Virchows Archiv (1994) 424:495–502

ORIGINAL ARTICLE

Vibeke Ravn · Ulla Mandel · Birgit Svenstrup
Erik Dabelsteen

Type-1 chain histo-blood group antigens (Le^a, monosialosyl-Le^a, disialosyl-Le^a, Le^b, and H) in normal and malignant human endometrium

Received: 31 December 1993 / Accepted: 10 March 1994

Abstract Type-1 chain histo-blood group antigens such as the Lewis (Le)^a, monosialosyl-Le^a, Le^b and H antigens show an increased expression in endometrial carcinomas. However, the possibility that these antigens are expressed under genetic or hormonal influence in endometrial carcinomas has not been considered. In the present study, the expression of type-1 chain carbohydrate antigens in normal and malignant endometrium was evaluated by immunohistochemistry and related to both genetic and hormonal factors. The glands of normal, non-secretory endometria expressed, in contrast with surface epithelial cells, Le^a, Le^b, disialosyl-Le^a, and H determinants infrequently. Adenomatous hyperplasias and endometrial carcinomas showed an increased expression of type-1 chain carbohydrates that was qualitatively influenced by the erythrocyte Lewis phenotype and the secretor status. Whereas Le^{a+b-} non-secretors mainly accumulated Le^a antigen, and only limited amounts of Le^b antigen, Le^{a-b+} secretors expressed H, Le^b and Le^a antigens. The expression of type-1 chain antigens showed no association with the serum-oestrogen level or to the hormone-receptor status. Thus the Lewis secretor status has a qualitative influence on the increased expression of type-1 chain antigens, which, however, seem to be unrelated to hormonal factors. Our findings suggest an increased activity of the *Se*-gene-defined or a closely related fucosyl-transferase in neoplastic endometrial epithelial cells.

Key words ABO blood-group antigen · Lewis blood-group antigen · Type-1 chain carbohydrates · Human endometrium · Endometrial carcinomas

V. Ravn (✉)
Department of Pathology, Rigshospitalet,
9 Blegdamsvej, DK-2100 Ø Denmark

U. Mandel · E. Dabelsteen
Department of Oral Diagnostics, School of Dentistry,
University of Copenhagen, Denmark

B. Svenstrup
Department of Clinical Biochemistry, Statens Seruminstitute,
Copenhagen, Denmark

Introduction

Essentially all human cancers show abberant glycosylation, and changes in histo-blood group ABH and Lewis (Le) antigens are common alterations in human cancers [7]. An increased expression of H and the related Le^b and Le^y antigens is found in many carcinomas and is believed to be due to an enhanced fucosylation in addition to deletion of A and B blood group antigens [5, 17, 19, 28, 39].

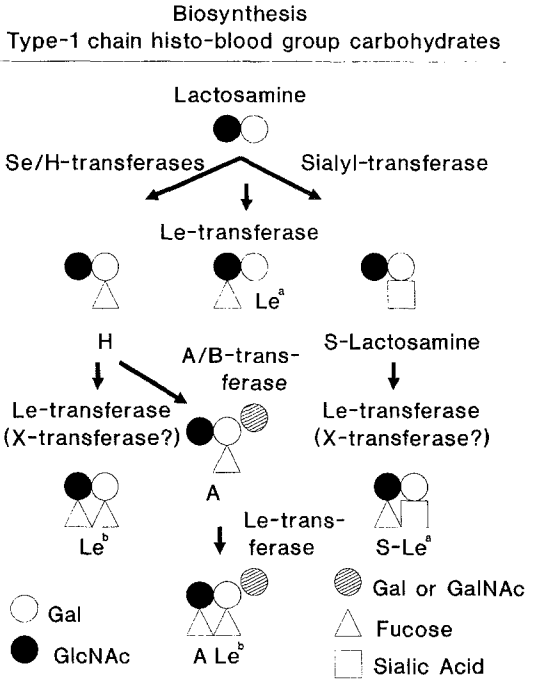

Fig. 1 The possible biosynthetic pathway for synthesis of type-1 chain fucosylated carbohydrate structures in human endometrium. The expression of H and Lewis (Le)^b antigens in malignant endometria from erythrocyte Le^{a+b-} individuals and of Le^a antigen in malignant endometria from erythrocyte Le^{a-b-} individuals indicate that fucosyltransferases other than the *Le*-gene- and *Se*-gene-encoded transferases may be involved in synthesis of these structures

The peripheral core structure of carbohydrates with blood-group specificity forms highly immunogenic structural isomers, the type-1, –2, –3, and –4 isomers, on which chain-elongation associated with synthesis of carbohydrates with blood-group specificity takes place. In normal epithelial tissues, the expression of type-1 chain blood-group antigens is qualitatively influenced by the genetic background of the individual in terms of the ABO, Lewis blood-group and the secretor status [13, 23, 24]. In general, only individuals possessing the genes responsible for synthesis of the specific glycosyltransferases needed for the step wise built-up of the growing carbohydrate chain can express the terminal carbohydrate antigen (Fig. 1).

In the endometrium, the type-1 chain histo-blood group antigens Le^a, monosialosyl-Le^a (msLe^a), disialosyl-Le^a (dsLe^a), Le^b and H have a limited but genetically and possibly also hormonally influenced expression in normal cycling endometrium [20, 32, 36]. Most of these carbohydrates show an increased expression in endometrial carcinoma cells [9, 10, 11, 12, 18, 21, 36, 37, 41]. However, the possibility, that the accumulation of type-1 chain antigens may be related to genetic or hormonal factors, as recently suggested for the isomere type-2 chain antigens [34], has not been considered. In the present paper we report our immunohistochemical findings with monoclonal antibodies (mAb) to type-1 chain histo-blood group carbohydrates, the H, Le^b, Le^a, msLe^a, and dsLe^a antigens in the same material of normal, premalignant, and malignant endometrial tissues recently studied for expression of type-2 chain antigens [34].

