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ANATOMICAL PATHOLOGY

FOXA1 is expressed in ovarian mucinous neoplasms

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Summary

FOXA1 is a transcription factor essential for the binding and action of other transcription factors on the chromatin. It is the major regulator of endoderm differentiation. It has important roles in breast, prostate and endometrial cancer. It has never been studied in ovarian tumours. The aim of this study was to investigate its expression in ovarian epithelial neoplasms.

A total of 195 primary ovarian epithelial borderline or malignant tumours were immunohistochemically studied for the expression of FOXA1.

Nineteen percent of the tumours strongly and diffusely expressed FOXA1. Of these, 75.7% belong to the mucinous category ($p < 0.0001$). Seventy-five per cent of mucinous borderline tumours and 46.7% of mucinous carcinomas overexpressed FOXA1. Brenner tumours also expressed FOXA1. FOXA1 was rarely expressed in serous (6/115) and endometrioid tumours (1/11). Clear cell tumours were completely negative (0/16). Of normal structures, ciliated tubal cells, Walthard nests and transitional metaplasias of the tubal-mesothelial junction, all strongly expressed FOXA1.

In conclusion, FOXA1 is found in ovarian mucinous and Brenner tumours.

Key words: Histogenesis; ovarian neoplasm; endoderm; serous; clear cell; endometrioid; Brenner.

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INTRODUCTION

Ovarian cancer is a heterogeneous disease consisting of tumours with different histology, histogenesis and prognosis. The absence of a normal counterpart inside the ovary for epithelial ovarian tumours has long been an intriguing mystery and an origin from the mesothelial lining has been the principal theory for years. However, nowadays we know that at least a significant proportion of serous neoplasms are of tubal origin, and that endometrial, clear cell and seromucinous tumours probably arise from foci of endometriosis.[1,2]

The histogenesis of mucinous neoplasms, however, remains a mystery. These tumours show a characteristic gastrointestinal differentiation and, in contrast to serous neoplasms, they rarely spread into the peritoneum or result in distant metastasis. No cell of origin or the pathway of their pathogenesis has been established. This is also true for Brenner tumours, and these two types are a puzzling issue as they show a non-müllerian phenotype, in contrast to serous, endometrioid and clear cell tumours.[1] An assumed common origin for mucinous and Brenner tumours from Walthard nests has been proposed;[2] a part of the mucinous tumours, mostly those accompanied by pseudomyxoma ovarii, could also arise from ovarian teratomas.[2]

Given the gastro-intestinal differentiation of mucinous tumours, we considered that a factor regulating the differentiation of endoderm during embryogenesis could also participate in the development of ovarian mucinous tumours. A major factor regulating differentiation of endoderm-derived organs is the Forkhead-box A1 transcription factor (FOXA1).[3] Moreover, FOXA1 has been recently implicated in the pathogenesis of hormone-dependent cancers like breast, ovarian and endometrial cancer, as a transcription factor necessary for the binding of hormone receptors onto chromatin.[4]

However, to the best of our knowledge, the expression of FOXA1 in ovarian tumours has never been studied. Thus, the aim of this study was to investigate the expression of FOXA1 in a large series of ovarian epithelial tumours.

MATERIAL AND METHODS

Study population

Specimens from 195 patients diagnosed with an ovarian borderline or malignant tumour, treated surgically without neoadjuvant chemotherapy, were included in the study. Clinical information was collected through the electronic medical records. The local ethics committee of the University Hospital of St-Etienne approved the study.

Histopathological evaluation and immunohistochemical analysis

All available slides from the formalin fixed, paraffin embedded tissues were histopathologically re-evaluated. Complementary immunohistochemical staining was performed in selected cases in order to establish the correct diagnosis. This mostly included p53 and WT1 for high grade serous carcinoma, ER, PR, CK7 and CK20 for mucinous or endometrioid tumours and WT1, ER, PR, CK7, CK20 for seromucinous tumours. Tumour classification was carried out in accordance with the 2014 World Health Organization (WHO) Classification[5] and staging was performed according to the TNM and FIGO classification.[6–8] Metastatic mucinous tumours were originally excluded on the basis of their morphology (bilateral, surface involvement, pseudomyxoma ovarii/peritoneii), immunohistochemistry and also on clinical grounds.[9]

Four-μm thick full tumour sections were used for immunohistochemistry, which was performed using an automated staining system (Leica Biosystems, UK). Positive immunoreactions were visualised using 3,3′-diaminobenzidine as the chromogenic substrate. Goat polyclonal antibody against FOXA1 [1:50, clone HNF-3α/β (C-20): sc-6553; Santa Cruz Biotechnology, USA] was used as the primary antibody. FOXA1 positive breast carcinoma was

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used as positive control and the same tissue with omission of the primary antibody was used as negative control.

FOXA1 expression was exclusively nuclear and it was recorded semi-quantitatively as previously suggested:[10] intensity of the staining, and area of tumour cells with positive staining were recorded. Staining intensity was graded from 0 (no staining) to 3 (strong staining). Proportion of stained tumour cells was graded as 0, 1 (<10%), 2 (10–50%) and 3 (>50%). A staining index was calculated as the product of intensity and staining area. As previously suggested, a score index from 0–4 was considered of low FOXA1 expression, while a score of 5 or higher was considered of high FOXA1 expression.

