AIB1 Polymorphisms Predict Aggressive Ovarian Cancer Phenotype

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Abstract

Purpose: The androgen receptor (AR) harbors a polymorphic CAG repeat sequence in exon 1, coding for a polyglutamine tract whose length inversely correlates with AR transactivation function. AIB1, an AR coactivator, expresses a similar polymorphic glutamine sequence within the carboxyl-terminal coding region. We hypothesized that genotypic variations in the androgen-signaling pathway correlate with estrogen-dependent transcription (5, 10). The AIB1 gene also harbors a glutamine tract encoded by a polymorphic CAG/CAA repeat sequence, although the biological function of these repeats is not established (5, 11). However, the analogous region of the steroid receptor coactivator SRC-1 harbors a polymorphic CAG trinucleotide repeat region coding for a polyglutamine tract, whose length inversely correlates with AR transactivation function. AIB1, an AR coactivator, expresses a similar polymorphic glutamine sequence within the carboxyl-terminal coding region. We hypothesized that genotypic variations in the androgen-signaling pathway correlate with AR transactivation function. AIB1, an AR coactivator, also mediates AR transactivation function in tumor biology (1-3). Ovarian cancers also overexpress AIB1 and ARA70 (AR-associated protein), coactivators that may enhance the transactivational potential of AR >10-fold (4-6). Androgens may also promote the progression and recurrence of disease through modulation of tumor growth factor-β receptor expression, which might disrupt normal tumor growth factor-β-mediated growth inhibition (7).

Androgen signaling is mediated through androgen binding to AR. The AR gene harbors a polymorphic CAG trinucleotide repeat region coding for a polyglutamine tract, and the length of the CAG repeat sequence has been shown to inversely correlate with AR transcriptional activity (8, 9). Steroid receptor coactivators, including AIB1, also mediate AR transactivation function. AIB1, also known as SRC-3, belongs to the SRC family of transcriptional coactivators involved in the control of estrogen-dependent transcription (5, 10). The AIB1 gene also harbors a glutamine tract encoded by a polymorphic CAG/CAA repeat sequence, although the biological function of the repeats is not established (5, 11). However, the analogous region of the steroid receptor coactivator SRC-1 interacts with AR to enhance signaling, and rare CAA/CAG sequence patterns have been reported in a significantly higher proportion of breast cancer cell lines and primary tumors (12, 13).

We previously reported that polymorphisms of the CAG repeat sequence in AR modulate tumor biology in epithelial ovarian cancers. Short AR alleles (with <19 CAG repeat lengths) are associated with decreased surgical cytoreducibility (at initial exploratory laparotomy) and poor overall survival (3). To further explore our hypothesis that enhancement of androgen signaling promotes aggressive epithelial ovarian cancer biology, we sought to examine the glutamine polymorphism in AIB1. The objectives of this study were to characterize AIB1 genotypes in a cohort of women with epithelial ovarian carcinoma and to determine the influence of AIB1 on clinical outcome.

Experimental Design

Under an Institutional Review Board–approved protocol, the Gynecologic Oncology Laboratory at Cedars-Sinai Medical Center routinely collects malignant and benign tissue specimens from consenting women undergoing surgical exploration. One hundred and forty-two patients were diagnosed with stages II and IV papillary serous epithelial ovarian carcinoma between 1995 and 2000 at our institution; we queried our database to identify consecutive patients who had available banked serum from their initial cytoreductive surgery. Patients with tumors of low malignant potential were excluded from this study. All patients had undergone primary surgical staging by a gynecologic oncologist with the intent of optimal tumor cytoreduction (defined as residual disease after surgical resection to <1 cm). Patients who received neoadjuvant chemotherapy and interval...
surgical cytoreduction were excluded. Following surgical staging, all patients received platinum-based chemotherapy. Patient data were abstracted from medical records, and included surgical and pathologic findings, time to recurrence, and death.

