Histochemical Analysis of Estrogen and Progesterone Receptors and Gastric-Type Mucin in Mucinous Ovarian Tumors with Reference to Their Pathogenesis

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Presented at the 49th meeting of the Japan Society of Obstetrics and Gynecology, Tokyo, Japan, April 7, 1997.

Supported in part by a grant from the Ministry of Education, Science, Sports and Culture of Japan (Grant No. 06671636).

The authors thank Dr. Robert E. Scully, Professor Emeritus, Massachusetts General Hospital, Boston, Massachusetts, for his critical review of this article.

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Received December 30, 1996; revision received April 14, 1997; accepted April 14, 1997.

BACKGROUND. Mucinous tumors of the ovary have been thought to originate in two ways: by müllerian-type metaplasia of surface epithelium, and as monodermal teratomas. To gain a better understanding of their pathogenesis, the authors analyzed these tumors for their expression of estrogen receptors (ER) and progesterone receptors (PR) as markers of müllerian-type differentiation and for their content of gastric-type mucin as a marker of gastric differentiation.

METHODS. The histochemical expression of ER, PR, and gastric-type mucin was studied in 10 specimens of the cervix with normal endocervical glands (as a representative of müllerian-derived mucin-containing cells), 3 ovary specimens with surface epithelial inclusion cysts that contained endocervical-like mucin-containing cells (representing müllerian-type metaplasia), and 47 mucinous tumors of the ovary (29 benign, 8 with low malignant potential, and 10 malignant).

RESULTS. Normal endocervical glands expressed ER and PR and rarely expressed gastric-type mucin. Ovarian inclusion cysts showed strong expression of ER and PR in the cuboidal cells and drastically reduced expression in the endocervical-like mucin-containing cells. The cuboidal cells were negative for gastric-type mucin, but the endocervical-like mucin-containing cells expressed gastric-type mucin. Endocervical-like mucinous cells in benign and borderline mucinous tumors showed expression of PR and/or gastric-type mucin in all cases.

CONCLUSIONS. The staining results for the inclusion cysts support the thesis that the endocervical-like mucinous cells encountered in the ones that express ER and PR weakly or not at all and have histochemical properties of normal gastric epithelium have their origin in metaplasia of müllerian-type epithelium. Application of the same staining methods to benign ovarian tumors and those with low malignant potential suggests strongly that similar müllerian-type metaplasia is a major pathway in their pathogenesis. ***Cancer* 1997;80:908–16.**
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KEYWORDS: mucinous ovarian tumor, steroid receptor, gastric mucin, organoid differentiation, pathogenesis.

Mucinous tumors of the ovary are classified as surface epithelial tumors. However, the pathogenesis of these tumors remains controversial because they possess two types of mucin-containing cells. One type is a tall columnar cell with a basal nucleus resembling endocervical gland cells (endocervical-like).[1,2] The other is a goblet cell that may be accompanied by other gastrointestinal-type epithelial cells, such as absorptive, argyrophil, or argentaffin cells (intestinal-type).[1,2] The finding of a morphologic transition between the tumor cells and surface epithelial cells of the ovary has suggested müllerian-type metaplasia in the pathogenesis of this tumor (metaplasia the-

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ory).[2,3] However, an occasional association of a mucinous tumor with a teratoma and its frequent content of gastrointestinal-type cells have suggested a clonal overgrowth of gastrointestinal cells in a teratoma (teratoma theory).[2,3] A report of gastric-type mucin in endocervical-type cells as well as gastrointestinal-type cells in mucinous cystadenomas of the ovary has caused further confusion with regard to the pathogenesis of this tumor.[4]

To obtain a better understanding of the pathogenesis of mucinous tumors of the ovary, we analyzed a series of them using two parameters, expression of ovarian steroid hormone receptors as a marker of müllerian-type differentiation[5–8] and expression of gastric-type mucin as a marker of gastric differentiation.[9] By an immunohistochemical technique using antibodies against ER and PR, the expression of estrogen receptors (ER) and progesterone receptors (PR) was studied in benign, borderline, and malignant mucinous tumors of the ovary and in ovarian surface epithelial inclusion cysts lined by both mucin-containing cells and mucin-free cuboidal cells. Gastric-type mucin was identified by a histochemical technique.

