Advances in Brief

Association of 17q21-q24 Gain in Ovarian Clear Cell Adenocarcinomas with Poor Prognosis and Identification of PPMID and APPBP2 as Likely Amplification Targets

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Abstract

Purpose: Although tumor stage is considered a prognostic feature for ovarian clear cell adenocarcinomas (OCCA), it is not likely to fully account for the clinical and biological variability characteristic of the disease. The aim of this study was to investigate aberrations of DNA copy number in OCCA tumors and identify genetic markers that would increase our understanding of the pathogenesis of OCCA and assist in more accurately predicting the outcome for an individual patient with this disease.

Experimental Design: We determined copy number aberrations among 20 primary OCCA tumors by means of comparative genomic hybridization and investigated their relationship to clinicopathological data. We also measured expression levels of candidate target genes within critical regions by quantitative real-time reverse transcription-PCR and compared those data with copy number status and patient outcomes.

Results: We identified several nonrandom chromosomal aberrations among the 20 primary OCCA tumors examined. Among them, gain of DNA at 17q21-q24 showed significantly negative correlation with disease-free and overall survival (P = 0.0012 and 0.0039, respectively, log-rank test). This correlation held even for patients with stage I tumors. Among 15 candidate genes within the 17q21-q24 region, we found significantly elevated expression of PPMID and APPBP2, and their heightened expression correlated negatively with disease-free survival (P = 0.0090, log-rank test adjusted for multiple comparisons).

Conclusions: Information gained from our relatively large panel of OCCA tumors suggested that 17q21-q24 gain and consequent overexpression of two potential targets, PPMID and APPBP2, are associated with malignant phenotypes of this tumor and may be useful predictors for prognosis.

Introduction

Although ovarian cancer is the third most common malignancy of the female genital tract, survival rates for this disease are the lowest among such tumors; it is the fifth most common cause of death from any cancer among women. Despite all therapeutic efforts, the overall 5-year survival rate is less than 30% (1, 2), and this discouraging statistic has remained largely unchanged for many years. Primary ovarian cancer is a morphologically and biologically heterogeneous disease; its four major types are classified on the basis of morphological criteria (3) as serous, mucinous, endometrioid, and clear cell adenocarcinomas. OCCA (4) appears to constitute only 3.7–12.1% of ovarian adenocarcinomas, but it exhibits distinctly different clinical behavior from other types of this disease (4–10). A number of studies have noted a particularly unfavorable prognosis for patients with OCCA, even when corrected for tumor stage (4–10). The low responsiveness of OCCA to conventional platinum-based chemotherapy may be at least partially associated with its poor prognosis (5–8), although the mechanisms underlying the malignant phenotypes of OCCA are not yet understood.

Because OCCA exhibits a wide spectrum of clinical behavior from one patient to another, accurate prognostic indicators are needed to distinguish high-risk patients from others so that optimal therapeutic protocols can be designed on a case-by-case basis. As with other types of ovarian cancer, the clinical
Table 1: Clinicopathological characteristics and summary of chromosomal changes in 20 patients with OCCA

