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Amplification of the ch19p13.2 NACC1 locus in ovarian high-grade serous carcinoma

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On the basis of digital karyotyping, we have identified a new, discrete amplified region at ch19p13.2 in a high-grade ovarian serous carcinoma. To further characterize this region, we determined the frequency and biological significance of ch19p13.2 amplification by analyzing 341 high-grade serous carcinomas from The Cancer Genome Atlas (TCGA) and found an increased DNA copy number at this locus in 18% of cases. We correlated the DNA and RNA copy number by analyzing the TCGA data set for all amplified genes and detected seven genes within ch19p13.2 that were significantly correlated ($R \geq 0.54$) and were, in fact, listed as the top 100 potential 'driver' genes at a genome-wide scale. Interestingly, one of the seven genes, *NACC1*, encoding NAC1 was previously reported to be involved in the development of tumor recurrence in ovarian serous carcinoma and to have a causal role in the development of paclitaxel resistance. Therefore, we selected *NACC1* for validation in an independent cohort. On the basis of fluorescence *in situ* hybridization, we found that 35 (20%) of 175 high-grade serous carcinomas had an increased DNA copy number at the *NACC1* locus, and those amplified cases were associated with early disease recurrence within 6 months ($P = 0.013$). A significantly high level of NAC1 protein expression based on immunohistochemistry was detected in amplified tumors as compared with non-amplified tumors ($P < 0.005$). In summary, our data suggest that amplification at the ch19p13.2 *NACC1* locus, leading to NAC1 overexpression, is one of the molecular genetic alterations associated with early tumor recurrence in ovarian cancer.
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Epithelial ovarian cancer is the most aggressive gynecologic malignancy. Ovarian cancer is composed of a diverse group of tumors that can be classified according to their distinctive morphologic and molecular features.[1] Among them, high-grade serous carcinoma represents the major tumor type associated with frequent tumor recurrence and high mortality. In contrast to other subtypes, high-grade serous carcinomas are highly aggressive, evolve rapidly, and almost always present at advanced stage. Several studies have analyzed global DNA copy number alterations specifically in different types of ovarian epithelial carcinomas.[2–8] The results from these reports indicate that high-grade serous carcinomas are characterized by higher levels of subchromosomal gains and losses than clear cell carcinoma, low-grade serous carcinomas, and their precursor lesions, serous borderline tumors. This finding suggests that chromosomal instability is more pronounced in high-grade serous carcinomas than other types of ovarian cancer.

To identify new cancer-associated genes that may participate in the pathogenesis of ovarian high-grade serous carcinoma, we have previously applied digital karyotyping[9] and, subsequently, SNP arrays to analyze somatic genome-wide DNA copy number alterations in purified ovarian high-grade serous carcinoma samples.[2,4,10] As a result, in addition to several previously known amplified chromosomal regions containing *CCNE1*, *AKT2*, and *PIK3CA* loci, we identified several new amplified loci including chromosome (ch)11q13.5 harboring *Rsf-1*

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(*HBXAP*),[11] chr19p13.12 harboring *NOTCH3*,[12] and regions at chr12p13, chr8q24, and chr19p13.2. Those regions containing known tumor-associated genes were validated using dual-color fluorescence *in situ* hybridization (FISH) in an independent set of ovarian carcinomas.[4] The purpose of this report is to study a previously uncharacterized amplified region at ch19p13.2. On the basis of digital karyotypic analysis in a limited set of clinical samples, we detected a discrete amplicon located at ch19p13.2, which has not been previously reported in ovarian cancer. This amplicon appears to be relatively large, encompassing 1.92 Mb and containing at least 60 coding sequences, which poses challenges for further investigation to identify the cancer 'driver' gene(s) within this amplified region. Several genome database resources such as The Cancer Genome Atlas (TCGA) have recently become available for public access, providing a hitherto unavailable opportunity for molecular genetic discovery in cancer. In this study, we take the advantage of the emerging data set of the TCGA to simultaneously analyze mRNA and genomic DNA copy numbers for all genes within the digital karyotyping-defined ch9p13.2 amplicon in a large set of high-grade serous carcinomas. This approach enabled us to distinguish cancer 'driver' genes from co-amplified 'passenger' genes. We then validated our results using FISH and immunohistochemistry in an independent set of ovarian carcinomas.