MATERIALS AND METHODS

Histologic Specimens

Three ovary specimens (containing surface epithelial inclusion cysts that were lined by mucinous cells as well as mucin-free cuboidal cells), 47 mucinous tumors of the ovary, and 10 specimens of normal human endocervix were selected from the pathology files of Shinshu University Hospital. The ovarian tumor tissues were obtained from patients who had undergone oophorectomy with or without hysterectomy. The ages of the patients ranged from 20 to 77 years, with mean age of 46.1 years. Normal endocervical tissue was obtained from 10 patients (3 in the proliferative phase of the cycle, 4 in the secretory phase, and 4 postmenopausal) who had undergone a hysterectomy for leiomyoma. The specimens were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Serial sections were made from each tissue block for hematoxylin and eosin (H & E) and histochemical staining.

Of the 47 mucinous tumors, 29 were cystadenomas (MA), 8 were tumors of low malignant potential (M-LMP), and 10 were adenocarcinomas (MCa). Six of the 10 MCa were Grade 1, 2 were Grade 2, and 2 were Grade 3. According to the morphology of the lining epithelium, MA and M-LMP were classified into three types:[10] 1) pure endocervical-like, when the lining epithelium was composed of a monotonous arrangement of mucin-filled columnar cells with basal nuclei (MA, 18 cases; M-LMP, 1 case); 2) pure intestinal-type, when the lining epithelium consisted of intestinal type-cells, such as goblet type-cells; and 3) mixed type, if the tumor contained both intestinal-type and endocervical-like cells (MA, 11 cases; M-LMP, 7 cases). The histopathologic features of the tumors examined in this study are summarized in Table 1.

TABLE 1
Histopathology of Ovarian Surface Epithelial Inclusion Cysts and Mucinous Tumors of the Ovary

Pathology (Total no. of cases)	No. of cases	Histology
Ovarian surface epithelial inclusion cyst (3 cases)	3	Mucin-free cuboidal cells and endocervical-like cells
Cystadenoma (29 cases)	18	Pure endocervical-like cells
	11	Mixed-type cells
Low malignant potential (8 cases)	1	Pure endocervical-like cells
	7	Mixed-type cells
Adenocarcinoma (10 cases)	6	Grade 1
	2	Grade 2
	2	Grade 3

Mixed-type cells: endocervical-like cells and intestinal-type cells.

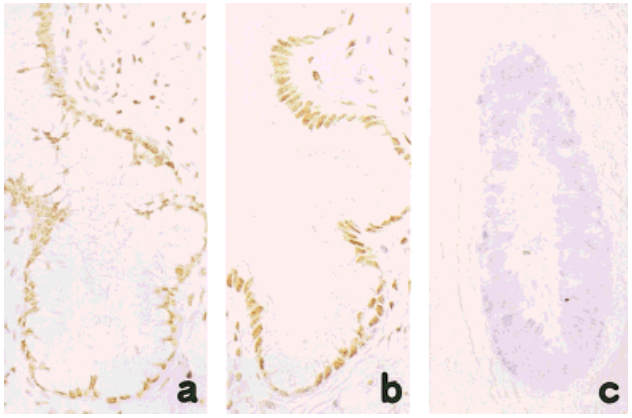

FIGURE 1. Normal cervical glands show positive staining for estrogen receptors (a) and progesterone receptors (b) in the nucleus. By galactose oxidase cold thionin Schiff–paradoxical Concanavalin A staining (GOCTS-PCS), a few glands show weakly positive staining for GOCTS (c). (original magnification ×135)

Immunostaining for ER and PR

Immunohistochemical staining for ER and PR was performed on paraffin sections with the primary antibodies anti-ER (ER1D5) and anti-PR (PR10A) (Immunotech, Marseille, France), by the avidin-biotin-peroxidase complex method, using a Histofine SAB-PO detector kit (Nichirei Co., Tokyo, Japan). In brief, the sections were deparaffinized in xylene, then rehy-

TABLE 2
Expression of ER/PR and GOCTS-PCS in Ovarian Surface Epithelial Inclusion Cysts

		Mucin-free cuboidal cells				Endocervical-like cells			
				GOCTS-PCS				GOCTS-PCS	
Patient no.	Age (yrs)	ER	PR	GOCTS	PCS	ER	PR	GOCTS	PCS
1	44	++	++	−	−	+ or −	+ or −	++	++
2	51	++	++	−	−	+ or −	+ or −	+	++
3	53	++	++	−	−	+ or −	+ or −	−	+

ER: estrogen receptors; PR: progesterone receptors; GOCTS: galactose oxidase cold thionin Schiff; PCS: paradoxical Concanavalin A staining.

drated through graded alcohols. They were subsequently treated in a microwave oven in 0.01M citrate buffer (pH 6) for 15 minutes. Then, 0.03% hydrogen peroxide was applied to block endogenous peroxidase activity, and 10% normal rabbit serum was applied to reduce nonspecific binding. The sections were incubated with specific primary antibodies or control nonimmunized mouse serum at 4 ° C overnight. Biotinylated antimouse immunoglobulin G was used as a linker. After the sections were washed, streptoavidin-peroxidase complex was applied, and staining was carried out with diaminobenzidine. Weak counterstaining was performed with hematoxylin.

