American Journal of Pathology, Vol. 142, No. 1, January 1993

Changes in Expression of Differentiation Markers Between Normal Ovarian Cells and Derived Tumors

C.C. Van Niekerk,* F.C.S. Ramaekers,† A.G.J.M. Hanselaar,‡ J. Aldeweireldt,‡ and L.G. Poels*

*From the *Department of Cell Biology and Histology, Faculty of Medical Sciences, University of Nijmegen, The Netherlands; The †Department of Molecular Cell Biology and Genetics, University of Limburg, Maastricht, The Netherlands; and the ‡Department of Pathology, University Hospital, Nijmegen, The Netherlands*

The marker profile of 18 samples of normal human ovarian tissues and 138 samples of their derived tumors was established using 51 monoclonal antibodies directed against intermediate filaments, ovarian carcinoma-specific antigens, general tumor-associated antigens and MHC-I/II antigens. Our data show that vimentin and keratins 7, 8, 18, and 19 were found in both epithelial and some nonepithelial ovarian tumors. Several tumor samples contained additional keratins 4, 10, 13, and 14, as well as desmin. BW 495/36 and to a lesser extent HMFG-2 were usually found in all ovarian tumors that contained simple epithelial keratins, except the absence of HMFG-2 in gonadal tumors as well as in dysgerminomas. In contrast to the keratin antibodies, these two panepithelial antibodies were negative in normal mesothelial cells and granulosa cells of the ovarian follicles. In general, the marker TAG-72 appeared useful for its discrimination between positively stained mucinous adenomas, the ovarian carcinomas as well as germ cell tumors, and the negatively stained gonadal tumors, serous adenomas, and cystomas. OV632 appeared useful in the distinction between negatively stained serous adenomas and positively stained serous carcinomas. In contrast, the monoclonal antibodies OC 125, OV-TL 3, OV-TL 16, and MOv 18 can be considered as pan-ovarian carcinoma markers, however without the discriminative capability as seen for OV632. These ovarian carcinoma-associated antigens were hardly found expressed in gonadal and germ cell tumors, except in the group of endodermal sinus tumors. HLA-I was found to be expressed in almost all nucleated cells, although loss of HLA-I expression was seen in areas of tumor cells. HLA-DR was negative in normal ovarian tissue, but heterogeneous expression was noticed in most of the epithelial tumors. (Am J Pathol 1993, 142:157–177)

In the Western world ovarian cancer is the most lethal of all gynecological neoplasms and responsible for approximately 50% of all deaths resulting from malignancies of the gynecological tract.[1]

Ovarian tumor histogenesis can be understood on the basis of elements that constitute the normal ovary, ie, the surface epithelium, the supporting stroma, and the germ cells. During embryogenesis the coelomic epithelium is thought to contribute to the formation of the Müllerian duct from which the upper genital organs develop.[2] Epithelial ovarian tumors are thought to arise from this germinal epithelium, which consists of modified peritoneal mesothelial cells covering the surface of the ovary.[2,3] Different types of epithelial ovarian tumors can be distinguished. The epithelium of the serous tumors resembles the epithelium of the fallopian tube, that of the mucinous tumors resembles the endocervical epithelium, and the epithelium of the endometrioid and clear cell tumors resembles that of the uterine endometrium. The Brenner tumor resembles the urothelium, and therefore it appears that cells of the ovarian surface epithelium also have the potential for differentiation along Wolffian lines.[2] Among the epithelial ovarian carcinomas, serous and endometrioid cystadenocarcinomas are most frequently observed, whereas mucinous and clear cell adenocarcinomas are the least common.[1] Sex cord-stromal tumors, which account for less than 10% of all ovarian

Accepted for publication July 7, 1992.

Address reprint requests to Dr. L. G. Poels, Department of Cell Biology and Histology, Faculty of Medical Sciences, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

tumors, may show differentiation toward female or male structures. Germ cell tumors (constituting 20% of all ovarian tumors[1]) may proliferate as undifferentiated neoplastic germ cells derived from primordial germ cells. These tumors may develop both in the gonads or at extra gonadal sites along the line of migration of these cells.

Monoclonal antibodies (Mabs) specific for ovarian cancer, and general tumor or tissue differentiation antigens can be used in diagnostic pathology to establish the nature of ovarian neoplasia. It is important to distinguish the different primary ovarian tumors, of epithelial, sex cord-stromal or germ cell origin, because the therapeutic modalities used for treatment of disseminated ovarian tumors differ between the different types and different stages of (pre)malignancy[3,4] as well from that used in the treatment of other malignancies.[1] Furthermore, the prognosis of the different ovarian tumors differs considerably.[2] The purpose of this study is to review the specificity and sensitivity of monoclonal antibodies to antigens of normal ovarian cells, ovarian tumor-associated antigens, and general tissue markers in immunohistochemically distinguishing ovarian tumors and for the detection of early changes in the transformation of normal ovarian cells to tumor cells.

Materials and Methods

Normal and neoplastic human ovarian tissue samples were obtained at the time of surgery from 156 patients. Small fresh tissue blocks were immediately snap-frozen in liquid nitrogen and stored at −80 C. Tissue sections (4 to 7 μm thick) were fixed in acetone (10 minutes at room temperature) and used in the immunoperoxidase technique as described previously.[5]

The following antibodies were used in this study:

As antibodies against intermediate filament proteins, mouse monoclonals 6B10 against keratin 4[6]; OV-TL 12/30 and RCK 105 against keratin 7[7,8,9]; M20[10], LE41[10], and CAM 5.2[11] against keratin 8; RKSE 60[11] and DE-K10[12] against keratin 10; 1C7 and 2D7[6] against keratin 13; LLOO1[13], LL002,[10,13] and RCK 107[14] against keratin 14; RGE53, RCK 106, CK 18–2, and 2C8[7,10,15] against keratin 18; LP2K[16] against keratin 19; the broad-spectrum keratin antibodies RCK 102 against keratins 5 and 8[7,8]; and OV-TL 12/5 against keratins 5, 7, 14, 19[5,7] were used. RCK 103[11] is a mouse monoclonal antibody that stains several types of glandular and squamous epithelia and transitional epithelium. Most important is its reaction with basal cell compartments and myoepithelial cells. Because such cells have been described to contain keratins 5 and 14, one of these two proteins may be among the candidates of being antigens for RCK 103. Besides a reaction in epithelial cells, neural tissues are stained also by this antibody. Vimentin was detected by RV202,[8,11] BV1118,[17] V9,[18] and polyvimentin,[17,19] whereas desmin was stained by RD 301,[11,20] polydesmin,[21] and D33.[22]

As antibodies against cell surface antigens, mouse monoclonals BW 495/36[4,23,24] against an epithelial cell type 200-kd glycoprotein, antibody HMFG-2,[25] directed against a human milk fat globule antigen, B72.3 reactive with a high molecular weight mucin-like glycoprotein complex TAG (tumor-associated glycoprotein)-72[26] and BW 431/31 [5,27] directed against a CEA epitope were used.

As antibodies against ovarian carcinoma tissues, mouse monoclonals OV-TL 3 directed against an ovarian carcinoma surface antigen,[5,28,29] and OV-TL 10 directed against a cytoplasmic antigen, present in about 60% of serous ovarian carcinomas[5] were used. OV-TL 15,[30] OV-TL 30,[30] and OV-TL 31[30] were obtained after immunizing mice with undifferentiated human ovarian cystadenoma extract and are directed against human ovarian carcinomas.

OV-TL 16,[31] OV-TL 17,[31] OV-TL 19,[31] OV-TL 22,[31] OV-TL 23,[31] OV-TL 24,[31] and OV-TL 27,[31] directed against human ovarian carcinomas, have been prepared by intrasplenic immunization of ovarian tumor derived cyst fluids.

OC 125 is directed against the CA 125 antigenic determinant and was obtained after immunizing mice with the OVCA 433 ovarian carcinoma cell line.[32]

MOv 18 is directed against an ovarian cancer-associated antigen, identified as a high-affinity folate-binding protein,[33] whereas OV632 is secreted by a hybridoma cell line that was produced from mice immunized with cyst fluid from a serous cystadenocarcinoma of the ovary.[34]

For the MHC class antigens, HLA-I (Sera lab clone W6–32), directed against nucleated cells was used. The anti-HLA-DR antigen marker (Becton Dickinson clone L-243, Etten-Leur, The Netherlands), is directed against a human monomorphic MHC class II antigen (molecular mass 28 to 34 kd with haplotypic variation). Anti-HLA-DR does not cross-react with HLA-DQ or HLA-DP molecules.

The immunoperoxidase reaction was arbitrarily scored and expressed as the number of positive cases over the total number of cases tested. Samples were considered as positive when the number of positive cells ranged between 10% and 100% in a section (without symbol); +/−, less than 10% positive tumor cells; ±, >10% weakly positive cells.

Results

The immunocytochemical reactivity patterns of the monoclonal antibodies in three groups of ovarian tumors and the corresponding normal ovarian cells are described below, summarized in Tables I through III and depicted in Figures 1 through 5.

Germinal Epithelium and Derived Tumors

Table 1 shows that the normal surface epithelium of the ovary and all stages of both benign and malignant transformation contain the simple epithelial keratins 7, 8, 18, and 19 (Figure 1A). This holds also true for the one Brenner and two mixed Müllerian tumor cases (results not shown). The keratin 7 antibodies OV-TL 12/30 and RCK 105 showed an identical reactivity except for one OV-TL 12/30 sporadically reactive adenocarcinoma sample and one partly positive endometrioid carcinoma sample, and matched its keratin 19 profile, except for one keratin 19 negative endometrioid carcinoma sample.

In the group of three keratin 8 antibodies, LE41 showed slightly immune reactivity in five samples, whereas in the group of three keratin 18 antibodies, 2C8 scored slightly less. Both keratin 4 (one Mab) and keratin 13 (two Mabs), being characteristic for noncornifying stratified epithelium, were found in a considerable number of samples of serous carcinomas as well as in mucinous adenomas and carcinomas in varying numbers of cells (Figure 1B, 1C), without, however, histological signs of stratification.

The expression in the other groups of benign and malignant epithelial tumors was much less pronounced. The normal mesothelial cells were devoid of these keratins. Strikingly in one case of a mucinous cystadenoma, keratin 13 positive metaplastic cells were found.

Keratin 10 was found in cell groups within approximately one-third of the mucinous tumor cases (Figure 1D), whereas no or hardly any expression was found in any of the other epithelial ovarian tumor groups, nor mesothelium. To exclude aspecificity, the RKSE 60 positive samples and three negative samples were reassessed with the antikeratin 10 monoclonal antibody DE-K10 and the results appeared identical.

Keratin 14 showed an expression pattern similar to keratin 4 and 13 in most tumor cases (Figure 1E). Whereas LL002-defined keratin 14 was found in more than 40% of the serous carcinomas, RCK 107 detected keratin 14 in only 1 of 16 serous carcinomas. Both monoclonal antibodies, however detected equally well the keratin 14 in about one-third of the mucinous tumor groups. The presence of keratin 14 was confirmed on all positive mucinous tumor samples by an additional keratin 14 monoclonal antibody, LL001, showing identical results as LL002. As illustrated in Figure 1E, the latter antibodies to keratins 10 and 14 showed both positive and negative areas in several of the positive cases. On basis of these and other finding we conclude that these rather unexpected findings are not a result of aspecific binding.

As expected, all samples ranging from mesothelium to ovarian carcinomas reacted positively with both broad-spectrum antikeratin Mabs (Figure 1F), although one serous cystadenoma sample and two endometrioid carcinoma samples showed less than 10% positive cells, whereas one serous carcinoma sample only stained weakly positive with RCK 103. One endometrioid carcinoma sample showed no reactivity with the broad-spectrum keratin antibody OV-TL 12/5.