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (yrs)</th>
<th>FIGO stagea</th>
<th>TNMb classification</th>
<th>Performance statusc</th>
<th>Peritoneal cytology</th>
<th>Endometriosis</th>
<th>Type of therapyd</th>
<th>Outcomee</th>
<th>No. of chromosomal changesf</th>
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<tr>
<td>1</td>
<td>60</td>
<td>I</td>
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<td>Negative</td>
<td>OC</td>
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<td>7</td>
<td>1 8</td>
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<td>38</td>
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<td>4</td>
<td>5 9</td>
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<td>3 6</td>
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<td>4</td>
<td>61</td>
<td>III</td>
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<td>Negative</td>
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<td>Death</td>
<td>2</td>
<td>1 3</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
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<td>Positive</td>
<td>OC</td>
<td>Death</td>
<td>1</td>
<td>2 3</td>
</tr>
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<td>6</td>
<td>65</td>
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<td>OC</td>
<td>Death</td>
<td>7</td>
<td>3 10</td>
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<td>51</td>
<td>IV</td>
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<td>OC</td>
<td>Death</td>
<td>1</td>
<td>1 2</td>
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<tr>
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<td>Negative</td>
<td>Positive</td>
<td>OC</td>
<td>Death</td>
<td>1</td>
<td>2 3</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>IV</td>
<td>1a x 1 0</td>
<td>Positive</td>
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<td>O</td>
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<td>3 9</td>
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<tr>
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<td>0 1</td>
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<td>1</td>
<td>2 3</td>
</tr>
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<td>13</td>
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<td>Negative</td>
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<td>Alive</td>
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<td>0 0</td>
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<td>14</td>
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<td>0 0</td>
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<tr>
<td>15</td>
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<td>1</td>
<td>0 1</td>
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<td>Positive</td>
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<td>Alive</td>
<td>3</td>
<td>0 3</td>
</tr>
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<td>57</td>
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<td>Positive</td>
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<td>Alive</td>
<td>1</td>
<td>0 1</td>
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<td>Negative</td>
<td>OC</td>
<td>Alive</td>
<td>2</td>
<td>2 4</td>
</tr>
<tr>
<td>19</td>
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<td>I</td>
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<td>Negative</td>
<td>Negative</td>
<td>OC</td>
<td>Alive</td>
<td>8</td>
<td>2 10</td>
</tr>
</tbody>
</table>

a FIGO, the International Federation of Gynecology and Obstetrics.
b TNM, tumor-node-metastasis.
c Classified according to the performance status criteria of the Eastern Cooperative Oncology Group.
d O, operation only; OC, operation and chemotherapy.
e Death, dead of cancer.
f Number of chromosomal changes were determined by CGH (see Fig. 1).

stage is generally relied on for patient management, but patients with tumors of identical stages frequently show different outcomes and/or responses to therapy (8). Histological measures of differentiation are not reliable prognostic indicators for OCCA as they can be in other types of ovarian neoplasms (11), and too few markers are available to fully account for the observed clinical and biological variability of OCCA. Thus, improved classifications of OCCA and additional indicators are needed so that clinicians can identify patients who should be considered for alternative methods of treatment.

Like other solid tumors, OCCA may develop and progress through multiple genetic alterations that occur sequentially in a cell lineage (12). Because the biological behavior of any tumor is affected primarily by such changes, genetic abnormalities identified in OCCAs could serve as useful indicators for tumor classification. To date, very little is known about the molecular pathobiology of OCCA; thus, identification of genetic changes and information about how they may correlate with clinicopathological findings in clinical cases of OCCA may provide novel insights and lead to effective strategies for identifying tumors with more malignant potential. Because nonrandom chromosomal aberrations in cancer cells tend to mirror functional gains of oncogenes and losses of tumor suppressor genes that accumulate during tumorigenesis, comprehensive documentation of chromosomal copy number alterations is considered an effective approach to identifying altered genes that are involved in the pathogenesis of specific tumors (13).

The technique of CGH has allowed assessment of consistent sites of chromosomal imbalance in a wide variety of solid tumors (14). Although many primary ovarian cancers and cell lines derived from them have already been analyzed using CGH (15–20), only small numbers of OCCA tumors were included in former studies because the incidence of this type of ovarian cancer is relatively low. It is always difficult to determine the biological and/or clinical significance of genetic alterations when information is so limited.

In the present study, we undertook CGH experiments in 20 primary tumors to define the spectrum of genetic abnormalities associated with OCCA, assessed their clinicopathological importance, and identified specific abnormalities that may be associated with cancer progression. Our relatively large panel of cases showed frequent gains at 17q21-q24, which might be associated with the malignant phenotype and poor prognosis of this disease. Moreover, by assessing the expression levels of 15 candidate genes located at 17q21-q24, we identified two putative target genes in the amplicon, each of which was overexpressed in consequence of the genomic amplification mechanism.