Genomic DNA was isolated from banked serum using standard procedures (14). Genotype analysis was done with PCR amplification of the polymorphic CAG/CAA trinucleotide region beginning at residue 3930 in the AIB1 coding sequence. Amplified products encompassed a (CAG)₉ CAA CAG (CAG)₂ CAA sequence, corresponding to an allele with 29 glutamine repeats as previously reported (15, 16). The primers used were: 5'-AGT-CAC ATT ACG AGG TGG GC-3' (forward) and 5'-TTC CGA CAA CAG AGG GTG G-3' (reverse) as published by Rebbeck et al. (17). Primers were labeled with fluorescein aminomethane to determine sequence length using laser-activated fluorescent dye technology (ABI 377 PRISM and associated software; Applied Biosystems, San Mateo, CA). Representative PCR products were independently sequenced to confirm the number of CAG/CAA repeat lengths and product identity. Unlike the CAG repeat expansion in neurodegenerative diseases, the polyglutamine length in AIB1 remains relatively stable, consistent with published reports implicating AIB1 as one of the major modifiers of breast and prostate cancer risk (18-20). Using the log-rank test with a two-sided significance level of 5% and a calculated power of 80%, 88 patients would be needed to perform the study with the 28/30 and 29/29 genotype (n = 12); level three included those with the 28/29 genotype (n = 38), and level four included those with the 28/30 and 29/29 genotype (n = 15). Kaplan-Meier analysis identified a statistically significant trend for overall survival favoring the longer AIB1 genotypes (P = 0.03; Fig. 4). Median survival was 63.0 months for patients in level one and 33.0 months for those in level two; median survival was not yet reached for those in levels three and four. Comparison of survival analyses examining the cohort as two versus four levels using the Akaike Information Criteria indicated a superior model fit when examining genotype length as a dichotomous variable.

**Results**

Eighty-nine patients with epithelial ovarian carcinoma underwent genotype analysis of the polymorphic CAG repeat sequence in AIB1. The number of glutamine codons ranged from 26 to 30; the alleles in this cohort contained 26, 28, 29, and 30 CAG/CAA repeats. The genotypes identified included 26/26, 26/28, 26/29, 28/28, 28/29, 28/30, and 29/29. The distribution and frequency of AIB1 genotypes is shown in Fig. 1. Following a cutoff of 28 CAG/CAA repeats established by other investigators in breast and prostate cancer, we defined a short AIB1 genotype as ≤28 glutamine codons in each allele, and a long AIB1 genotype as ≥29 glutamine codons in at least one allele. Clinicopathologic characteristics of established prognostic factors in epithelial ovarian carcinomas for the short and long groups are shown in Table 1. No differences were seen between ages of diagnosis, incidence of stage II, III, and IV disease, high-grade histology, or incidence of optimal surgical tumor resection.

To determine the potential influence of AIB1 genotype length on disease course, we performed Kaplan-Meier survival analyses comparing patients with short and long AIB1 genotypes. Patients with a short AIB1 had a statistically shorter time to disease recurrence compared with those with a long genotype (15.0 versus 30.0 months; P = 0.01; Fig. 2). A short AIB1 genotype also correlated with decreased overall survival (57.0 months) compared to those with a long genotype (median survival not yet reached; P = 0.02; Fig. 3).

Although other investigators support the clinical significance of this cutoff of 28 glutamine codons in AIB1, the division of the cohort into two groups remains somewhat arbitrary. We thus explored the effect of AIB1 genotype length as a semicontinuous variable using four levels. Level one included patients harboring the 26/26, 26/28, and 26/29 genotypes (n = 24); level two included those with the 28/28 genotype (n = 12); level three included those with the 28/29 genotype (n = 38), and level four included those with the 28/30 and 29/29 genotype (n = 15). Kaplan-Meier analysis identified a statistically significant trend for overall survival favoring the longer AIB1 genotypes (P = 0.03; Fig. 4). Median survival was 63.0 months for patients in level one and 33.0 months for those in level two; median survival was not yet reached for those in levels three and four. Comparison of survival analyses examining the cohort as two versus four levels using the Akaike Information Criteria indicated a superior model fit when examining genotype length as a dichotomous variable.
To ascertain the effect of \( AIB1 \) genotype length on overall survival in the context of established prognostic factors, multivariate analysis was done using the Cox regression hazards model (Table 2). After controlling for age, stage, grade, and optimal cytoreduction, the presence of an \( AIB1 \) allele harboring 26 glutamine codons remained an independent poor prognostic factor for overall survival (hazard ratio, 1.28; \( P = 0.05 \)). Whereas age and grade were not significant prognosticators in this cohort, stage (hazard ratio, 2.09; \( P = 0.02 \)) and residual disease >1 cm after surgical resection (hazard ratio, 2.57; \( P = 0.04 \)) also retained prognostic significance.