Histochemical Staining of Gastric-Type Mucin

To detect gastric-type mucin, galactose oxidase cold thionin Schiff (GOCTS)–paradoxical Concanavalin A staining (PCS),[9] a dual staining method, was used. Surface gastric mucin is stained dark blue by GOCTS and glandular gastric mucin, and dark brown by PCS. The staining procedures were performed as previously described.[9] Normal gastric mucosa was used as a positive control for GOCTS-PCS.

Interpretation of the Staining

Staining was evaluated according to the percentage of stained cells as follows: −: negative (no stained cells); +: focally positive (less than 5% of the cells stained); ++: regionally positive (5–50% of the cells stained); and +++: diffusely positive (more than 50% of the cells stained). The staining intensity of ER and PR in mucinous tumors was compared with that of endocervical glands, and the results were described as either strong (intensity as strong as that of endocervical glands) or weak (intensity weaker than that of endocervical glands).

RESULTS

Normal Endocervical Glands

Normal cervical glands in all 10 cases were strongly stained for ER and PR irrespective of the phase of the menstrual cycle (Fig. 1a, b). Three were focally stained for GOCTS (Fig. 1c). No glands were positive for PCS.

Ovarian Epithelial Inclusion Cysts

Three ovarian specimens of surface epithelial inclusion cysts (Table 2) contained both mucin-free cuboidal cells resembling the surface epithelium of the ovary and mucin-containing cells resembling endocervical gland cells. The morphology of the mucin-containing cells suggested metaplasia of the cuboidal cells derived from the surface epithelium into endocervical-like mucin-containing cells. The cuboidal cells were strongly stained for ER and PR (Figs. 2a, 3a) and negative for GOCTS-PCS (Figs. 2b, 3b). However, the cuboidal cells with faint mucin production showed staining for PCS (Fig. 2b). The mucin-containing cells were either weakly positive or negative for ER and PR (Figs. 2a, 3a) and were positive after dual staining for GOCTS-PCS (Fig. 2b) or focally positive for PCS (Fig. 3b). Therefore, gastric-type mucin was detected in the mucin-containing cells of ovarian surface epithelial inclusion cysts.

Pure Endocervical-Like Mucinous Tumors

Of 19 mucinous tumors of pure endocervical-like type (Table 3), 3 (15.8%) were weakly stained for ER (Fig. 4a), and 16 (84.2%) were weakly positive for PR (Fig. 4b).

All 19 tumors showed staining for GOCTS (16 cases, 84.2%), PCS (16 cases, 84.2%), or both (13 cases, 68.4%). In the dual staining, GOCTS staining was frequently observed in the surface linings of the cysts and/or the upper portions of the crypts, and PCS was frequently observed in the basal portions of crypts (Fig. 5). Observations on serial sections revealed that ER and/or PR positive cells were intermingled in the portions stained for GOCTS-PCS. One M-LMP was negative for ER and PR and positive for GOCTS-PCS.

