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Telomeres and Genomic Instability from Precancerous Lesions to Advanced Cancer – Understanding Through Ovarian Cancer

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Abstract

Genetic instability plays an important role in ovarian carcinogenesis. 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. Telomere shortening appears to take place in most human preinvasive epithelial lesions: short telomeres are found in up to 88% of early precancerous conditions of the bladder, cervix, colon, esophagus, or prostate. However, little is known about ovarian carcinogenesis and telomere shortening. Recent evidence has shown that the fallopian tube may be the origin of ovarian cancer. A new tubal carcinogenic sequence has been described with precancerous lesions that could metastasize to the ovary and result in invasive ovarian cancer. In this review, we will describe the degree of telomere shortening and genomic instability (estimated by the expression of DNA damage response proteins, such as H2AX, Chk2, ATM, 53BP1, p53, and TRF2, and by array comparative genomic hybridization) in early preinvasive stages of ovarian cancer (serous tubal intraepithelial carcinoma (STIC)), ovarian high-grade serous carcinoma, and benign controls. Given that STICs have the shortest telomeres, they could be in a telomere crisis phase preceding genomic stabilization due to telomerase activation (see appended diagram). Concordant results were obtained in immunohistochemical and molecular studies. The expression of all DNA damage proteins increased from benign fallopian tubes to STICs suggesting an early activation of the DNA damage response (DDR) pathways in STICs and indicating that genomic instability may occur early in the precancerous lesions of high-grade serous ovarian cancer (HGSC). In this chapter, we propose to review current knowledge about the function of human telomeres and telomerase and their relevance in genomic instability in cancer and to focus on specific results for ovarian cancer.

Keywords: Telomere, genomic instability, ovarian cancer, dysplastic lesion, STIC

1. Introduction

1. 1. Telomeres, telomerases, and p53 interaction

Genomic instability is probably one of the first phases in carcinogenesis and would thus facilitate the accumulation of multiple mutations. This instability was first described in connection with the discovery of microsatellite instability with the hereditary non-polyposis colon cancer (HNPCC) or Lynch syndrome for example. In most cancers, there is another form of genomic instability, that is, the chromosomal instability (CIN), which we will describe in this review [1]. At molecular level, this instability could result in chromosome rearrangements (number and structure), duplication or segregation anomalies during mitosis, along with DNA repair system dysfunction [1–4]. The CIN may be explained by the mutator hypothesis in hereditary cancers (germline mutations in DNA repair genes as *TP53* or *BRCA* genes for instance, beginning in the precancerous lesions) and by the oncogene-induced DNA replication stress in sporadic (non-hereditary) cancers [1]. Because CIN precedes *TP53* and DNA repair genes mutations in sporadic cancer, genomic instability would be the result of activated oncogenes or antioncogenes (growth signaling pathways...) [1]. However, in both cases (sporadic and genetic cancers), CIN may be due also to telomere erosion and is likely linked to multifactorial molecular events.

Telomere dysfunction has been described to be one of the initial phases in genomic instability. Telomeres are found at the tips of the chromosomes and consist of a large number of hexamer (TTAGGG)_n repeats capped with the multiprotein shelterin complex. Their main function is to protect chromosomal ends from fusion and nucleotidic degradation, which ensures chromosomal stability. Telomeres are regularly shortened during cell division due to incomplete replication of telomeric DNA. Excessive shortening (a “telomere crisis”) activates DNA repair at telomeres leading to CIN and finally cell death. In tumors, telomeres are usually shortened, which makes the cell unstable, but the activation of telomerase (telomere elongation complex generally found only in stem, progenitor, and tumor cells and not in normal somatic cells) maintains the telomere length at a certain level, enabling the survival of these cells [2–4]. These unstable cells can then continue to proliferate and this results in cancer. The interaction between telomeres and p53 would thus represent one of the trigger events in carcinogenesis [5]. In physiological terms, telomere shortening represents a real “genotoxic stress” resulting in activation of the DNA damage signaling pathway (and consequently p53 tumor suppressor protein), finally leading to apoptosis or cycle arrest. When p53 is inactivated by mutations, as is the case in ovarian high-grade serous carcinoma, the telomere dysfunction is neither repaired nor signaled, resulting in genomic instability with the accompanying chromosome aberrations, which are a prelude to cancer [2–6].