Materials and Methods

Patients. Tissue specimens were obtained and frozen at the time of surgery from 20 patients with OCCA who were treated at the Keio University Hospital from 1984 to 1999 (21), after obtaining written consent from each patient in the formal style and after approval by the local ethics committee. The mean age of the patients was 51 years (range, 38–65 years); clinicopathological data for all cases are shown in Table 1. All patients underwent complete surgical staging including i.p. cytology, bilateral salpingo-oophorectomy, hysterectomy, omentectomy,
and pelvic/para-aortic lymphadenectomy. Aggressive cytoreductive surgery was performed in patients with advanced disease. Most patients (18 of 20 patients, 90%) received platinum-based chemotherapy after surgery.

One gynecological pathologist classified all 20 tumors by histology according to the WHO criteria. Surgical staging was based on the International Federation of Gynecology and Obstetrics (FIGO) staging system: stage I, 13 patients; stage II, 2 patients; stage III, 3 patients; and stage IV, 2 patients. All tumor specimens were composed of at least 70% neoplastic cells. DNA was successfully extracted from all 20 tumors, and total RNA was successfully extracted from 11 of them, according to standard procedures (22). The duration of disease-free and overall survival was calculated for each patient from the date of primary surgery to the date of the last follow-up visit or death. The median follow-up period has been 36.0 months (range, 12.6–207.0 months) for the 11 patients who are alive at the time of this writing.

**CGH Analysis.** CGH was performed by using fluorochrome-conjugated DNA as described by Kallioniemi et al. (14), with minor modifications (22). Briefly, tumor and normal DNAs labeled with Spectrum Green-DUTP and Spectrum Orange-DUTP (Vysis, Chicago, IL), respectively, by nick translation were denatured and hybridized to normal male metaphase chromosome spreads together with Cot-1 DNA (Invitrogen, Carlsbad, CA). The slides were washed and counterstained with 4',6-diamidino-2-phenylindole. Shifts in CGH profiles were rated as gains or losses if they reached at least the 1.2 or 0.8 threshold, respectively (22). Overrepresentations were considered to be HLGs when the fluorescence ratios exceeded 1.5 (22). Heterochromatic regions near the centromeres as well as the entire Y chromosome were excluded from the analysis.

**Real-Time Quantitative PCR and RT-PCR.** Quantification of genomic DNA copy numbers and expression levels of mRNAs in 11 primary tumors was carried out using a real-time fluorescence detection method (23, 24). Single-stranded cDNAs were generated from total RNA using Superscript II Reverse Transcriptase (Invitrogen) following the manufacturer’s directions. Real-time quantitative PCR experiments were performed using Light-cycler (Roche Diagnostics, Tokyo, Japan) with CYBR Green according to the manufacturer’s protocol. The β2-microglobulin gene (B2M), which is located at 15q21-q22.2 and is rarely involved in OCCA, either in our panel or in previous studies (17, 18), served as the endogenous control for genomic DNA copy number; the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was the endogenous control for mRNA expression levels. Copy numbers in each tumor sample were normalized by dividing them by the corresponding B2M value and recorded as copy number ratios. Levels of mRNA expression in each tumor sample were normalized on the basis of the corresponding GAPDH level and recorded as a relative expression level. PCR amplification was performed in triplicate for each sample; the primer sequences for each gene are available on request.

**Analysis for TP53 Mutations.** We looked for mutations in exons 5–8 of the TP53 gene by directly sequencing PCR-amplified products with an ABI377 sequencer (PE Applied Biosystems, Foster City, CA) as described previously (25).

**Statistical Analysis.** Fisher’s exact test was performed to analyze differences in the frequencies of individual chromosomal changes within subgroups of tumors. Differences between groups with respect to the total number of copy number aberrations and in the expression level of each gene/transcript were tested by the nonparametric Mann-Whitney U test and by the exact nonparametric test using ranks adjusted for multiple comparisons by the permutation minP tests of Westfall and Young (26, 27), respectively. Probabilities of survival were calculated by the Kaplan-Meier method, and statistical differences between groups were evaluated by log-rank tests. Univariate and multivariate prognostic effects were evaluated under the Cox proportional hazards model. The relationship between the relative copy number and the relative expression level of each gene/transcript was calculated using Spearman’s test, with determination of correlation coefficients and associated probabilities (P). For the survival analysis and correlation analysis, Bonferroni-Holm’s method was used to control for multiple comparisons if necessary. Two-sided P < 0.05 was required for significance.