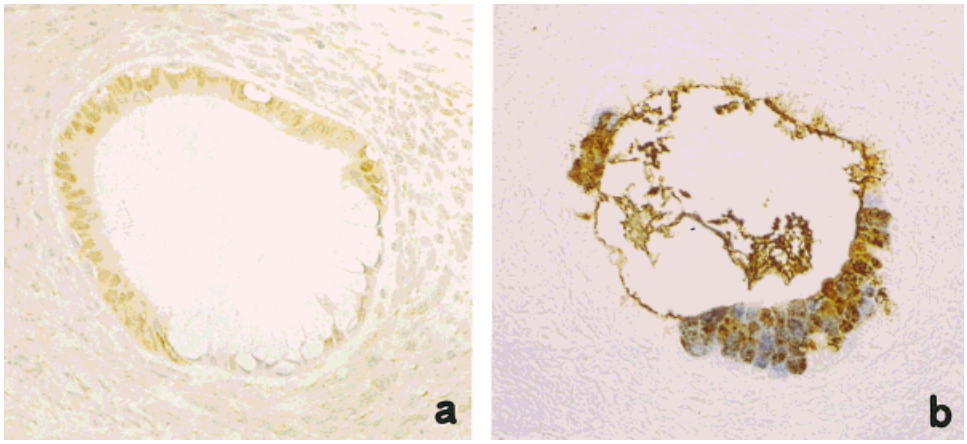

FIGURE 2. An ovarian surface epithelial inclusion cyst, lined by mucin-free cuboidal cells, cuboidal cells with faint mucin, and mucin-containing cells resembling endocervical gland cells, shows staining for estrogen receptors (ER) (a) in the cuboidal cells, and weakly positive or no staining for ER (a) in the mucin-containing cells. By galactose oxidase cold thionin Schiff–paradoxical Concanavalin A staining (GOCTS-PCS) (b), the mucin-containing cells show dual staining for GOCTS and PCS, and the cuboidal cells with faint mucin show positive staining for PCS (original magnification ×175).

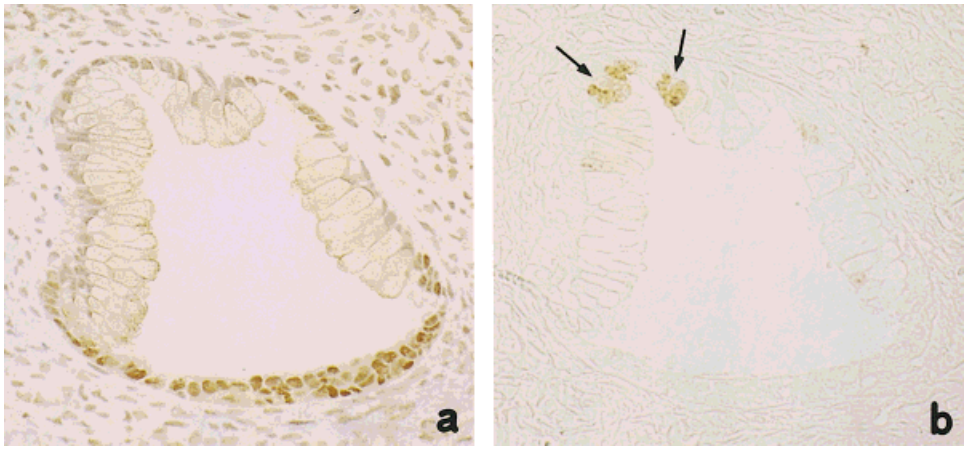

FIGURE 3. An ovarian surface epithelial inclusion cyst, lined by mucin-free cuboidal cells resembling the surface epithelial cells of the ovary and mucin-producing cells resembling endocervical gland cells, shows positive staining for progesterone receptors (PR) (a) in the cuboidal cells, but weakly positive or no staining for PR (a) in the mucin-containing cells. By galactose oxidase cold thionin Schiff–paradoxical Concanavalin A staining (GOCTS-PCS) (b), focal staining for PCS exists in the mucin-containing cells (arrows) (original magnification ×175).

Mixed-Type Mucinous Tumors

Of 18 mixed-type mucinous tumors (Table 4), 2 showed ER staining. One was MA and the staining for ER was confined to the endocervical-like cells. The other was M-LMP and both endocervical-like and intestinal-type cells exhibited ER staining (Fig. 6a). In contrast, 11 tumors (6 MA and 5 M-LMP) showed PR staining. In 7 of the 11 cases, the staining was confined to the endocervical-like cells (Fig. 6b); and in the remaining 4 cases, both the endocervical-like and intestinal-type cells were stained, although the intensity of PR staining appeared weaker in the intestinal-type cells. Only one M-LMP showed both ER and PR staining not only in the endocervical-like cells, but also in the intestinal-type cells. However, in general, there was no apparent difference between MA and M-LMP in their staining patterns for ER and PR.

Seventeen of 18 mixed-type mucinous tumors showed staining for GOCTS (16 cases, 88.9%), PCS (14 cases, 77.8%), or both (13 cases, 72.2%) in the endocervical-like cells. In contrast, the intestinal-type cells were negative for PCS, and only 3 tumors showed weak, focal reactivity for GOCTS. Only one M-LMP was negative for GOCTS-PCS in both the endocervical-like and intestinal-type cells. However, in general, no apparent difference in the staining pattern for GOCTS-PCS was observed between MA and M-LMP. As described for pure endocervical-like tumors, PCS reaction was often observed in the basal portions of the crypts, whereas the upper portions were stained for GOCTS. Observations on serial sections revealed that ER and/or PR positive cells were intermingled in the portions stained for GOCTS-PCS.