DNA double-strand breaks can be the result of telomere dysfunction and are one of the most critical DNA alterations at the origin of genomic instability. One of the first response mechanisms aimed at repairing these breaks is activation of the DNA damage checkpoints. These checkpoints represent the transition state between two cell phases that may be inhibited by genotoxic lesions. There is then a slowing or complete halt in the progression from one phase to the next (checkpoint G1/S or G2/M) or during replication (checkpoint intra-S) [7].

The phosphorylation of ATM (a member of the phosphoinositide 3-kinase or PI3 kinase) occurs first. Then ATM phosphorylates Chk2 p53, H2AX, and 53BP1. Chk2 also phosphorylates p53 (Figure 1) [8–12]. Histone H2AX phosphorylated on residue serine 139, also called γ H2AX, is a marker for cellular DNA double-strand breaks. Phosphorylation of H2AX results in the condensation of chromatin each side of the break, which makes γ H2AX detectable at nuclear level by immunohistochemistry.

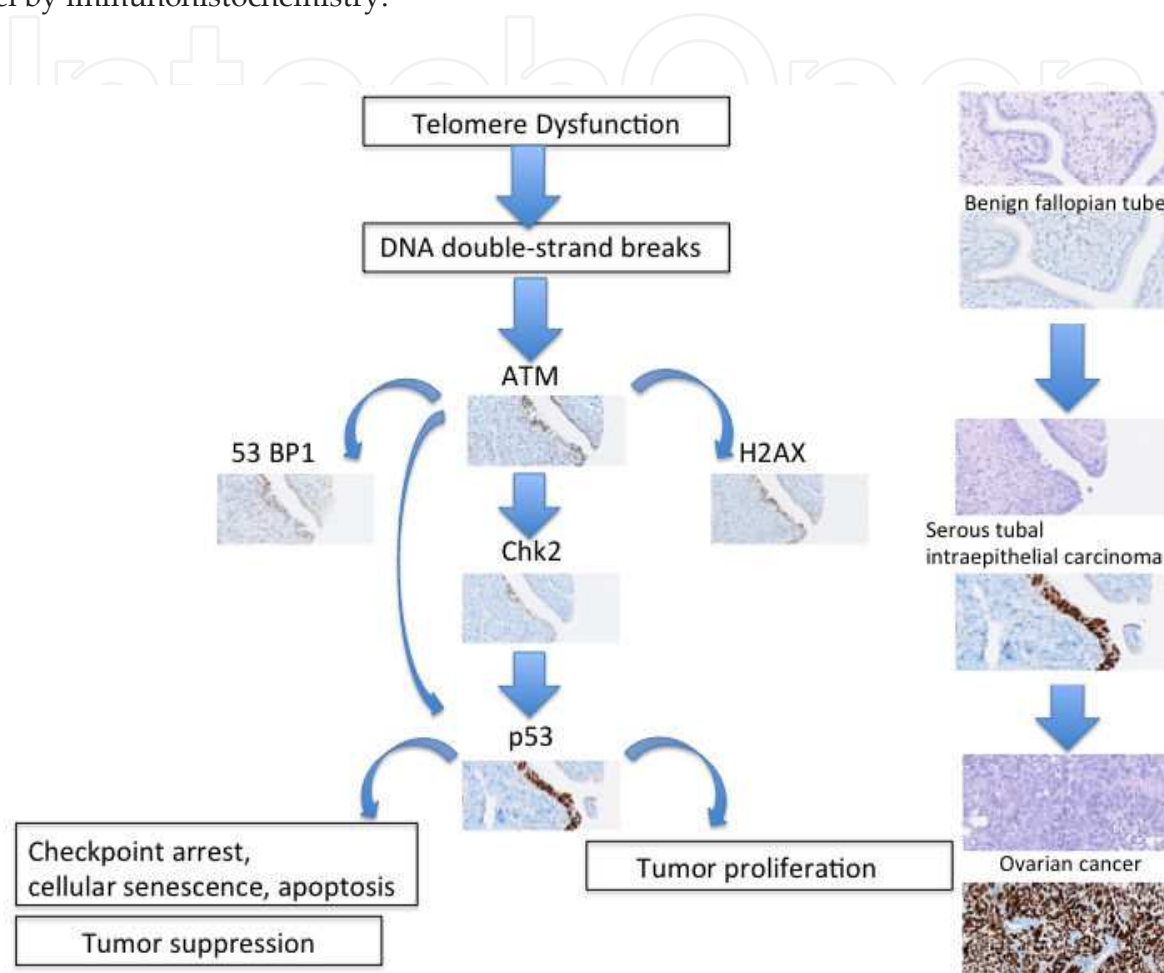


Figure 1. Relationship between telomeres and the DNA damage response pathway. DNA double-strand breaks may be the result of telomere dysfunction with activation of the DNA damage checkpoints. The apical kinase ATM is first activated. Then ATM activates the DNA damage mediators, H2AX and 53BP1. ATM also phosphorylates the downstream kinase Chk2, which phosphorylates p53. ATM may directly activate the effector p53. In non-cancerous situation, this pathway leads to checkpoint arrest, cellular senescence, or apoptosis. In cancerous situation, this cascade may activate tumor proliferation to invasive carcinoma. On the left side, you can see the serous carcinogenic sequence from benign fallopian tube (no p53 mutation) to serous tubal intraepithelial carcinoma (the precancerous lesion with p53 signature) and after that ovarian cancer (with also *TP 53* mutation) [15, 17].

Strong expression of γ H2AX was detected in most precancerous lesions and was considered to represent an antitumor function. Experimental studies have shown that H2AX-deficient mice were at greater risk of immunodeficiency, infertility, and were also more sensitive to ionizing radiation, all of which can be explained by increased chromosomal instability related to reduced capability of the DNA repair systems. When these mice were also p53 deficient (knock-out), there was an increased risk of tumors [13, 14].

As it has been demonstrated that telomere shortening occurs early during carcinogenesis (being found in 89% of preinvasive lesions of the bladder, cervix, colon, and also the esophagus) [5], we investigated genetic instability in precancerous ovarian lesions, which has been described very recently.

2. Ovarian cancer: A model for greater insight into genetic instability

This review concerns high-grade serous ovarian cancer (HGSC) characterized by a higher genomic instability (see below) and very frequent *TP53* gene mutations. Germline mutations in breast cancer susceptibility 1 (BRCA 1) or BRCA 2 may lead to hereditary HGSC, whereas 90% of HGSC are sporadic (no BRCA mutations but frequent BRCA inactivation or modulation with promoter hypermethylation, loss of heterozygosity in over 50% of cases) [15].

