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Morphological and immunohistochemical pattern of tubo-ovarian dysplasia and serous tubal intraepithelial carcinoma

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ABSTRACT

Objective: Histopathological examination of material from prophylactic salpingo-oophorectomies performed in patients at genetic risk of ovarian cancer can reveal abnormalities interpreted as possible pre-cancerous "ovarian dysplasia" and tubal precursors lesions. We sought to study the morphological features and immunohistochemical expression patterns of neoplasia-associated markers in prophylactically removed ovaries and fallopian tubes (pBSO) in comparison with a group of serous tubal intraepithelial carcinoma (STIC) and non-cancerous controls.

Study design: Morphological features and immunohistochemical expression patterns of Ki-67 (for proliferation biomarker), p53 (key pathway of mullerian serous tumorogenesis), Bcl2 (anti-apoptotic), γH2AX (a double-strand breaks marker) and ALDH1 (a stem cell marker significantly associated with early-stage ovarian cancer) were blindly evaluated by two pathologists in 111 pBSO, 12 STICs and 116 non-cancerous salpingo-oophorectomies (control group) (nBSO).

Results: Morphological ovarian and tubal dysplasia scores were significantly higher in the pBSO than in controls (respectively, 8.8 vs 3.12, $p < 0.0001$, for ovaries and 6.54 vs 1.58, $p < 0.0001$ for tubes). Increased γH2AX expression was observed in the pBSO and STICs compared with the controls whereas expression patterns of Ki67, p53 and bcl2 were low to moderate in the pBSO group. STICs overexpressed Ki67 and p53 while bcl2 expression was low; Interestingly, ALDH1 expression was low in non dysplastic epithelium, high in dysplasia and constantly low in STICs.

Conclusion: The morphological and immunohistochemical profile of tubo-ovarian dysplasia and STICs might be consistent with progression toward neoplastic transformation in the Serous Carcinogenesis Sequence. These changes may be pre-malignant and could represent an important phase in early neoplasia. ALDH1 activation in pBSO samples and its extinction in STICs should be considered as a target for prevention.

Introduction

Histopathological study of material from prophylactic oophorectomies performed for a genetic predisposition for ovarian cancer can reveal cytological and architectural abnormalities considered to be pre-cancerous manifestations, and termed "ovarian dysplasia" by analogy with the pre-invasive lesions described for the genital tract (vulva, vagina, cervix, endometrium) [1]. This suggests that ovarian dysplasia could represent precursor lesions of invasive ovarian carcinoma [2,3].

Similarly, Serous Tubal Intraepithelial Lesions (STILs, a spectrum of epithelial changes ranging from normal appearing tubal epithelium to lesions with cytologic atypia and dysplasia) in prophylactically removed Fallopian tubes of women predisposed to developing ovarian cancer have recently been described [3].

On the other hand, morphologic, immunohistochemical and molecular studies have identified a lesion designated as "Serous Tubal Intraepithelial Carcinoma" which could be between STIL/ovarian dysplasia and high grade ovarian serous carcinoma on the Carcinogenic Serous Sequence as recently described [2–4].

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The aim of this study was to investigate the occurrence of preinvasive neoplastic changes in prophylactically removed ovaries and fallopian tubes of women with a genetic predisposition to female adnexal cancer (the pBSO group) and to assess the expression of proliferation and differentiation related proteins (Ki67, p53, blc-2, γH2AX and ALDH1) in comparison with a cohort of STICs as positive control and with a control group which is a negative control. To the best of our knowledge, this is the first study assessing the analysis of ALDH1 as a potential marker of tubo-ovarian precursor lesions.

Materials and methods

This study was approved by the regional ethics committee.

Patients

Between January 2003 and April 2010, we selected three groups of patients:

- **Group A: Prophylactic salpingo-oophorectomies (pBSO) group.** We recruited 111 consecutive patients in whom non cancerous ovaries and tubes had been prophylactically removed, including 51 pBSO with BRCA1 gene mutation, 22 pBSO with BRCA2 gene mutation and 38 pBSO with a strong family history of ovarian and/or breast cancer. We defined a positive family history as follows: (1) at least 1 first-degree relative with ovarian cancer and at least 1 first- or second-degree relative with breast cancer and/or a personal history of breast cancer or (2) at least 2 first- or second-relatives with breast cancer with a personal history of breast cancer. We have distinguished patients with BRCA mutations (Group A1, $n = 73$) from patients without BRCA mutation (family history, group A2, $n = 38$). Incidentally found carcinoma were excluded from the morphological dysplasia study as the morphological analysis was designed to identify potential premalignant lesions.
- **Group B: STICs group (positive control)**: 12 morphologically defined STICs (abnormal chromatin pattern and marked pleomorphism and epithelial stratification and loss of polarity and nuclear molding) were obtained from 12 patients with primary high grade serous carcinoma of ovary (stage III). None of them had a BRCA mutation. They were excluded from the morphological dysplasia study.
- **Group C: Control group (negative control).** We selected a spontaneously fertile population of matching age, with no personal nor family history of gynaecological neoplasia (breast, ovary, endometrium), who underwent adnexectomy for which the histopathological examination concluded that the ovaries and tubes showed no sign of cancerous nor borderline pathology and salpingitis: 116 controls were included in the study.

Histopathological criteria

Evaluation of morphological features in groups A and C

The ovaries and the tubes were sampled in totality, formalin fixed and embedded in paraffin. Morphological studies were processed on three micron paraffin sections stained with standard haematoxylin phloxin safran (HPS). The number of sections available for review for each case (ovary and tube) ranged from eight to eleven in both study groups.

The histopathology slides for groups A and C were all read in blind fashion by two pathologists (FPL & IR) in order to obtain an average score. When several slides were available, the one with the highest score was retained.

In the event of obvious disagreements between pathologists, a further examination at multiheaded microscope was carried out to reach a consensus.

Ovarian dysplasia: Our definition of ovarian atypia was based on previous studies of ovarian dysplasia, i.e. dysplasia described in ovaries from patients with a genetic risk (prophylactic oophorectomy for BRCA1/2 mutations) [5–7], in the normal appearing areas adjacent to an ovarian cancer [8,9], in the normal appearing contralateral ovary in case of unilateral ovarian cancer [10,11], and in stimulated ovaries [12,13]. This scoring system (eleven histopathological criteria) was designed in our previous studies about the relationship between ovarian dysplasia, ovulation induction, and genetic risk (OP for BRCA 1/2 mutations) [1,12]:

epithelial multilayering,
tufting,
surface papillomatosis,
nuclear chromatin irregularity,
nuclear contour irregularity,
cellular pleomorphism,
nuclear size,
inclusion cysts,
deep epithelial cortical invaginations,
psammoma,
stromal hyperplasia.

In each case, the abnormal areas were scored between zero and two (0 = normal, 1 = moderately abnormal, 2 = frankly abnormal), whether located on the surface or in an inclusion cyst.

An overall ovarian score was then obtained for each patient by simply adding the scores for each of the 11 items (total range: 0 to 22).

Tubal dysplasia: Our definition was based on previous studies of tubal precursor lesions (named "Serous Tubal Intraepithelial Lesions" STILs) described in Fallopian tubes from patients with a genetic risk (prophylactic oophorectomy for BRCA1/2 mutations) [3,14,15], and we have designed a scoring system with seven histopathological criteria:

epithelial pseudostratification,
tufting,
loss of nuclear polarity,
increase in nuclear density,
nuclear atypia,
nucleomegaly,
loss of ciliation.

In each case, the abnormal areas were scored between 0 and 2 (0 = normal, 1 = moderately abnormal, 2 = severely abnormal).

An overall tubal score was then obtained for each patient by simply adding the scores for each of the 7 items (total range: 0 to 14).

Evaluation of immunostaining in groups A, B and C

Immunohistochemistry was performed on 3 micron sections, on silanised slides dried overnight at 56 °C.

Ki67 (1:100; clone MIB-1, Dako®), p53 (1:200; clone DO-7, Dako®), ALDH1 (1:400; clone 44/ALDH, Biosciences®), Bcl2 (1:50; clone 124, Dako®), γH2AX (1:50; clone JBW301, Millipore®) immunostaining were performed with a Benchmark XT immunostainer (Ventana Medical Systems, Illkirch, France).

For Ki67, Bcl2, γH2AX and P53, immunostaining was evaluated semiquantitatively and independently by two pathologists using a scoring protocol previously described [2]: an immunoreactive score (IRS) ranging from 0 to 12 was defined as the product of staining intensity (0 to 3) and the percentage of cells with nuclear staining (0 to 4).