Vimentin was found strongly positive in all mesothelial cells, in most cystomas and serous adenomas, but to a lesser extent in serous carcinomas (Figure 1G). Half of the mucinous adenomas and almost all mucinous carcinomas expressed vimentin heterogeneously in up to 50% of the cells. In the remaining group of carcinomas, vimentin expression was scattered or focal, in more than 50% of the samples. The presence of vimentin was again reassayed in 12 positive mucinous tumor samples using a polyclonal antibody to vimentin and two monoclonal antibodies, ie, BV1118 and V9. The results did not deviate from the data obtained with the RV202 antibody.

Desmin was found in the tumor cells of half of the mucinous adenomas (Figure 1H), half of the mucinous carcinomas, and one cystoma sample. The results of RD301 antibody were confirmed with a polyclonal antibody to desmin and the monoclonal antibody D33 in all 12 samples tested.

The panepithelial marker BW495/36, described earlier [5] for its reaction in a smaller group of tumors, as well as HMFG-2 antibody, did not react with normal mesothelial cells, and showed heterogeneous positive and negative reactions with ovarian cysts, and strong reactions with all adenomas and carcinomas (Figure 2, A and B), except for a weak BW495/36 reactivity in metaplastic cells in one case of a mucinous adenoma. HMFG-2 showed a weak reactivity with one mucinous adenoma, two endometrioid carcinomas, and a negative HMFG-2 reaction with one endometrioid carcinoma sample.

Antibody B72.3 discriminated between negative reacting mesothelial cells, cystomas, serous adenomas (Figure 2D) and positively stained serous

Table 1. *Intermediate Filament Patterns and Tumor Marker Profile of Normal Ovarian Epithelium and Germinal Epithelial Tumors*

Monoclonal Antibody	Antigen	Normal Mesothelium	Benign Cystoma	Serous		Mucinous		Endometrioid Carcinoma	Clear Cell Carcinoma	Adeno-carcinoma NOS
				Benign cyst-adenoma	Carcinoma*	Benign cyst-adenoma	Carcinoma***			
6B10	Keratin 4	0/7	2/16	2/6	9/17 +/−6/17*	10/19 +/−2/19 ±1/19	4/12* +/−5/12*	2/14 +/−5/14	1/11	2/10 +/−3/10
OV-TL 12/30[A] RCK 105	Keratin 7	7/7	16/16	6/6	17/17	19/19	12/12	14/14[A]	11/11	10/10[A]
M20[A] LE41[B,C] CAM 5.2[D]	Keratin 8	7/7	16/16	6/6[A,B]	17/17[B,D]	19/19[D]	12/12	14/14[B,C]	11/11[C]	10/10[B]
RKSE 60	Keratin 10	0/7	0/16	1/6	+/−2/17	4/19 +/−1/19 ±3/19	4/12* +/−2/12*	+/−2/14	0/11	+/−1/10
1C7	Keratin 13	0/7	+/−1/16	0/6	+/−8/17*	7/19 +/−1/19	7/12** +/−2/12	+/−4/14 ±1/14	±1/11	1/10 +/−1/10
2D7	Keratin 13	0/7	1/16	0/6	2/17 +/−5/17* ±1/17	8/19 +/−3/19 ±1/19	8/12** ±1/12	+/−5/14	±1/11	1/10 +/−2/10
LL002	Keratin 14	0/7	2/16	0/6	7/17 +/−6/17*	6/19 +/−2/19	4/12* +/−2/12*	2/14 +/−5/14	1/11 +/−1/11 ±1/11	2/10 +/−2/10
RCK 107	Keratin 14	0/7	2/16	0/6	1/17 +/−1/17	7/19 +/−3/19	5/12* +/−3/12*	+/−5/14	1/11 +/−1/11	+/−4/10
RGE53 RCK 106 CK 18–2 2C8[C,D]	Keratin 18	7/7	16/16	6/6[C]	17/17[D]	19/19	12/12	14/14[C]	11/11[D]	10/10[D]
LP2K	Keratin 19	6/6	16/16	6/6	17/17	19/19	12/12	13/14	11/11	10/10
RCK 102	Keratin 5 + 8	7/7	16/16	6/6[A]	17/17[B]	19/19	12/12	14/14[A,C]	11/11	10/10
OV-TL 12/5[C]	Keratin 5 + 7 + 14 + 19									
RCK 103[A,B]	Keratin									
RV 202	Vimentin	7/7	14/16	5/6 ±1/6	12/17* +/−5/17	12/19 +/−2/19 ±2/19	11/12 +/−1/12	10/14 +/−4/14	9/11 +/−2/11	8/10 +/−2/10
RD 301	Desmin	0/6	1/16	0/6	+/−1/17	9/19	6/12* +/−2/12*	+/−2/14	+/−1/11	+/−1/10
BW 495/36	Panepithelial 200 kd	0/7	11/15 ±3/15	6/6	17/17	19/19	12/12	14/14	11/11	10/10
HMFG-2	HMFG	0/7	7/15 ±1/15	6/6	17/17	18/19 ±1/19	12/12	11/14 ±2/14	11/11	9/10 +/−1/10
B 72.3	TAG-72	0/6	2/15	0/6	13/17 +/−3/17*	16/19 +/−2/19	12/12	11/14 +/−1/14 ±1/14	8/11 +/−2/11	7/10 +/−3/10
BW 431/31	CEA	2/6 ±2/6	9/15 ±1/15	6/6	9/17* +/−3/17 ±1/17	17/19	10/12*** +/−1/12	4/14 +/−5/14 ±1/14	8/11 +/−3/11	4/10 +/−1/10 ±1/10

OV-TL3	OA 3	±6/7	13/15 ±1/15	6/6	16/17* ±1/17	16/19 ±2/19	12/12	13/14 ±1/14	9/11 ±2/11	9/10 ±1/10
OV-TL 10	OA 10	1/6 ±2/6	8/14 ±3/14	3/6 ±3/6	7/17* +/−2/17 ±2/17	13/19 ±2/19	6/12* +/−3/12* ±2/12*	6/14 ±4/14	6/11 +/−1/11	4/10 +/−1/10 ±1/10
OV-TL 15	OA 15	0/7	5/16 ±1/16	6/6	17/17	12/19 +/−2/19 ±1/19	8/12* ±1/12*	11/14 ±1/14	8/11 +/−1/11	8/10 +/−1/10
OV-TL 16	OA 16	2/6 ±1/6	8/15 ±5/15	4/6 ±2/6	16/17* ±1/17	19/19	12/12	13/14	9/11 ±2/11	8/10 ±2/10
OV-TL 17	OA 17	±2/6	1/13 ±8/13	2/4 ±2/4	14/17* ±3/17	13/15 ±1/15	10/11** ±1/11*	7/8 +/−1/8	3/6 +/−1/6	6/10 ±3/10
OV-TL 19	OA 19	±2/7	±5/14	±3/4	14/17 +/−2/17* ±1/17	9/15 +/−1/15 ±1/15	5/9* +/−3/9* ‡	5/8 +/−1/8 ±2/8	3/6	8/10 +/−1/10 ±1/10
OV-TL 22	OA 22	0/5	1/14 ±3/14	4/4	15/17 +/−2/17*	7/15 +/−2/15 ±1/15	6/10* +/−2/10 ‡	5/8 +/−2/8	2/6 +/−1/6	5/10 +/−4/10
OV-TL 23	OA 23	±2/6	2/15 ±3/15	6/6	14/17 +/−1/17* ±2/17	11/19 ±1/19	6/12* +/−2/12	10/14 +/−2/14 ±1/14	8/11	6/10 +/−3/10
OV-TL 24	OA 24	0/6	1/13 ±4/13	3/4 +/−1/4	13/17 +/−4/17*	5/15 +/−2/15 ±2/15	6/10* ±2/10* ‡	6/8 +/−1/8	2/6 +/−2/6	6/10 +/−2/10
OV-TL 27	OA 27	1/6	6/13 ±1/13	4/4	17/17	8/15 +/−3/15	8/10** ‡	5/8 +/−2/8 ±1/8	3/6 +/−2/6	7/10 +/−1/10 ±1/10
OV-TL 30	OA 30	3/6 ±1/6	8/16 +/−2/16	3/6	15/17* +/−1/17	9/19 +/−4/19 ±1/19	7/12** +/−3/12*	9/14 +/−4/14 ±1/14	8/11 +/−1/11 ±1/11	8/10 +/−2/10
OV-TL 31	OA 31	2/6 ±1/6	4/15 ±1/15	5/6 +/−1/6	11/17 +/−3/17 ±2/17	7/19 ±1/19	4/12* +/−2/12 ±1/12	3/14 +/−4/14 ±2/14	3/11 +/−2/11 ±2/11	3/9 +/−3/9 ±2/9
OC 125	CA 125	±2/7	8/14 ±2/14	6/6	17/17	10/19 ±1/19	10/12** +/−1/12	11/14 +/−1/14	6/11 ±1/11	7/10 +/−1/10 ±1/10
MOv 18	Glycoprotein 38–40 kd	0/7	6/14 +/−1/14	6/6	17/17	16/19 +/−2/19	9/12** +/−2/12 ±1/12	13/14	11/11	9/10
OV632	Nonmucinous ovarian carcinoma	0/6	±1/15	+/−2/6	14/17 +/−2/17	9/19 +/−1/19 ±1/19	9/12**	6/14 +/−2/14	3/11	7/10 ±1/10
HLA-I	MHC-I	6/6	14/15 ±1/15	6/6	17/17	19/19	12/12	13/14	7/11 +/−3/11 ±1/11	9/10 ±1/10
HLA-DR	MHC-II	+/−1/5	9/15	5/6 +/−1/6	16/17* +/−1/17	16/19	11/12**	13/14 +/−1/14	5/11 +/−4/11 ±1/11	9/10 +/−1/10

Values are the number of positive cases over the total number of cases tested in an immunoperoxidase assay.

No symbol: 10% to 100% positive tumor cells in a section; +/−, less than 10% of positive tumor cells; ±, weakly positive cells in more than 10% of the tumor cell population. NOS, not otherwise specified.

* In 1 case, ** in 2 cases, or *** in 3 cases: borderline malignant tumor.

[A] In 1 or 2 cases +/−; [B] 1 or 2 cases ±; [C] 1 or 2 cases −; [D] in 1 to 4 cases +/− or ±.

‡ Only 2 borderline malignant tumors investigated.