Materials and methods

Analysis of TCGA Database

Copy number variations of the ch9p13.2 amplicon in 343 ovarian tumor samples and paired normal samples were characterized using the Affymetrix SNP6.0 array by the Broad Institute (Boston, MA, USA). The intensity and $\log_2$ ratios for each probe set on the SNP array were downloaded from TCGA Data Portal (http://tcga-data.nci.nih.gov/tcga/). Somatic copy number alterations were analyzed using the Circular Binary Segmentation algorithm[13] with R package 'DNACopy' (version 1.14.0, default setting). Regions with focal somatic copy number alterations were identified by GISTIC analysis in GenePattern (http://www.broadinstitute.org/cancer/software/genepattern/).[14] Expression analysis of 377 ovarian tumor samples was performed using the Affymetrix Human Exon 1.0 ST Array by the Lawrence Berkeley Laboratory. The CEL files were downloaded from the TCGA Data Portal. The probe set signals were summarized as RMA scores with Affymetrix Power Tools (http://www.affymetrix.com/partners_programs/programs/developer/tools/powertools.affx). The locations of all probe sets were obtained from the Affymetrix annotation file (HuEx-1_0-st-v2.na29.hg18.probeset.csv). A total of 8101 core probe sets (representing 590 genes) located at the regions with focal somatic copy number alterations were selected for expression-copy number correlation analysis. Copy number data and exon-array data were available for 341 cases; therefore, this sample set was used for analysis in this study. For these samples, Pearson correlation coefficients were calculated using the expression of each probe set and its corresponding copy number across the 341 cases. The distribution of correlation coefficients of all 8101 probe sets is bimodal, separating the genes (probe sets) with no correlation (correlation coefficient below 0.2) from those that show correlations between DNA copy number and RNA expression.

Fluorescence *In Situ* Hybridization

A total of 175 ovarian high-grade serous carcinomas were analyzed for DNA copy number within the ch19p13.2 region using two-color FISH. Stage III or IV tumor tissues from patients treated at the Johns Hopkins Hospital were originally retrieved from the Department of Pathology at the Johns Hopkins Hospital. The acquisition of the anonymous tissue specimens for this study was approved by the Johns Hopkins Institutional Review Board. The tissues were arranged in tissue microarrays to facilitate FISH and immunohistochemistry procedures. BAC clones (RP11-356L15 and CTD-2508D10) containing the genomic sequences of the ch19p13.2 amplicon were purchased from Bacpac Resources (Childrens' Hospital, Oakland, CA, USA) and Invitrogen (Carlsbad, CA, USA). Bac clones located at ch19p12 (CTD-2518O18) were used to generate reference probes. The method for FISH was previously described.[15] The hybridization signals were counted by two individuals. Signal ratios of experimental probe/reference probe >2.5 were considered as gain, and signal ratios of experimental probe/reference >3.5 were considered as high-fold amplification. At least 50 nuclei were counted for each specimen.

Immunohistochemistry

The NAC1 mouse monoclonal antibody used for immunohistochemistry was purchased from Novus Biologicals (Littleton, CO, USA). The specificity of the antibody was previous demonstrated.[16] Paraffin sections from the same tissue microarrays as used for FISH were deparaffinized and pretreated with low pH citrate buffer in a microwave oven for antigen retrieval. Tissue sections were incubated with the anti-NAC1 antibody at a dilution of 1:100 at 4 °C overnight. Visualization was performed using the EnVision™+ peroxidase system (DakoCytomation, Glostrup, Denmark). Positive controls consisted of an ovarian carcinoma shown to be positive in a pilot study. Negative controls were stained with an isotype-matched mouse myeloma protein. Immunoreactivity was scored by two investigators who were blinded to the patient clinical data. Nuclear localization was interpreted as

positive staining. Staining intensity was scored on a scale of 0–3, corresponding to undetectable, weak, moderate, and intense immunoreactivity in tumor cells.[16] At least 500 tumor cells were counted for each specimen.