**Results**

**DNA Copy Number Aberrations Detected by CGH in OCCAs.** An overview of the chromosomal aberrations we detected among 20 primary tumors of OCCA is shown in Fig. 1 and Table 1. Seventeen of the 20 tumors (85%) showed detectable chromosomal imbalances. On average, 4.1 genetic changes were observed per tumor, including 2.5 gains (range, 0–8 gains) and 1.6 losses (range, 0–5 losses). Minimal common regions for the most frequent copy number gains were at 17q21-q24 (8 of 20, 40%), 20q13 (5 of 20, 25%), and 8q23-qter (5 of 20, 25%); the most common losses were observed at 9q22 (5 of 20, 25%) and 9q32-qter (5 of 20, 25%). HLGs indicative of gene amplification were detected at three different regions among the 20 cases: (a) 8q (2 cases); (b) 17q21-q23 (1 case); and (c) 5p13-qter (1 case).

**Relationship between DNA Copy Number Aberrations and Clinicopathological Parameters in OCCA.** The average number of chromosomal aberrations for primary OCCA tumors showed no significant difference between early tumors (stage I, mean = 3.85) and advanced tumors (stages II–IV, mean = 4.43; P = 0.7185) or between patients who are alive (mean = 3.36) and patients who had died of cancer (mean = 4.89; P = 0.3368; Fig. 2). These results suggested that the overall degree of genomic or chromosomal instability is not as important as DNA copy number changes at specific chromosomal regions in terms of involvement in development and/or progression of OCCA. Therefore, we evaluated the relationship between recurrent chromosomal abnormalities and clinicopathological features. As summarized in Table 2, the frequencies of chromosomal abnormalities involving gains in three regions (17q21-q24, 20q13, and 8q23-qter) and losses in two regions (9q22 and 9q32-qter) were compared among different subtypes of OCCA according to their clinicopathological features. Gains on 17q21-q24 and 20q13 occurred more frequently in tumors from older women (≥60 years; P = 0.0144 and 0.0320, respectively) and in patients with positive peritoneal cytology (P = 0.0014 and 0.0379, respectively). An association was also observed between poorer outcome and gain of DNA at 17q21-q24 or 20q13;
gains in those regions were more frequent in tumors from patients who had died of cancer ($P = 0.0045$ and $0.1273$ at respective sites) as well as in stage I tumors ($P = 0.0140$ and $0.0035$, respectively), although the association between gains on 20q13 and outcome of all patients did not reach statistical significance. On the other hand, gain at 8q23-qter or losses at 9q22 or 9q32-qter showed no significant correlation with any clinicopathological feature (Table 2). These results suggested that gains on 17q21-q24 and 20q13 might be significantly associated with a more malignant phenotype of OCCA, including invasiveness to the peritoneal cavity and poorer prognosis.

Fig. 3 shows Kaplan-Meier survival curves for all 20 OCCA patients (Fig. 3, A and B) and for the 13 cases of grade I OCCA (Fig. 3, C and D). Patients whose tumors showed...
17q21-q24 gains had significantly shorter disease-free and overall survival times than did patients without 17q21-q24 gains in their tumors (\(P = 0.0012\) and 0.0039, respectively; Fig. 3, A and B). The influence of 17q21-q24 gain on disease-free and overall survival time remained significant by the log-rank test (\(P = 0.0043\) and 0.0064, respectively; Fig. 3, C and D) when the outcomes of only stage I patients were stratified according to 17q21-q24 copy number status. Status of 20q13, on the other hand, did not reflect significant differences in disease-free and overall survival (\(P = 0.0708\) and 0.2624, respectively) or in overall survival between patients with or without 17q gain (data not shown). The log-rank tests for the Kaplan-Meier survival curves showed that patients with advanced tumors (stages II–IV), patients with higher performance status (scores 1–3), or patients with positive peritoneal cytology showed significantly shorter disease-free and overall survival times than did women with early tumors (stage I; \(P = 0.0044\) and 0.0040, respectively), patients with lower performance status (score 0; \(P = 0.0058\) and 0.0034, respectively), or patients with negative peritoneal cytology (\(P = 0.0078\) and 0.01149 respectively; data not shown).