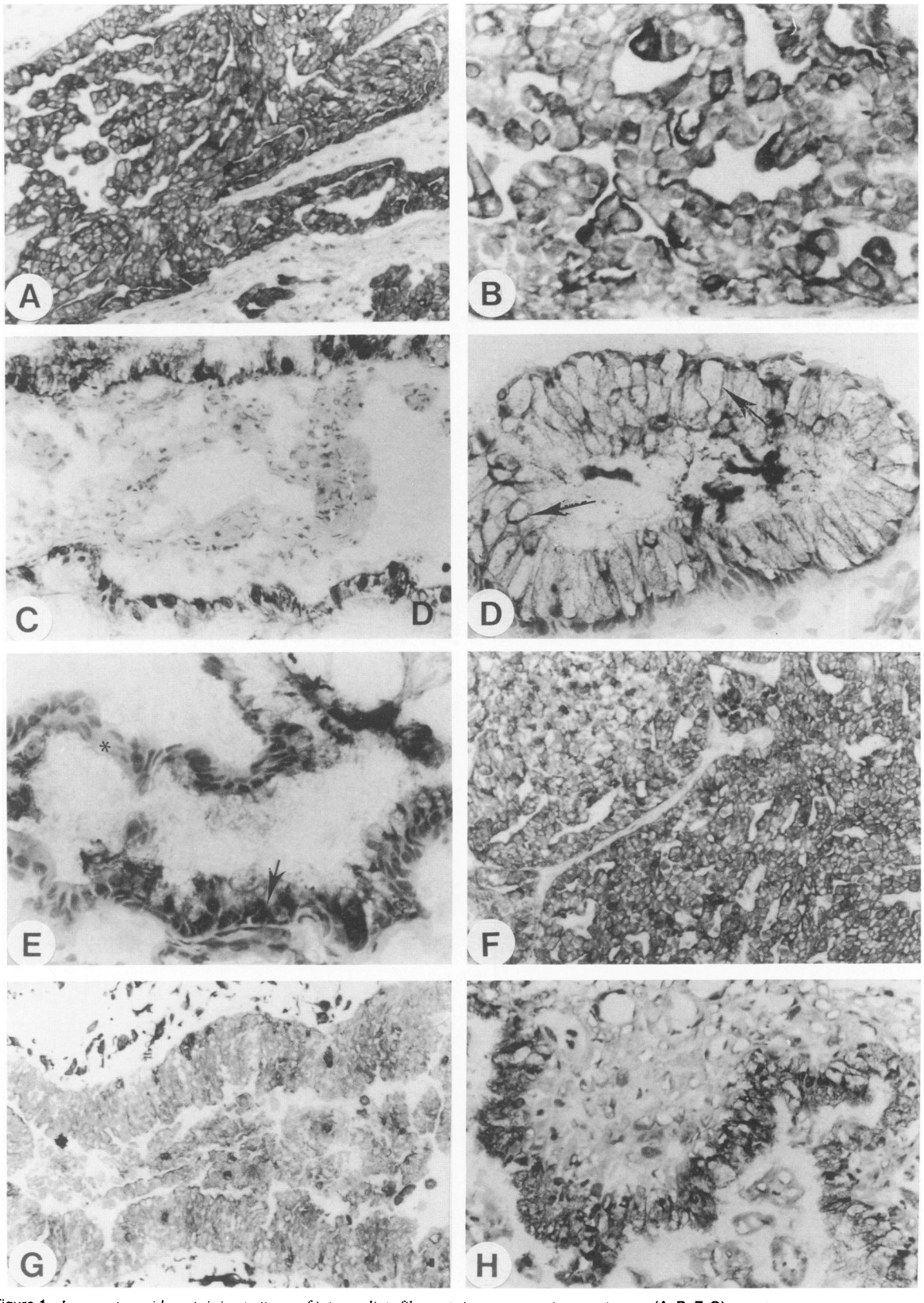

Figure 1. *Immunoperoxidase staining patterns of intermediate filaments in serous ovarian carcinomas* (A, B, F, G), *mucinous ovarian carcinomas* (C, D, E), *and a mucinous ovarian adenoma* (H), *incubated with monoclonal antibodies, antikeratin 7, OV-TL 12/30* (A); *antikeratin 4, 6B10* (B); *antikeratin 13, 2D7* (C); *antikeratin 10, DE-K10* (D); *note the positive staining of the tumor cells (arrow); antikeratin 14, LL001* (E), *note the positive staining (arrow) and negative staining (asterisk) of the tumor cells; antikeratins 5+8, RCK 102* (F); *antivimentin, RV 202* (G); *and antidesmin, RD 301* (H).

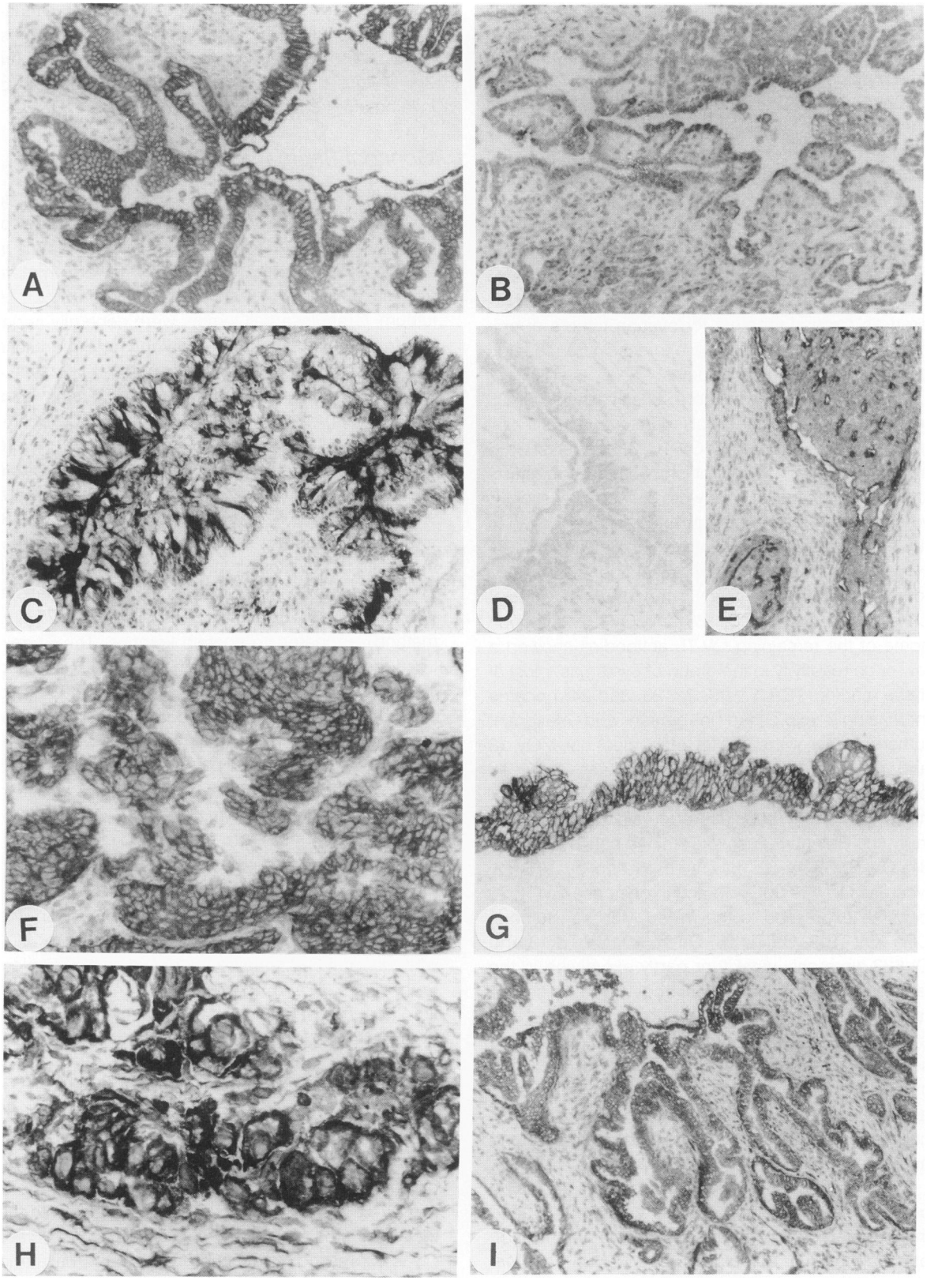

Figure 2. *Immunoperoxidase staining patterns for tumor markers and HLA markers of serous ovarian carcinomas* (A, B, I), *mucinous ovarian adenoma* (C, G), *serous adenoma* (D), *mixed serous/endometrioid ovarian carcinoma* (E, F), *and a clear cell carcinoma* (H), *incubated with panepithelial marker BW495/36* (A), *HMFG-2* (B), *anti-TAG-72, B72.3* (C, D); *antiovarian tumor antigen OC 125* (E), *OV-TL 3* (F), *OV-TL 30* (G); *anti-MHC-I and II antigens HLA-I* (H) *and HLA-DR* (I).

carcinomas. Only two (slightly) positive cystomas were observed. The antibody did not differentiate between positive mucinous adenomas (Figure 2C) and mucinous carcinomas.

Because CEA was expressed heterogeneously throughout all categories of samples, with a slightly stronger expression in the mucinous tumors, the marker was not considered as discriminative for any subtype of ovarian epithelial tumors.

The tumor markers OC 125 (Figure 2E), OV-TL 3, OV-TL 16, and MOv 18 reacted with an increasing number of samples when going from cystomas to ovarian carcinomas. Therefore, they can be considered as general markers for ovarian tumors of epithelial origin, with increasing expression from benign epithelial tumors to carcinomas.

Antibody MOv 18 was more sensitive than OC 125 in that it stained considerably more ovarian epithelial tumors. Both antibodies OV-TL 3 and OV-TL 16 showed similar immunohistochemical reactivity patterns. Mesothelial cells were stained only slightly, whereas most of the benign and malignant ovarian tumors were positive for both antibodies (Figure 2F). However, the OA 3 and OA 16 antigens were also found (weakly) expressed in most ovarian stromal cells in contrast to OC 125 and OV632. Although some of the remaining OV-TL antibodies showed a low or no reactivity in the group of cystomas, most of these markers (OA 17, 19, 22, 23, and 24) became increasingly expressed in benign and malignant tumors. None of the OV-TL antibodies, nor MOv 18 and OC 125, discriminated completely between benign ovarian tumors and carcinomas (Figure 2G), nor between any subclass of carcinomas. Interestingly, the Brenner tumor was stained only by BW431/31, OV-TL 3, OV-TL 16, OV-TL 19 and by TAG-72, whereas OC 125, MOv 18 and in one case OV-TL 30 became expressed in the mixed Müllerian tumors. CEA, OV-TL 3, OV-TL 16, OV-TL 10, OV-TL 19 and OV632 were only weakly or partly expressed in the latter tumor. Although the antibody OV632 discriminated between negative-reacting mesothelial cells, cystomas, serous adenomas, and positively stained serous carcinomas, it did not distinguish between mucinous adenomas and mucinous carcinomas, and the antibody was negative with a considerable number of samples of endometrioid, clear cell, and adenocarcinomas.

HLA-I remained expressed in almost all nucleated cells, except for one negative endometrioid and three sporadically positive clear cell carcinoma samples (Figure 2H). Sometimes, loss of HLA-I expression was seen in foci of tumor tissue, and in a number of cases areas of negative tumor cells were observed.

HLA-DR was not expressed in normal ovarian mesothelium, but was found heterogenously in most cases of the benign and malignant samples (Figure 2I), while a direct correlation with the presence of inflammatory cells was not observed.

Gonadal Stroma and Derived Tumors

Although follicle cells are not considered primarily as epithelial cells, keratins 8, 18, and 19 (and traces of keratin 7, as stained with OV-TL 12/30) were found in ovarian follicle cells, diminishing toward mature granulosa cells in Graafian follicles and corpus luteum. Granulosa cell tumors were generally negative for keratin, although remarkably one granulosa cell tumor was stained with both antikeratin 7 antibodies. Scattered stromal cells in normal ovaries were found positive with some keratin antibodies. In one fibroma sample keratins 7, 8, 18, and 19 were also found weakly or sporadically expressed (Table 2). Normal thecal cells were devoid of keratins, but in some fibrothecomas expression of keratins 7, 8, 18, and 19 was found. A similar positive keratin expression pattern was found in the granulosa-theca cell tumor (Figure 3A), in which both cell populations (identified by silver staining and sudan fat staining method) appeared to contain keratins. The Sertoli-Leydig cell tumors contained keratins 7, 8, 18, and 19 (Figure 3B), whereas strikingly, again, the Leydig cell tumor displayed keratin 7 positivity only. No other keratins than the simple epithelial types were found. Vimentin was expressed in both keratin-positive and keratin-negative normal and tumor cells. Desmin expression remained limited to two cases of fibroma-thecomas.

Except for the negative-reacting Leydig cell tumor, the expression of the panepithelial BW495/36 marker again followed the keratin expression within the group of malignant gonadal tumors (Figure 3C), whereas HMFG-2 remained negative on all samples. Both HMFG-2 and BW495/36 did not react with fibromas. TAG-72 showed no reactivity within the group of gonadal tumors or the corresponding normal ovarian cells. Most tumor markers were negative in gonadal stromal tumors, but OV-TL 3 and OV-TL 16 were moderately positive in most samples and MOv 18 reacted with two fibrothecomas and the Leydig cell tumor. OV632 reacted with two out of three granulosa cell tumors. Four Mabs (MOv 18, OV-TL 15, OV-TL 22, and BW495/36) reacted also with the zona pellucida of the oocyte (Figure 3D). HLA class I was expressed in (almost) all nucleated cells and HLA-DR was found (weakly) expressed in some primary and primordial follicle cells, in corpus luteal cells, and in granulosa cell tumors.