Materials and methods

The material, which has been described in detail previously, included normal and malignant formalin-fixed paraffin-embedded endometrial tissues from hysterectomies [34] (Tables 1 and 2). The ABO and Lewis blood type was established on erythrocytes in all cases, and a ABH-saliva-secretor status was determined in 69 of the 112 cases (Table 1) [30, 34]. A serum-hormone analysis was performed on 54 women (Table 1). This included measurements of oestrone (E_1), 17β-oestradiol (E_2), oestrone sulphate, sex hormone-binding globulin (SHBG), non-protein-bound E_2 (free E_2), and non-SHBG-bound E_2 [35].

The oestrogen (ER) and progesterone receptor (PgR) status was determined on three of seven adenomatous hyperplasias and 30/36 carcinomas from Le^{a-b+} secretors by using the Abbott ER- and PgR-ICA staining kits on sections of frozen tissue. The immuno-histochemical assays for ER and PgR were performed as described in detail previously [33]. The receptor status, which was defined as negative when less than 10% of the neoplastic cells were stained [35] is given in Table 2. All three and two of three adenomatous hyperplasias were ER and PgR positive, respectively.

For immunohistochemistry mouse mAb with specificity to the type-1 chain structures Le^b, Le^a, msLe^a, and dsLe^a were used. The specificity, source, immunoglobulin subclass, and reference for the mAb used is given in Table 3. As no antibody has proven specificity to type-1 H, we used anti-H mAb A583 (DAKO, Denmark) reactive with both types-1 and 2 H-determinants [25]. The A583 and anti-Le^a/Le^b mAb were available as affinity-purified ascites and were used diluted 1: 50, and 1: 200, respectively. The other mAb were hybridoma culture supernatants and were used undiluted. Neuraminidase type X (Sigma) from *Clostridium perfringens* (0.1 unit per ml acetate buffer with 0.04 M calcium chloride added for 2 h at 37° C) was used to remove sialic acid from antigens possibly masked by sialic acid [6]. Peroxidase-conjugated rabbit anti-mouse immunoglobulin, diluted 1: 20, was used as second layer antibody (Dako, P260).

After deparafinisation and rehydration, some of the serially cut sections were preincubated with neuraminidase and all subsequently stained by an indirect two layer standardized immunoperoxidase staining technique using overnight incubations of the first layer anti-

Table 1 The number of endometria investigated in relation to morphology and blood group-status

Morphology[a]	Number of specimens	Blood group ABO/Lewis (Le) status					Saliva status[h]	Hormone Analysis available (number)	Hormone Treatment[j]	Age (years) median (range)
		O		A/B		ABO				
		Le^{a-b+}	Le^{a+b-}	Le^{a-b+}	Le^{a+b-}	Le^{a-b-}				
Atrophic[b]	17	6	1	9	1		15	12	2	63 (46–76)
Weakly proliferative	10	3	0	5	2		8	6	0	50 (31–52)
Normal proliferative[c]	7	2	0	5	0		4	2	1	47 (40–50)
Irregular proliferative	9	3	0	4	2		9	5	2	49 (43–63)
Adenomatous hyperplasia[d]	13	1	2	6	3	1[f]	7	7	2	63 (51–67)
Endometrial carcinoma[e]:										
grade I	33	12	2	11	3	5[g]	16	13	11	66 (47–84)
grade II	17	5	1	6	1	4[g]	8	6	2	65 (58–88)
grade III	6	1		1	2	2[g]	2	3	1	65 (53–71)
Total	112	36	7	52	17	12	69	54	21	31–88

[a] Based on conventional criteria [4]
[b] Includes inactive endometria
[c] The findings in normal cycling endometrium have been published in a separate report [32]
[d] AH all grades (2 slight, 4 moderate, 7 severe) included
[e] Graded in accord with the commendations of WHO [29] and FIGO [3]
[f] Saliva secretor
[g] Saliva status determined on 7 of 12 Le^{a-b-} individuals included
[h] The ABH secretor status was determined on saliva from 69 of the 112 women and was performed as described in detail previously [30]; if no secretor status was available, the erythrocyte Le phenotype was used to predict secretor status in Le^{a-b+} (secretors) and Le^{a+b-} (non-secretors) individuals [8, 27, 42]
[j] The number of women in hormonal substitution therapy at the time of hysterectomy or during the last month prior to hysterectomy (11 were on sequential and 1 on a combined estradiol – progestagen hormone treatment regime, 7 women recieved pure estradiol and 2 women recieved pure progestagens) [34]

Table 2 Histological grade, oestrogen (*ER*)- and progesterone receptor (P*g*R) status, and FIGO stage of carcinomas investigated

FIGO stage	Histological grade			All grades
	1 (<5% solid)	2 (5–50% solid)	3 (>50% solid)	
1A	5	1	0	6
1B	21	8	0	29
1C	2	3	2	7
2	5	3	0	8
>2	0	2	4	6
All	33	17	6	56[b]
ER positive[a]	20/23	3/5	0/2	26/33
PgR positive[a]	18/23	3/5	1/2	24/33

[a] Le^{a-b+} individuals only
[b] Fifty carcinomas were pure endometrioid (13 showed focal secretory or mucinous carcinoma). The remaining 6 carcinomas included 1 adenosquamous carcinoma, 4 endometrioid carcinomas with components of clear cell carcinoma or serous carcinoma (3 cases) and 1 pure serous carcinoma

Table 3 Specificity of antibodies used to demonstrate type-1 chain mono- and difucosylated carbohydrate antigens (*ms*Le^a monosialosyl Le^a, *ds*Le^a disialosyl Le^a