Mucinous Adenocarcinoma

Of 10 cases of MCa (Table 5), only one (10.0%) showed staining for ER, but the staining was regional and weak (Fig. 7a). Three tumors (30.0%) showed staining for

TABLE 3
Expression of ER/PR and GOCTS-PCS in Pure Endocervical-Like Mucinous Tumors of the Ovary

Patient no.	Age (yrs)	Pathology	ER	PR	GOCTS-PCS	
					GOCTS	PCS
1	28	MA	++	++	−	+
2	21	MA	++	++	++	−
3	25	MA	−	++	−	+++
4	36	MA	−	++	+++	−
5	37	MA	−	++	+	++
6	45	MA	−	++	+	++
7	50	MA	−	++	+++	−
8	37	MA	+	+	+	++
9	21	MA	−	+	+	++
10	35	MA	−	+	−	++
11	35	Ma	−	+	+	++
12	48	MA	−	+	+	++
13	53	MA	−	+	++	+
14	58	MA	−	+	+	++
15	64	MA	−	+	++	+
16	77	MA	−	+	+	++
17	49	MA	−	−	+	+++
18	60	MA	−	−	+	++
19	61	M-LMP	−	−	+	++

ER: estrogen receptors; PR: progesterone receptors; GOCTS: galactose oxidase cold thionin Schiff; PCS: paradoxical Concanavalin A staining; MA: mucinous adenoma; M-LMP: mucinous tumor of low malignant potential.

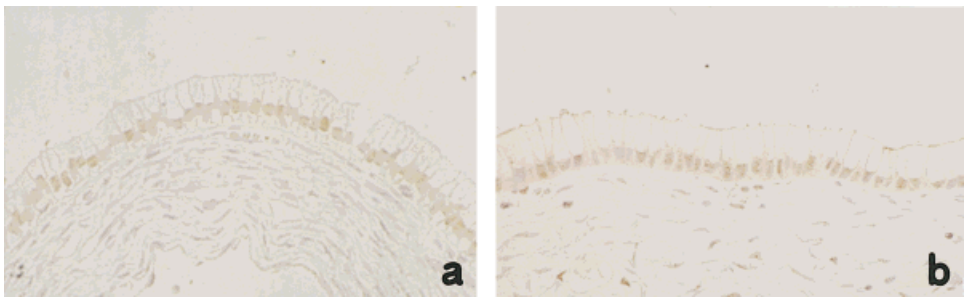

FIGURE 4. Pure endocervical-like mucinous cystadenoma of the ovary shows weak staining for estrogen receptors (a) and progesterone receptors (b) in the endocervical-type cells. (original magnification ×175)

PR; in one of them the staining was diffuse and strong (Fig. 7b).

Of 10 cases of MCa, 7 showed staining for GOCTS (7 cases, 70.0%), PCS (3 cases, 30.0%), or both (3 cases, 30.0%). The localization of the staining for GOCTS and PCS was random, and the structure specific staining for GOCTS-PCS observed in MA and M-LMP was not identified (Fig. 8). In addition, no apparent correlation between the expression of gastric-type mucin and steroid receptors was observed.

DISCUSSION

The current study has demonstrated that, as a representative of müllerian-derived, mucin-containing cells, normal endocervical glands constantly express both ER and PR and are rarely stained for GOCTS (dual staining for GOCTS-PCS). GOCTS reacts strongly with gastric surface mucin and less intensely or faintly with the mucin of the transverse colon and lung,[9] and PCS reacts specifically with the mucin of gastric gland cells.[11] We have recently demonstrated that among the neoplasms of the cervical glands, minimal deviation adenocarcinoma shows loss of the expression of ER and PR and focal expression of gastric-type mucin (GOCTS and PCS).[12] These results indicate that the müllerian-derived endocervical epithelium can acquire histochemical properties of normal gastric epithelium under pathologic conditions. This staining pattern was also observed in ovarian epithelial inclusion cysts that showed metaplasia of the cuboidal cells