Table 2. *Intermediate Filament Patterns and Tumor Marker Profile of Gonadal Stroma and Gonadal Stromal Tumors*

		Normal			Benign		Malignant			
			(Pre)anthral follicle* corpus luteum**							
Monoclonal antibody	Antigen	Primord/prim follicle	Granulosa cells	Theca cells	Fibroma	Fibrothecoma	Granulosa cell tumor	Granulosa theca cell tumor	Sertoli-Leydig cell tumor	Leydig cell tumor
6B10	Keratin 4	0/14	0/11	0/11	0/3	0/11	0/3	0/2	0/2	0/1
OV-TL 12/30	Keratin 7	+/−2/13 ±7/13	1/11** ±7/11	±1/11*	±1/3	4/11 +/−3/11 ±1/11	1/3 +/−1/3 ±1/3	1/2 ±1/2	1/2	1/1
RCK 105	Keratin 7	2/15 ±1/15	0/11	0/11	±1/3	+/−1/11 ±8/11	1/3	1/2	2/2	1/1
M20	Keratin 8	15/15	11/11	0/11	+/−1/3	1/11 +/−4/11 ±1/11	+/−1/3	2/2	2/2	0/1
LE41	Keratin 8	6/16 ±2/16	±1/11*	0/11	0/3	0/11	0/3	+/−1/2 ±1/2	1/2	0/1
CAM 5.2	Keratin 8	16/16	5/10 ±5/10*	0/11	+/−1/3	1/11 ±1/11	0/3	2/2	2/2	0/1
RKSE 60	Keratin 10	0/15	0/11	0/11	0/3	0/11	0/3	0/2	0/2	0/1
1C7/2D7	Keratin 13	0/15	0/11	0/11	0/3	0/11	0/3	0/2	0/2	0/1
LL002	Keratin 14	0/16	0/11	0/11	0/3	0/11	0/3	0/2	0/2	0/1
RCK 107 RGE53	Keratin 18	13/13	6/11 +/−1/11* ±4/11	0/11	0/3	1/11	0/3	2/2	2/2	0/1
RCK 106	Keratin 18	14/15	10/11 +/−1/11*	0/11	+/−1/3	1/11 +/−2/11	+/−1/3	2/2	2/2	0/1
CK 18–2	Keratin 18	15/15	11/11	0/11	0/3	1/11 +/−2/11	+/−1/3	2/2	2/2	0/1
2C8	Keratin 18	3/16 ±6/16	±3/11*	0/11	0/3	0/11	0/3	+/−1/2	1/2	0/1
LP2K	Keratin 19	8/15 ±4/15	0/11	0/11	+/−1/3	1/11 +/−1/11	+/−1/3	2/2	2/2	0/1
RCK 102	Keratin 5 + 8	12/13	11/11	0/11	0/3	1/11 +/−1/11	+/−1/3	2/2	2/2	0/1
OV-TL 12/5	Keratin 5 + 7 + 14 + 19	8/13 ±3/13	0/11	0/11	0/3	1/11 +/−1/11	0/3	2/2	1/2	0/1
RCK 103	Keratin	1/16	0/11	0/11	0/3	0/11	0/3	1/2 ±1/2	1/2	0/1
RV 202	Vimentin	15/15	11/11	11/11	3/3	11/11	3/3	2/2	2/2	1/1
RD 301	Desmin	0/16	0/11	0/11	0/3	2/11	+/−1/3	±1/2	0/2	0/1
BW 495/36	Panepithelial 200 kd	7/13‡ ±3/13‡	1/11**	±2/11	0/3	1/11	1/3	2/2	2/2	0/1
HMFG-2	HMFG	0/15	0/11	0/11	0/3	0/11	0/3	0/2	0/2	0/1
B 72.3	TAG-72	0/15	0/10	0/10	0/3	0/11	0/3	0/2	0/2	0/1

Table 2. *Continued*

BW 431/31	CEA	0/15	0/10	0/10	0/3	±1/11	0/3	+/−1/2	0/2	0/1
OV-TL 3	OA 3	4/12 ±1/12	3/11** ±8/11*	±8/11*	2/3	4/11 ±4/11	3/3	1/2 +/−1/2	0/2	1/1
OV-TL 10	OA 10	0/16	0/10	0/10	0/3	0/11	0/3	0/2	±1/2	0/1
OV-TL 15	OA 15	3/15‡ ±4/15‡	±1/11**	0/11	0/3	0/11	0/3	0/2	0/2	0/1
OV-TL 16	OA 16	2/13 ±2/13	1/10** ±9/10	±7/10*	1/3	3/11 ±4/11	3/3	1/2	1/2	1/1
OV-TL 17	OA 17	±2/9	1/10** ±5/10	0/10	0/1	+/−5/9	+/−1/3 ±1/3	1/1	ND	ND
OV-TL 19	OA 19	±1/11	1/10**	0/10	1/1	1/9	0/3	0/1	ND	ND
OV-TL 22	OA 22	4/13‡	0/9	0/9	0/1	0/9	0/3	0/1	ND	ND
OV-TL 23	OA 23	5/15 ±1/15	±1/10**	0/10	0/3	0/11	0/3	0/2	0/2	0/1
OV-TL 24	OA 24	1/11‡	0/10	0/10	0/1	0/9	0/3	0/1	ND	ND
OV-TL 27	OA 27	1/10‡ ±2/10	4/10* ±2/10	0/10	±1/1	±5/9	2/3	+/−1/1	ND	ND
OV-TL 30	OA 30	9/15 ±3/15	0/11	9/11 ±2/11	0/3	1/11 +/−1/11	+/−1/3	±2/2	±2/2	0/1
OV-TL 31	OA 31	±5/15	±2/11*	0/11	0/3	1/11 ±1/11	0/3	0/2	0/2	0/1
OC 125	CA 125	0/15	0/10	0/10	0/3	0/11	0/3	0/2	0/2	0/1
MOv 18	Glycoprotein 38–40 kd	11/15‡ ±1/15‡	1/10* 1/10**	0/10	±1/3	2/11	0/3	±1/2	0/2	1/1
OV632	Nonmucinous ovarian carcinoma	0/16	1/11** ±4/11*	0/11	0/3	±1/11	2/3	0/2	0/2	0/1
HLA-I	MHC-I	11/12 ±1/12	7/11 ±4/11*	11/11	1/3 ±1/3	5/11 +/−6/11	3/3	2/2	2/2	1/1
HLA-DR	MHC-II	±5/13	2/11**	0/11	1/3	4/11 +/−4/11	2/3	1/2	±1/2	0/1

Values are the number of positive cases over the total number of cases tested in an immunoperoxidase assay.
No symbol, 10% to 100% positive tumor cells in a section; +/−, less than 10% of positive tumor cells; ±, weakly positive cells in more than 10% of the tumor cell population.
* (Pre)anthral follicle (8 samples); ** corpus luteum (3 samples).
‡ Only zona pallucida positive.

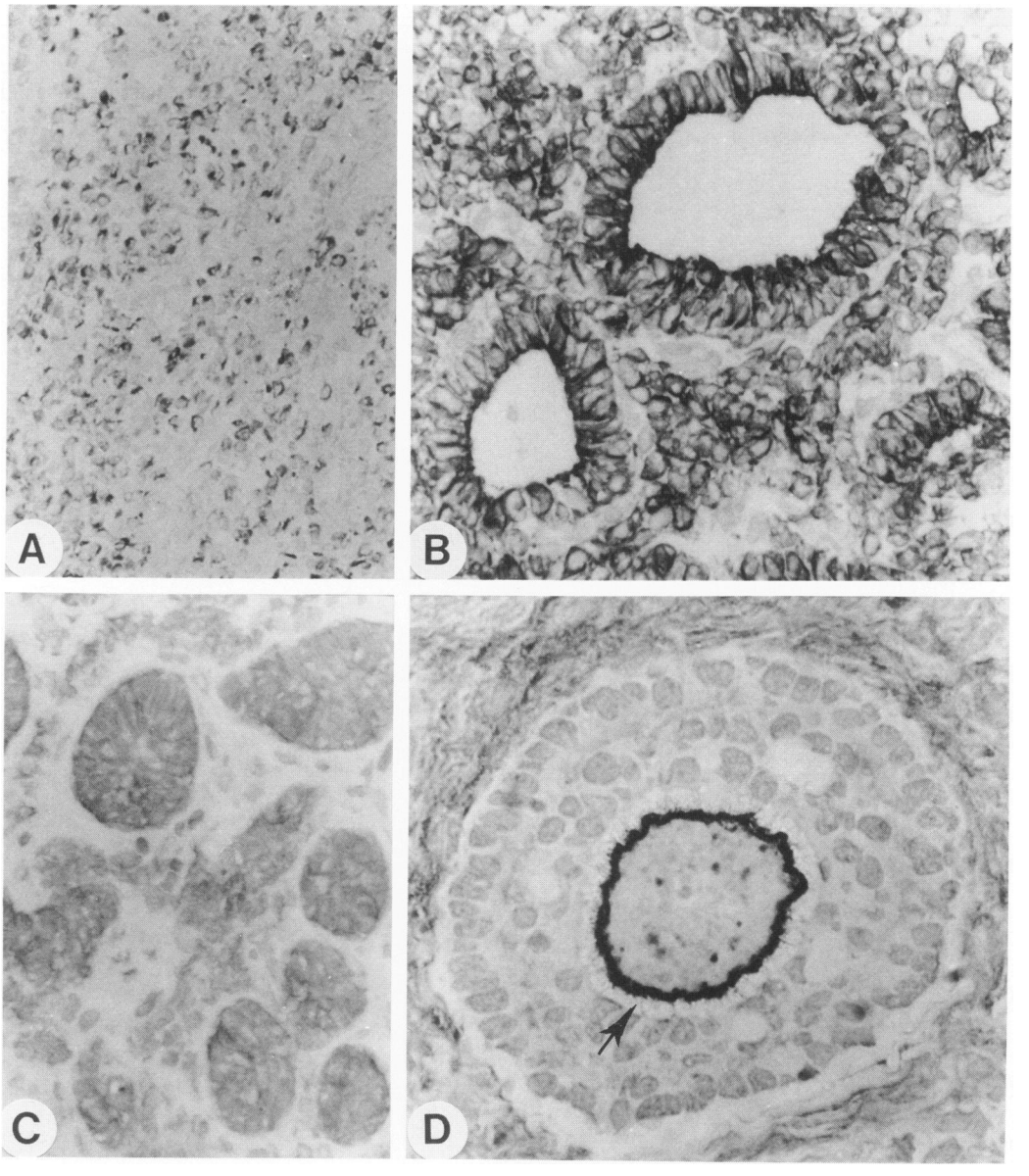

Figure 3. *Immunoperoxidase staining patterns of ovarian granulosa theca cell tumor* (A), *ovarian Sertoli-Leydig cell tumor* (B, C), *and (pre)anthral follicle, with positive zona pellucida* (D→), *incubated with monoclonal antikeratin antibodies 5+8, RCK 102* (A); *keratin 18, CK 18–2* (B); *antipanepithelial marker BW495/36* (C), *and antiovarian carcinoma marker MOv 18* (D).

Germ Cell Tumors

The group of ovarian teratomas consisted of four cases of dermoid cysts, two containing simple epithelium, one containing (cornified) squamous epithelium, and one with possible epithelial elements (Table 3).