Antigen	Antigen determinant	Antibody/Ig-class	Reference
Le^a	Galβ1→3[Fucα1→4]GlcNAcβ1→R	Lea, Chemb/IgM	Yuan et al. [44]
MsLe^a*	NeuAcα2→3Galβ1→3[Fucα1→4]GlcNAcβ1→R	Anti-CA19-9/IgG1	Magnani et al. [15]
DsLe^a*	NeuAcα2→3Galβ1→3[NeuNAcα2→6][Fucα1→-4]GlcNAcβ→R	FH7/IgG3	Nudelman et al. [22]
Le^b**	Fucα1→2Galβ1→3[Fucα1→4]GlcNAcβ1→R	Leb, Chemb/IgM	Yuan et al. [44] and Mandel [16]
Chain-1 H***	Fucα1→2Galβ1→3GlcNAcβ1→R	A583, DAKO/IgM	Ørntoft et al. [25]

* FH7 antibody is reactive with ms $Le^a{}_{II}$ also
** The anti-Le^b antibody is, in addition to Le^b, reactive with type-1 H, but no evidence for anti-Le^y reactivity was found [16, 27]
*** The antibody used is reactive with type-1 and -2 chain H [25]

bodies and AEC (0.04% 3-amino-9-ethyl carbazole) as chromogen, as previously described in detail [30, 31, 32, 34].

Staining was controlled by replacing the primary antibody with diluent buffer, a mAb of irrelevant specificity but with the same isotype, and culture supernatant. Cervical tissues with known ABO, Le blood-group and saliva-secretor status served as positive and negative tissue controls [40]. Neuraminidase treatment was controlled as previously described [30, 31, 32].

Staining was scored semi-quantitatively in arbitrary staining scores as follows: score 0, no stain; score 1, <10% of the cells stained; score 2 between 10% and 25%; score 3 between 25% and 75%; score 4 >75% of the cells stained. This scoring was shown to be reproducible by use of kappa statistics [30], and by comparing staining in different blocks of tissue from the same hysterectomy specimen (own unpublished data).

The computer programme Medstat (Astra, Copenhagen) was used to perform the statistics, which included Spearman's rank correlation and the Mann-Whitney tests. A P value <0.05 was chosen as the level of significance.

Results

The mAb to H (A583) stained endothelial cells, most frequently in endometria from O and A_2 individuals. Apart from this stromal cells were unstained. Staining of epithelial cells was mainly heterogeneous in carcinomas. This intraindividual variation displayed itself inside individual tissue sections, and staining did not differ between morphologically similar tissue blocks from the same hysterectomy specimen. Staining in endometria from women on hormone therapy was similar to staining in endometria from women who were not receiving hormones.

In epithelial cells the expression of the carbohydrates investigated varied, but distinct patterns of staining relating to the genetic background, layer, or morphology were evident. In the normal endometrium, Le^a, Le^b, ms- and dsLe^a antigens were predominantly expressed by surface epithelial cells. Some of the cells stained were ciliated cells. Staining for H antigen was infrequent in all layers of normal endometrium. The staining results for epithelial cells in the functionalis in relation to the genetic background and the morphology are described in detail below and in Figures 2–4. In normal endometrium, the carbohydrate determinants were almost exclusively demonstrated at apical membranes. In contrast, both the apical membranes and the cytoplasm stained for type-1 chain structures in malignant endometrial cells.

Le^b and H antigens were demonstrated infrequently in the normal endometrium. Both carbohydrates were found most frequently in atrophic endometria from 0 secretors. Neither staining for Le^a antigen nor the increased expression of Le^b and H antigens found in premalignant and malignant endometria was related to the ABO blood-group status.

Le^a and Le^b antigens were demonstrated regardless of the Le erythrocyte phenotype in normal (mainly surface

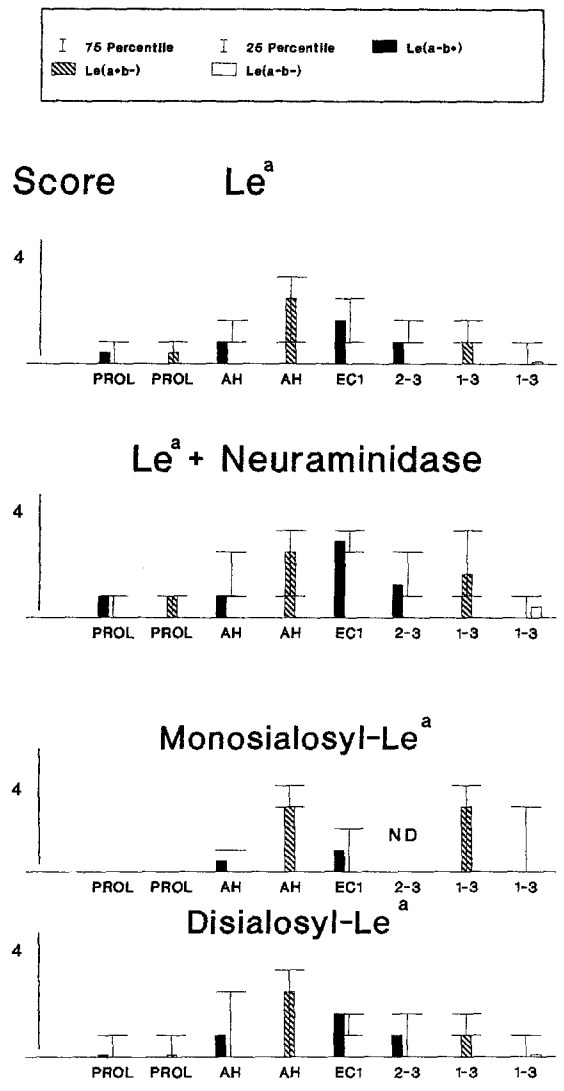

Fig. 2 Staining scores for Le^a and sialosyl-Le^a and in human endometrium in relation to histology and the erythrocyte Le phenotype. (*Prol* proliferative, *AH* adenomatous hyperplasia, *EC* endometrial carcinoma, figures indicate the histological grade), *ND* not performed