Univariate analysis demonstrated that tumor stage, performance status, peritoneal-cytology status, and 17q21-q24 status all correlated with both disease-free survival and overall survival (Table 3). Multivariate analysis using a stepwise Cox proportional hazard regression procedure in which tumor stage, performance status, and peritoneal-cytology status were included with 17q21-q24 status revealed that 17q21-q24 status was identified as the only selective factor for disease-free survival in both forward and backward analyses (\(P = 0.0068\) for both). Performance status and 17q21-q24 amplification were identified as the independently selected predictive factors for overall survival in both forward and backward analyses (\(P = 0.0297\) and 0.0258, respectively). 17q21-q24 status remained statistically the most significant indicator with respect to disease-free survival of patients with OCCA (risk ratio, 8.93; 95% confidence interval, 1.83–43.48). In addition, 17q21-q24 status remained significant for overall survival when the data were stratified for multivariate analysis with respect to performance score, indicating that 17q21-q24 status is an independent predictor of overall survival of OCCA patients (risk ratio, 6.54; 95% confidence interval, 1.25–34.48).

**Evaluation of Candidate Target Genes for 17q21-q24 in OCCA.** We focused on the amplicon at 17q, especially 17q21-q24, because this region was most frequently amplified in our panel of tumors (Fig. 1) and because this abnormality seemed to correlate with poor prognosis in primary tumors of OCCA even in the earlier stages (Fig. 3; Tables 2 and 3), suggesting that dosage effects of genes located in this region might play important roles in the progression of OCCA. Gains of copy number or amplification of this region, as well as some potential amplification targets, had already been reported in breast and gastric cancers (28–33). These lines of evidence strongly suggested that 17q might harbor one or more genes whose gain in copy number renders them oncogenic in OCCA. To explore targets of the 17q gain observed in OCCAs, we first analyzed expression of transcripts located at 17q21-q24 in the 11 tumors from which high-quality RNA was available. We based this strategy on the commonly accepted criterion that putative targets for amplification are overexpressed in consequence (28–34). Listed in Table 4 are 15 transcripts (13 known genes and 2 uncharacterized transcripts) that we selected as...
positional and functional candidates on the basis of published work involving breast and gastric cancers (28–33) and on positional information from the genome databases archived by the University of California at Santa Cruz4 and the National Center for Biotechnology Information.5

Among the 15 transcripts we examined, only PPM1D and APPBP2 were differentially expressed between tumors with and without 17q21-q24 gain, with statistical significance (P < 0.05, adjusted for multiple comparisons; Table 4). Relative expression levels of these two transcripts were significantly correlated with genomic DNA copy number ratios determined by real-time quantitative PCR and were clearly higher in the five tumors having ≥2.0 copy number ratio of those genes and gain at 17q21-q24 than in the six tumors averaging <1.5 copy number ratio and normal 17q21-q24 status (Fig. 4A). For both genes, Kaplan-Meier analysis revealed a significantly negative influence of their heightened expression on disease-free survival (P = 0.0090, adjusted for multiple comparisons by Bonferroni-Holm’s method; Fig. 4B) and a marginal significance on overall survival (adjusted P = 0.0684; Fig. 4B). When the outcomes of only stage I patients were stratified according to expression status of PPM1D and APPBP2, the influence of their heightened expression on disease-free and overall survival time was significant by the log-rank test (adjusted P = 0.0164 and 0.0386, respectively; data not shown), even though only 7 cases were analyzed. Taken together, our results, albeit preliminary due to the limited number of cases for analysis, suggest that PPM1D and/or APPBP2 may be targets for 17q gain in OCA tumors and might participate in the progression of this disease.