Results

As a continuing effort to understand the molecular etiology of ovarian cancer, we have previously analyzed DNA copy number changes in different types of ovarian carcinoma. On the basis of digital karyotyping[9] in affinity-purified, high-grade ovarian serous carcinomas, we detected a discrete, novel amplified region located at ch19p13.2 in one of seven specimens (Figure 1a). This amplified locus was estimated to have ~17 copies of haploid genome and spanned 12 735 244–14 655 263, containing at least 60 genes. On the basis of this preliminary finding, we decided to determine the frequency of increased copy number at this region in a large set of high-grade serous carcinomas. First, we analyzed DNA copy number in 341 high-grade serous carcinomas from the TCGA ovarian cancer genome data set. The results indicated that 18% of 341 high-grade serous carcinomas showed increased DNA copy numbers (>2.5) based on SNP arrays. If a higher cutoff value (>3.5) was applied, the fraction of amplified cases was 3%. To identify potential cancer 'driver' genes within the ch19p13.2 amplicon, we correlated mRNA expression levels and DNA copy number at a genome-wide scale in the same set of carcinomas. The rationale for this analysis is that, when amplified, 'driver' genes almost always upregulate their expression, which enables their oncogenic functions. On the basis of this analysis, we listed the top 100 amplified genes with the highest correlation coefficient between DNA copy number and mRNA expression levels (as determined by Pearson correlation analysis (Table 1)). Using this approach, we identified several amplicons harboring well-known amplified oncogenes, including *CCNE1* (Farley *et al*[17]), *AKT2* (Cheng *et al*[18]), *Pak1* (Schraml *et al*[5]), *Rsf-1* (Choi *et al*[19]), and *Paf1* (Chaudhary *et al*[20]). Interestingly, chromosome 19 contained a very high number of candidate driver oncogenes. Among the top 100 genes, 54 genes were located at discrete subchromosomal regions of chromosome 19, indicating that frequent structural re-arrangement may occur in this chromosome in high-grade ovarian serous carcinoma (Figure 1b). Importantly, the ch19p13.2 region harbored seven genes, which showed the most significant correlation between DNA copy number and RNA expression level.