Statistical analysis

Data were analysed using the StatView software (Abacus Concepts, USA). Relationship between two groups was investigated using chi-square test for categorical data. Survival probability was estimated by Kaplan–Meier analysis with log-rank product limit estimation. For all analyses, statistical significance was indicated at a p value of < 0.05.

RESULTS

Patient characteristics are presented in Table 1. Table 2 shows FOXA1 expression in ovarian tumours studied. FOXA1 expression was exclusively nuclear. Only epithelial cells stained for FOXA1. Of the 195 tumours studied, 37 (19%) overexpressed FOXA1. Of these, 75.7% belong to the mucinous category (Fig. 1). Seventy-five percent of mucinous borderline tumours and 46.7% of mucinous carcinomas overexpressed FOXA1. Only five non-mucinous carcinomas (three serous, one endometrioid and one seromucinous) overexpressed FOXA1, corresponding to 4.2% of the serous carcinomas, 9% of the endometrioid and 33.3% of the seromucinous carcinomas, respectively. None of the clear cell carcinomas expressed FOXA1. Three (6.2%) of the serous borderline tumours expressed FOXA1 (Fig. 2). All endometrioid or clear cell borderline components of the corresponding carcinomas were negative. Brenner borderline tumour was strongly and diffusely positive for FOXA1. The Brenner component of a mucinous carcinoma was similarly positive.

When comparing the expression of FOXA1 in the mucinous group, a reduction in expression was found with progression of the tumour (75% of the borderline versus 46.7% of the carcinomas, $p = 0.06$, $\chi^2 = 3.5$). In FOXA1 positive borderline mucinous tumours, all components inside the same tumour (benign and borderline) were positive; similarly, in positive mucinous carcinomas, all components inside the same tumour (benign, borderline and carcinoma) were positive. In negative mucinous carcinomas, the benign/borderline components, when visible, were positive, but this percentage was not taken into account when assessing a carcinoma. In negative borderline tumours, even the benign component was negative. As for the type of infiltration in mucinous carcinomas,[11,12] this was destructive in two cases (one positive, one negative for FOXA1) and expansive in the remaining thirteen. In these cases, the presence of one expansive region or multiple such regions was recorded (5 and 8 cases, respectively). No difference in regards to FOXA1 expression was noted ($p = 0.4$, $\chi^2 = 0.5$).

After these results, ten benign mucinous and five benign Brenner tumours (pure or combined with mucinous neoplasia) were also stained to reveal all similarly diffuse and strong expression. In contrast, ten benign serous tumours were negative, while one of five cases of endometriosis was positive.

It should be mentioned, in regards to the cut-off point that leaves tumours with weak or very limited staining in the 'negative category', that most tumours in the 'negative' category were completely negative (with the exception of benign/bordeline component for mucinous carcinomas as mentioned earlier), while the positive tumours (mostly mucinous) were always strongly and diffusely positive.

No association between FOXA1 expression and prognosis (overall or progression-free survival) was found in the whole sample ($p = 0.1$, $\chi^2 = 1.8$ and $p = 0.4$, $\chi^2 = 0.6$, respectively) or separately the mucinous tumours ($p = 0.9$, $\chi^2 = 0.001$).

Table 1 Patient and tumour characteristics

Characteristic	*n*	%
Stage of disease		
I	115	59
II	19	9.7
III	57	29.3
IV	4	2.1
Histological type		
Serous borderline tumour	43	22.1
Mucinous borderline tumour	28	14.4
Seromucinous borderline tumour	5	2.6
Clear cell borderline tumour	1	0.5
Brenner borderline tumour	1	0.5
Low grade serous carcinoma	2	1
High grade serous carcinoma	70	35.9
Endometrioid carcinoma	11	5.6
Clear cell carcinoma	15	7.7
Mucinous carcinoma	15	7.7
Seromucinous carcinoma	4	2
Follow up, months		
Range (median)	4–310 (44)	
Overall survival, months		
Range (median)	4–310 (44)	
Progression-free survival, months		
Range (median)	4–310 (36.5)	
Status		
Alive	150	76.9
Dead	30	15.4
FOXA1		
Negative	37	19
Positive	158	81

Table 2 FOXA1 expression by tumour type

Tumour type	FOXA1 positive ($n = 37$) *n* (%)	FOXA1 negative ($n = 158$) *n* (%)	p (χ^2)
Serous borderline tumour	3 (8.1)	40 (25.3)	<0.0001 (93.3)
Mucinous borderline tumour	21 (56.8)	7 (4.4)	
Seromucinous borderline tumour	0	5 (3.2)	
Clear cell borderline tumour	0	1 (0.6)	
Brenner borderline tumour	1 (2.7)	0	
Low grade serous carcinoma	0	2 (1.3)	
High grade serous carcinoma	3 (8.1)	67 (42.4)	
Endometrioid carcinoma	1 (2.7)	10 (6.3)	
Clear cell carcinoma	0	15 (9.5)	
Mucinous carcinoma	7 (18.9)	8 (5.1)	
Seromucinous carcinoma	1 (2.7)	3 (1.9)	

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