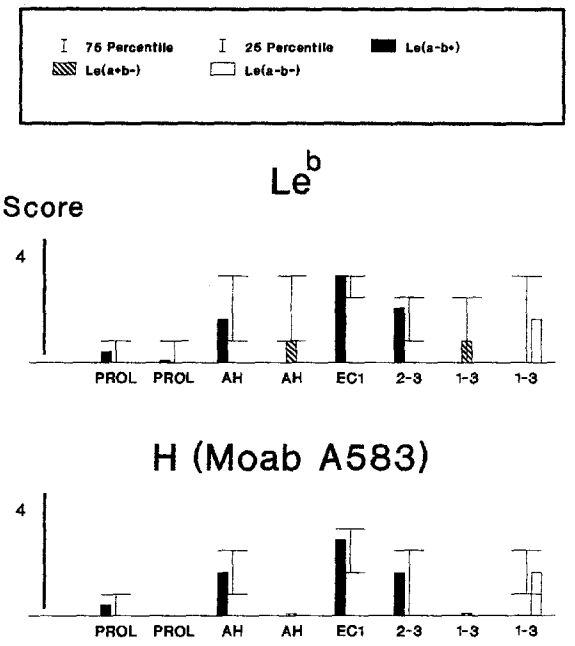

Fig. 3 Staining scores for Le^b and H antigens as per Figure 2

epithelial cells), premalignant and malignant endometrial cells (Figs. 2, 3). However, endometria from erythrocyte Le^{a-b-} individuals expressed Le^a and Le^b antigens less frequently than endometria from Le^{a+b-} or Le^{a-b+} individuals (Table 4, Figs. 2, 3), and $msLe^a$ antigen showed the highest expression in adenomatous hyperplasias and endometrial carcinomas from Le^{a+b-} individuals (Fig. 2). Adenomatous hyperplasias and grade 1 endometrial carcinomas from Le^{a-b-} or Le^{a-b+} secretors expressed H-determinants in 32 of 34 cases, whereas H antigen was expressed by only one of ten corresponding cases from non-secretors (Fig. 3).

Functional glands of normal endometrium stained infrequently for type-1 chain carbohydrate determinants (Figs. 2, 3). Le^a and Le^b antigens were expressed most frequently in the atrophic endometrium (few cells or single glands were stained for Le^a in 7/12 and for Le^b in 7/11 atrophic endometria, respectively). Neuraminidase pretreatment had no significant influence on staining for Le^a antigen, and mAb to $msLe^a$ and $dsLe^a$ antigens stained few cells in less than half of the specimens. Normal proliferative endometrium expressed Le^a, $msLe^a$, $dsLe^a$ or Le^b antigens in less than half the cases and if so in very few cells (Figs. 2, 3).

The glands in 17 of 37 normal endometria stained focally (score ≤ 1) for H antigen. Apical membranes of very few cells, mainly in atrophic endometria were stained (Fig. 3). Neuraminidase pretreatment was performed as spot-testing and did not significantly increase staining in normal non-secretory endometria.

In neuraminidase untreated sections of adenomatous hyperplasia, the mAb to Le^a stained most cells in four of five specimens from non-secretors (Le^{a+b-}), but typically few cells only in adenomatous hyperplasias from Le^{a-b+} secretors (Fig. 2). After neuraminidase pretreatment, more cells were stained by the mAb to Le^a compared to staining in untreated sections in 5 of the 13 specimens. In accord with this, the mAb to $msLe^a$ (CA 19-9) stained two of six and all four, and the mAb to $dsLe^a$ (FH7) stained one of six and four of five, adenomatous hyperplasias from Le^{a-b+} secretors and Le^{a+b-} non-secretors, respectively.

Le^b antigen was expressed in 12/13 adenomatous hyperplasias (including one Le^{a-b-}, saliva-secretor). The expression varied. The median staining score was higher in adenomatous hyperplasias from Le^{a-b+} secretors than in adenomatous hyperplasias from Le^{a+b-} non-secretors (Fig. 3).

The H-determinant (mAb A583) was expressed by six of seven adenomatous hyperplasias from Le^{a-b+} secretors (Fig. 3). When expressed, staining was focal except for

