Virchows Arch [Pathol Anat] (1986) 408:395–402

Virchows Archiv A
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O-acylated sialic acid variants in mucinous tumours of the ovary*

G. Lapertosa, P. Baracchini, E. Fulcheri, and R. Tanzi

Institute of Pathological Anatomy and Histology, (Director Prof. A. Giampalmo) University of Genoa, Via De Toni 14, I-16132 Genova, Italia

Summary. O-acylated sialic acid variants (site 8) can be demonstrated histochemically by the PB/KOH/PAS method. They are secreted by goblet cells of the lower gastrointestinal tract, by colorectal adenocarcinomas, and by their metastases. Since the metastases are positive only when the primary tumour is positive, O-acylated sialomucins can be considered to be specific markers of colorectal adenocarcinomas if identified in metastases of a tumour of unknown origin. In our histochemical study we evaluated 29 mucinous cystomas of the ovary (23 benign and 6 malignant). We found that six cases were positive to PB/KOH/PAS. The positivity was observed in a limited number of cells and only in areas which presented an intestinal type epithelium. It was also more evident in malignant cystomas than in benign ones. We therefore think that the PB/KOH/PAS positivity can not only be considered a marker of colorectal adenocarcinomas, but also of all neoplasms which originate from an intestinal epithelium or appear to an "intestinal type epithelium".

Key words: Mucinous tumours – Ovary – Histochemistry – Mucins – O-acylated sialic acids

Introduction

Methods used to detect different sites of O-acylated sialic acid were identified during various investigations on histochemical properties of mucins in the human gastrointestinal tract (Culling et al. 1971; Reid et al. 1973; Culling et al. 1974; Culling et al. 1976; Reid et al. 1984). O-acetylated variants (sites 7, 8) arise or are secreted only by goblet cells of the lower gastrointestinal tract (especially by the mucosa of the terminal ileum, right colon

* This work is partially supported by the M.P.I. (40%)

Offprint requests to: G. Lapertosa at the above address

and ano-rectal region), by a primary tumour in this region, and by its metastases. Many authors (Dawson et al. 1978; Filipe and Fenger 1979; Reid et al. 1980; Filipe and Lake 1983) investigated the behaviour of O-acylated variants in normal and malignant states of the small and large intestine. In previous studies (Fulcheri et al. 1984; Lapertosa et al. 1984), we found 54% of positive cases in 91 colorectal carcinomas but did not find a relationship between the site of neoplasia and the staining intensity related to the grading of the tumour, as suggested by Culling et al. (1977), Montero and Segura (1980), Culling et al. (1981).

A very interesting property of O-acetylated variants is that the metastasis is positive only when the tumour is positive: in a study on 89 carcinomas and 177 metastases Culling et al. (1975) did not find any positive metastasis when the primary tumour was negative.

In a study by Fulcheri et al. (1984) on 31 adenocarcinomas in Dukes' C stages, 45% of metastases were positive if the primary was positive. Therefore, O-acetylated variants can be used as a specific marker of a colorectal adenocarcinoma if a metastasis from a carcinoma of a unknown origin is found to be positive.

In order to verify the reliability of the use of the O-acetylated variants as markers of colorectal carcinoma, we examined many metastases of adenocarcinomas from different sites and found one metastasis from a mucinous adenocarcinoma of the ovary to be positive. This unexpected result suggested that we widen our histochemical study on mucinous cystomas of the ovary. The histogenesis of these tumours is still controversial. It has been proposed that they may arise as monophyletic teratomas in which endodermal elements alone have persisted, or, as coelomic epithelial inclusion cysts via metaplasia (Fox et al. 1964; Scully 1970; Fenoglio et al. 1975; Blaunstain 1977; Scully 1979; Louwerens et al. 1983). This last theory is the most readily accepted at present. On the basis of their histological pattern mucinous tumours of the ovary may be classified (Fenoglio et al. 1975) as: 1) "endocervical" in type, if they are totally composed of endocervical type cells and if the epithelium presents an homogeneous, orderly array of tall columnar cells with basal nuclei and finely granular cytoplasm; 2) "intestinal" in type, if they are composed only of intestinal type epithelial cells including goblet cells, absorptive cells, and argentaffin cells, 3) "mixed" in type, if they contain both intestinal and endocervical type epithelium.