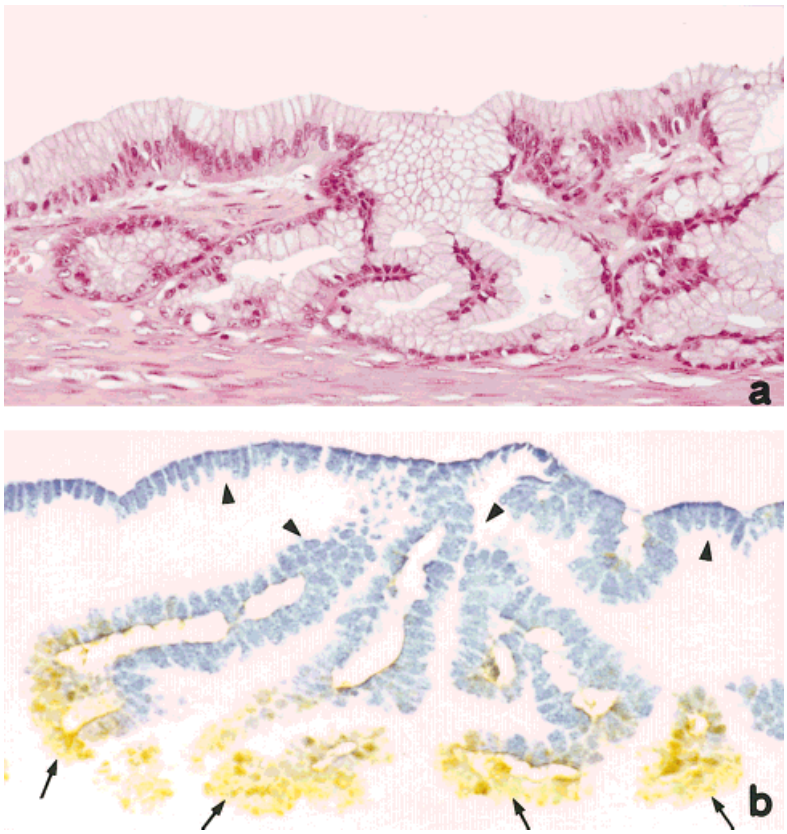

FIGURE 5. Pure endocervical-like mucinous cystadenoma of the ovary, with (a) H & E staining and (b) galactose oxidase cold thionin Schiff–paradoxical Concanavalin A staining (GOCTS-PCS). GOCTS staining (arrow head, dark blue) can be seen in the surface and upper portions of the crypts, and PCS (arrows, dark brown) can be seen in the basal sites of the crypts. The staining pattern suggests gastric-type ''organoid'' differentiation. (original magnification ×175)

TABLE 4
Expression of ER/PR and GOCTS-PCS in Mixed-Type Mucinous Tumors of the Ovary

			Endocervical-like cells				Intestinal-type cells			
					GOCTS-PCS				GOCTS-PCS	
Patient no.	Age (yrs)	Pathology	ER	PR	GOCTS	PCS	ER	PR	GOCTS	PCS
1	73	MA	++	++	+	++	−	−	+	−
2	49	MA	−	++	+	+	−	+	−	−
3	29	MA	−	+	++	+	−	+	−	−
4	30	MA	−	+	++	+	−	+	−	−
5	49	MA	−	+	++	−	−	−	+	−
6	72	Ma	−	+	++	−	−	−	−	−
7	33	MA	−	−	++	+	−	−	−	−
8	36	MA	−	−	++	+	−	−	−	−
9	45	MA	−	−	+	−	−	−	−	−
10	45	MA	−	−	+	++	−	−	−	−
11	55	MA	−	−	++	+	−	−	−	−
12	37	M-LMP	++	++	−	−	+	+	−	−
13	20	M-LMP	−	++	+	++	−	−	−	−
14	58	M-LMP	−	++	+	++	−	−	−	−
15	66	M-LMP	−	++	+	+	−	−	+	−
16	42	M-LMP	−	+	−	++	−	−	−	−
17	41	M-LMP	−	−	+	++	−	−	−	−
18	56	M-LMP	−	−	+	++	−	−	−	−

Mixed-type: endocervical-like cells and intestinal-type cells; ER: estrogen receptors; PR: progesterone receptors; GOCTS: galactose oxidase cold thionin Schiff; PCS: paradoxical Concanavalin A staining; MA: mucinous adenoma; M-LMP: mucinous tumor of low malignant potential.

