Early Telomere Shortening and Genomic Instability in Tubo-Ovarian Preneoplastic Lesions

Gautier Chene1,2,3,4, Andrei Tchirkov2, Eleonore Pierre-Eymard2, Jacques Dauplat3, Ines Raoello2, and Frederique Penault-Llorca1

Abstract

Purpose: Genetic instability plays an important role in ovarian carcinogenesis. We investigated the level of telomere shortening and genomic instability in early and preinvasive stages of ovarian cancer, serous tubal intraepithelial carcinoma (STIC), and tubo-ovarian dysplasia (TOD).

Experimental Design: Fifty-one TOD from prophylactic salpingo-oophorectomies with BRCA1 or 2 mutation, 12 STICs, 53 tubo-ovarian high-grade serous carcinoma, and 36 noncancerous controls were laser capture microdissected from formalin-fixed, paraffin-embedded sections, analyzed by comparative genomic hybridization (array CGH) and for telomere length (using quantitative real-time PCR based on the Cawthon's method). TOD and STICs were defined by morphologic scores and immunohistochemical expressions of p53, Ki67, and γH2AX.

Results: TOD showed marked telomere shortening compared with noncancerous controls (P < 10−7). STICs had even shorter telomeres than TOD (P = 0.0008). Ovarian carcinoma had shorter telomeres than controls but longer than STICs and dysplasia. In TOD, telomeres were significantly shorter in those with BRCA1 mutation than in those with BRCA2 mutation (P = 0.005). In addition, γH2AX expression in TOD and STIC groups with short telomeres was significantly increased (P < 10−7). In dysplastic epithelium, we found subtle genomic alterations, in contrast to more important genomic imbalances in STICs. The total number of genetic alterations was the highest in ovarian cancers.

Conclusions: These findings suggest that genetic instability occurs in early stages of ovarian tumorigenesis. STICs and noninvasive dysplasia are likely an important step in early serous ovarian neoplasia.

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Introduction

Ovarian serous cancer is the most life-threatening gynecologic malignancy. A better understanding of the molecular basis of the disease and the preinvasive stages would be helpful for defining means of prevention or early detection. Genetic instability is one of the characteristics shared by most human cancers and seems to exist (at various levels) at all stages of the disease, from precancerous lesions to advanced cancer. It is possible that this instability is one of the first trigger events which would facilitate the subsequent establishment of all the other cancer hallmarks (1, 2).

Recent meticulous and exhaustive histopathologic analysis of specimens from prophylactic adnexectomy in BRCA gene mutation carriers revealed in up to 17% of patients occult cancers, 57% to 100% of which were located in the distal portion of the tubes (called serous tubal intraepithelial cancer or STIC; refs. 3–8). Similar lesions have also been revealed in series of sporadic ovarian or peritoneal cancer, suggesting that serous ovarian carcinogenesis could have a tubal origin. In the serous ovarian carcinogenic sequence, histopathologic anomalies termed tubal dysplasias (or tubal intraepithelial lesions in transition: TILT or p53 proliferation signature) along with ovarian anomalies have been described and could precede STIC lesions (9–13). They are characterized by cytologic and architectural abnormalities (see Materials and Methods), an overexpression of p53, a low proliferation index (Ki67 between 10% and 40%), and evidence of DNA damage manifested by immunopositivity for H2AX. Nonproliferative p53 signatures are earlier entities. They exhibit no abnormal histologic features and have an immunohistochemical definition with at least 12 consecutive secretory cells that are p53-positive and have a low proliferation index (Ki67 < 10%). They have also evidence of DNA damage by staining with γH2AX (14).

In the present study, we examined the genetic instability of these probably precancerous tubo-ovarian lesions at
Translational Relevance
The study of genomic instability in tubo-ovarian pre-malignant lesions could have important implications in the understanding and the early diagnosis of serous ovarian carcinoma.