Four hundred and eighty-nine HGSC were recently studied by the Cancer Genome Atlas Research Network [16]. *TP53* mutations were present in 96% of cases followed by mutations in *NB1*, *BRCA1*, *BCA2*, *RB1*, and *CDK12* genes. Homologous recombination (and the DNA damage signaling pathway) is defective in about half of the cancers analyzed [16]. Taken together, all these findings can explain why ovarian cancer may be a relevant model to explore genetic instability.

Recent histopathologic and molecular studies enabled to describe a model of tumorigenesis comprising two pathways [15]:

- Type I tumors: These are low-grade tumors for which the pathogenesis would consist of a sequence of cystadenomas/adenofibromas, then borderline tumors and finally progression towards cancer. A lesional continuum has indeed been found, that is, molecular mutations that are common to benign, borderline, and invasive lesions (BRAF and KRAS mutations in over 60% of cases, less frequently mutated PTEN (20%–46%) and β -catenin ($\approx 30\%$). Note also the recent discovery of ARID1A gene mutations in endometriosis and cancers associated with endometriosis (clear cell and endometrioid cancers) [17].
- Type II tumors: These are high-grade tumors from the outset. At the molecular level, *TP53* gene mutations are found in 50%–80% of cases. These tumors show high genomic instability and are essentially represented by HGSC. This histologic subtype is the most frequent and consequently has been the subject of many studies, and a tubal carcinogenic sequence has been identified [15].

True occult intraepithelial cancerous lesions of the tube (serous tubal intraepithelial carcinoma (STIC)) have been reported unusually often in the distal portion of tubes obtained after prophylactic adnexectomy for BRCA mutation, prompting systematic and meticulous examination of these tubes [18–23]. They show epithelial stratification, nuclear atypicalities with an increase in the nucleocytoplasmic ratio, loss of nuclear polarity, nuclear pleiomorphism, and loss of ciliated cells. Immunohistochemical analysis may reveal intense and diffuse expression of *p53* (the p53 signature) and a high proliferative index (Ki67 > 40%). There could be a high level of genetic instability, as demonstrated by the high positive level of γ H2AX [15, 16, 18–23].

In other words, high-grade ovarian serous carcinoma could originate in the fallopian tube, and all these findings encouraged us to study STIC lesions and genomic instability.

We started by assessing the telomere length in 12 STICs, 36 non-cancerous controls, and 43 high-grade serous cancers [24, 25]. Laser microdissection of paraffin-embedded samples was used in all cases. A DNA extraction was followed by purification step. Telomere length was measured by real-time quantitative polymerase chain reaction (PCR) according to Cawthon's method [26].