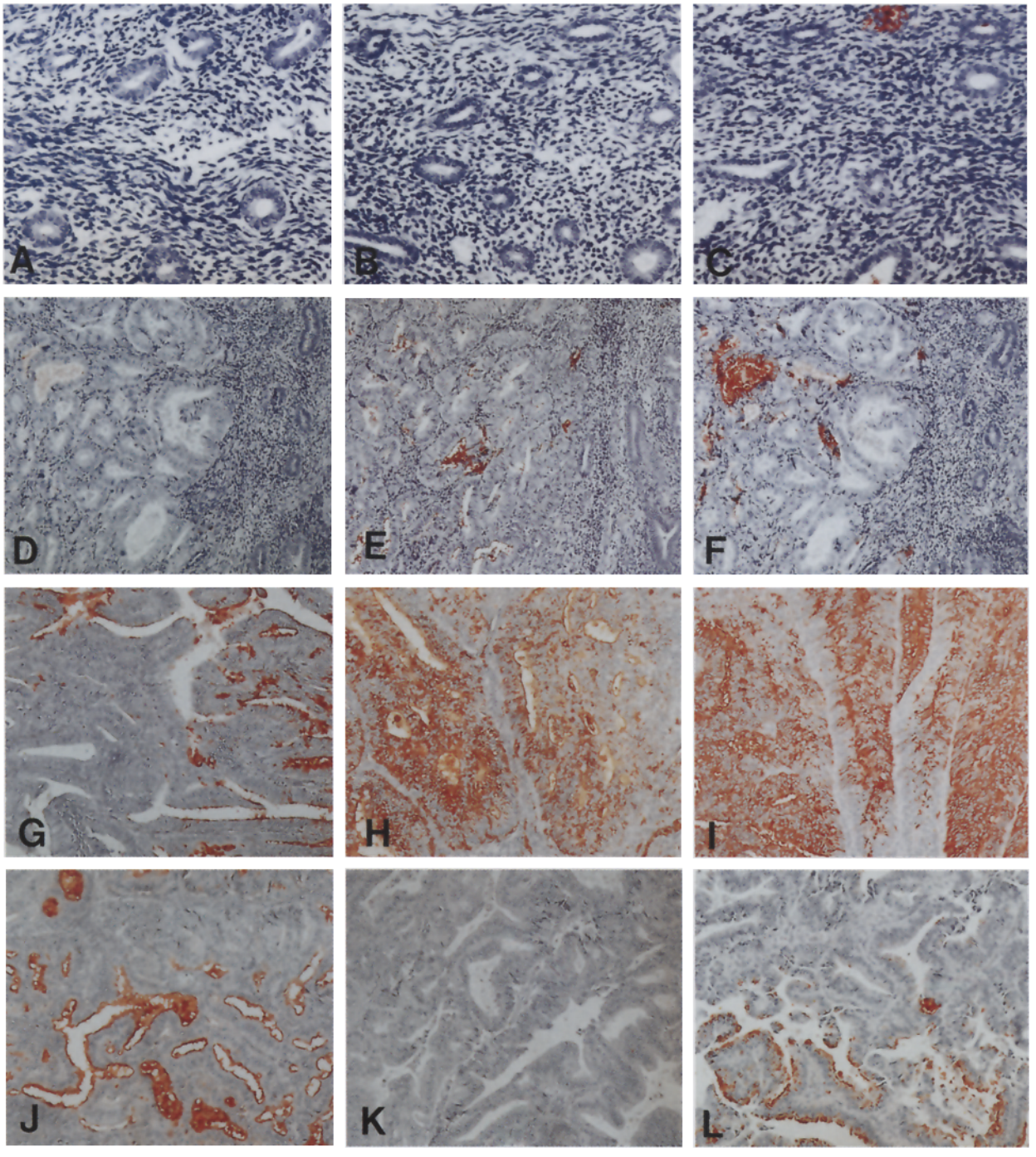

Fig. 4 Immunohistochemical staining for type-1 chain carbohydrates in normal and malignant endometrium. Atrophic endometrium stained for Le^a (**A**), H (**B**), and Le^b (**C**). Adenomatous hyperplasia from an A_1B Le^{a-b+} secretor stained immunohistochemically for monosialosyl (ms)Le^a (**D**), H (**E**), and Le^b antigens (**F**). Well differentiated endometrioid carcinoma from a blood group O Le^{a-b+} secretor stained immunohistochemically for Le^a (**G**), H (**H**), and Le^b (**I**) antigens. Well differentiated endometrioid adenocarcinoma from a blood group A_1 Le^{a+b-} non-secretor stained immunohistochemically for sialosyl-Le^a with mAb CA 19-9 (**J**), H (**K**), and Le^b (**L**) antigens

Table 4 The influence of the erythrocyte Le phenotype on staining for type-1 chain carbohydrates in adenomatous hyperplasias and endometrial carcinomas [*NS* non-significant (i.e., p>0.05 by the two-sided *t*-test]

Antigen	Difference in staining in relation to Le type (Mann Whitney test)	*P*-values (two-sided) Le^{a+b-} vs Le^{a-b+}	Le^{a+b-} vs Le^{a-b-}	Le^{a-b+} vs Le^{a-b-}
Le^a	Le^{a+b-}*>Le^{a-b+}>Le^{a-b-}	0.01429	0.00152	0.00245
Le^a (with neuraminidase pretreatment	La^{a-b+}~Le^{a+b-}>Le^{a-b-}	NS	0.00288	0.00029
H	Le^{a-b+}>Le^{a-b-}>Le^{a+b-}	<0.00001	0.00133	0.03077
Le^b	Le^{a-b+}>Le^{a+b-}~Le^{a-b-}	0.00561	NS	0.01242

* A significant difference in staining for Le^a between endometria from Le^{a+b-} and Le^{a-b+} individuals was observed in adenomatous hyperplasias only

one case in which all cells stained. An increase in the fraction of cells stained was found in three of seven cases from secretors after neuraminidase pretreatment, but in none of the five specimens from non-secretors that remained unstained.

Staining for Le^a antigen was pronounced in grade 1 carcinomas from erythrocyte Le^{a-b+} or Le^{a+b-} individuals (Fig. 2). Fewer cells were stained in grade 2 and 3 carcinomas, but staining scores showed no significant association with the histological grade. More cells were stained (1 score level) after neuraminidase pretreatment and 21/28 and 23/28 grade 1 carcinomas from women with the erythrocyte Le^{a-b+} or Le^{a+b-} phenotype were found to express msLe^a (mAb 19-9) and dsLe^a (mAb FH7) antigens, respectively (Fig. 2). Staining showed no significant relationship to the tumour grade. Endometrial carcinomas from erythrocyte Le^{a-b-} individuals expressed Le^a or sialosyl-Le^a in 5 of 11 cases (Fig. 2).

All grade 1 carcinomas from Le^{a-b+} individuals, four of five grade 1 carcinomas from Le^{a+b-} individuals, and four of five grade 1 endometrial carcinomas from Le^{a-b-} individuals stained for Le^b antigen (Fig. 3). Grade 1 carcinomas showed a pronounced expression of Le^b antigen. Among Le^{a-b+} secretors, staining scores were related to the tumor grade [*P* (two-tailed)=0.026, Fig. 3].

All 23 grade 1 carcinomas from Le^{a-b+} secretors, one of five carcinomas from Le^{a+b-} and four of five carcinomas from Le^{a-b-} individuals were found to express H-determinants (Fig. 3). The staining scores declined with increasing grade [*P* (two-tailed)=0.044], and whereas staining was found mainly at apical membranes in grade 1 carcinomas, grade 3 carcinomas expressed H-determinants in the cytoplasm mainly (Fig. 3). Neuraminidase pretreatment had no demonstrable influence on staining in grade 1 carcinomas.