Material and Methods
Patients
This retrospective study was conducted on tissue samples provided by the Pathology Departments of the Jean Perrin Anticancer Center (Clermont-Ferrand, France) and Croix-Rousse Hospital (Lyon, France) and obtained in 3 patient and 1 control groups

- Dysplasia: A total of 51 cases (20 ovarian and 31 tubal dysplasias) obtained after 36 prophylactic adnexectomies in BRCA1 gene carriers and 15 prophylactic adnexectomies in BRCA2 gene carriers.
- STICs: Twelve cases, none of these have a BRCA mutation.
- Cancer: Fifty-three high-grade serous tubo-ovarian cancers (16 cancers of tubal and 37 cancers of ovarian origin).
- Controls: Thirty-six adnexectomies in a spontaneously free of any cancerous or borderline pathology.

The diagnosis of STICs complied with the criteria of Kuhn and colleagues (22): nonciliated cells exhibiting 3 or more of the following features: (i) abnormal chromatin pattern, (ii) nuclear enlargement, (iii) marked nuclear pleomorphism, (iv) epithelial stratification and/or loss of polarity, and (v) nuclear molding.

Finally, we investigated the presence of chromosomal rearrangements in these early lesions.

Histopathological criteria
We defined the cases of ovarian and tubal dysplasia on the basis of the morphologic and immunohistochemical criteria described recently in the literature (9, 10, 14, 18–21). In brief, ovarian dysplasia was defined by the following morphologic features: epithelial multilayering, tufting, surface papillomatosis, nuclear chromatin irregularity, nuclear contour irregularity, cellular pleomorphism, increased nuclear size, epithelial inclusion cysts, psammoma bodies, deep cortical invaginations, and cortical stromal hyperplasia. Tubal dysplasia was defined by the following morphologic dysplasia: epithelial multilayering, tufting, loss of nuclear polarity, increase in nuclear density, nuclear atypia, increased nuclear size, and loss of ciliation. Both tubal and ovarian dysplasia had p53 expression, low Ki67 proliferative index, and immunopositivity for γH2AX.

The diagnosis of STICs complied with the criteria established by Hu and Sedlis (23, 24).

An immunohistochemical analysis of p53 and Ki67 was carried out (respectively DAKO, DO-7, 1:200 and DAKO, MIB-1, 1:100) to help with the diagnosis of tubo-ovarian dysplasia (TOD) and STICs. In addition, γH2AX (Millipore, JBW301, 1:50) was used on 3-mm paraffin sections on dry silanized slides for one night at 56°C. In brief, antibody staining was carried out in a Benchmark XT and revealed using the Ultraview DAB kit (Roche Ventana).

Semiquantitative assessment (quick score) of γH2AX was made by 2 pathologists (IR/FPL) using an optical microscope.

Quantification of telomere length using quantitative real-time PCR
The telomere length measurement assay was adapted from the published original quantitative real-time PCR (qRT-PCR) method (27, 28). This method measures the relative average telomere lengths in gDNA by determining the ratio of telomere repeat copy number to single gene copy number (T/S ratio) in experimental samples relative to a reference sample. Previously, it has been shown that the results obtained using this method correlate very well with results of classical terminal restriction fragment (TFR) length by Southern blot technique (27, 28). Telomere repeated sequences and glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH) amplification was carried out for each DNA sample by real-time PCR. GAPDH was used as
cycles of 95 °C for 10 minutes to activate Taq polymerase, followed by 35 cycles of 94 °C for 30 seconds, 62 °C for 30 seconds, and 72 °C for 15 seconds. A melting curve was generated on every run to confirm the specificity of the amplification. The amplification efficiency for both GAPDH and telomere DNA was examined with a set of serial dilutions of a DNA sample (10 ng) on the same reaction plate, and amplifications were carried out under the same PCR conditions to eliminate the interplate variation. Thermal cycling was carried out according to the following protocol: 95 °C for 10 minutes to activate Taq polymerase, followed by 35 cycles of 95 °C for 10 seconds, 62 °C for 15 seconds, and 72 °C for 15 seconds. A melting (dissociation) curve was generated on every run to confirm the specificity of the reaction.

The amplification efficiency for both GAPDH and telomere DNA was examined with a set of serial dilutions of a reference human DNA (a pooled normal blood DNA samples from 5 healthy donors) and found to be >90% with correlation coefficients of ≥99% for both primer sets. The ΔCt (threshold cycle number) method was used for the calculation of T/S ratios. All samples were measured at least in duplicate.