**Table 2. Multivariate Cox proportional hazards analysis of prognostic factors on overall survival**

<table>
<thead>
<tr>
<th><strong>Hazard ratio (95% confidence interval)</strong></th>
<th><strong>( P )</strong></th>
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<tbody>
<tr>
<td>Short ( AIB1 )</td>
<td>1.28 (1.01-1.66)</td>
</tr>
<tr>
<td>Age</td>
<td>1.00 (0.98-1.03)</td>
</tr>
<tr>
<td>Stage</td>
<td>2.09 (1.14-3.84)</td>
</tr>
<tr>
<td>Grade</td>
<td>2.42 (0.81-7.20)</td>
</tr>
<tr>
<td>Suboptimal cytoreduction</td>
<td>2.57 (1.06-0.22)</td>
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**Discussion**

We have hypothesized that enhanced androgen signaling promotes aggressive epithelial ovarian cancer biology. To test this hypothesis, we studied the polymorphic glutamine codon repeat sequence in the AR coactivator \( AIB1 \). Although the biological significance of this repeat in \( AIB1 \) is not established, we hypothesized that a functional relationship exists between length and activity, similar to that shown in AR. We identified a significant association between short \( AIB1 \) genotype and decreased time to recurrence and overall survival, a finding confirmed in analyses examining \( AIB1 \) length both as a dichotomous and semicontinuous variable. Furthermore, multivariate analyses confirmed the prognostic significance of a short \( AIB1 \) genotype in predicting overall survival, after controlling for established prognostic factors.

Limited studies have examined \( AIB1 \) in epithelial ovarian cancers; however, existing data suggests the potential role of \( AIB1 \) genotype in predicting overall survival, after control-

![Figure 4. Association of \( AIB1 \) genotype length with overall survival when examined as a semiquantitative variable. Four levels were examined: level one included patients harboring the 26/26, 26/28, and 26/29 genotypes (n = 24); level two included those with the 28/28 genotype (n = 12); level three included those with the 28/29 genotype (n = 38); and level four included those with the 28/30 and 29/29 (n = 15) genotypes. A statistically significant trend was identified for overall survival, favoring the longer \( AIB1 \) genotypes (\( P = 0.03 \)).](image)

Despite the lack of molecular evidence correlating \( AIB1 \) genotype length with \( AIB1 \) functional activity, data identifying the influence of \( AIB1 \) glutamine codon length on risk of carcinogenesis in other hormone-responsive organ sites suggests an inverse relationship between polyglutamine length and \( AIB1 \) transactivation function similar to that seen in AR. Hsing et al. reported that men with homozygous \( AIB1 \) alleles of 28 glutamine codons or less showed an 81% excess risk of prostate cancer (20). In the breast, however, short \( AIB1 \) genotype seems to reduce risk; data from Rebbeck et al. and Kadouri et al. revealed a risk reduction of breast carcinogenesis in women with \( BRCA \) mutations who harbor \( AIB1 \) alleles of <28 polyglutamine repeats (17, 19). Interestingly, these same relationships have been reported for the AR polyglutamine polymorphism; short \( AR \) alleles are associated with increased risk of prostate cancer, and with decreased risk of both sporadic and \( BRCA \)-associated breast cancers (25-27). Taken together, these data strongly suggest a functional role for polyglutamine length in \( AIB1 \) similar to that found in \( AR \).

These data add to the growing body of evidence linking heightened androgenicity to the pathogenesis and tumor biology of epithelial ovarian cancers. Our findings suggest genetic polymorphisms in \( AIB1 \) influence disease outcome, potentially through differential activation of \( AR \) and enhanced androgen signaling. Functional studies confirming an inverse relationship between \( AIB1 \) polyglutamine length and \( AR \) activity may identify specific mechanisms by which androgens function in ovarian cancer biology. Multi-institutional cohorts examining both \( AIB1 \) and \( AR \) genotype are also under way to further examine the influence of these factors on ovarian cancer biology.
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References

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