A weak, inverse correlation between staining scores for H antigen (mAb A583) in carcinomas from erythrocyte Le^{a-b+} individuals and the FIGO stage [3] was found [Spearman's rank correlation test, $R(S)=-0.332$, $P=0.04814$ (*t*-test)]. The expression of Le^a and Le^b antigens showed no relation to the FIGO stage.

Staining scores for Le^a, Le^b and H antigens showed no significant association to serum-hormone levels nor to the hormone receptor status (data not shown). Meanwhile, ER-positive carcinomas from Le^{a-b+} secretors tended to express higher levels of H-determinants than ER-negative carcinomas (Mann-Whitney test, $P=0.07$).

Discussion

This study has shown that the histo-blood group related type-1 chain carbohydrates, the H, Le^a, dsLe^a, and Le^b antigens are "tumour-associated" antigens in endometrial tissue. Our present findings support the view that the expression of type-1 chain antigens in the endometrium is influenced by the genetic status of the individual in terms of the Le and secretor status. However, although a genetic influence was demonstrated it seems not to follow the pattern seen in erythrocytes.

The H, Le^b and Le^a antigens are expressed at low levels – in formalin-fixed paraffin embedded specimens at almost undetectable levels – in the glands of normal endometrium. A difference in expression of Le^b antigen in normal cycling endometrium between blood group O and A individuals was evident only in sections of frozen tissue, in which immunoreactivity was stronger than in sections of formalin-fixed paraffin embedded tissue [32]. The ABO blood group status seems not, however, to have any influence on the increased expression of types-1 and –2 H or Le^b determinants in adenomatous hyperplasias and endometrial carcinomas [34]. This agrees with the finding that the A/B transferases, which catalyze synthesis of A/B determinants from H-precursor structures in A/B individuals, in general, have a low expression in adenomatous hyperplasias and endometrial carcinomas.

The increased expression of both type-1 and –2 H antigens in malignant transformed endometrial cells is, however, influenced by the Le/secretor phenotype; the H determinant was infrequently expressed in carcinomas from Le^{a+b-}, or Le^{a-b-} non-secretors, when compared with the expression in most carcinomas from Le^{a-b+} secretors. Similar results were obtained with the mAb Be2 with a restricted specificity to type-2 H antigen [34]. The stronger staining with the mAb A583, which is reactive with both types-1 and –2 H determinants, indicate that both types-1 and 2 H antigens accumulate in neoplastic endometrial cells. The closely linked *H* and *Se* genes (Fig. 1) each codes for a distinct α-2-fucosyltransferase which works preferentially but not exclusively on types-2 and –1 precursor chains, respectively [1, 13, 23, 24]. An increased activity of the *Se* gene-encoded fucosyltransferase has been demonstrated in other carcinomas [26]. The increased expression of both types-1 and –2 H antigens in malignant endometrial cells from secretors, suggest an increased activity of the *Se* gene-defi-

ned or a related α1–2-fucosyltransferase in endometrial carcinoma cells too [34, 43].

The mechanisms involved in the regulation of expression of Le^a and Le^b antigens seem to be alike in normal and malignant endometrial tissues. In agreement with previous findings [32], staining for Le^a and Le^b antigens showed some but no close correlation with the Le erythrocyte phenotype; both Le^a and Le^b antigens were demonstrated in adenomatous hyperplasias and endometrial carcinomas regardless of the erythrocyte Le phenotype. However, Le^a antigen showed a more infrequent expression in endometria from erythrocyte Le^{a-b-} individuals than in corresponding endometria from women with the Le^{a-b+} or Le^{a+b-} phenotype.

There are several possible explanations for our findings. These include a tissue-specific regulation of Lewis antigen expression in endometrium that differs from that in erythrocytes. This could be due to the action of other glycosyl-transferases, for example the *X*- and *H*-gene encoded transferases [2, 14, 23, 43]. In addition, a change in the erythrocyte Le phenotype to "non-genuine" Le^{a-b-} in individuals with endometrial carcinoma, as has been found in other malignancies, may also be involved [27]. In our prospectively sampled material, women treated by hysterectomy for endometrial carcinoma had an increased frequency of the erythrocyte Le^{a-b-} phenotype when compared with women operated on for other conditions (personal unpublished observation). The Le genotype (le/le) inferred from the Le^{a-b-} erythrocyte Le phenotyping may thus be incorrect in some carcinomas.

An alternative but less likely explanation for our findings is cross-reactivity of the mAb with related carbohydrate determinants. The anti-Le^b antibody also reacts with the chain 1 H determinant, but does not react with Le^y [16, 27]. However, the mAb A583, reactive with both types-1 and –2 H determinants [25], did not stain Le^{a+b-} endometria at all or stained less frequently than anti-Le^b stained. Moreover, the fact that Le^a antigen was demonstrated in erythrocyte Le^{a-b-} endometria with all the three anti-Le^a mAb employed supports further the validity of our results, that Le antigens are inappropriately expressed in human endometrium.

The increased expression of type-1 chain antigens in neoplastic endometrial cells seems, however, to be unrelated to hormonal factors. Expression is limited in normal endometrial glands and confined almost exclusively to atrophic, luteal and menstrual endometria, which are characterized by either low levels of E_2 (atrophic) or low ER and PgR levels (luteal and menstrual) [33, 35, 38]. $MsLe^a$ antigen seems to be a marker of secretory transformation in normal endometrial epithelial cells [20, 21, 32]. However, the inverse relation between hormone receptor levels and expression of $msLe^a$ in normal endometrium was lost in endometrial carcinomas [21]. No significant association between either serum E_2 levels or the ER/PgR status and expression of type-1 chain antigens was demonstrable, even though most adenomatous hyperplasias and endometrial carcinomas investigated in the present study were associated with low levels of E_2 and were ER and PgR positive.

