Telomerase Activity in Benign and Malignant Epithelial Ovarian Tumors

Minghong Wan, Wei-Zhi Li, Bridgette D. Duggan, Juan C. Felix, Yanle Zhao, Louis Dubeau*

Background: Ovarian epithelial tumors include benign lesions lacking invasive and metastatic abilities (cystadenomas) in addition to malignant lesions (carcinomas). An intermediate category, called tumors of low malignant potential (LMP), is also recognized. The merit of this classification is being challenged because the clinical behavior of LMP tumors appears closer to that of cystadenomas than to that of carcinomas. Purpose: To verify our hypothesis that the expression of the enzyme telomerase distinguishes these two categories of ovarian epithelial tumors, we examined and compared such expression in ovarian cystadenomas and carcinomas. By examining the expression of telomerase in LMP tumors, we then sought to determine if these tumors were more closely related to cystadenomas or to carcinomas with regard to telomerase expression. Methods: We examined a total of 64 consecutive ovarian tumors subdivided into 20 carcinomas, 17 LMP tumors, and 27 cystadenomas. We subsequently discarded three of the 27 cystadenomas because of the presence of admixed normal ovarian stroma in those specimens. Tumor subtyping was done without knowledge of the telomerase results, and telomerase assays were likewise interpreted without knowledge of tumor types. Telomerase activity was determined by use of the TRAP (i.e., telomeric repeat amplification protocol) assay. Differences between the proportions of tumors expressing this enzyme in each subgroup were evaluated by use of Fisher’s exact test (two-sided). Results: Telomerase activity was detected in all 20 carcinomas and in 17 LMP tumors. In contrast, it was not detected in 19 of the 24 cystadenomas. These differences between rates of telomerase expression in either carcinomas or LMP tumors and those in cystadenomas were statistically significant (P<.0001). All five of the telomerase-positive cystadenomas belonged to a variant called papillary cystadenomas, whereas none of the telomerase-negative cystadenomas belonged to this variant (P<.0001). Conclusions and Implications: The presence of telomerase activity in ovarian LMP tumors supports the merit of continuing to separate these tumors from cystadenomas, in spite of their apparent benign clinical course. The finding of telomerase expression in papillary cystadenomas suggests that such tumors may be mechanistically related to LMP tumors and should perhaps be reclassified as variants of LMP tumors. Lack of telomerase expression in ovarian cystadenomas raises questions about the alleged immortality of these tumors because expression of this enzyme is thought to be essential for continuous growth in adult tumors. [J Natl Cancer Inst 1997;89:437-41]

Ovarian epithelial tumors are among the most lethal of the gynecologic cancers. Not all ovarian epithelial tumors, however, are malignant. A benign category in which invasive and metastatic abilities are absent is another common form. Tumors belonging to the latter category are called cystadenomas because they are made up of secretory cells that line cystic cavities in which secreted fluids accumulate (1). They are considered neoplastic because of their apparent incessant growth, which may result in large tumors associated with significant morbidity. Recent data (2) indicate that these tumors are of monoclonal origin, further supporting their neoplastic nature.

In addition to the clearly benign and clearly malignant lesions described above, ovarian epithelial tumors also include a third entity called tumors of low malignant potential (LMP) (1,3). The merit of maintaining this classification is presently the subject of much debate among pathologists. Ovarian LMP tumors often show disorganized growth patterns reminiscent of those of carcinomas and, under rare circumstances, may show limited invasive and metastatic abilities. These tumors, however, are not invasive in their typical form, and their apparent benign clinical behavior has led to the suggestion that they should be regarded as variants of cystadenomas (4).

In this study, we examined the expression of the enzyme telomerase as a means of better defining the similarities between ovarian LMP tumors and either cystadenomas or carcinomas. This enzyme is a ribonucleoprotein that elongates telomeric sequences (5-7). These sequences are specialized structures with characteristic repetitive DNA sequences that form specialized nucleoprotein complexes at the ends of eukaryotic chromosomes (6,7). They are thought to be important for DNA replication and chromosome protection. They become progressively shorter during each cell division (8). It is thought that chromosomes are unable to undergo additional rounds of replication once their telomeres have shortened below a critical size (6,7). This hypothesis implies that dividing cells can undergo only a limited number of replication cycles unless they activate the telomerase enzyme, which can restore the length of their telomeres. In support of this concept, Kim et al. (9) showed that telomerase, which is otherwise present in germ cells and stem cells but usually undetectable in somatic cells, is generally expressed in malignant tumor cells.