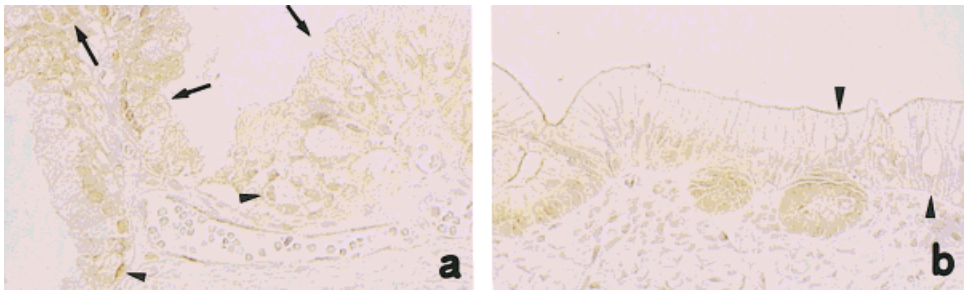

FIGURE 6. Immunostaining for estrogen receptors (ER) and progesterone receptors (PR) is shown. (a) A mixed-type mucinous tumor with low malignant potential shows weakly positive staining for ER in endocervical-like cells (arrows) and goblet type cells (arrowheads). (b) A mixed-type mucinous cystadenoma shows weak staining for PR in the endocervical-like cells, but goblet type cells are negative for PR (arrowheads) (original magnification ×175).

TABLE 5
Expression of ER/PR and GOCTS-PCS in Mucinous Adenocarcinomas of the Ovary

Patient no.	Age (yrs)	Histologic grade	ER	PR	GOCTS-PCS	
					GOCTS	PCS
1	33	G1	+	+++	++	+
2	65	G1	−	++	++	+
3	62	G1	−	+	−	−
4	44	G1	−	−	+	−
5	49	G1	−	−	+++	−
6	54	G1	−	−	++	+
7	29	G2	−	−	+++	−
8	42	G2	−	−	−	−
9	52	G3	−	−	+	−
10	62	G3	−	−	−	−

ER: estrogen receptors; PR: progesterone receptors; GOCTS: galactose oxidase cold thionin Schiff; PCS: paradoxical concanavalin A staining.

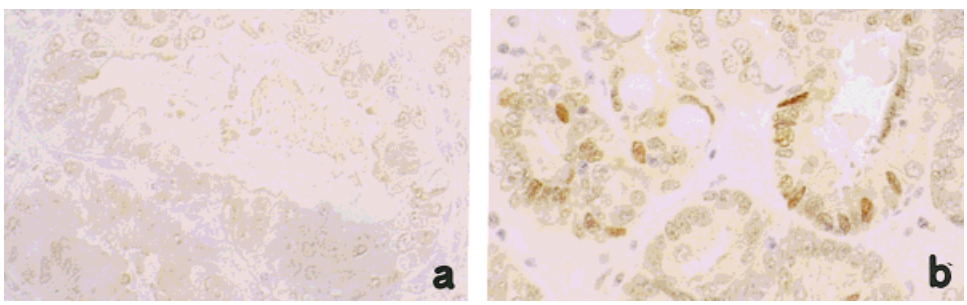

FIGURE 7. Immunostaining for estrogen receptors (ER) (a) and progesterone receptors (PR) (b) is shown in a mucinous adenocarcinoma of the ovary (Grade 1). There is weak staining for ER in the nuclei of the tumor cells. Cells strongly stained for PR are intermingled with weakly stained cells (original magnification ×175).

of surface epithelial origin into endocervical-like mucin-containing cells, i.e., weak or absent ER and PR expression and acquisition of normal gastric-type mucin stained by GOCTS and/or PCS. These results strongly suggest that acquisition of histochemical properties of normal gastric epithelium and gradual disappearance of sex steroid receptors are fundamental in the process of mucinous metaplasia of müllerian-type epithelium.

Application of the same staining methods to pure endocervical-like and mixed-type (endocervical-like cells and intestinal-type cells) mucinous tumors of the ovary (benign and low malignant potential) revealed that the endocervical-like cells in these tumors expressed PR, gastric-type mucin, or both in all cases. It is noteworthy that the intestinal-type cells rarely expressed gastric-type mucin. Conversely, almost all the endocervical-like mucinous epithelium in the benign and low malignant potential mucinous tumors had a staining pattern of sex steroid receptors and