The increased membraneous expression of type-1 chain structures (as for example the Le^a and Le^b antigens in neoplastic endometrial cells) [9, 10, 11, 36, 37], is accompanied by a lowered expression of the isomere type-2 chains, the Le^x and Le^y antigens [34]. This suggest a "tumour-associated" shift in predominant peripheral core structure on which fucosylation associated with synthesis of carbohydrates with blood group specificity takes place. Le^b and H antigens are prognostic markers for other carcinomas [19]. The grade and stage are prognostic markers for endometrial carcinomas, and expression of both H and Le^b antigens was related to tumour grade, and expression of H antigen weakly correlated to the FIGO stage. More extended studies including long-term follow-up are needed to clarify any independent prognostic significance of H/Le^b antigen expression for endometrial carcinomas.

In conclusion, the present study has substantiated the view that the type-1 chain antigens, Le^a, $dsLe^a$, Le^b and H are "tumour-associated" antigens of endometrial tissue. The increased expression of type-1 chain carbohydrates in premalignant and malignant endometrium seem to be unrelated to hormonal factors, but is qualitatively influenced by the genetic background of the individual, in that non-secretors express Le^a, ms- and $dsLe^a$ antigens predominantly, whereas secretors, in addition to Le^a structures, express H and Le^b antigens. Our findings suggest a change in predominant peripheral core structure on which fucosylation takes place, and an increased activity of the *Se*-gene-encoded or a closely related fucosyltransferase in neoplastic endometrial cells.

Acknowledgements The financial support from the Danish Cancer Society, Ferdinand and Ellen Hindsgauls Fund, director Jacob Madsen and hustru Olga Madsens Fund, Mogens Svarre Mogensens Fund, Wedell Erichsens Fund, and the Danish Medical Research Council is acknowledged.

References

1. Ball SP, Tongue N, Gibaud A, Le Pendu J, Mollicone R, Girard G, Oriol R (1991) The human chromosome 19 linkage group FUT1 (H), FUT2 (SE), LE, LU, PEPD, C3, APOC2, D19ST and D19S9. Ann Hum Genet 55: 225–233
2. Clausen H, Hakomori S (1989) ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. Vox Sang 56: 1–20
3. Commentaries (1989) Revised FIGO staging for gynaecological cancer. Br J Obstet Gynaecol 96: 889–890
4. Dallenbach-Hellweg G (1987) Histopathology of the endometrium, 4th edn. Springer, Berlin Heidelberg New York
5. Dennis JW (1992) Changes in glycosylation associated with malignant transformation and tumor progression. In: Fukada M (ed) Cell surface carbohydrates and cell development. CRC Press, New York
6. Drezeniek R (1973) Substrate specificity of neuraminidases. Histochem J 5: 271–290
7. Hakomori S (1984) Philip Levine award lecture: blood group glycolipid antigens and their modifications as human cancer antigens. Am J Clin Pathol 82: 635–648
8. Hartman G (1941) Group antigens in human organs. A discussion of the "secretor" "non-secretor" phenomenon. Munksgaard, Copenhagen