Array comparative genomic hybridization

We made a high-resolution array comparative genomic hybridization (aCGH) study using oligonucleotide microarrays (Agilent 180 K) of 10 adnexectomies in dysplasia group, 12 STICs, and 15 high-grade serous ovarian cancers using databases: UCSC Genome Browser and the database developed by the Western France genomic center at Nantes (http://cardioserve.nantes.insERM.fr/madtools/home/).

Finally, the results were compared with known polymorphic CNVs (Database of Genomic Variants). The nonpolymorphic regions of each case were then listed, i.e. the levels dysplastic cases from the subgroup carrying BRCA1 mutations.

We used a 180K CGH oligonucleotide microarray (Agilent Technologies). Each case was hybridized twice, marked once with Alexa 3 and once with Alexa 5 in the DNA mixtures during hybridization.

Briefly, 1 to 2 µg of purified DNA (sample and sex-matched reference) was chemically labeled respectively with 25 µL d’Alexa Fluor 3 Oligo aCGH 2X Reaction Mix and Alexa Fluor 5 Oligo aCGH 2X Reaction Mix at 37 °C for 120 minutes according to the manufacturer’s instructions (Bioprime Total Genomic Labelling Kit, Invitrogen). Labeled samples were purified (Purification Module B with Pure link, Invitrogen), mixed with human Cot-1 DNA and denatured at 95 °C (Oligo aCGH hybridization kit). The mixture was applied to microarrays and hybridization was conducted at 65 °C for 24 hours. After hybridization, the microarrays were washed in Oligo aCGH wash buffer 1 at room temperature for 5 minutes and wash buffer 2 at 37 °C for 1 minute. After drying, the microarrays were scanned using a DNA microarray scanner G2565BA (Agilent) and data (log2) were extracted from raw microarray image files using Feature Extraction software (version 9.2, Agilent). Then, data were analyzed by DNA Analytics software (version 3.4, Agilent) with default filter settings: this software allows a dye-flip analysis, taking log2 ratios > or < 0.25 and −0.25 for 3 consecutive spots as thresholds for the detection of a copy number gain and loss, respectively.

Areas with less than 3 consecutive spots or that were not confirmed by dye-flip analysis in the 2 tests were eliminated.

The altered chromosomal regions were then checked using databases: UCSC Genome Browser and the database developed by the Western France genomic center at Nantes.

Finally, the results were compared with known polymorphic CNVs (Database of Genomic Variants). The nonpolymorphic regions of each case were then listed, i.e. the

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*Figure 1. Individual and mean values of relative telomere length (TL) in investigated groups. The statistical comparison was carried out using the Kruskal–Wallis H test. A, significant differences in telomere length between normal tubal/ovarian tissue and samples obtained from ovarian/tubal dysplasia, STICs, and tubo-ovarian cancer. B, shorter telomeres in BRCA1-mutated ovarian and tubal dysplasia cases than in BRCA2-mutated cases.*
regions found with a ratio > 0.58 [log (2) 3/2] or <−1 [log (2) 1/2], for 3 consecutive nonpolymorphic spots. Each of the arrays was independently analyzed and evaluated for genetic alterations.

Statistical analysis

Molecular analysis of the results by CGH array was descriptive (small cohort). Telomeric and immunohistochemical parameters obtained in different groups were compared with nonparametric Wilcoxon or Kruskal–Wallis $H$-tests. The relationship between variables was tested using Pearson correlation analysis.

Results

Telomere lengths and immunohistochemical analysis

The $T/S$ ratio obtained for each sample reflects the relative telomere length. We compared the $T/S$ values between control, dysplasia, STIC, and cancer groups. In addition, we evaluated the impact of genetic characteristics (BRCA1 and BRCA2 status) and expression of p53 and $γH2AX$ proteins.