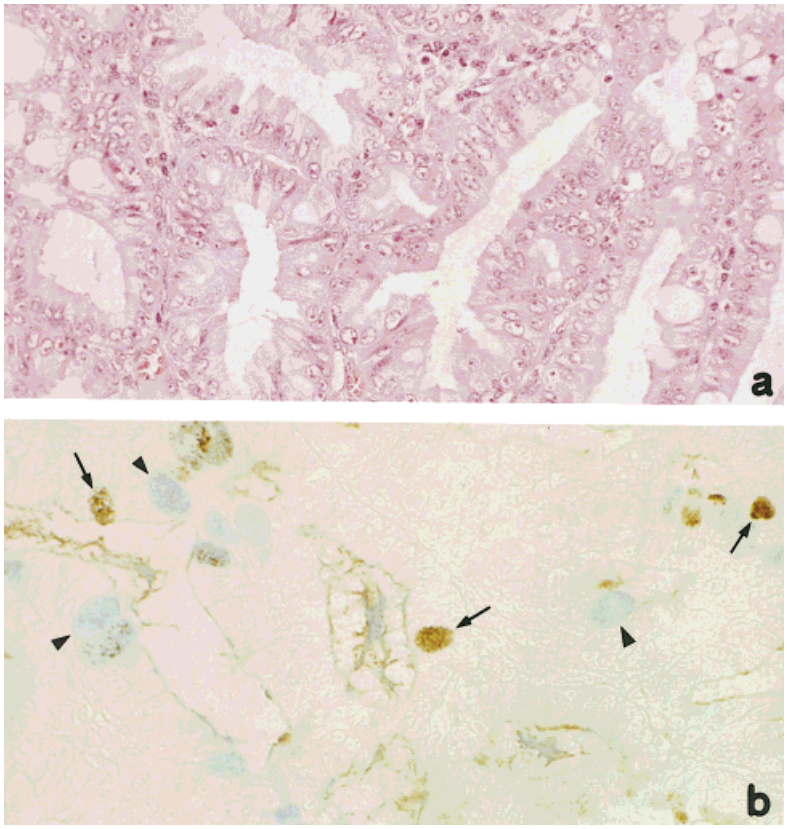

FIGURE 8. Mucinous adenocarcinoma of the ovary (Grade 1) is shown, with (a) H & E staining and (b) galactose oxidase cold thionin Schiff–paradoxical Concanavalin A staining (GOCTS-PCS). Sporadic distribution of GOCTS positive cells (arrowheads) and PCS positive cells (arrows) are noted. ''Organoid'' differentiation is not observed (original magnification ×175).

gastric-type mucin resembling that of the metaplastic mucinous epithelium in ovarian epithelial inclusion cysts. This finding suggests strongly that metaplasia of müllerian-type epithelium is a major pathway in the pathogenesis of mucinous tumors of the ovary. A recent report that gastric-type mucin is stained in mucin-producing cells adjacent to ciliated cells (a phenotypic feature of müllerian-derived epithelium) in ovarian mucinous tumors[13] supports this thesis. However, we cannot rule out the possibility that some mucinous tumors have a monodermal-teratoma nature, because pure intestinal-type mucinous tumors were not examined in this study.

GOCTS-PCS dual staining can demonstrate two types of gastric mucin in the same tissue section. In normal gastric mucosa, the mucinous cells on the surface and in the gland ''neck'' portion are stained for GOCTS, and those in the basal portion of the glands for PCS.[9] It is noteworthy that in this study an almost identical staining pattern was observed in benign and borderline malignant mucinous tumors of the ovary, i.e., gastric-type ''organoid'' differentiation, was observed in the endocervical-like epithelium of these tumors. This gastric-type organoid differentiation has been reported to exist in early gastric carcinomas,[14] in adenocarcinoma of the large intestine,[15] and in metaplastic epithelium that is regarded as precancerous in the pancreas.[16] In this study, staining for both GOCTS and PCS was observed in 3 of 10 mucinous ovarian carcinomas, but it was disorganized. Therefore, gastric-type differentiation seems to be destroyed in advanced stages of malignant mucinous tumors of the ovary.

In summary, through the analysis of the expression of steroid receptors as well as gastric-type mucin, acquisition of histochemical properties of normal gastric epithelium and gradual disappearance of sex steroid receptors are considered a fundamental feature of mucinous metaplasia of müllerian-type epithelium and are demonstrated in almost all benign and low malignant potential mucinous ovarian tumors. This finding suggests that the development of mucinous ovarian tumors operates via müllerian-type metaplasia, which in turn is a prerequisite for gastric-type differentiation in mucinous tumors as they dedifferentiate toward malignant histiotypes.

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