*Affiliations of authors: M. Wan, W.-Z. Li, J. C. Felix, Y. Zhao, L. Dubeau (Department of Pathology), B. D. Duggan (Department of Gynecologic Oncology), University of Southern California, USCNorris Comprehensive Cancer Center, Los Angeles, CA.

Correspondence to: Louis Dubeau, M.D., Ph.D., USCNorris Comprehensive Cancer Center, 1441 Eastlake Ave., Los Angeles, CA 90033.

See “Notes” following “References.”
Although the activity of telomerase in ovarian cystadenomas has not been reported to our knowledge, we hypothesized that it may be absent in these tumors because it was not detected in other benign neoplasms such as colorectal polyps (9, 10). We examined and compared the expression of telomerase in ovarian cystadenomas and carcinomas to verify our hypothesis that such expression distinguishes these two categories of ovarian epithelial tumors. By examining the expression of the same enzyme in LMP tumors, we then sought to determine if these tumors were more closely related to cystadenomas or to carcinomas with regard to telomerase expression.

Materials and Methods

Sources and Processing of Tumor Specimens

Tumor specimens were obtained either from the USC/Norris Comprehensive Cancer Center of the University of Southern California or from the Women’s Hospital of the Los Angeles County Medical Center. Two of the original 27 cystadenomas (three were subsequently discarded), three of the 17 LMP tumors, and 10 of the 20 carcinomas were obtained from the USC/Norris Comprehensive Cancer Center. Only one tumor specimen from each patient was examined. All investigations and procedures involving human tissues were performed after approval by the University of Southern California School of Medicine Research Committee in accord with an assurance filed with and approved by the U.S. Department of Health and Human Services.

All tumors were of primary ovarian epithelial origin. Diagnostic evaluations and tumor subtyping or grading were done independently by two of us (L. Dubeau and J. C. Felix), who are practicing surgical pathologists experienced in ovarian histopathology. These diagnostic evaluations were done blindly, without knowledge of telomerase results. Although not all histologic evaluations were completed before the telomerase assays were initiated, they were done independently and by different individuals. Clinical information was obtained from hospital records. All the surgically removed tumor tissues were stored at −80 °C until they were subjected to the telomerase assay. The criteria used for tumor staging were those recommended by the International Federation of Gynecology and Obstetrics [see (1)].

Tumor sample collections were done consecutively. The number of cases studied for each tumor subtype was determined arbitrarily and depended primarily on the number available when the study was initiated. The only cases discarded were three cystadenomas that, upon further review, were found to contain admixed normal ovarian tissues (see “Results” section).

Assay of Telomerase Activity

Telomerase assays were performed and interpreted blindly, without knowledge of the tumor types. Each sample of frozen tissue (50–100 mg) was rinsed and stored at −80 °C in dry ice–ethanol and stored at −80 °C.

Materials and Methods

**Statistical Analyses**

Fishér’s exact test was used to compare the rates of telomerase positivity in the different tumor subgroups. All P values quoted are two-sided.

**Results**

**Telomerase Activity in Ovarian Carcinomas**

We first looked for the presence of telomerase activity in 20 different ovarian carcinomas. Although the presence of such activity was previously reported in this tumor type (9, 11), we sought to determine the frequencies of telomerase expression in our tumor samples in order to better evaluate potential differences between ovarian carcinomas and other ovarian epithelial tumor types. One of the 20 carcinomas was stage I, three were stage II, 11 were stage III, and five were stage IV. Eleven were subclassified as serous, five as endometrioid, two as clear-cell carcinomas, and two as homologous, mixed müllerian tumors.