Across the groups, the mean telomere length differed significantly (Fig. 1). Dysplasia group showed telomere shortening, as compared to the controls. Telomeres were even shorter in STICs. Interestingly, cancer group showed longer telomeres than dysplasia and STIC cases but shorter than those of the control group. No significant difference was found depending on the type of organ (ovary or tube) whether for the control, dysplasia, or cancer cases (Table 1). However, the telomere length was significantly altered in case of BRCA mutation ($P = 0.005$): $T/S$ ratio = 0.56 in case of BRCA1 mutation versus 0.80 in case of BRCA2 mutation, so the telomere length was shorter in subgroup BRCA1.

Regarding immunohistochemical characteristics (Figs. 2 and 3), the expression of $γH2AX$ was significantly higher in case of dysplasia and STICs than in cancers (Fig. 2A, $P < 10^{-7}$). There was a significant inverse correlation (Fig. 2C, $P = 0.00013$) between $γH2AX$ expression and telomere length:

telomeres were shorter in cases with higher levels of $γH2AX$. The level of p53 expression was higher in STIC and cancer groups compared to dysplasia group (Fig. 2B, $P < 10^{-5}$).

Within dysplasia and STIC groups, the level of p53 correlated inversely with the telomere length (Fig. 2D, $P = 0.0016$). Of note, the level of p53 was homogenously and highly (mean score near 100%) elevated in cancers. The Ki-67 labeling showed high levels in cancer (81.0% ± 4.9%) and STIC (52.5% ± 16.3%) groups, whereas the values seen in ovarian (6.4% ± 4.7%) and tubal dysplasia (8.0% ± 6.3%) were much lower and not different from each other. There was a highly significant difference between the 3 groups: cancer, STIC, and dysplasia ($P < 10^{-6}$, $H$ test).

Table 1. The relative telomere length in normal dysplastic and cancerous tubal/ovarian epithelium

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of samples</th>
<th>Mean (SEM)</th>
<th>$P$ (ovary vs. tube)</th>
<th>$P$ (dysplasia, STIC, cancer vs. control ovary or tube)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>1.892 (0.20)</td>
<td>0.56 (NS)</td>
<td>—</td>
</tr>
<tr>
<td>Ovary</td>
<td>24</td>
<td>1.691 (0.21)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dysplasia</td>
<td></td>
<td>20</td>
<td>0.647 (0.10)</td>
<td>0.77 (NS)</td>
</tr>
<tr>
<td>STIC</td>
<td>12</td>
<td>0.613 (0.08)</td>
<td></td>
<td>0.000013</td>
</tr>
</tbody>
</table>
| Ovary     | 31             | 0.241 (0.11) | —                    | 1q (3 of 9, 33.3%). No common gain was found in ovaries. The most common DNA copy number losses were detected in tubes at chromosomes 1p (5 of 9 cases, 55.5%), 15q (5 of 9, 55.5%), 6p (4 of 9, 44.4%), 8p (4 of 9, 44.4%), 11q (4 of 9, 44.4%), 12q (4 of 9, 44.4%), 12p (4 of 9, 44.4%), and 1q (3 of 9, 33.3%). In ovaries, common losses involved chromosomes 1q (2 of 5, 40%), 8p (2 of 5, 40%), 14q (2 of 5, 40%), and 15q (2/5, 40%).
| Tube      | 16             | 1.168 (0.24) | 0.92 (NS)            | 0.000023                                               | 0.046                                                   |
| STIC      | 12             | 0.953 (0.09) | —                    | 1q (3 of 9, 33.3%). No common gain was found in ovaries. The most common DNA copy number losses were detected in tubes at chromosomes 1p (5 of 9 cases, 55.5%), 15q (5 of 9, 55.5%), 6p (4 of 9, 44.4%), 8p (4 of 9, 44.4%), 11q (4 of 9, 44.4%), 12q (4 of 9, 44.4%), 12p (4 of 9, 44.4%), and 1q (3 of 9, 33.3%). In ovaries, common losses involved chromosomes 1q (2 of 5, 40%), 8p (2 of 5, 40%), 14q (2 of 5, 40%), and 15q (2/5, 40%).

aCGH

Among the 10 adnexectomies in the dysplasia group with genetic risk, it was possible to study only 5 ovaries and 9 tubes due to insufficient material. Twelve STICs and 15 high-grade serous ovarian cancers were also analyzed. The most common changes found in these subgroups are shown in Fig. 3 and detailed in Supplementary Tables S1–S3.