Material and methods

The tissues used in this study were obtained from 29 mucinous cystomas of the ovary (23 benign and 6 malignant). The mean age of the patients was 50 years in benign and 60 years in malignant cases.

All tissues were fixed in 10% formol-saline and embedded in paraffin wax. From each, 5-μm serial sections were cut, stained with haematoxylin and eosin and by using the following histochemical techniques to assess the amount and types of mucins: diastase-periodic acid-Schiff (D-PAS) (Pearse 1968) to detect vic-glycol groups in neutral and some sialomucins; Alcian blue pH 2.5 (AB) for acid mucins (Pearse 1968); high iron-diamine (HID) and HID followed by Alcian blue pH 2.5 (HID-AB) to discriminate between sialo- and sulphomucins (Filipe and Lake 1983, modified from Spicer 1965); periodate-borohydride-potassium hydrox-

ide-PAS technique (PB/KOH/PAS) to reveal O-acylated sialic acids, sites 7,8 (Reid et al. 1973; Culling et al. 1974); periodic acid-thionin Schiff-saponification-borohydride reduction-periodic acid-basic fuchsin Schiff (PAT/KOH/Bh/PAS) to reveal O-acetylated sialic acids, site 8 (red), O-acylated sialic acids sites 4, 7, 9 (blue), N-acylated sialomucins (blue) (Reid et al. 1984) and a mixture of O-acylated sialic acids (site 8), of O-acylated sialic acid (sites 4, 7, 9), of N-acylated sialomucins (purple); Grimelius silver method (Grimelius 1968) to detect argyrophil cells; Masson-Fontana method (Masson 1938) to identify argentaffin cells. Paneth's cells were studied using the immunohistochemical method (PAP-anti lysozyme) (Stenberger et al. 1979; Stenberger 1979).

Results

The 29 mucinous cystomas studied were classified into 3 categories according to Fenoglio et al. (1975); (1) endocervical, (2) mixed, and (3) intestinal type (Table 1).

The cystomas were classified as endocervical when the lining epithelium resembled a typical endocervical epithelium consisting of a single and uniform row of tall mucin-filled columnar cells with basal nuclei and finely granular cytoplasm. Neither ciliated cells nor argentaffin cells were seen. As seen in the Table 1 we found 18 benign and 2 malignant endocervical type cystomas.

The cystomas were classified as intestinal type when the lining epithelium totally resembled that of the intestine having goblet cells scattered among numerous absorptive cells, argentaffin cells, and occasionally Paneth's cells. No pure intestinal type cystoma was found. The cystomas were classified as mixed type if they presented both intestinal and endocervical type epithelium. In these types of cystomas the epithelium often presented a disordered mixture of both types of cells (endocervical and intestinal): sometimes only absorbent cells were seen between the endocervical cells and in other cases only goblet cells were found. The lining epithelium of a glandular lumen might be completely composed of endocervical cells, while the adjacent glandular lumen was lined by intestinal type cells. Argentaffin cells and argyrophil cells were present above the basement membrane (Fig. 1). We classified nine cystomas (five benign and four malignant) as mixed type cystomas.

As seen from the Table 2 different cell types were identified in benign and malignant cystomas. In the latter the classification was possible because they were mainly well differentiated, and for the constant presence of argyrophil cells (Fig. 2).

The endocervical type cystomas both benign and malignant showed a large amount of neutral mucins (Table 3) (85% of cases); sialomucins were present in 90% of cases, the sulphomucins in 60% showing a prevalence of sialomucins over the sulphomucins, especially in benign cystomas. Benign and malignant cases were negative to PB/KOH/PAS staining. The PAT/KOH/Bh/PAS confirmed the absence of O-acylated (site 8) detecting only blue stained cells. The mucous secretion of the goblet cells in benign mixed cystomas showed abundant neutral mucins (100%). The sialomucins were always identified, while sulphomucins were present in 80% of the cases. Three of five cases (60%) were positive to PB/KOH/PAS. The positivity

Table 1. Classification of the histological type of the mucinous ovarian tumours

Histological classification	Histological type			No of Tot. cases
	Endocervical	Intestinal	Mixed	
Benign	18	0	5	23
Malignant	2	0	4	6