Both simple epithelial cysts contained keratins 7, 8, 14, 18, and 19, whereas keratins 4 and 13 were found in one case and in less than 10% of the cells in a second case. Vimentin was expressed weakly in cystic epithelium. Desmin found in these teratomal tissues was present in mucin epithelial cells in one case and further limited to muscular cell types. The epithelial character of the cells was supported by a positive staining with BW495/36, HMFG-2, BW431/31, and TAG-72 in one case. The epithelial cells were not stained by OC 125 and OV632, but some staining was observed with MOv 18, OV-TL 3, OV-TL 10, OV-TL 15, OV-TL 16, OV-TL 19, and OV-TL 23.

The squamous cells contained keratin 14 and likely keratin 5, because RCK 102 (recognizing keratins 5+8) stained the epithelial cells, whereas keratin 8 was not detected. The cornified epithelium contained keratins 5, 14, and 10. Vimentin was not found in squamous epithelium. The markers BW495/36,

Table 3. *Intermediate Filament Patterns and Tumor Marker Profile of Ovarian Germ Cell Derived Tumors*

Monoclonal Antibody	Antigen	Benign: Dermoid cyst (terato)	Malignant	
			Dysgerminoma	Endodermal sinus tumor
6B10	Keratin 4	$^{A}1/4$ $+/-^{A}1/4$ $^{B3}0/4$ $^{C}0/4$	0/2	+/−1/1
OV-TL 12/30* RCK 105	Keratin 7	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	2/2*	1/1
M20 LE41* CAM 5.2	Keratin 8	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	2/2*	1/1
RKSE 60	Keratin 10	$+/-^{A}1/4$ $^{B2}1/4$ $^{B1}0/4$ $^{C}0/4$	0/2	+/−1/1
1C7 2D7	Keratin 13	$^{A}1/4$ $+/-^{A}1/4$ $^{B3}0/4$ $^{C}0/4$	0/2	1/1
LL002	Keratin 14	$+/-^{A}2/4$ $^{B1}1/4$ $^{B2}0/4$ $^{C}0/4$	0/2	0/1
RCK 107	Keratin 14	$^{A}2/4$ $^{B3}1/4$ $^{C}0/4$	0/2	0.1
RGE53 RCK 106 CK 18–2/2C8**	Keratin 18	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	2/2**	1/1
LP2K	Keratin 19	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	0/2	1/1
RCK 102	Keratin 5 + 8	$^{A}2/4$ $^{B3}1/4$ $^{C}0/4$	2/2	1/1
OV-TL 12/5	Keratin 5 + 7 + 14 + 19	$^{A}2/4$ $^{B3}1/4$ $^{C}0/4$	±1/2	1/1
RCK 103	Keratin	$^{A}2/4$ $^{B3}1/4$ $+/-^{C}1/4$	0/2	1/1
RV 202	Vimentin	$+/-^{A}1/4$ $\pm^{A}1/4$ $^{B3}0/4$ $^{C}1/4$	2/2	1/1
RD 301	Desmin	$^{A}1/4$ $^{B3}0/4$ $^{C}0/4$	0/2	0/1
BW 495/36	Panepithelial 200 kd	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	2/2	1/1
HMFG-2	HMFG	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	0/2	1/1
B72.3	TAG-72	$^{A}1/4$ $^{B3}0/4$ $^{C}0/4$	2/2	1/1
BW 431/31	CEA	$^{A}2/4$ $^{B3}0/4$ $^{C}0/4$	0/2	+/−1/1
OV-TL 3	OA 3	$^{A}1/4$ $^{B3}0/4$ $^{C}1/4$	+/−2/2	+/−1/1
OV-TL 10	OA 10	$+/-^{A}1/4$ $^{B3}0/4$ $^{C}0/4$	0/2	0/1
OV-TL 15	OA 15	$^{A}1/4$ $B^{3}0/4$ $^{C}0/4$	0/2	1/1
OV-TL 16	OA 16	$^{A}1/4$ $\pm^{A}1/4$ $^{B3}0/4$ $^{C}0/4$	0/2	0/1

Table 3. *Continued*

Monoclonal Antibody	Antigen	Benign: Dermoid cyst (terato)	Malignant: Dysgerminoma	Malignant: Endodermal sinus tumor
OV-TL 17	OA 17	^{A}0/3 ^{B1}1/3 ^{B2}0/3 ^{C}1/3	+/−1/1	ND
OV-TL 19 OV-TL 22‡	OA 19 OA 22	±^{A}1/3‡ ^{B3}0/3 ^{C}0/3	0/1	ND
OV-TL 23	OA 23	^{A}1/4 +/−^{A}1/4 ^{B3}1/4 ^{C}0/4	0/2	1/1
OV-TL 24	OA 24	^{A}0/3 ^{B3}0/3 ±^{C}1/3	0/1	ND
OV-TL 27	OA 27	^{A}0/3 ^{B3}0/3 +/−^{C}1/3	0/1	ND
OV-TL 30	OA 30	^{A}0/4 ^{B3}0/4 ^{C}1/4	1/2 ±1/2	1/1
OV-TL 31‖ OC 125	OA 31 CA 125	^{A}0/4 ^{B3}0/4 ^{C}0/4	0/2	1/1‖
MOv 18	Glycoprotein 38/40 kd	^{A}1/4 ^{B3}0/4 ^{C}0/4	0/2	1/1
OV632	Nonmucinous ovarian carcinoma	^{A}0/4 ^{B3}0/4 ^{C}0/4	0/2	0/1
HLA-I	MHC-I	^{A}2/4 ^{B1}1/4 ^{B2}0/4 ^{C}1/4	2/2	1/1
HLA-DR	MHC-II	+/−^{A}1/4 ±^{A}1/4 ^{B3}0/4 ^{C}1/4	1/2 ±1/2	0/1

Values are the number of positive cases over the total number of cases tested in an immunoperoxidase assay.
No symbol, 10% to 100% positive tumor cells in a section; +/−, less than 10% of positive tumor cells; ±, weakly positive cells in more than 10% of the tumor cell population.
^{A} cysts; ^{B1} squamous epithelium; ^{B2} keratinized squamous epithelium; ^{B3} = ^{B1} + ^{B2}; ^{C} possibly some epithelial elements.
* In 2 cases, ±; ** in 2 cases, +/−; ‡ negative, ‖ in 1 case, ±.

HMFG-2, and TAG-72 did not react with both cell types. None of the tumor markers, except for OV-TL 17 and OV-TL 23, was expressed. HLA-I was expressed in the group of ovarian teratomas except in the keratinized squamous epithelium, whereas the HLA-DR was found weakly positive or positive in 5% to 10% of the cells in the epithelial cysts, negative in the squamous epithelium, and positive in the suspected epithelial component). The two cases of dysgerminoma present expressed the simple epithelial keratins 7, 8, and 18, but not 19 and others (Figure 4A). Vimentin was coexpressed with keratin. The epithelial character was further supported by a positive staining for BW495/36 (Figure 4B) and TAG-72 (Figure 4C), but not by HMFG-2. Of all tumor markers, only OV-TL 30 reacted with the dysgerminoma cells, whereas OV-TL 3 and OV-TL 17 only stained some cells sporadically. HLA-I and HLA-DR were focally expressed in these tumors. In the case of malignant endodermal sinus tumor keratins 7, 8, 18, and 19 (Figure 4D), were found, in addition to keratin 13, whereas keratins 4 and 10 were expressed in less than 10% of the cells. Vimentin was only focally expressed. The epithelial character was confirmed by a positive reaction with BW495/36 (Figure 4E), and a heterogeneously positive reaction for TAG-72 and HMFG-2. The tumor was heterogeneously stained with OC 125, MOv 18 (Figure 4F), OV-TL 30, OV-TL 23, OV-TL 15, but sporadically with OV-TL 3 (Figure 4G) and BW431/31. In the endodermal sinus tumor HLA-I antigen was expressed, whereas HLA-DR was negative.

Discussion

This study describes the marker profile of normal ovarian cells and tumors derived from the different ovarian cell types. Three categories of ovarian

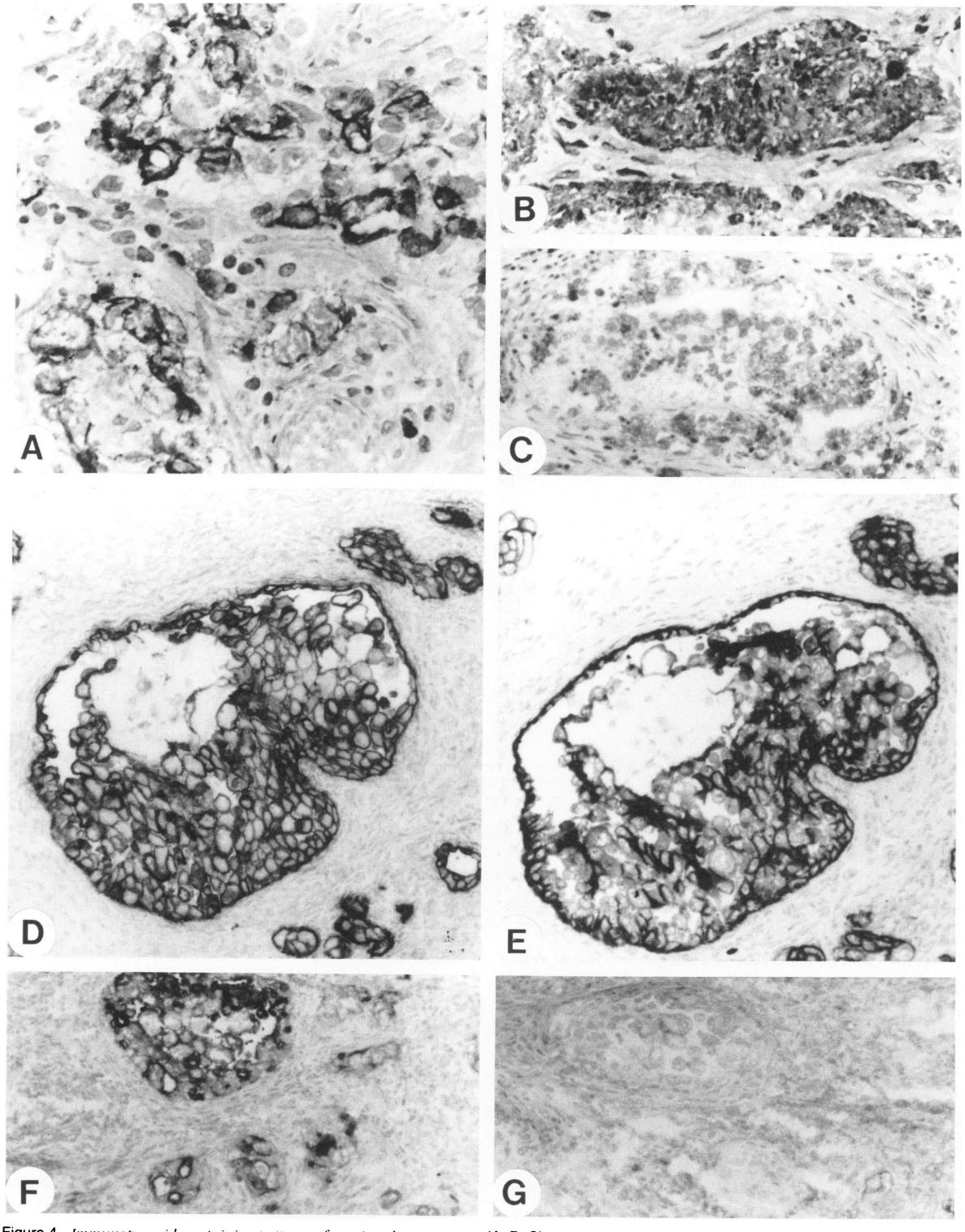

Figure 4. *Immunoperoxidase staining patterns of ovarian dysgerminoma* (A, B, C) *and ovarian endodermal sinus tumor* (D, E, F), *incubated with monoclonal antibodies directed against keratin 18, RCK 106* (A); *panepithelial marker BW495/36* (B); *TAG-72 marker* (C); *antikeratin 19, LP2K* (D); *panepithelial marker BW495/36* (E); *antiovarian carcinoma marker MOv 18* (F); *and OV-TL 3* (G).

tumors have been studied: 1) tumors derived from germinal epithelium, 2) gonadal stromal tumors, and 3) tumors derived from germ cells. A marker profile as defined by a selected panel of monoclonal antibodies can contribute to our understanding of the cellular origin of the individual tumor types, their progression behavior during transformation, and may also be of help in the differential diagnosis of ovarian tumor (sub)classes. For the sake of clarity we have summarized the main immunohistochemical results in the diagram in Figure 5, showing the (gradual) changes during early transformation of normal ovarian surface epithelium into cystoma, adenoma and carcinoma.