STICs had the shortest telomeres ($p=0.0008$). Telomeres of invasive cancer were shorter than those in benign controls but longer than telomeres found in STICs. A significant correlation was also found between overexpression of p53 and H2AX proteins and shortened telomeres in STICs ($p<10^{-7}$) [24]. Kuhn *et al* [27] used fluorescence in situ hybridization technique to analyze telomere length of a series of 22 STICs in comparison with non-cancerous controls and high-grade cancers: 82% of STICs had the shortest telomeres, followed by the cancers. The controls had the longest telomeres. These findings suggest that STIC lesions are the most unstable genetically and are likely to represent one of the first steps in tubo-ovarian carcinogenesis.

We also studied these STICs by array comparative genomic hybridization (aCGH) using oligonucleotide microarrays (Agilent 180 K) [24].

The size of rearrangements in STICs was high, with an average of 2363.37 Kb (488–8161).

We found common gains at chromosomes 19q (6/12, 50%), 16p (5/12, 41.6%), 12q (5/12, 41.6%), 10q (5/12, 41.6%), 11p (4/12, 33.3%), 4p (3/12, 25%), and 8q (3/12, 25%), and common losses at chromosomes 3q (6/12, 50%), 2q (5/12, 41.6%), 11q (4/12, 33.3%), 6p (4/12, 33.3%), 22q (4/12, 33.3%), 18q (3/12, 25%), 19p (3/12, 25%), 20p (3/12, 25%), and 2 p (2/12, 16.6%) [24]. These early rearrangements could be key steps in these first phases of carcinogenesis, and the corresponding candidate genes could be involved directly in the tumor triggering process, meaning that they could represent diagnostic and/or treatment targets. More studies are required.

Finally, in another study, we investigated the level of DDR activation in STICs by immunohistochemistry (pATM, pChk2, γ H2AX, 53BP1, and TRF2) [28]. We constructed a tissue microarray, including 21 benign fallopian tubes, 21 STICs, and 30 HGSCs. We demonstrated that the expression of all DDR proteins increased from benign fallopian tubes to STICs. Analysis of staining variance within cases showed that 53BP1, γ H2AX, pATM, pCHK2, and TRF2 expressions were significantly higher in STICs than in HGSC [28].

Taken together, all our results have shown evidence of genomic instability in the precancerous lesions known as STICs. Of note among the frequent chromosomal breakpoints in STICs, more than half occurred at terminal bands, which is characteristic for a telomere crisis with the occurrence of telomere fusions, leading to chromosomal aberrations.

It could also be interesting to study human telomerase reverse transcriptase (hTERT) expression, which is a determinant for telomerase activity, and also the telomere architecture proteins (shelterin complex proteins TRF1, TRF2, and POT1) in STICs and HGSC [29–33]. TRF1 and TRF2 proteins are involved in negative regulation of telomere lengthening and interact with

telomerase [29, 30]. POT1 appears to play a dual antagonist role, depending on cell conditions, acting as positive or negative regulator of telomere length depending on telomerase [6, 34]. In our study [28], the level of expression of TRF2 was increased in STICs in comparison with HGSC ($p=0.012$). It has been shown that TRF2 may shorten telomeres without telomerase inactivation [12]. TRF2 is also phosphorylated by ATM to enable DNA damage repair in response to DNA damage [12].

Telomerase would probably be activated after telomere shortening at the invasive stage and would thus counteract further telomere shortening: stabilization of telomere length at this stage would moreover represent an advantage in terms of tumor proliferation and escaping apoptosis [3].

Wang *et al* [35] investigated the relationship between telomere length and telomerase activity in 15 ovarian cancers. The authors found telomeric dysfunction in 9/15 (60%) and telomerase activation in 11/15 (73.3%) ovarian cancers. However, they did not study precancerous lesions.

Other authors demonstrated that telomerase activity was higher in ovarian carcinoma than in borderline tumors (considered as premalignant tumors) and normal ovary [36–38]. This was confirmed in cell cultures [39]. However, telomerase activity in STICs has not yet been studied.

3. Conclusion

Telomere shortening has been proven to be one of the potential precursors of ovarian cancer indicative of genomic instability. This could lead to significant preventive strategies such as prophylactic salpingectomy in patients with a genetic risk of ovarian cancer in order to avoid the malignant transformation of benign fallopian tube into STIC [40].

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