aCGH results for tubo-ovarian dysplasia samples. Both DNA copy number gains and losses were found (Figs. 4 and 5). The mean number of genomic changes was 4.25 (2–7) in ovaries and 6.5 (4–11) in tubes. The most common gains were detected in tubes at chromosomes 4q (6 of 9 cases, 66.6%), 6q (2 of 9, 22.2%), 14q (2 of 9, 22.2%), 20q (2 of 9, 22.2%), and 3q (1 of 9, 11.1%). No common gain was found in ovaries. The most common DNA copy number losses were detected in tubes at chromosomes 1p (5 of 9, 55.5%), 15q (5 of 9, 55.5%), 6p (4 of 9, 44.4%), 8p (4 of 9, 44.4%), 11q (4 of 9, 44.4%), 12q (4 of 9, 44.4%), 12p (4 of 9, 44.4%), and 1q (3 of 9, 33.3%). In ovaries, common losses involved chromosomes 1q (2 of 5, 40%), 8p (2 of 5, 40%), 14q (2 of 5, 40%), and 15q (2/5, 40%).

The mean size of rearrangements was 404.44 kb (72–1318) for ovarian dysplasia and 758.85 kb (44–6452) for tubal dysplasia cases. The minimal regions of interest in the
common losses and gains are presented in Supplementary Tables S1 and S2.

**aCGH results for STIC samples.** The mean number of rearrangements was greater than in dysplasia cases (8.6, 4–17; Fig. 4). The most common gains were detected at chromosomes 19q (6 of 12, 50%), 16p (5 of 12, 41.6%), 12q (5 of 12, 41.6%), 10q (5 of 12, 41.6%), 11p (4 of 12, 33.3%), 4p (3 of 12, 25%), and 8q (3/12, 25%). The most
common losses were at chromosomes 3q (6 of 12, 50%), 2q (5 of 12, 41.6%), 11q (4 of 12, 33.3%), 6p (4 of 12, 33.3%), 22q (4 of 12, 33.3%), 18q (3 of 12, 25%), 19p (3 of 12, 25%), 20p (3 of 12, 25%), and 2p (2 of 12, 16.6%). The size of rearrangements in STICs was also greater than for dysplasia samples, with an average of 2,363.37 kb (488–8,161). The minimal regions of interest in the common losses and gains are given in Supplementary Table S3.

Discussion

We have revealed telomere shortening and chromosomal rearrangements in the potential precursors of ovarian cancer: TOD and STICs, indicative of genomic instability. TODs and STICs have shorter telomeres than noncancerous controls. High-grade serous ovarian cancer telomeres are shorter than noncancerous controls but longer than TOD and STIC precursors. The correlation of telomeres shortening with the BRCA gene status and activation of proteins involved in DNA damage response (γH2AX) provide additional evidence for the existence of genomic instability in these preinvasive conditions.

Telomeres are located at the extremity of the chromosomes and consist of multiple hexameric repeats 5'-TTAGGG-3'. Their main function is to ensure chromosome stability. Telomeres are regularly shortened during cell division. The critical shortening ('telomere crisis') results in irreversible chromosomal instability, which is a hallmark of cancer.
in chromosomal instability, activation of the DNA repair processes, then cellular death. In tumors the telomeres are usually shortened, rendering the chromosomes unstable, without reaching the critical length which results in cell death, activation of telomerase, which is able to regenerate telomeres, generally limited to stem cells, progenitor cells, and tumor cells, but not present in normal somatic cells, enables the continuous proliferation of genetically unstable cancer cells (15–17).

Telomere dysfunction has been described as one of the first phases in genomic instability (29–31). Telomere shortening appears to take place in most human preinvasive epithelial lesions: telomeres shorten in 88% of early precancerous conditions of the bladder, cervix, colon, esophagus, or prostate (15, 32).

Telomerase activation probably takes place later during the invasive stage and would thus counterbalance the telomere shortening: stabilization of the telomere length at this stage would moreover represent an advantage in terms of tumor proliferation and escaping apoptosis (16).