Epithelial ovarian tumors are thought to arise from the ovarian surface epithelium or mesothelium.[2] The epithelial origin of four major types of ovarian tumors, ie, serous, mucinous, endometrioid, and clear cell, was supported by the presence of the so-called simple keratins in pairs of keratins 8 and 18, and mostly keratins 7 and 19, confirming our previous data.[5] The higher positive score for keratin 19 in the present study as compared with our previous results[5] and also noticed for some other markers, may, to some extent, be related to a higher Mab concentration or sensitivity of detection procedures in combination with the use of acetone-fixed frozen sections instead of unfixed sections. The fact that keratins 5 and 14, 4 and 13, and 10, normally markers for squamous differentiation, were not found in ovarian mesothelial cells, only incidentally in cystomas and serous cystadenomas, but in a considerable number in serous carcinomas, mucinous adenomas, and carcinomas, suggests that the expression of these keratins is related to serous or mucinous differentiation rather than to the remaining category of ovarian cancers, largely lacking these latter keratins. Although Moll and coworkers[35] reported the absence of stratification related keratins 4, 5, 13, 17 in particular mucinous tumors, we now clearly demonstrate the presence of keratins 4 and 13, on the basis of a large study of 31 mucinous tumor samples. In addition, we demonstrate the presence of keratins 10 and 14 in about one-third of the mucinous tumors by using two to three different monoclonal antibodies for each type of these keratins. Our observation that stratification-related keratins were found in ovarian tumors without histological evidence of squamous cell differentiation confirms and extents the suggestion of Moll and coworkers[35] for keratin 4 that was found in polar columnar cells of several simple and complex epithelia and in adenocarcinomas of the lung.

The keratin 7 antibodies OV-TL 12/30 and RCK 105 were found to react with virtually all 109 of the epithelial ovarian neoplasms (Figure 5). Therefore, the keratin 7 antibodies can be used with more confidence in the differential diagnosis of the primary ovarian epithelial cancer *versus* metastases to the ovary, in particular those types of gastrointestinal tract cancers, largely missing keratin 7.

Coexpression of vimentin and keratin was reported for normal mesothelial cells[22,24,36,37] granulosa cells and rete ovarii cords of the human ovary.[38] High vimentin coexpression in mesothelial cells as well as in mesotheliomas[36] is in line with the finding that mesothelial-derived ovarian cystomas and adenomas still express vimentin. The fact that vimentin coexpression is not limited to cells in culture or ascites, but also occurs in benign and malignant ovarian tumor cells of all subclasses, illustrates that vimentin cannot be considered as an (exclusive) marker for mesenchymal-derived (tumor) cells. Although sporadic vimentin and keratin coexpression in ovarian carcinomas has been reported earlier,[39–42] we now demonstrate that a relatively high number of all subclasses of the ovarian epithelial tumors show (focal) vimentin staining by using four different vimentin antibodies (Figure 5). Coexpression of different types of intermediate filaments is a more common phenomenon in human tissues, either normal or malignant, than previously realized.[22] High vimentin coexpression also has been suggested to be associated with the proliferative activity of cells.[43] Viale and associates[44] showed that most serous tumors (80%), some endometrioid adenocarcinomas, and all clear cell carcinomas investigated exhibited a variable number of neoplastic cells cosynthesizing keratins and vimentin, whereas only one of 29 mucinous tumors and none of the Brenner tumors displayed vimentin-immunoreactive cells. In our study, however, 23 of 31 cases of mucinous neoplasias exhibited expression of both keratins and vimentin. The discrepancy between our present high vimentin score in mucinous ovarian tumor cells on frozen sections and the low score in the report by Viale and coworkers[44] is probably due to the use of formalin-fixed paraffin-embedded material in their study.

BW495/36 and, to a lesser extent, HMFG-2 discriminate between negatively reacting mesothelial cells and positively reacting carcinoma cells, with a heterogenous expression in the group of cystomas (Figure 5). This finding again favors the idea that ovarian carcinoma develops and progresses from mesothelial cells. Using BW495/36, the differential diagnosis between mesothelial cells and carcinomas in cytodiagnosis of ascites can be greatly facilitated. Similarly, the combined markers for keratins and

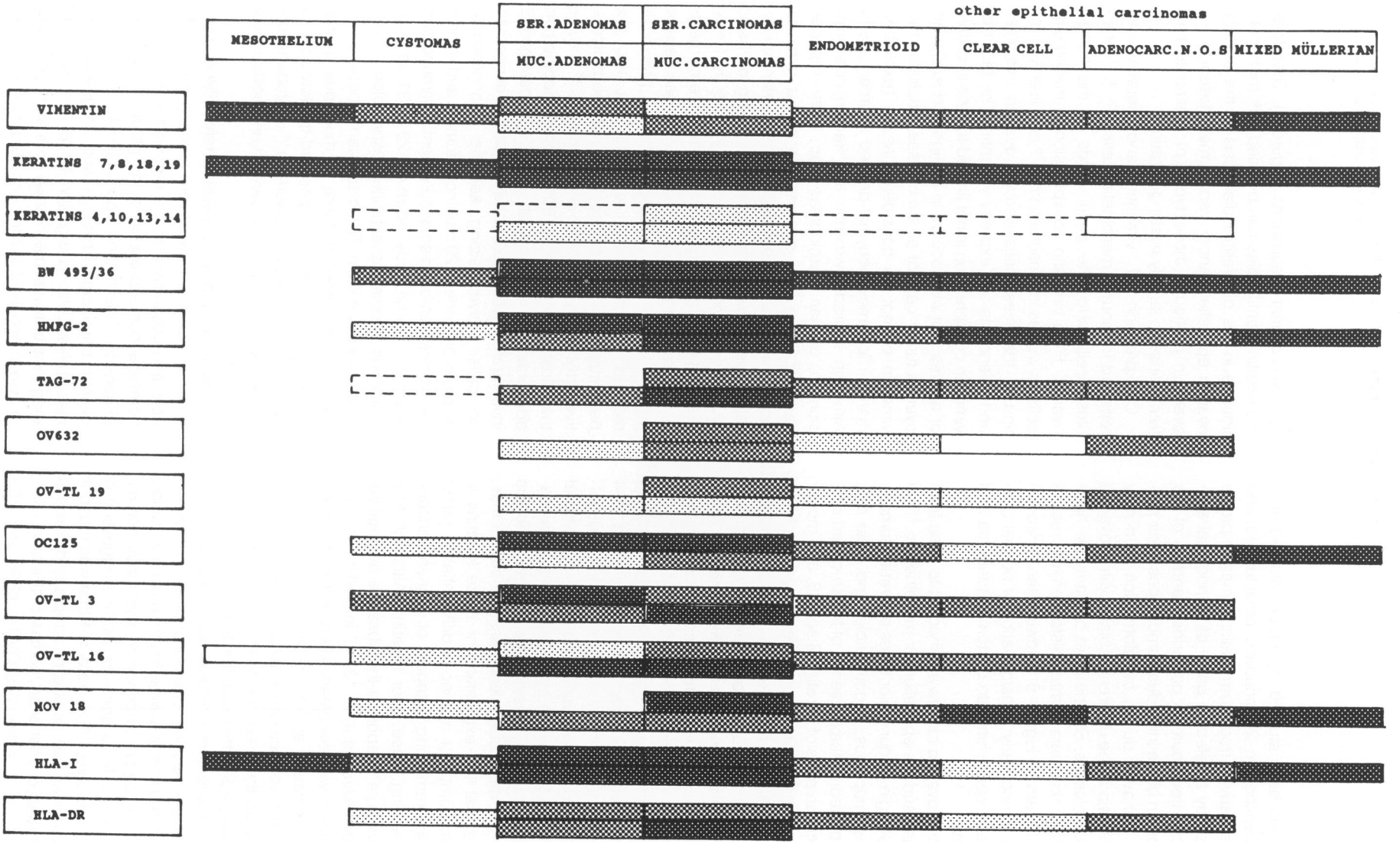

Figure 5. *Diagram of the main immunophenotypic changes occurring during the transformation of normal ovarian surface epithelium into cystoma, adenoma, and carcinoma. The increasing density of the bars represents an increasing number of positively stained samples.*

BW495/36 were successfully applied in the differential diagnosis of mesothelial cells *versus* endometrioid cells in case of endometriosis.[45,46]

The marker TAG-72, assumed to differentiate between benign and malignant tumors,[47] appeared in our study to discriminate between negatively stained mesothelial cells, most cystomas and serous adenomas versus positively stained mucinous adenomas and most carcinomas (Figure 5). The antibody thus marks progression from serous adenomas to serous carcinomas. Therefore, it is tempting to speculate that the two TAG-72 positive cystomas (of 15) might progress into mucinous adenomas, rather than into serous adenomas.

The monoclonal antibodies against ovarian carcinoma-associated antigens such as the OV-TL antibodies, OC 125, and MOv 18 can be considered as panovarian tumor markers for epithelial-derived tumors because they showed an increasing reactivity in the progression from benign tumors to carcinomas, but did not discriminate between benign and malignant tumors (Figure 5). The Mabs OV-TL 15, OV-TL 30, and OV-TL 31 have been studied earlier by Boerman and coworkers[30] in immunoradiometric assays, and the corresponding ovarian carcinoma antigens were found in much lower concentrations in cystic fluid in benign than in malignant ovarian tumors, whereas other antigen levels, such as CA 125, were found to be much less discriminative. The OV632 antibody, however, discriminated between negative mesothelial cells, cystomas, serous adenomas, and positive mucinous adenomas, mucinous carcinomas, and serous carcinomas, in this way being a marker for progression within the serous subgroup. The lower positive score in the group of other ovarian carcinomas, however, limits the applicability of this antibody.

HLA-I remained expressed in normal and tumor cells (Figure 5). HLA-DR was hardly found in mesothelial cells but was expressed usually in more than half of the epithelial tumors. MHC class I and II antigens play an important role in the interaction between tumor cells and the immune system. As described earlier,[48–52] decreased expression of MHC class I antigens has been suggested to correlate with increased tumorigenesis for some tumors. Our results are in line with those of Valea and associates,[53] who described positive expression of HLA-I antigen by ovarian epithelial neoplasms, although in our study loss of HLA-I expression was seen in some areas of tumors. In some human malignancies the presence of MHC class II correlates with the presence of inflammatory cells and with a better prognosis,[48,54,55] whereas other investigators reported the absence of any correlation between the expression of MHC class II antigens and the induction of an anti-tumor immune response *in vitro*.[48,56,57] The expression of HLA class II antigens reported by Ruiter and co-workers[48] in 15% to 100% of primary cutaneous melanoma lesions tends to be associated with the presence of lymphocytic infiltrates and suggests that in primary melanomas the expression of HLA class II antigens may be induced by locally produced γ-interferon. However, in our study, a direct correlation with the presence of inflammatory cells was not observed. Our results are in line with the positive HLA-DR antigen expression of 11 of 21 samples of ovarian carcinoma cells (ranging between 30% and 95%) as described by DiBello and associates.[56]

In conclusion, our data suggest that during early transformation from normal ovarian surface epithelium (mesothelium) into cystomas, adenomas, and carcinomas, a series of different antigens mark these changes, and can therefore be useful in the differential diagnosis in surgical pathology.