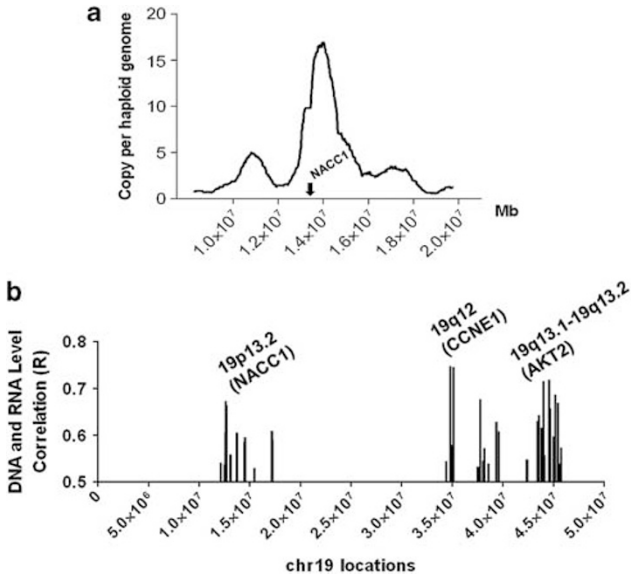

Figure 1 Amplification at the ch19p13.2 region harboring *NACC1*. (**a**) A focused view of the digital karyotyping result demonstrates a discrete amplicon spanning from nucleotide position 12 735 244 to 14 655 263 in a high-grade serous carcinoma. *NACC1* is located within the amplicon. (**b**) Analysis of the TCGA ovarian cancer data set reveals 100 top genes showing the highest correlation coefficient between DNA copy number and RNA expression levels. Among them, 54 genes are located on chromosome 19. Their physical map is shown. Of note, three amplified chromosomal regions, 19p13.2, 19q12, and 19q13.1-19q13.2 harbor *NACC1*, *CCNE1*, and *AKT2*, respectively.

To validate the above results obtained from *in silica* analysis, we applied two-color FISH and immunohistochemistry to an independent set of tumors composed of 175 high-grade serous carcinomas collected at our institution. We focused on one of the seven candidate 'driver' genes at ch19p13.2, *NACC1*, because as compared with the other six genes, the biological role of *NACC1* has been previously reported. NAC1, encoded by *NACC1*, is a nuclear protein involved in stemness of embryonic stem cells[21] and in the pathogenesis of human cancer.[16] NAC1 has been demonstrated to participate in the development of chemoresistant recurrent tumors.[22,23] In this study, we further determined if amplification of the *NACC1* locus was related to early disease recurrence in high-grade ovarian serous carcinomas. We designed FISH probes that hybridized to the *NACC1* coding region and found that 35 (20%) of 175 carcinomas demonstrated gene copy number gain (>2.5) at ch19p13.2. Furthermore, 8 (5%) of 175 tumors exhibited high level amplification (>3.5) in DNA copy number at this locus. An example of FISH in a high-grade serous carcinoma is shown in Figure 2. Immunohistochemistry was used to estimate semi-quantitatively the protein expression levels of NAC1 based on immunostaining intensity in the same set of 175 tumor tissues. We observed that amplified tumors exhibited a significantly higher level of NAC1 expression than those without amplification ($P<0.005$, Fisher's exact test). As shown in Table 2, the percentage of tumors with an immunostaining score of 3+ was 63, 26, and 13% in specimens showing high amplification, low level gain, and no amplification

Table 1 The top 100 amplified potential 'driver' genes in ovarian high-grade serous carcinomas[a]