In our study, STIC samples showed the shortest telomeres, in agreement with the results of Kuhn and colleagues (22), followed by dysplasia and then cancer samples. So there appears to be a gradual shortening of telomeres during transition from dysplasia to STIC phase. Given that STICs have the shortest telomeres, they could be in a telomere crisis phase preceding genomic stabilization due to telomerase activation. Analysis of the expression of telomerase would most probably help to confirm this hypothesis.

In dysplasia group, no difference was found in terms of telomere length according to the type of organ (ovary or tube). However, a significant difference appeared between the BRCA1 and BRCA2 mutation statuses (the TOD was even more unstable when there was a BRCA1 type mutation). The BRCA1 and 2 genes play a key role in DNA signaling and repair (33): BRCA1, BRCA2 (via hRAD51), and p53 interact in cooperation in the DNA repair (mainly by homologous recombination). DNA repair does not take place in mice with BRCA1 mutation: the result is activation of p53 and cell-cycle arrest (34). So a BRCA1 mutation would contribute to enhanced instability (35).

BRCA1 and 2 were shown to be telomere-modifying genes. BRCA1 may regulate telomeres by multiple mechanisms, including regulation of telomerase activity and additional telomerase-independent mechanisms related to 3' G-strand telomeric overhang length and possibly also to telomere degradation (36). BRCA2-mediated homologous recombination reactions contribute to the maintenance of telomere length by facilitating telomere replication (37, 38). An association between shorter telomeres in peripheral blood leucocytes and increased risk of ovarian cancer, particularly for younger women, was reported in families carrying BRCA1/2 mutations as well as in sporadic cases (39). Our data suggest that BRCA1 mutations might have greater impact on telomere shortening than BRCA2 mutations in both ovarian and tubal dysplasia, and further studies are necessary to confirm this finding and to investigate possible underlying mechanisms. BRCA1 gene seems to be involved in multiple telomere maintenance mechanisms, which could perhaps explain that its deficiency has stronger impact on the telomere length, at least in ovarian and tubal tissue.

The telomere length of normal individuals is high heterogeneous. The variation in the T/S ratios observed in controls is most likely related to various degree of degradation of DNA obtained from microdissected fixed tissue samples. In the case of peripheral blood, it was noted that T/S ratios were higher in dried blood spots, which may be partially degraded, than in freshly obtained blood samples (40). In our study, samples from control, dysplasia, and cancer tissues were collected and extracted in exactly the same way, and this similarity of sample treatment enabled us to compare the results.

High expression of γH2AX (surrogate marker of genetic instability signaling double-stranded breaks in DNA) has been evidenced in most human precancerous lesions (40, 41). Experimental studies have shown that H2AX-knockout mice were at greater risk of immune deficiency, infertility and were also more sensitive to ionizing radiation, all of which can be explained by increased chromosomal instability related with decreased efficiency of the DNA repair. When these mice were also knockouts for p53, the risk of developing tumors was also increased (42, 43). Recent studies have also revealed activation of the DNA damage responses in most early precancerous conditions in humans (44, 45).

In our study, we found strong expression of γH2AX in cases of TOD. Concerning STICs, strong and diffuse nuclear marking was found to be present. Finally, telomere dysfunction caused by telomere shortening was strongly correlated with overexpression of γH2AX.

These results prove that DNA repair mechanisms are activated in these early conditions and are definitely an additional indication of genomic instability.

Our final molecular approach to the genomic instability inherent to these early lesions was a global analysis of DNA copy number gains and losses using aCGH (46, 47). In sporadic cancers of the ovary, the rearrangements most frequently found are located in regions 1q, 2p, 2q, 3q, 6q, 7q, 8q, 12p, 17q, 18q, 20q, ch X for gains and in ch4, 6q, 8p, 13q, 16q, 18q, 4q for losses (48). It has even been shown that these profiles are distinctly different between sporadic ovarian cancers and ovarian cancers of a hereditary type due to BRCA mutation (49–51): genomic imbalances appear to be more numerous in case of genetic risk, probably because there is less DNA repair (one of the functions of BRCA) and the genome is thus more fragile.