Our conclusions with respect to the epithelial ovarian tumors can be summarized as follows:

1. None of the monoclonal antibodies against ovarian carcinoma-associated antibodies discriminated completely between benign ovarian epithelial tumors and carcinomas, nor between the subclasses of carcinomas when applied in this immunohistochemical study.
2. Using panovarian carcinoma markers OV-TL 3, OV-TL 16, OC 125, and MOv 18, the epithelial-derived ovarian tumors can be identified, without discrimination, between benign and malignant ovarian tumors.
3. The marker BW495/36 and to a lesser extent HMFG-2 can be used to confirm the epithelial character of the tumors and to discriminate them from mesothelial cells.
4. The keratin antibodies can be used to specify the grade of differentiation of the tumor (adeno-/squamous) and in the differential diagnosis of the primary ovarian epithelial cancer *versus* metastases to the ovary.
5. The antibodies TAG-72, OV632, and, to a lesser extent, OV-TL 19 can be used to subtype within the serous/mucinous tumors and carcinomas.
6. HLA-DR in general distinguishes normal mesothelium from benign and malignant lesions.

Both mesenchymal cells and coelomic epithelium[38] have been suggested to give rise to ovarian gonadal tumors (sex cord-stromal tumors). As reported earlier[5,38] primary follicle cells show strong expression of keratins 8 and 18 in all samples and

keratin 19 in half of the samples. Keratin 7 expression was limited to traces in some samples. The expression of these keratins was reduced in Graafian follicles and corpus luteum. For human granulosa cells it was reported that the synthesis of keratins 8 and 18, and, to a lesser extent, keratins 7 and 19 could be suppressed by treating the cells with HCG.[58] The keratin expression pattern of primary follicle cells as well as maturing granulosa cells confirm the epithelial nature of these cells. Strikingly, traces of keratin 7 were sometimes detected scattered throughout ovarian stroma and thecal cells. One fibroma sample expressed keratins 7, 8, 18, and 19 weakly or sporadically, in contrast to the results of Benjamin and associates,[59] whereas some fibrothecoma tumors and both granulosa-theca cell tumors were found positive for keratins 7, 8, 18, and 19. The finding of keratins in these stromal tumors agree, however, with our (unpublished) observations of occasional keratin expression in normal activated stromal cells, and does not point to an other histogenetic origin of these tumors.[22] Vimentin was expressed in all gonadal tumors and corresponding normal cells as shown previously.[38,59] The finding of desmin in two fibromas is consistent with the observation of Czernobilsky.[38] The (almost) negative staining reactions of both BW495/36 and HMFG-2 markers in both the follicle cells (mature granulosa) cells and mesothelial cells as well as the rather similar keratin profile of both cell types point to a close relation of the granulosa cells with mesothelial cells, supporting the granulosa cell histogenesis from epithelial cell types (coelomic epithelium or rete ovarii cords) as described by Czernobilsky.[38] Interestingly, the negative score of TAG-72 in granulosa cells and mesothelial cells also underlines the relationship with mesothelial cells, because TAG-72 has been reported to be negative in malignant mesotheliomas as well as in benign mesothelial proliferations.[60]

No coexpression of BW495/36, HMFG-2, and TAG-72 with keratins was found in ovarian stromal cells, theca cells, and fibromas. However, one fibrothecoma, one granulosa cell tumor, and both granulosa theca cell tumors were found positive for BW495/36, but negative for HMFG-2 and TAG-72. Explanation needs further confirmation in a larger number of samples.

The finding that the one Leydig cell tumor was negative for BW495/36, HMFG-2, and TAG-72, but positive for keratin 7 does not disprove the mesenchymal origin of that tumor because keratin 7 was also found in the above-mentioned fibrothecomas. Thus, expression of keratins as well as the positive reaction with BW495/36 in Sertoli-Leydig cell tumors may indicate an epithelial character of the Sertoli cell tumor compartment but not of the Leydig cells. With respect to the keratin expression pattern in this Leydig and granulosa tumor type, it is striking that we observed only keratin 7 positivity in the Leydig cell tumor, but no reaction with any of the other Mabs, whereas keratins 8, 18, and 19 were sporadically expressed in one granulosa cell tumor. In general, keratins form pairs, with keratin 7 normally occurring in conjunction with keratin 19 or keratin 8 and 18. Although positive with both keratin 7 Mabs, further studies will have to reveal whether or not the keratin 7 reaction patterns are specific or result from a shared epitope on a completely different protein. Reversely, we have to exclude also epitope masking for any of the other keratins in these malignancies.

The germ cell tumors are thought to arise from primitive germ cells. These tumors may develop both in the gonads or at extragonadal sites along the line of migration of the primitive germ cells. The remarkable homology between the various germ cell tumor types in male and female is in agreement with the theory about the histogenesis of these neoplasias as described by Teilum.[61] Mature teratomas (dermoid cysts) usually contained ectodermal structures. The presence of the simple epithelial keratins 7, 8, 18, and 19 in dermoid cysts was supplemented with keratins 4, 13, and 14. The squamous cell types contained keratins 5, 10, and 14, but not keratins 8, 18, 4, and 13. This indicates that these squamous epithelial tumor cells are of the dermoid (cornifying stratified) type, because in normal human keratinizing squamous epithelia, keratins 5, 14, and 10 are found. In this study, we confirmed, in essence, the results of Czernobilsky and coworkers,[62] but because we stained with a more extended panel of monoclonal antibodies, we were able to identify the localization of various other keratin polypeptides in the different dermoid cysts. Vimentin was coexpressed with keratins in the simple dermoid cyst but not in squamous cell types. The markers BW495/36, HMFG-2, and TAG-72 in general confirmed the epithelial character of the simple dermoid cysts, but were negative in the case of squamous cell differentiation in line with their specificity in normal adult human tissues.

Also in case of dysgerminoma and endodermal sinus tumor the immunostaining pattern supported the epithelial nature of the tumor cells. Although similarity of endodermal sinus tumor and dermoid cysts has been suggested,[63] and is supported by the identical keratin pattern of BW495/36 and TAG-72 staining, both tumors can be distinguished on the basis of the reactivity of the cysts with ovarian carcinoma markers.

With respect to the nonepithelial ovarian cancers, our data suggest that although the gonadal stromal tumors and germ cell tumors are not thought to originate from epithelial cell types, most corresponding tumors express one or more keratins as well as BW495/36. In general, gonadal tumors could be distinguished from germ cell tumors by the absence of TAG-72 and HMFG-2 in the former, whereas germ cell tumors in general expressed TAG-72 and, to a lesser extent, also HMFG-2. A proper combination of these markers might be very helpful in diagnostic pathology of ovarian malignancies.

With respect to the discrimination between the three categories of ovarian tumors (epithelial, gonadal stromal, and germ cell tumors) we suggest that:

1. .TAG-72 and HMFG-2 in general also distinguish between positive epithelial ovarian tumors and germ cell tumors *versus* negatively reacting gonadal stromal tumors, whereas keratins and the marker BW495/36 were found more or less in all three categories of ovarian tumors.

2. Of the ovarian tumor-associated markers OC 125, OV-TL 3, OV-TL 16, and MOv 18, the latter three Mabs did not discriminate sufficiently between epithelial, gonadal, and germ cell tumors, whereas OC 125, OV-TL 15, OV-TL 23, and, to a lesser extent, OV-TL 31 distinguished between positive epithelial ovarian tumors and negatively reacting gonadal tumors. Because of the limited number of samples tested in the groups of gonadal and germ cell tumors, these suggestions for the nonepithelial tumors will need further confirmation.

Acknowledgments

We thank Prof. Dr. P. Kenemans and coworkers (Nijmegen and Amsterdam, The Netherlands), Dr. P. M. Arkenbout and Dr. W. B. K. N. V. de Goey (Canisius Wilhelmina Hospital, Nijmegen) for providing biopsy material, and Drs. F. Smedts (Delft, The Netherlands) for valuable discussions concerning tumor diagnosis. We also thank Dr. K. Bosslet (Behringwerke, Marburg, Germany) for the BW series of antibodies, Dr. V. R. Zurawski, Jr. (Harvard Medical School, Boston, MA, and Centocor, Malvern, PA) for the OC 125 antibody, Dr. M. I. Colnaghi (Milan, Italy) for the MOv 18 antibody, Dr. G. van Muijen (Nijmegen, The Netherlands) for the keratin antibodies 6B10, 1C7, 2D7, and M20, Dr. Birgit Lane (Dundee, UK) for the antibodies LE41, LLOO2, and LP2K, and Dr. Joyce Taylor-Papadimitrian (London, UK) for the HMFG-2 antibody. We also thank Dr. A. L. H. Stols for his help with the layout of the manuscript.

References

1. Fleuren GJ: Ovarian cancer. In: Ruiter DJ, Fleuren GJ, Warnaar SO, eds. Application of Monoclonal Antibodies in Tumor Pathology. Boston, Martinus Nijhoff, 1987, pp 237–254
2. Scully RE: Tumors of the ovary and maldeveloped gonads. In: Hartman WH, ed. Atlas of Tumor Pathology Second series, fascicle 16. Washington DC, Armed Forces Institute of Pathology, 1979
3. Berek JS: Epithelial ovarian cancer. Nonepithelial ovarian and tubal cancers. In: Berek JS, Hacker NF, eds. Practical Gynecologic Oncology. Baltimore, Williams & Wilkins, 1989, pp 327–382
4. Berek JS: Nonepithelial ovarian and tubal cancers. In: Berek JS, Hacker NF, eds. Practical Gynecologic Oncology. Baltimore, Williams & Wilkins, 1989, pp 365–390
5. Van Niekerk CC, Boerman OC, Ramaekers FCS, Poels LG: Marker profile of different phases in the transition of normal human ovarian epithelium to ovarian carcinomas. Am J Pathol 1991, 138:455–463
6. Van Muijen GNP, Ruiter DJ, Franke WW, Achtstatter T, Haasnoot WHB, Ponec M, Warnaar SO: Cell type heterogeneity of cytokeratin expression in complex epithelia and carcinomas as demonstrated by monoclonal antibodies specific for cytokeratins nos 4 and 13. Exp Cell Res 1986, 162:97–113
7. Ramaekers FCS, van Niekerk CC, Poels LG, Schaafsma E, Huysmans A, Robben H, Schaart G, Vooijs GP: Use of monoclonal antibodies to keratin 7 in the differential diagnosis of adenocarcinomas. Am J Pathol 1990, 136:641–655
8. Ramaekers FCS, Huysmans A, Schaart G, Moesker O, Vooijs GP: Tissue distribution of keratin 7 as monitored by a monoclonal antibody. Exp Cell Res 1987, 170:235–249
9. Niekerk van CC, Jap PHK, Ramaekers FCS, Molengraft van de F, Poels LG: Immunohistochemical demonstration of keratin 7 in routinely fixed paraffin embedded human tissues. J Pathol 1991, 165:145–152
10. Schaafsma HE, Ramaekers FCS, Muijen GNP van, Lane EB, Leigh IM, Robben H, Huijsmans A, Ooms ECM, Ruiter DJ: Distribution of cytokeratin polypeptides in human transitional cell carcinomas with special emphasis on changing expression patterns during tumor progression. Am J Pathol 1990, 136:329–343
11. Smedts F, Ramaekers F, Robben H, Pruszczynski M, Van Muijen G, Lane B, Leigh I, Vooijs GP: Changing patterns of keratin expression during progression of cervical intraepithelial neoplasia. Am J Pathol 1990, 136:657–668
12. Ivanyi D, Ansink A, Groeneveld E, Hageman PC, Mooi WJ, Heintz AP: New monoclonal antibodies recognizing epidermal differentiation-associated keratins in formalin-fixed, paraffin-embedded tissue. Keratin 10 expression in carcinoma of the vulva. J Pathol 1989, 159:7–12