Gene	*Chr*	*Start*	*End*	*correlation*	*fCN>2.5*	*fCN>3.5*
MRPS15	chr1	36693996	36702545	0.54	0.157	0.017
NDUFS5	chr1	39264593	39272842	0.548	0.190	0.029
NFYC	chr1	40950961	41009843	0.555	0.201	0.029
CTPS	chr1	41221558	41250797	0.561	0.201	0.026
ANKRD17	chr4	74159477	74262083	0.544	0.055	0.020
TAF2	chr8	120812677	120914224	0.551	0.475	0.050
DERL1	chr8	124095009	124123533	0.674	0.484	0.055
C8orf76	chr8	124301438	124322767	0.545	0.487	0.061
FAM91A1	chr8	124849893	124894217	0.547	0.490	0.067
TRMT12	chr8	125532260	125534217	0.585	0.501	0.073
RNF139	chr8	125556194	125569280	0.609	0.501	0.073
TATDN1	chr8	125569937	125600266	0.573	0.504	0.073
NDUFB9	chr8	125620609	125631394	0.67	0.507	0.076
SQLE	chr8	126080726	126103690	0.566	0.510	0.085
KIAA0196	chr8	126105711	126173112	0.563	0.510	0.082
NSMCE2	chr8	126173357	126448429	0.577	0.513	0.082
FAM84B	chr8	127633871	127639562	0.534	0.539	0.085
TRAPPC9	chr8	140742588	141468678	0.53	0.487	0.061
EIF2C2	chr8	141610518	141714814	0.552	0.504	0.079
PTK2	chr8	141738135	142080483	0.594	0.519	0.093
TSTA3	chr8	144765938	144770015	0.592	0.464	0.055
ZNF623	chr8	144802992	144809553	0.54	0.466	0.055
PUF60	chr8	144970693	144983194	0.589	0.472	0.055
EXOSC4	chr8	145133522	145135551	0.526	0.469	0.055
GPAA1	chr8	145209555	145213055	0.546	0.469	0.055
CYC1	chr8	145222009	145224324	0.636	0.469	0.055
MAF1	chr8	145231324	145234469	0.554	0.472	0.055
DGAT1	chr8	145510769	145521317	0.54	0.478	0.050
CPSF1	chr8	145589295	145605344	0.544	0.481	0.055
CYHR1	chr8	145660034	145661276	0.61	0.481	0.055
RPL8	chr8	145986002	145988284	0.589	0.461	0.050
ZNF7	chr8	146025232	146039406	0.55	0.461	0.050
C8orf33	chr8	146277824	146281416	0.532	0.443	0.058
UVRAG	chr11	75268572	77468762	0.538	0.187	0.012
PRKRIR	chr11	75744310	75740093	0.56	0.184	0.023
C11orf30	chr11	75835631	75939155	0.584	0.190	0.026
PAK1	chr11	76721458	76781171	0.581	0.213	0.029
CLNS1A	chr11	77004989	77026476	0.638	0.222	0.029
RSF1	chr11	77055016	77056050	0.558	0.224	0.032
C11orf67	chr11	77209888	77261043	0.56	0.222	0.032
INTS4	chr11	77306760	77306854	0.542	0.227	0.032
NDUFC2	chr11	77457655	77468762	0.537	0.233	0.035
ALG8	chr11	77489671	77528282	0.612	0.233	0.035
TM7SF3	chr12	27018069	27058508	0.55	0.248	0.023
MED21	chr12	27066787	27073856	0.585	0.248	0.023
CCDC91	chr12	28410133	28703099	0.528	0.222	0.020
ZNF136	chr19	12134940	12159758	0.541	0.169	0.020
ZNF564	chr19	12497231	12500487	0.537	0.172	0.026
ZNF791	chr19	12582753	12601047	0.605	0.169	0.023
C19orf56	chr19	12639907	12640414	0.672	0.175	0.023
MORG1	chr19	12645066	12647612	0.549	0.175	0.023
DHPS	chr19	12647536	12653522	0.617	0.178	0.023
FBXW9	chr19	12660756	12668379	0.541	0.178	0.023
TNPO2	chr19	12671568	12695172	0.603	0.178	0.023
C19orf43	chr19	12702580	12706471	0.563	0.181	0.020
ASNA1	chr19	12709344	12719395	0.664	0.181	0.020
TRMT1	chr19	13076853	13088417	0.55	0.216	0.032
NACC1	chr19	13108094	13112537	0.558	0.216	0.032
STX10	chr19	13115892	13122076	0.557	0.216	0.032
CCDC130	chr19	13723435	13735101	0.605	0.230	0.041
C19orf53	chr19	13746303	13750255	0.561	0.233	0.041
DNAJB1	chr19	14486600	14490026	0.585	0.224	0.029
NDUFB7	chr19	14537895	14543869	0.595	0.227	0.029
AKAP8	chr19	15464337	15490603	0.529	0.251	0.029
USE1	chr19	17187243	17191604	0.608	0.207	0.023
NR2F6	chr19	17203747	17216995	0.542	0.210	0.023
C19orf62	chr19	17239287	17251013	0.589	0.210	0.023
UQCRFS1	chr19	34390062	34395865	0.544	0.222	0.070
POP4	chr19	34797996	34798411	0.747	0.257	0.111

