal antibodies OV-TL 12/5 (keratins 5, 7, 14, and 19); RCK 102 (keratins 5 and 8); OV-TL 12/30 and RCK 105 (keratin 7); RGE53, RCK 106, CK 18-2, 2C8 (all four keratins 18) CAM 5.2, and M20 (keratin 8).

From these results we conclude that normal ovarian mesothelium and ovarian carcinomas contain keratins 7, 8, 18, and 19 as their epithelial intermediate filament constituents in accordance with earlier reports in the literature.[17,18] The ovarian cystomas and adenomas, however, also containing keratins 7, 8, and 18, are variable in the expression of keratin 19.

Although keratin 19, as detected by LP2K, was found in all mesothelial cells, expression in cysts and cystadenomas was less (ie, two of eight and four of eight cases positive, respectively). All ovarian carcinomas were found (heterogeneously) positive for LP2K.

Although keratin 4 and 13 were not found in simple ovarian mesothelial cells, both keratins were expressed in scattered cells or groups of cells in several samples of the cystadenomas and carcinomas, while in one of nine ovarian carcinomas all tumor cells were strongly positive with the keratin 4 antibody. These keratins are characteristic of complex epithelia.[11,17]

Whether the expression of keratins 4 and/or 13 in some cystoma simplex samples might indicate an early metaplasia of benign epithelial cells remains to be elucidated.

As shown previously,[6] the antibody BW495/36 recognizing a 200-kd glycoprotein perfectly discriminated between ovarian mesothelial cells negative for BW 495/36 and ovarian carcinomas positive for BW 495/36. We have now extended this observation by showing that also all ovarian adenomas stained strongly positive with BW 495/36. Within the group of ovarian cysts, however, some samples were positive, while other samples showed only focal positive staining or a completely negative reaction. This suggests that generation of adenoma from cystic epithelium is marked by the initiation expression of the 200-kd glycoprotein defined by the BW 495/36. Although antibodies HMFG1 and HMFG2 have been described to discriminate between mesothelial cells (negative) and ovarian carcinoma cells (positive), the expression on ovarian cysts and adenomas (distinctive by proliferation of glandular elements), was much less marked than shown here for BW 495/36.[18]