9. Inoue M, Sasagawa T, Saito J, Shimizu H, Ueda G, Tanizawa O, Nakayama M (1987) Expression of blood group antigens A, B, H, Lewis-a, and Lewis-b in fetal, normal, and malignant tissues of the uterine endometrium. Cancer 60: 2985–2987
10. Inoue M, Sasagawa T, Shimizu C, Shimizu H, Saito J, Ueda G, Tanizawa O, Nakayama M (1988) Altered expression of Lewis antigens associated with malignant transformation in endometrial tissues. Nippon Sanka Fujinka Gakkai Zasshi 40: 1425–1431
11. Inoue M, Nakayama M, Tanizawa O (1990) Altered expression of Lewis blood group and related antigens in fetal, normal adult and malignant tissues of the uterine endometrium. Virchows Arch [A] 416: 221–228
12. Iwamori M, Sakayori M, Nozawa S, Yamamoto T, Yago M, Noguchi M, Nagai Y (1989) Monoclonal antibody-defined antigen of human uterine endometrial carcinomas is Leb. J Biochem (Tokyo) 105: 718–722
13. Le Pendu J, Cartron JP, Lemieux RU, Oriol R (1985) The presence of at least two different H-blood-group-related β-D-Gal α-2-L-fucosyltransferases in human serum and the genetics of blood group H substances. Am J Hum Genet 37: 749–760
14. Macher BA, Holmes EH, Swiedler SJ, Stults CLM, Srnka CA (1991) Human a1–3 fucosyltransferases. Glycobiology 1: 577–584
15. Magnani JL, Nilsson BL, Brockhaus M, Zopt D, Steplewsky Z, Kropowski H, Ginsburg V (1982) A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentose II. J Biol Chem 257: 14365–14369
16. Mandel U, Ørntoft TF, Holmes EH, Sørensen H, Clausen H, Hakomori S, Dabelsteen E (1991) Lewis blood group antigens in salivary glands and stratified epithelium: lack of regulation of Lewis antigen expression in ductal and buccal mucosal lining epithelia. Vox Sang 61: 205–214
17. Mandel U, Langkilde NC, Ørntoft TF, Therkildsen MH, Karkov J, Reibel J, White T, Clausen H, Dabelsteen E (1992) Expression of histo-blood group A/B gene defined glycosyltransferases in normal and malignant epithelia: correlation with A/B carbohydrate expression. Int J Cancer 52: 7–12
18. Memom RS, Yonezawa S, Hasui K, Sato E (1990) Expression of blood group antigens and lectin binding in adenocarcinomas of the endometrium. Acta Pathol Jpn 40: 507–516
19. Miyake M, Taki T, Hitomi S, Hakomori S (1992) Correlation of expression of H/Le^y/Le^b antigens with survival in patients with carcinoma of the lung. N Engl J Med 327: 14–18
20. Neunteufel W, Breitenecker G (1989) CA19-9, CA125 and CEA in endometrial mucosa during the menstrual cycle, in atypical hyperplasia and endometrial carcinoma. Cancer Lett 48: 77–83
21. Neunteufel W, Bieglmayer C, Breitenecker G (1988) CA19-9, CA125 and CEA in endometrial carcinoma tissue and its relation to hormone receptor content and histological grading. Arch Gynecol Obstet 244: 47–52
22. Nudelman E, Fukushi Y, Levery SB, Higuchi T, Hakomori S (1986) Novel fucolipids of human adenocarcinomas: disialosyl Le^a antigen ($III^4FucIII^6NeuAc\text{-}IV^3NeuAcLc_4$) of human colonic adenocarcinoma and the monoclonal antibody(FH7) defining this structure. J Biol Chem 261: 5487–5495
23. Oriol R (1990) Genetic control of the fucosylation of ABH precursor chains. Evidence for new epistatic interactions in different cells and tissues. J Immunogenet 17: 235–245
24. Oriol R, Mollicone R, Coullin P, Dalix A-M, Candelier J-J (1992) Genetic regulation of the expression of ABH and Lewis antigens in tissues. APMIS Suppl 27; 100: 28–38
25. Ørntoft TF, Wolf H, Clausen H, Hakomori S-I, Dabelsteen E (1987) Blood group ABO and Lewis antigens in fetal and normal adult bladder urothelium: immunohistochemical study of type-1 chain structures. J Urol 138: 171–176
26. Ørntoft TF, Wolf H, Watkins WM (1988) Activity of the human blood group ABO, Se, H, Le, and X gene-encoded glycosyltransferases in normal and malignant bladder urothelium. Cancer Res 48: 4427–4433
27. Ørntoft TF, Holmes EH, Johnson P, Hakomori S, Clausen H (1991) Differential tissue expression of the Lewis blood group antigens; enzymatic, immunohistologic and immunochemical evidence for Lewis a and b antigen expression in Le(a–b–) individuals. Blood 77: 1389–1396
28. Passaniti A, Hart GW (1988) Cell surface sialylation and tumor metastases. Am J Biol Chem 263(16):7591–7603
29. Poulsen HE, Taylor SW, Sobin LH (1975) Histological typing of female genital tract tumours. International histological classification of tumours (vol 13). World Health Organization, Geneva, pp 64–65
30. Ravn V, Stubbe Teglbjærg C, Visfeldt J, Bock JE, Sørensen H, Dabelsteen E (1992) Mucin-type carbohydrates (type 3 chain antigens) in normal cycling human endometrium. Int J Gynecol Pathol 11: 38–46
31. Ravn V, Teglbjærg CS, Mandel U, Dabelsteen E (1992) The distribution of type-2 chain histo-blood group antigens in normal cycling human endometrium. Cell Tissue Res 270: 425–433
32. Ravn V, Teglbjærg CS, Mandel U, Dabelsteen E (1993) The distribution of type-1 chain ABO and related histo-blood group antigens in normal, cycling human endometrium. Int J Gynecol Pathol 12: 70–79
33. Ravn V, Rasmussen BB, Højholt L, Barfoed M, Heiberg I, Svenstrup B, Thorpe SM (1993) Reproducibility of subjective immunohistochemical estrogen and progesterone receptor determination in human endometrium. Pathol Res Pract 189: 1015–1022
34. Ravn V, Mandel U, Svenstrup B, Dabelsteen E (1994) Expression of type-2 histo-blood group carbohydrate antigens (Le^x, Le^y and H) in normal and malignant human endometrium. Virchows Arch [A] (in press)
35. Ravn V, Rasmussen BB, Højholt L, Barfoed M, Heiberg I, Svenstrup B, Thorpe SM (1994) Estrogen and progesterone receptors in normal cycling endometrium as studied by endpoint titration. Cell Tissue Res (in press)
36. Scharl A, Grombach G,Vierbuchen M, Gohring U, Gottert T, Holt JA (1991) Antigen 19-9: presence in mucosa of nondiseased müllerian duct derivatives and marker for differentiation in their carcinomas. Obstet Gynecol 77(4):580–585
37. Shiozawa T, Tsukahara Y, Nakayama J, Ishii K, Katsuyama T (1991) Immunohistochemical localization of blood group substances in normal and neoplastic endometrial tissues with special reference to type-1 core chain expression. Gynecol Obstet Invest 32(3):185–188
38. Snijders MPML, Goiij AFPM de, Debets-Te Baerts MJC, Rousch MJM, Koundstall J, Bosman FT (1992) Immunocytochemical analysis of oestrogen receptors and progesterone receptors in the human uterus throughout the menstrual cycle and after menopause. J Reprod Fertil 94: 363–371
39. Stellner K, Hakomori S, Warner GS (1973) Enzymic conversion of "H_1-glycolipid" to A- or B-glycolipid and deficiency of these enzyme activities in adenocarcinoma. Biochem Biophys Res Commun 55: 439–445
40. Torrado J, Gutierrez Hoyos A, Blasco E, Larraz J, Fernandez Rivas JL (1990) Immunohistochemical patterns of blood group ABO and type-1 chain (Lewis a Lewis b) and type-2 chain (H2, Y) antigens in normal uterine cervix. Tissue Antigens 36: 8–11
41. Tsuji Y, Yoshioka M, Ogasawara T, Takemura T, Isojima S (1987) Identification of an H antigen-like blood group antigen in sera of cancer patients using a novel monoclonal antibody raised against endometrial carcinoma. Cancer Res 47: 3543–3550
42. Watkins WM (1966) Blood group substances. Science 152: 172–181
43. Yazawa S, Nakamura J-I, Asao T, Nagamachi Y, Sagi M, Matta KL, Tachikawa T, Akamatsu M (1993) Aberrant $\alpha 1 \rightarrow 2$fucosyltransferases found in human colorectal carcinoma involved in the accumulation of Le^b and Y antigens in colorectal tumors. Jpn J Cancer Res 84: 989–995
44. Yuan M, Itzkowitz SH, Palekar A, et al. (1985) Distribution of blood group antigens A, B, H, $Lewis^a$, and $Lewis^b$ in human normal, fetal, and malignant colonic tissue. Cancer Res 45: 4499–4511