Table 2. Different types of cells present in the mixed cystomas

Histological classification	Presence of				
	Absorbent cells	Goblet cells	Argentaffin cells	Paneth's cells	Argyrophil cells
Benign					
1	+	+	+	+	+
2	+	+	0	+	+
3	+	+	+	0	+
4	+	+	0	0	0
5	+	+	0	0	0
Malignant					
1	0	+	0	0	+
2	0	+	+	0	+
3	0	+	+	0	+
4	+	+	0	0	+

Table 3. Pattern of mucins in the endocervical and mixed type cystomas

Pattern of mucins		Histological classification			
		Endocervical type cystomas		Mixed type cystomas	
		Benign tot. cases 18	Malignant tot. cases 2	Benign tot. cases 5	Malignant tot. cases 4
		N° of cases	N° of cases	N° of cases	N° of cases
Neutral mucins	+	16	1	5	4
	0	2	1	0	0
Sialomucins	+	16	2	5	4
	0	2	0	0	0
Sulphomucins	+	10	2	4	3
	0	8	0	1	1
O-acylated sites 7, 8 acid variants	+	0	0	3	3
	0	18	2	2	1

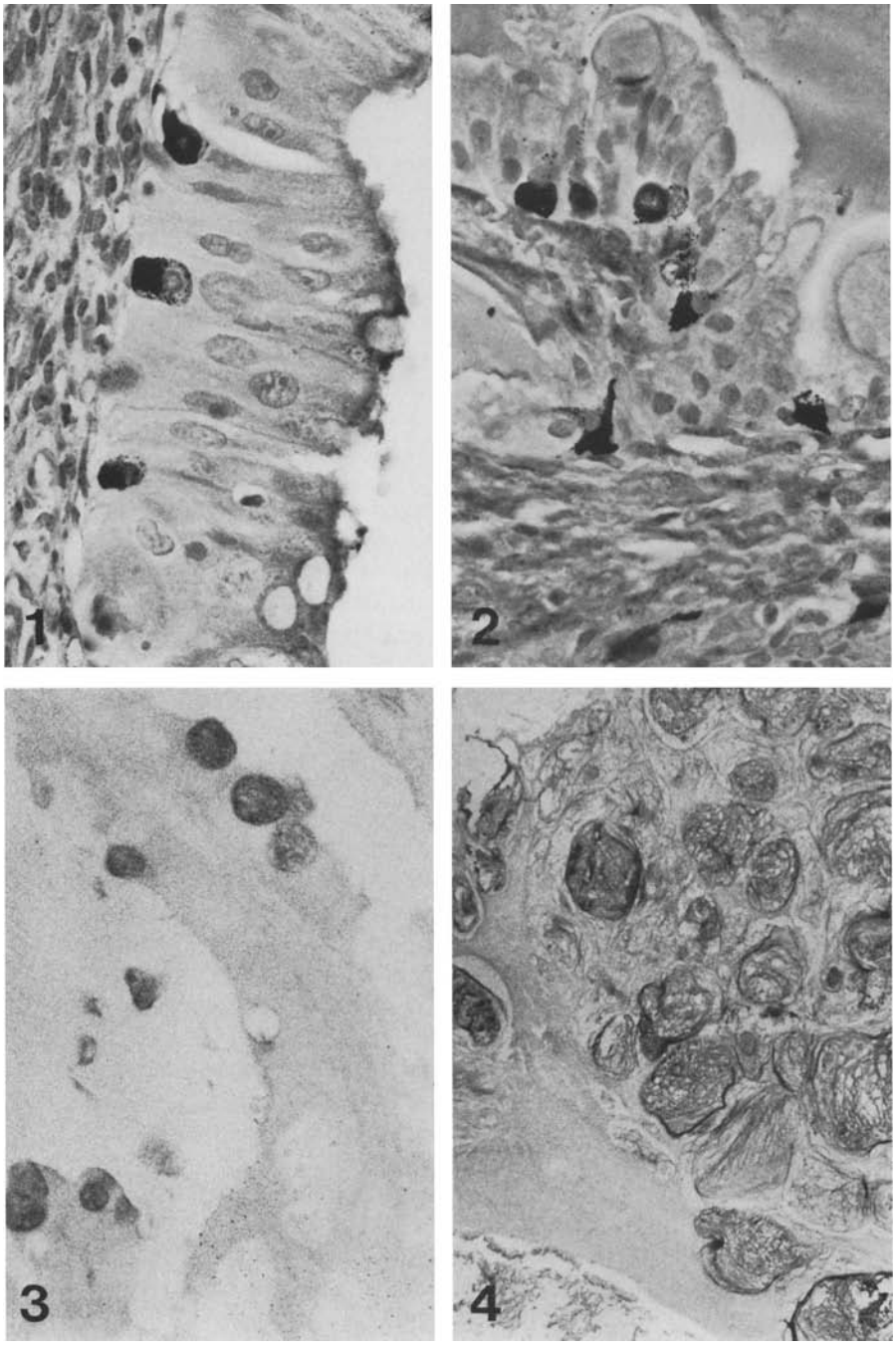

Fig. 1. Benign mucinous cystoma: intestinal type epithelium with some argyrophil cells located above the basement membrane (Grimelius stain, ×400)

Fig. 2. Malignant mucinous cystoma: papillary process which contains goblet and argyrophil cells (Grimelius stain, ×400)

Fig. 3. Benign mucinous cystoma (mixed type): some goblet cells containing O-acylated sialomucins (PB/KOH/PAS stain, ×400)

Fig. 4. Malignant mucinous cystoma (mixed type) showing large extracellular pools of O-acylated sialomucins (PB/KOH/PAS stain, ×100)

was detected in isolated goblet cells or in clustered goblet cells (groups of 2–3 cells) (Fig. 3). All goblet cells positive to PB/KOH/PAS were also positive to PAT/KOH/Bh/PAS, detecting red and/or red-purple stained cells. All malignant mixed cystomas contained abundant neutral mucins (100%) and abundant sialomucins (100%). Sulphomucins occurred in 75% of the cases. Three cases resulted positive to PB/KOH/PAS. The positivity was observed in clustered cells or in extracellular pools (Fig. 4). The same cells which were positive to O-acylated sialic acid variants (sites 7, 8) were red and/or red-purple stained to the PAT/KOH/Bh/PAS method.

Discussion

Results from our histochemical study showed that all benign and malignant cystomas contained an abundant amount of neutral and acid mucins and generally presented a prevalence of sialomucins over sulphomucins.