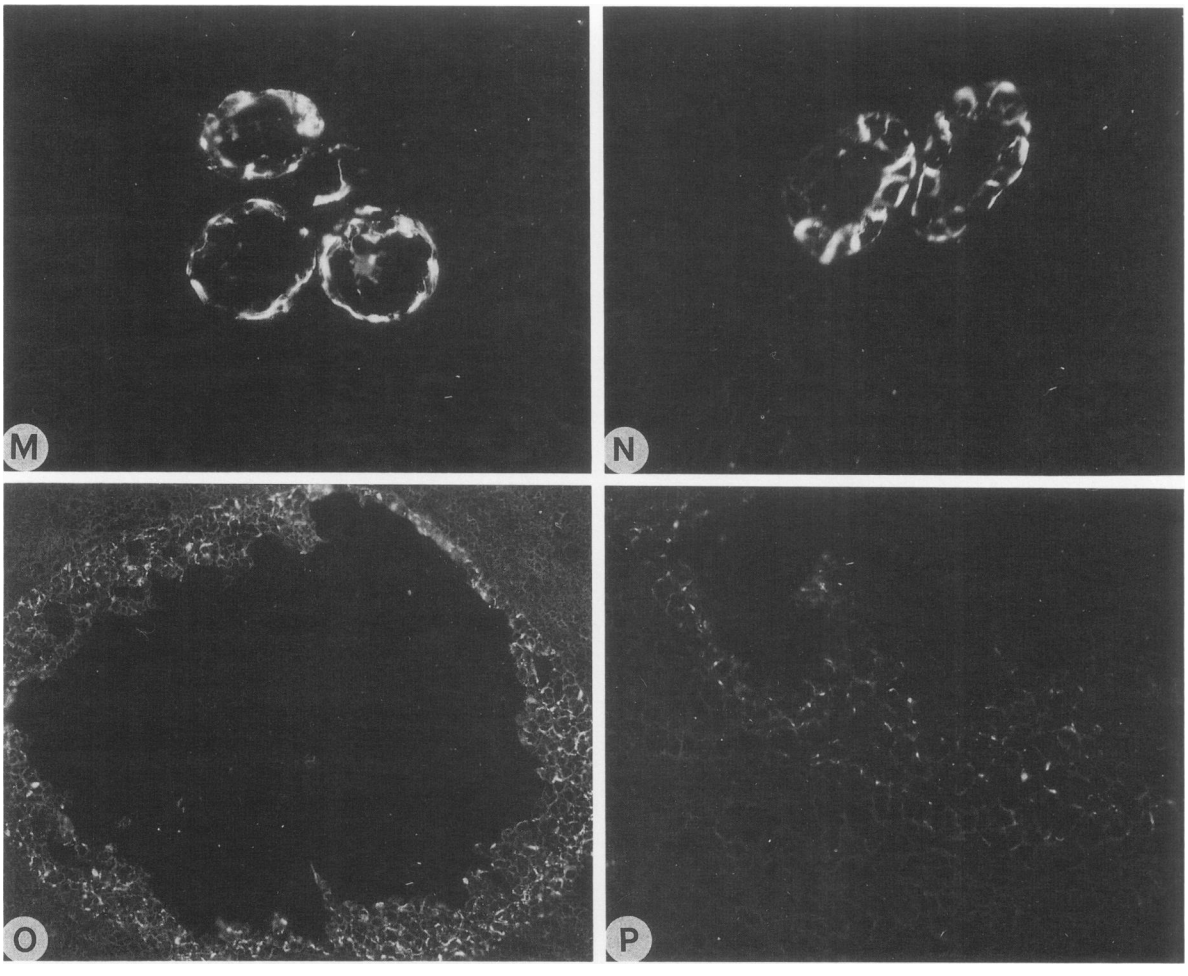

Figure M–P: *Normal ovarian follicles.* **M:** *Primary follicle positive with anti-keratins 5 and 8 (RCK 102);* **N:** *Growing follicle positive with anti-keratins 5, 7, 14 and 19 (OV-TL 12/5);* **O:** *Graafian follicle positive with anti-keratins 5 and 8 (RCK 102);* **P:** *Graafian follicle weakly positive with anti-keratins 5 and 8 (RCK 102).*

As a result, the latter antibody seems to be a better candidate to discriminate between normal and atypical mesothelial cells on the one hand and (pre)malignant ovarian cells on the other hand, in, for example, cytologic specimens of patients.

The ovarian carcinoma-associated antigens OA3, CA 125, and the MOv 18-defined antigen all were strongly expressed in more than 90% of the ovarian carcinomas examined, while the reactivity with OV-TL 10 reached a level of approximately 60%. The number of positively scored samples was also high in the group of cysts and adenomas, although the immunofluorescence reaction usually was weak. The mesothelial cells lining the ovary showed in one or more samples only weak reactivity with antibodies OV-TL 3, OC 125, MOv 18, and OV-TL 10. One can argue, therefore, that these markers are increasingly expressed in the transformation of mesothelial cells through adenoma to carcinoma rather than being expressed exclusively in ovarian cancers. Local invagination and proliferation of the germinal epithelium might, therefore, be considered as the time of initiation of tumor antigen expression, as was suggested originally for CA 125.[18]

Carcinoembryonic antigen expression was not discriminative between all stages from mesothelial cells to ovarian carcinomas. Its expression may reflect differentiating and/or proliferative state of cells by analogy with the expression of CEA in transitional epithelial cells[19] and by mesothelial cell strains in culture.[6]

A limited number of granulosa cell tumors was investigated and found to be negative for all the keratin monoclonal antibodies examined. Also the BW 495/36 antigen could not be detected. Although this observation confirms the assumption that granulosa cell tumors are not carcinomas, our study has indicated that the corresponding normal granulosa cells at least transiently expressed keratins 8 and 18 and traces of keratin 7 and 19 in a single sample. These keratins gradually disappeared on

maturation of the granulosa cells into Graafian follicles, thus extending the results described by Czernobilsky et al[1] and Ben-Ze'ev and Amsterdam[20] using different monoclonal keratin antibodies. In addition to the absence of keratins in granulosa cell tumors, the panepithelial marker BW 495/36 gave hardly any detectable reaction in both granulosa cell tumors as well as in normal granulosa cells or any other cell type in the ovary.

As discussed by Czernobilsky,[1] granulosa cells could develop from the coelomic epithelium (= mesothelium), the ovarian mesenchym, or the mesonephric tubules, which transform into the rete ovarii cords. All three cell types have been shown to coexpress keratins and vimentin,[1,21] thus making a granulosa cell histogenesis from epithelial cell types (= mesothelium or rete ovarii) plausible. The absence of BW 495/36 reactivity in both mesothelial and granulosa cells supports such a hypothesis. The keratin pattern of granulosa cells, however, was distinct partly from that of the mesothelial cells. In addition to keratins 8 and 18 in both cell types, mesothelial cells also expressed keratins 7 and 19. The absence of the latter two keratins in human granulosa cells might, however, be associated with steroid levels as recently has been shown by Ben-Ze'ev and Amsterdam[20] for human granulosa cells in culture in the presence of human chorionic gonadotrophin.

Comparing the changes from mesothelial cells to ovarian carcinomas with the changes of granulosa cells to corresponding tumors, the ovarian carcinomas activated the synthesis of the 200-kd glycoprotein as well as the production of several ovarian carcinoma-associated antigens. The granulosa cell tumors, on the other hand, deactivated the keratin synthesis and did not initiate any significant synthesis of the 200-kd glycoprotein or of the ovarian carcinoma-associated antigens.

In the future these findings may be used as diagnostic criteria, although it must be examined to what extent steroid levels influence the expression levels of all these antigens.

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