So in hereditary ovarian cancers related with BRCA mutations, very frequent chromosome amplifications at 1q, 2q, 3q, and 8q along with deletions at 9q and a chromosome 19 were often found (49–51). aCGH thus allows specific molecular profiles to be established. Moreover amplifications and deletions may include tumor suppressors and oncogenes. Different pathways involving kinase function, cell-cycle regulation, or apoptosis could be significantly affected. Genomic profiles may help to molecularly subtype cancers.
and lead toward individualized cancer-type–specific therapies as it has been shown with the Cancer Genome Atlas (52–54).

Yet there are no data of this nature concerning precancerous ovarian conditions: we postulated that CGH could target particular regions involved in the early stages of ovarian carcinogenesis. We found an increase in terms of both number and size between dysplasia lesions and STICs, confirming that genetic instability already exists in these very early stages (55).

It has even been shown that these profiles are distinctly different between sporadic ovarian cancers and ovarian cancers of a hereditary type due to BRCA mutation (49–51). And yet these secondary rearrangements—probably responsible for continued proliferation and the metastatic risk—do not necessarily have any direct link with the pathogenesis and tumorigenesis. The Cancer Genome Atlas project (54) has shown that high-grade serous ovarian cancers are characterized by TP53 mutations in 96% of all tumors as it is the case in STICs. However, other additional mutated genes with low prevalence have been shown in ovarian cancers (BRCA1, BRCA2, NF1, RB1, CDK12, CSM3D, FAT3, GABRA6), and we did not find them in our precancerous lesions. Conversely, the early alterations in precancerous lesions are certainly key stages in these initial phases of carcinogenesis, and the corresponding candidate genes could act directly in tumor initiation and represent targets for diagnosis and/or treatment. Some authors (50, 51) underline, for example, the importance of the long arm of chromosome 11 which carries several genes encoding for DNA repair proteins (such as H2AX); we did indeed find deletions at 11q not only in dysplasia but also in STICs. In our dysplasia series, certain rearrangements encompass genes involved in the DNA repair system and/or the transcription and regulation channels for various cytokines (HLMB1 or ING4 involved in the p53 signaling pathway at 12p13.31, MIM13SA2 at 12q23.1, RAG1, RAG2, C11orf74 at 11p12, PTHLH at 12p11.23, DOK5 at 14q32.2-q32.33, TRAF3 at 20q13.2, ITG83BP (co-activator of retinoic nuclear receptors) at 1p31.3). In STICs, rearrangements detected in the present study contain genes involved in transcriptional or translational regulation (MEIS1 at 2p24, NCK1 at 3q26.2-q26.31, GAK at 4p16.3, MIR202 at 10q26.3) as well as genes involved in the regulation of DNA damage response (OLA1 at 2q31.1- q31.2, RAD21 at 8q13.1-q24.3) and apoptosis (GAK at 4p16.3 and AXIN1 at 16p13.3). Other studies are needed to confirm the involvement of these gene candidates by considering regions common to cases of dysplasia, STICs, and cancers (at 3q, 11q at 12q) as priority targets.