Because wild-type p53 is considered a possible target for the oncogenic function of overexpressed PPM1D through inactivation of the p38 mitogen-activated protein kinase (35), we examined the TP53 gene for mutations in our series of OCA tumors. Although mutations in exons 5–8 of TP53 have been observed in ovarian cancers of other types (36, 37), we detected none in our panel of OCA tumors.

Discussion

Biological and clinical characteristics of individual tumors can be evaluated by a comprehensive CGH analysis of abnormalities in genomic DNA copy numbers in tumors (38–40). Profiles of such genomic anomalies yield information for identifying the locations of markers associated with aggressive behavior and poor prognosis or markers useful for improving diagnosis and treatment of specific tumors (38–40). However, such analyses are most fruitful when large numbers of tumors are available for a specific clinical and/or pathological category. Because it is difficult to obtain less common tumors such as OCA in large numbers, to date copy number aberrations associated with various phenotypes of OCA have been poorly understood, although possible nonrandom aberrations have been reported (17, 18). In the present study, we analyzed 20 cases of OCA by CGH and identified several regions that were frequently altered in this type of tumor. Among them, moreover, by means of a multivariate analysis using a stepwise Cox proportional hazard regression procedure, we identified gain at 17q21-q24 as the most powerful and independent genomic marker to be associated with poor prognosis of OCA. Although the number

4 http://genome.ucsc.edu/
observed no significant differences in the average number of tumor, probably reflecting the inherent genetic instability of prognosis of OCCA even in earlier stages of the disease. 17q21-q24 gain may be a novel and useful predictor for poor highly detailed statistical analysis, our results do suggest that of cases we used in this study was still too small to achieve a statistically significant value (adjusted expression level.

Table 4 Analysis of expression levels of candidate targets of the 17q21–q24 gain, as determined by quantitative real-time RT-PCR experiments

<table>
<thead>
<tr>
<th>Genes/transcript</th>
<th>Locus ID</th>
<th>Location</th>
<th>17q21–q24 gain (–)</th>
<th>17q21–q24 gain (+)</th>
<th>Adjusted P</th>
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<tr>
<td>ERBB2</td>
<td>2064</td>
<td>17q21.1</td>
<td>1.2 ± 0.4</td>
<td>2.4 ± 0.8</td>
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<td>PSMD3</td>
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<td>17q21.1</td>
<td>30.3 ± 3.3</td>
<td>68.5 ± 16.6</td>
<td>0.4091</td>
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<tr>
<td>KIAA0130</td>
<td>9862</td>
<td>17q21.1</td>
<td>32.0 ± 5.7</td>
<td>58.3 ± 21.8</td>
<td>0.8423</td>
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<tr>
<td>TOP2A</td>
<td>7153</td>
<td>17q21.2</td>
<td>38.7 ± 17.9</td>
<td>70.2 ± 40.8</td>
<td>0.8638</td>
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<td>CCR7</td>
<td>1236</td>
<td>17q21.2</td>
<td>3.5 ± 1.1</td>
<td>7.2 ± 3.2</td>
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<td>HBOAr</td>
<td>11143</td>
<td>17q21.33</td>
<td>4.7 ± 0.7</td>
<td>9.9 ± 2.5</td>
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<td>PRO1855c</td>
<td>55379</td>
<td>17q21.33</td>
<td>32.2 ± 8.5</td>
<td>93.4 ± 37.7</td>
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<td>ZNF147</td>
<td>7706</td>
<td>17q22</td>
<td>1.7 ± 0.3</td>
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<tr>
<td>VMPTr</td>
<td>81671</td>
<td>17q23.1</td>
<td>25.3 ± 7.2</td>
<td>56.0 ± 9.7</td>
<td>0.2232</td>
</tr>
<tr>
<td>RP56KB1</td>
<td>6198</td>
<td>17q23.1</td>
<td>38.9 ± 6.9</td>
<td>56.4 ± 18.1</td>
<td>0.9548</td>
</tr>
<tr>
<td>APPBP2r</td>
<td>10513</td>
<td>17q23.2</td>
<td>26.4 ± 6.9</td>
<td>66.7 ± 8.9</td>
<td>0.0475</td>
</tr>
<tr>
<td>PPM1D0c</td>
<td>8493</td>
<td>17q23.2</td>
<td>21.4 ± 6.7</td>
<td>105.0 ± 33.3</td>
<td>0.0475</td>
</tr>
<tr>
<td>TBX2</td>
<td>6909</td>
<td>17q23.2</td>
<td>4.5 ± 1.3</td>
<td>7.3 ± 3.3</td>
<td>0.9548</td>
</tr>
</tbody>
</table>