A decrease of mucin secretion was noted in relation to loss of cell differentiation (Garcia-Bunuel and Morris 1964; Louwerens et al. 1983). Furthermore, the 20 cases of endocervical type (benign and malignant) showed an absence of O-acetylated sialic acid (sites 7, 8) both to PB/KOH/PAS and to PAT/KOH/Bh/PAS; out of 9 mixed type cystomas (five benign and four malignant) we found three benign cystomas and three malignant cystomas containing O-acetylated (sites 7, 8). The PAT/KOH/Bh/PAS method confirmed that we are dealing with predominant O-acetylated site 8 (red cells) and/or O-acetylated sialic acids sites 4, 7, 9 plus O-acylated site 8 (red-purple cells).

The positivity was observed only in areas classified as intestinal in type on the basis of the presence of different types of cells, as seen in the Table 2. It was more evident in malignant cystomas than in benign ones.

Three mixed cystomas (two benign and one malignant) were negative to PB/KOH/PAS. Concerning the two benign mixed cystomas, we consider that the intestinal epithelium was not complete (there is an absence of argentaffin and argyrophil cells). Concerning the malignant mixed cystomas negative to PB/KOH/PAS a situation similar to that occurring in colorectal carcinomas may be encounted (Fulcheri et al. 1984).

In conclusion the results obtained from our histochemical study show that the intestinal type epithelium identified in mixed type ovarian cystomas often contains O-acylated sialic acid variants (sites 7, 8). However, we think that the positivity to the PB/KOH/PAS method alone cannot be considered to be a marker of colorectal adenocarcinoma. If a metastasis from a tumour of an unknown origin proves to be positive, it may arise not only from a carcinoma of the lower gastrointestinal tract, but also from a mucinous cystoma of the ovary.

The concept that O-acylated sialomucins are markers of colorectal adenocarcinoma, must be changed. The O-acylated sialomucins are typical of all neoplasms which originate from an intestinal epithelium or present an "intestinal type epithelium".

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Accepted July 31, 1985