The parallel with breast cancer and its precursor lesions is interesting. It has even been shown that these profiles are distinctly different between sporadic breast cancers and breast cancers of a hereditary type due to BRCA mutation (49–51). And yet these secondary rearrangements—probably responsible for continued proliferation and the metastatic risk—do not necessarily have any direct link with the pathogenesis and tumorigenesis. The Cancer Genome Atlas project (54) has shown that high-grade serous breast cancers are characterized by TP53 mutations in 96% of all tumors as it is the case in BRCA mutations. However, other additional mutated genes with low prevalence have been shown in breast cancers (BRCA1, BRCA2, NF1, RB1, CDK12, CSM3D, FAT3, GABRA6), and we did not find them in our precancerous lesions. Conversely, the early alterations in precancerous lesions are certainly key stages in these initial phases of carcinogenesis, and the corresponding candidate genes could act directly in tumor initiation and represent targets for diagnosis and/or treatment. Some authors (50, 51) underline, for example, the importance of the long arm of chromosome 11 which carries several genes encoding for DNA repair proteins (such as H2AX); we did indeed find deletions at 11q not only in dysplasia but also in breast cancers. In our breast cancer series, certain rearrangements encompass genes involved in the DNA repair system and/or the transcription and regulation channels for various cytokines (HLMB1 or ING4 involved in the p53 signaling pathway at 12p13.31, MIM13SA2 at 12q23.1, RAG1, RAG2, C11orf74 at 11p12, PTHLH at 12p11.23, DOK5 at 14q32.2-q32.33, TRAF3 at 20q13.2, ITG83BP (co-activator of retinoic nuclear receptors) at 1p31.3). In breast cancers, rearrangements detected in the present study contain genes involved in transcriptional or translational regulation (MEIS1 at 2p24, NCK1 at 3q26.2-q26.31, GAK at 4p16.3, MIR202 at 10q26.3) as well as genes involved in the regulation of DNA damage response (OLA1 at 2q31.1-q31.2, RAD21 at 8q13.1-q24.3) and apoptosis (GAK at 4p16.3 and AXIN1 at 16p13.3). Other studies are needed to confirm the involvement of these gene candidates by considering regions common to cases of breast dysplasia, STICs, and cancers (at 3q, 11q at 12q) as priority targets.

The relationship between CIS and invasive cancers due to the existence of identical chromosomal abnormalities in both cases (56).

In addition, specific profiles have been discovered that differ between hereditary and sporadic forms of breast cancer. Certain regions that are amplified (3q, 20q) or deleted (5q) and the corresponding genes (TM4SF1, BCAS4, RAD50) found in hereditary type tumors would suggest defects in the DNA repair system with a consequent installation of genomic instability (46, 56–58).

Ovarian/tubal dysplasia and STIC showed breakpoints in telomeric bands (Supplementary Tables). In particular, ovarian and tubal dysplasia had a common terminal breakpoint at chromosome 14q32.33. Tubal dysplasia had another telomeric breakpoint at 12q24.33. Among the frequent breakpoints at STICs, more than half occurred at terminal bands: 4p16.3; 8q24.3; 10q26.3; 11p15.5, 12q24.33; 16p13.3; 19q13.43; 20p13; and 22g13.33. This is in line with the study by Gisselsson and colleagues (59), showing the clustering of breakpoints at telomeric chromosomal bands at initial stages of genomic instability in cancer. These data also confirm that cells from STICs, which are characterized by the shortest telomeres, are undergoing a telomere crisis with the occurrence of end-to-end telomere fusions and breakage fusion bridge cycles involving many chromosomes.

By using CGH technique, it is not possible to take into account reorganizations or rearrangements of the genome. This technique only can envisage genome imbalances such as gains, losses, or amplification events. Unfortunately, array CGH analyses for genome-wide chromosomal imbalance using paraffin sections is a limitation because large amounts of DNA are required (60). This is why we were unable to analyze all the samples extracted from small ovarian and tubal epithelium specimens. These are therefore preliminary results and will need to be confirmed in larger cohorts. The next step will consist of screening and identification of the genes corresponding to the regions revealed by CGH array along with study of the correlation between telomere length and activation of telomerase.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: G. Chene, J. Dauplat, F.P. Llorca
Development of methodology: G. Chene, A. Tchirkov, E. Pierre-Eymard, F.P. Llorca
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G. Chene, A. Tchirkov, E. Pierre-Eymard, I. Raoelfils, A. Cayre, E. Watkin, P. Vago, F.P. Llorca
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G. Chene, A. Tchirkov, E. Pierre-Eymard, I. Raoelfils, A. Cayre, E. Watkin, P. Vago, F.P. Llorca
Writing, review, and/or revision of the manuscript: G. Chene, A. Tchirkov, E. Pierre-Eymard, J. Dauplat, I. Raoelfils, A. Cayre, E. Watkin, P. Vago, F.P. Llorca
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Tchirkov, E. Pierre-Eymard, I. Raoelfils, A. Cayre, E. Watkin, P. Vago
Study supervision: J. Dauplat, F.P. Llorca

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