13. Purkis PE, Steel JB, Mackenzie IC, Nathrath WBJ, Leigh IM, Lane EB: Antibody markers of basal cells in complex epithelia. J Cell Sci 1990, 97:39–50
14. Wetzels RHW, Kuijpers HJH, Lane EB, Leigh IM, Troyanovski SM, Holland R, Haelst UJGM van, Ramaekers FCS: Basal cell specific and hyperproliferation-related keratins in human breast cancer. Am J Pathol 1991, 138:751–763
15. Ramaekers FCS, Huysmans A, Moesker O, Kant A, Jap PHK, Herman CJ, Vooijs GP: Monoclonal antibodies to keratin filaments specific for glandular epithelia and their tumors: Use in surgical pathology. Lab Invest 1983, 49:353–361
16. Stasiak PC, Purkis PE, Leigh IM, Lane EB: Keratin 19: Predicted amino acid sequence and broad tissue distribution suggest it evolved from keratinocyte keratins. J Invest Dermatol 1989, 92:707–716
17. Schaart G, Viebahn C, Langmann W, Ramaekers F: Desmin and titin expression in early postimplantation mouse embryos. Development 1989, 107:585–596
18. van Muijen GNP, Ruiter DJ, van Leeuwen C, Prins F, Rietsema K, Warnaar SO: Cytokeratin and neurofilament in lung carcinomas. Am J Pathol 1984, 116:363
19. Ramaekers FCS, Puts JJG, Moesker O, Kant A, Huijsmans A, Haag D, Jap PHK, Herman CJ, Vooijs GP: Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumours: an overview. Histochem J 1983, 15:691–713
20. Pieper R, Schaart G, Krimpenfort PJ, Hendrik JB, Moshage HJ, Kemps A van de, Ramaekers FCS, Berns A, Bloemendal H: Transgenic expression of the muscle-specific intermediate filament protein desmin in non-muscle cells. J Cell Biol 1989, 108:1009–1024
21. Ramaekers FCS, Moesker O, Huijsmans A, Schaart G, Westerhof G, Wagenaar SjSc, Herman CJ, Vooijs GP: Intermediate filament proteins in the study of tumor heterogeneity: an-indepth study of tumors of the urinary and respiratory tracts. Ann NY Acad Sci 1985, 455:614–634
22. van Muijen GNP, Ruiter DJ, Warnaar SO: Coexpression of intermediate filament polypeptides in human fetal and adult tissues. Lab Invest 1987, 57:359–369
23. Bosslett K, Kanzy EJ, Luben G, Sedlacek HH: A homogeneously expressed pancarcinoma epitope. Symposium: New drugs in cancer therapy. Invest New Drugs 1987, 5:93
24. van Niekerk CC, Jap PHK, Thomas CMG, Smeets DFCM, Ramaekers FCS, Poels LG: Marker profile of mesothelial cells versus ovarian carcinoma cells. Int J Cancer 1989, 43:1065–1071
25. Taylor-Papadimitriou J, Peterson JA, Arklie J, Burchell J, Ceriani RL, Bodmer WF: Monoclonal antibodies to epithelium-specific components of the human fat globuline membrane: production and reaction with cells in culture. Int J Cancer 1981, 28:17–21
26. Johnson V, Schlom J, Paterson AJ, Bennett J, Magnani JL, Colcher D: Analysis of human tumor-associated glycoprotein (TAG-72) identified by monoclonal antibody B 72.3. Cancer Res 1986, 46:850–857
27. Bosslett K, Luben G, Schwarz A, Hundt E, Harthus HP, Seiler FR, Muhrer C, Kloppel G, Kayser K, Sedlacek HH: Immunohistochemical localization and molecular characteristics of three monoclonal antibody defined epitopes detectable on carcinoembryonic antigen (CEA). Int J Cancer 1985, 36:75–84
28. Poels LG, Peters D, Van Megen Y, Vooijs GP, Verheyen RNM, Willemen A, van Niekerk CC, Jap PHK, Mungyer G, Kenemans P: Monoclonal antibody against human ovarian tumor-associated antigens. JNCI 1986, 76:781–791
29. Boerman OC, van Niekerk CC, Makkink WK, Hanselaar AGJP, Kenemans P, Poels LG: A comparative immunohistochemical study of four monoclonal antibodies directed against ovarian carcinoma-associated antigens. Int J Gynecol Pathol 1991, 10:15–25
30. Boerman OC, Makkink WK, Thomas CMG, Hanselaar AGJM, Yedema CA, Kenemans P, Poels LG: Monoclonal antibodies that discriminate between human ovarian carcinomas and benign ovarian tumors. Eur J Cancer 1990, 26:117–127
31. Boerman OC, Makkink WK, Massuger LFAG, Thomas CMG, Kenemans P, Hanselaar AGJM, Poels LG: Monoclonal antibodies against ovarian carcinoma associated antigens, raised by immunization with cyst fluids. Anticancer Res 1989, 9:551–558
32. Bast RC, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC: Reactivity of a monoclonal antibody with human ovarian carcinomas. J Clin Invest 1981, 68:1331–1337
33. Campbell IG, Jones TA, Foulkes WD, Trowsdale J: Folate-binding protein is a marker for ovarian cancer. Cancer Res 1991, 51:5329–5338
34. Fleuren GJ, Coerkamp EG, Nap M, van de Broek LCJM, Warnaar SO: Immunohistological characterization of a monoclonal antibody (OV632) against epithelial ovarian carcinomas. Virchows Arch 1987, 410:481–486
35. Moll R, Pitz S, Levy R, Weikel W, Franke WW, Czernobilsky B: Complexity of expression of intermediate filament proteins, including glial filament protein, in endometrial and ovarian adenocarcinomas. Hum Pathol 1991, 22:(10) 989–1001
36. LaRocca PJ, Rheinwald JG: Coexpression of simple epithelial keratins and vimentin by human mesothelium and mesothelioma in vivo and in culture. Cancer Res 1984, 44:2991–2999
37. van de Molengraft F, Ramaekers FCS, Jap PHK, Vooijs P, Mungyer G: Changing intermediate-sized filament patterns in metastatic hepatocellular carcinoma cells of the guinea pig. Virchows Arch (Cell Pathol) 1986, 51:285–301
38. Czernobilsky B, Moll R, Levy R, Franke WW: Co-expression of cytokeratin and vimentin filaments in mesothelial, granulosa and rete ovarii cells of the human ovary. Eur J Cell Biol 1985, 37:175–190

39. Osborn M and Weber K: Biology of disease. Tumor diagnosis by intermediate filament typing: A novel tool for surgical pathology. Lab Invest 1983, 48:372–394
40. Puts JJG, Moesker O, Aldeweireldt J, Vooijs GP, Ramaekers FCS: Application of antibodies to intermediate filament proteins in simple and complex tumors of the female genital tract. Int J Gynecol Pathol 1987, 6:257–274
41. Miettinen M, Lehto VP, Virtanen I: Expression of intermediate filaments in normal ovaries and ovarian epithelial, sex cord-stromal, and germinal tumors. Int J Gynecol Pathol 1983, 2:64–71
42. Ramaekers FCS, Puts J, Moesker O, Kant A, Jap PHK, Vooijs GP: Demonstration of keratin in human adenocarcinomas. Am J Pathol 1983, 111:213–223
43. Connell ND, Rheinwald JG: Regulation of the cytoskeleton mesothelial cells: reversible loss of keratin and increase in vimentin during rapid growth in culture. Cell 1983, 34:245–253
44. Viale G, Gambacorta M, Dell-Orto P, Coggi G: Coexpression of cytokeratins and vimentin in common epithelial tumors of the ovary: an immunocytochemical study of eighty-three cases. Virchows Arch 1988, 413:91–101
45. Kruitwagen RFPM, Poels LG, Willemsen WNP, Jap PHK, de Ronde IJY, Hanselaar AGJM, Rolland R: Immunocytochemical marker profile of endometriotic epithelial endometrial epithelial, and mesothelial cells: a comparative study. Eur J Obstet Gynecol Reprod Biol 1991, 41:215–223
46. Kruitwagen RFPM, Poels LG, Willemsen WNP, de Ronde IJY, Jap PHK, Rolland R: Endometrial epithelial cells in peritoneal fluid during the early follicular phase. Fertil Steril 1991, 55:297–303
47. Johnston WW, Szpak CA, Rottich SC, Thor A, Schlom J: Use of monoclonal antibody (B72.3) as a novel immunohistochemical adjunct for the diagnosis of carcinomas in fine needle aspiration biopsy specimens. Hum Pathol 1986, 17:501–513
48. Ruiter DJ, Bröcker EB, Ferrone S: Expression and susceptibility to modulation by interferons of HLA class I and II antigens on melanoma cells. Immunohistochemical analysis and clinical relevance. J Immunogenet 1986, 13:229–234
49. Hammerling GJ, Klar D, Pulm W, Momburg F, Moldenhauer G: The influence of major histocompatibility complex class I antigens on tumor growth and metastasis. Biochim Biophys Acta 1987, 907:245–259
50. Durrant LG, Ballantyne KC, Armitage NC, Robins RA, Marksman R, Hardcastle JD, Baldwin RW: Quantitation of MHC antigen expression in colorectal tumors and its association with tumor progression. Br J Cancer 1987, 56:425–432
51. Momburg F, Ziegler A, Harpprecht J, Moller P, Moldenhauer G, Hammerling GJ: Selective loss of HLA-A or HLA-B antigen expression in colon carcinoma. J Immunol 1989, 142:352–358
52. Napolitano LA, Vogel J, Jay G: The role of major histocompatibility complex class I antigens in tumorigenesis: future applications in cancer therapy. Biochim Biophys Acta 1989, 989:153–162
53. Valea FA, Haskill S, Olafsson K, Fowler WC: Flow cytometric versus immunohistochemical analysis of ovarian cancer class I antigen expression: Differences may represent a defect in antigen expression. Gynecol Oncol 1990, 38:413–420
54. Heinemann D, Smith PJB, Symes MO: Expression of histocompatibility antigens and characterization of mononuclear cell infiltrates in human renal cell carcinomas. Br J Cancer 1987, 56:433–437
55. Miller TP, Lippman SM, Spier CM, Slymen DJ, Grogan TH: HLA-DR (Ia) immune predicts outcome for patients with diffuse large cell lymphoma. J Clin Invest 1988, 82:370–372
56. DiBello M, Lucchini V, Chiari S, Colleoni R, Colombo N, Mantovani A, Allavena P: DR antigen expression on ovarian carcinoma cells does not correlate with their capacity to elicit an autologous proliferative response. Cancer Immunol Immunother 1988, 27:63–68
57. Vanky F, Klein E, Willems J: DR antigens expressed on tumor cells do not contribute to the blastogenetic response of autologous T cells. Cancer Immunol Immunother 1985, 19:219–225
58. Ben-Ze'ev A, Amsterdam A: Regulation of cytoskeletal protein organization and expression in human granulosa cells in response to gonadotropin treatment. Endocrinology 1989, 124:1033–1041
59. Benjamin E, Law S, Bobrow LG: Intermediate filaments cytokeratin and vimentin in ovarian sex cord-stromal tumours with correlative studies in adult and fetal ovaries. J Pathol 1987, 152:253–263
60. Szpak CA, Johnston BW, Roggli V, Kolbeck J, Lottich C, Vollmer R, Thor A, Schlom J: The diagnostic distinction between malignant mesothelioma of the pleura and adenocarcinoma of the lung as defined by a monoclonal antibody (B72.3). Am J Pathol 1986, 122:252–260
61. Teilum G: Tumors of germinal origin. In: Gentil F, Junqueira AC, eds. Ovarian cancer, U.I.C.C. Monograph Series. Berlin, Springer-Verlag, 1968, 2:58–73
62. Czernobilsky B, Lifschitz-Mercer B, Luzon A, Jacob N, Ben-Hur H, Gorbacz S, Fogel M: Cytokeratin Patterns in the epidermis of human ovarian mature cystic teratomas. Hum Pathol 1989, 20:185–192
63. Ashley DJB: Evans' Histological Appearances of Tumors. London, Churchill Livingstone, 1978, pp 663–704