a Gene symbol and locus ID number of each gene are from the LocusLink (http://www.ncbi.nlm.nih.gov/LocusLink).

* Location of each gene was compiled from information archived by the University of California at Santa Cruz and the National Center for Biotechnology Information.

* Value of mRNA expression of each gene in each sample was normalized with that of GAPDH in the same sample and reported as a relative expression level.

* 17q21–q24 (+) versus (–). Ps were derived from exact nonparametric testing using ranks, adjusted for multiple comparisons by the permutational minP tests of Westfall and Young. Statistically significant values (adjusted P < 0.05) are in bold.

* Brackets indicate genes or transcript showing statistically significant overexpression in tumors with gains of DNA at 17q21–q24, compared with tumors showing no gains in that region.

of cases we used in this study was still too small to achieve a highly detailed statistical analysis, our results do suggest that 17q21–q24 gain may be a novel and useful predictor for poor prognosis of OCCA even in earlier stages of the disease. In general, the average number of sequence copy changes in cells of a given tumor parallels the aggressiveness of that tumor, probably reflecting the inherent genetic instability of malignant neoplasms (13, 40). In the present study, however, we observed no significant differences in the average number of copy alterations either between earlier (stage I) and advanced (stages II-IV) OCCA tumors or between tumors of patients who are alive and tumors of patients who had died of OCCA. On the other hand, copy number changes in specific chromosomal regions, specifically, gain of DNA at 17q21–q24, correlated significantly with patient outcome in our panel. One explanation for the disparity between other results (13, 40) and ours may be that certain malignancy-related oncogenes at 17q might be activated by amplification at an early stage of OCCA and thereby...
determine poor outcomes for patients whose tumors show gains in the region. As shown in Fig. 3, C and D, stage I patients with 17q21-q24 gain indeed exhibited poorer disease-free and overall survival than did those without 17q21-q24 gain.

In view of the strong correlation we found between 17q21-q24 gain and poor prognosis of OCCA, we speculated that this chromosomal abnormality might indicate the location of genes involved in cellular functions associated with the aggressive phenotype of this tumor. However, because the commonly gained region is large and relatively gene-rich, instead of screening all transcripts within the region we used a candidate gene approach to identify putative targets in the 17q21-q24 amplicon. Parts of 17q, especially 17q21 and q23, are of particular interest because amplification of these regions can be observed in a variety of cancers including breast and gastric carcinomas (28–33). With regard to those cancers, several outstanding candidate targets emerged from a survey of the literature (Table 4). Those genes had been identified by comparing expression levels with gene amplification and/or by functional characterization (28–33). Here we demonstrated a clear correlation between expression levels determined by quantitative real-time RT-PCR and 17q21-q24 status determined by CGH in only 2 of the 15 genes we tested. Moreover, we identified these two genes, PPM1D and APPBP2, as potential targets for 17q21-q24 gain because their expression not only was elevated through copy number gain but was likely to be correlated with prognosis even among the relatively small number (11 cases) of our OCCA tumors from which high quality RNA was available. Because of the limited number of cases available for the present analysis, further examination using larger sets of OCCA tumors will be necessary to confirm this result.