**Telomerase Activity in Ovarian Cystadenomas**

We next looked for the presence of telomerase activity in ovarian cystadenomas to determine if these benign tumors could be distinguished from ovarian carcinomas on the basis of such activity. A representative experiment based on the TRAP assay first developed by Kim et al. (9) is shown in Fig. 1. This assay is a two-step procedure, in which tissue extracts are first incubated in the presence of a synthetic oligonucleotide. If present, the telomerase enzyme synthesizes multiple copies of the basic telomeric sequence in tandem using the oligonucleotide as primer and its own RNA moiety as template. The next step consists of using the PCR to enzymatically amplify the telomeric sequences synthesized in the first step. The radiolabeled PCR products are then subjected to electrophoresis on polyacrylamide gels and visualized by autoradiography. The first lane (lane a) for cases 12, 13, and 19 in Fig. 1 shows a characteristic ladder that corresponds to various lengths of telomeric DNA synthesized by the enzyme. The absence of such a ladder in case 8 indicates absence of telomerase activity in this sample. Lanes b and c show the two control experiments that were performed with all assays. The telomerase assay was preceded by a digestion step with RNase in lanes b. As expected, this procedure resulted in the disappearance of detectable telomerase activity in all positive cases (Fig. 1) because telomerase, which is a ribonucleoprotein, is inactivated by RNase (J2). Small amounts of
tissue extracts from an ovarian carcinoma with known telomerase activity were added to the reaction mixes in lanes c, resulting in detectable telomerase activity in all cases (Fig. 1). These two control experiments indicate, respectively, that the activity detected in cases 12, 13, and 19 was authentic and rule out the possibility that absence of activity in case 8 was due to the presence of reaction inhibitors in this tissue sample.

We used the above approach to examine various histologic subtypes of ovarian cystadenomas, ranging in size from small ovarian cortical cysts less than 1 cm in diameter to large tumors with diameters up to 36 cm (Table 1). Three of the 27 tumors originally selected for these studies are not shown in Table 1 because, as we realized that some, but not all, of these tumors were telomerase positive, we re-examined histopathologic sections of all tissue samples to determine if the telomerase-positive tumors were different from the telomerase-negative ones. This re-examination led to the realization that the above three cases, all of which expressed telomerase activity, contained significant amounts of normal ovarian stroma in their cyst wall. We therefore obtained additional tissue samples from all of our 27 cystadenomas. Each sample was cut in half. The first half was examined for telomerase activity, and the remaining, adjacent portion was fixed in formalin and examined histologically to evaluate the presence or absence of normal ovarian tissues. Telomerase assays were interpreted without knowledge of the histologic findings, and histologic examinations were likewise done without knowledge of the telomerase status. These studies confirmed that the above three cases were telomerase positive and were the only ones among our 27 cystadenomas that showed normal ovarian stroma in the sampled portions. We therefore elected to delete these cases from Table 1 because the detectable levels of telomerase activity in these samples were probably not due to the neoplastic cells but rather were due to the presence of admixed normal ovarian stroma. Indeed, telomerase activity was previously reported in normal ovaries (9). Although the presence of telomerase activity in normal ovarian tissues adjacent to telomerase-negative cystadenomas may appear paradoxical, it should be remembered that the former contain a variety of different cell types not present in the latter. In particular, normal ovaries contain germ cells, which are known to express telomerase (9).

Telomerase activity and clinicopathologic characteristics are shown in Table 1 for each of the 24 remaining cystadenomas. Only five cystadenomas contained detectable levels of telomerase activity. Differences between the proportions of ovarian cystadenomas and carcinomas expressing the telomerase enzyme (five of 24 cystadenomas versus 20 of 20 carcinomas) were found to be significant when the two-sided P value was calculated by use of Fisher’s exact test (P<.0001).

**Distinction Between Papillary and Nonpapillary Cystadenomas**

We further examined the five cystadenomas that were not contaminated with normal ovarian stroma and in which telomerase was detected to determine if they were different from the telomerase-negative lesions. It is interesting that all five tumors belonged to a variant subtype called papillary (Table 1). Histopathologic differences distinguishing papillary cystadenomas from the more typical (nonpapillary) forms as well as from LMP tumors are shown in Fig. 2. Typical cystadenomas are composed...
Telomerase Activity in LMP Tumors

We next examined the expression of telomerase in ovarian LMP tumors to determine if levels of expression in these tumors would resemble those found in cystadenomas or carcinomas. As shown in Table 2, all 17 cases examined, which included tumors of stages I to III and of either serous or mucinous differentiation, were positive for this enzyme. Thus, LMP tumors resemble carcinomas and are clearly distinguishable from the most common (nonpapillary) forms of cystadenomas with regard to telomerase activity.

Discussion

The results of our experiments clearly show that the presence or absence of telomerase, which is an enzyme required for the maintenance of telomeric length, is highly specific for distinct subtypes of ovarian epithelial tumors. Activity of this enzyme was present in all ovarian carcinomas examined, confirming earlier reports (9,11) that it is expressed in a high proportion of malignant ovarian tumors. In contrast, telomerase activity was rarely found in ovarian cystadenomas, which are benign tumors arising from the same progenitor cells as carcinomas. Moreover, the few cystadenomas in which telomerase activity was observed had histopathologic features that distinguished them from typical cystadenomas. The fact that telomerase was present in all LMP tumors examined indicates that the frequent presence of this enzyme is a characteristic shared by both carcinomas and LMP tumors.