PPM1D (also known as WIP1) encodes a serine/threonine protein phosphatase. Its expression is induced in response to ionizing radiation in a p53-dependent manner (41). The oncogenic properties of overexpressed PPM1D have been demonstrated elsewhere (35, 42). For example, Li et al. (42) showed that overexpression of PPM1D confers two oncogenic phenotypes on cells in culture: attenuation of the apoptotic process induced by serum starvation; and transformation of wild-type p53-containing cells in cooperation with RAS. Overexpressed PPM1D inactivates p38 mitogen-activated protein kinase, reduces wild-type p53 phosphorylation at Ser33 and Ser46, abrogates RAS-induced apoptosis, and rescues cells from cell cycle arrest, promoting cell transformation in vitro (35). Bulavin et al. (35) also demonstrated that overexpressed PPM1D can expedite tumor formation in vivo after injection of RAS/E1A-transformed mouse embryonic fibroblasts into nude mice. Unlike other ovarian cancers, OCCAs rarely carry mutations in TP53 (36, 37). In addition, Li et al. (42) demonstrated the positive correlation among the genetic copy number, mRNA expression level, and protein expression level of PPM1D, indicating that increased copy number of PPM1D leads to its overexpression in both mRNA and protein levels. Together with these functional data, our molecular genetic findings strongly suggest that PPM1D, when activated by 17q gain, might contribute to the malignant progression of OCCA by inactivating wild-type p53.

The APPBP2 gene (also known as PAT1) encodes a microtubule-binding protein that mediates targeting of intracellular proteins such as the translocation amyloid precursor (43); thus far, its product has never been functionally linked to any cancers. Li et al. (42) did report that APPBP2 was amplified and overexpressed in some breast cancers but probably only as a
secondary consequence of its nearness (<100 kb) to PPM1D, because its overexpression did not induce an oncogenic phenotype. However, it is still possible that APPBP2 can play a functional role in the pathogenesis of OCCA because it might act synergistically with other amplified genes, including PPM1D, within 17q21-q24.

Among the 15 candidate genes we tested, RPS6KB1 is considered an outstanding candidate as a target of 17q23 amplification in breast cancers (44, 45). In our study, however, we detected no significant degree of overexpression of this gene by nonparametric statistical analysis. Even in breast cancers, Wu et al. (28) had demonstrated that amplification of RPS6KB1 often failed to result in its overexpression, which suggests that amplified genes need not necessarily be transcriptionally active. However, because activation of RPS6KB1 may occur in response to mechanisms other than amplification, it cannot at this point be eliminated as a candidate oncogene in OCCA.

Genes and/or uncharacterized transcripts within 17q21-q24 other than the 15 genes we tested might be targets of amplification in OCCA. Because the 17q21-q24 region is relatively gene-rich, and our selection of candidates was based on the results of studies using breast and gastric cancers (28–33), genes specific to ovarian cancer in general or to OCCA in particular might have been overlooked in our analysis. Genes that were differentially expressed in OCCA compared with other ovarian cancers were recently screened using a microarray technique (46). In that study, among the genes up-regulated in OCCA, several genes, including ERBB2, TOB1, and VATI, lay within 17q21-q24; however, their differential expression was not validated by RT-PCR experiments. Among the three genes mentioned, only ERBB2 is known to have oncogenic properties (47), but our results excluded ERBB2 as a target for 17q21-q24 gain in our panel of OCCA tumors. Although we identified PPM1D and APPBP2 as possible target genes for 17q21-q24 amplification in the present study, we are attempting to analyze a larger set of candidate genes that might affect the prognosis of OCCA patients and to evaluate the possibility that multiple genes other than PPM1D and APPBP2 may play important roles in OCCA carcinogenesis, malignant progression, and resistance to therapy.

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References


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Association of 17q21-q24 Gain in Ovarian Clear Cell Adenocarcinomas with Poor Prognosis and Identification of PPM1D and APPBP2 as Likely Amplification Targets

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