Ovarian LMP tumors are often regarded as intermediates between cystadenomas, which are clearly benign lesions, and carcinomas, which are malignant (1,3). Their designation as LMP implies a possibility of progression to malignancy. However, the usual lack of clinical recurrence that characterizes these tumors has raised questions about the merit of this classification (4). We recently showed that DNA methylation levels seen in LMP tumors were similar to those seen in carcinomas and different from those present in cystadenomas (2). In another study (13), allelic imbalances involving the long arm of chromosome X, which are frequent in LMP tumors as well as in high-grade carcinomas, were shown to be rare in cystadenomas. Our above results, therefore, constitute the third example of a molecular change shared by both LMP tumors and carcinomas that is absent in cystadenomas. It therefore seems appropriate to continue to regard LMP tumors as a distinct disease entity, despite their nonaggressive clinical behavior. Elucidation of their exact relationship to...
carcinomas awaits further progress in our understanding of the significance of the above molecular changes. All LMP tumors that we examined, most of which were of low stage, were typical and did not include the newly described and clinically more aggressive variant known as micropapillary serous carcinoma (14).

All five of our telomerase-positive cystadenomas belonged to a variant called papillary. Although such variants are currently classified as cystadenomas on the basis of their bland cytologic appearances as well as their typical lack of recurrence following surgical treatment, their tendency to form complex histologic patterns such as papillary structures is reminiscent of LMP tumors. The presence of telomerase expression in the papillary lesions may be an indication that such lesions are mechanistically more related to LMP tumors than to cystadenomas and should perhaps be reclassified as variants of LMP tumors. It is currently not known whether any of the other molecular genetic changes that appear to distinguish LMP tumors from cystadenomas are present in the papillary cystadenomas.

Telomerase is believed to be essential for continuous growth such as that associated with cancer cells because, in the absence of this enzyme, telomere lengths in dividing cells will inevitably become too short to allow further chromosomal replication (6, 7, 9, 15). This notion is supported by observations of telomerase activation during neoplastic progression (10, 16, 17) and led to the attractive idea that interference with telomerase function may represent an efficient and universal approach for cancer therapy (18, 19). The alleged importance of telomerase for the establishment of the malignant phenotype was recently challenged by the demonstration that childhood tumors such as retinoblastomas, which may carry long telomeric sequences, can develop in the absence of telomerase (20). However, the large sizes of some of the telomerase-negative cystadenomas included in our study (>20 cm in diameter in some cases), plus the fact that several of our patients with telomerase-negative cystadenomas were elderly, should preclude continuous growth of these tumors in the absence of an active mechanism to maintain telomeric length. Thus, the possibility that ovarian cystadenomas have a limited life span and are therefore not immortal merits some consideration.

The above idea is not supported by findings that cystadenomas are monoclonal (2) and thus probably neoplastic. However, it is consistent with a recent report (21) of ultrasonographic evidence that benign ovarian cysts frequently regress or remain unchanged in postmenopausal women. It is also compatible with current theories about the origin of ovarian cystadenomas. According to the currently favored theory, these tumors arise from invaginations of ovarian surface epithelium (1) and are therefore not necessarily the result of neoplastic transformation. The possibility that ovarian cystadenomas are not immortal neoplasms has implications for the clinical management of ovarian cystadenomas, which are common tumors in women of child-bearing age.

The fact that telomerase is expressed in most, if not all, ovarian carcinomas also has potential applications for the detection of these tumors in clinical specimens. Malignant ovarian tumors typically spread to the pelvic and abdominal cavities but, unlike most other malignant tumor types, rarely metastasize outside these cavities. Cytologic examination of ascites or of pelvic/abdominal washings is therefore frequently performed to evaluate minimal residual disease or tumor recurrences. It may be possible to use the presence of telomerase activity as a tumor-specific marker in such fluids in order to complement more conventional approaches to monitor disease status following therapy. Although the merit of this approach has not yet been tested experimentally, the high sensitivity of the TRAP assay and the apparent high specificity of telomerase for the neoplastic phenotype add to its potential attractiveness.

References


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Present address: M. Wan, Department of Genetics, Stanford University Medical Center, CA.
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