Table 1 Continued

Gene	*Chr*	*Start*	*End*	*correlation*	*fCN>2.5*	*fCN>3.5*
C19orf12	chr19	34883970	34891160	0.555	0.262	0.117
CCNE1	chr19	34994774	35007039	0.578	0.268	0.117
C19orf2	chr19	35125351	35198176	0.745	0.262	0.111
ZNF507	chr19	37529996	37542468	0.532	0.181	0.035
ZNF420	chr19	37569382	37620662	0.531	0.172	0.020
ANKRD27	chr19	37779935	37781120	0.676	0.187	0.038
CCDC123	chr19	38061790	38154709	0.545	0.175	0.029
RHPN2	chr19	38161367	38173389	0.572	0.175	0.026
PEPD	chr19	38569954	38594491	0.539	0.163	0.023
LSM14A	chr19	39355265	39355508	0.628	0.178	0.023
UBA2	chr19	39611147	39652626	0.607	0.175	0.023
ZNF585B	chr19	42368828	42369771	0.547	0.178	0.023
ZNF383	chr19	42409209	42418811	0.546	0.178	0.020
SPINT2	chr19	43447250	43474812	0.63	0.190	0.029
PSMD8	chr19	43557200	43566216	0.642	0.184	0.029
EIF3K	chr19	43806612	43819425	0.615	0.201	0.029
ACTN4	chr19	43830180	43912972	0.581	0.201	0.032
ECH1	chr19	43997942	44014245	0.715	0.195	0.035
SIRT2	chr19	44061065	44061778	0.535	0.204	0.050
SARS2	chr19	44097752	44113153	0.556	0.201	0.050
SAMD4B	chr19	44539177	44565771	0.6	0.198	0.052
PAF1	chr19	44568126	44573344	0.718	0.198	0.052
MED29	chr19	44573856	44582453	0.657	0.195	0.052
RPS16	chr19	44615726	44618471	0.556	0.195	0.052
SUPT5H	chr19	44628301	44659054	0.641	0.190	0.052
TIMM50	chr19	44662966	44672833	0.657	0.181	0.052
FBL	chr19	45016951	45023256	0.597	0.166	0.041
PSMC4	chr19	45168950	45179137	0.686	0.152	0.035
AKT2	chr19	45430106	45483092	0.668	0.128	0.023
SERTAD3	chr19	45638677	45640331	0.539	0.120	0.023
SHKBP1	chr19	45774650	45789026	0.573	0.111	0.020

Abbreviation: Chr: chromosome.
[a]These genes showed the best correlation between DNA and copy number and RNA expression levels with $R>0.526$ from the TCGA ovarian cancer data set. Different chromosomes are indicated by different color highlights. Genes within the chr19p13.2 amplicon are in red fonts.

at the *NACC1* locus, respectively. NAC1 immunoreactivity in representative amplified and non-amplified tumors is shown in Figure 2b.

Next, we asked whether *NACC1* amplification was associated with amplification of other chromosomal loci, including *CCNE1*, *RSF1*, *NOTCH3*, *AKT2,* and *PIK3CA,* which were frequently amplified in high-grade serous carcinoma.[4] The FISH results from the above genes were available in 146 cases and they were used for the correlation study. On the basis of FISH data and analysis using a 2×2 contingency table, we found that among these genes amplification of only the *NOTCH3* locus (ch19p13.12) was significantly correlated with amplification at the ch19p13.2 locus ($P=0.0044$) (Table 3). Among 175 high-grade serous carcinomas, we identified a subset of 52 primary tumors from patients whose follow-up information was available. These patients were selected also based on their similar clinical treatment outcome including optimal cytoreductive surgery (residual tumor <0.5 cm) followed by a similar regimen of carboplatin/paclitaxel therapy at the Johns Hopkins Hospital, Baltimore, MD, USA. We observed that an increase in DNA copy number in the *NACC1* locus significantly correlated with earlier recurrence (within 6 months after diagnosis) compared with those without amplification ($P=0.013$) (Table 4).

Discussion

In this study, we provide new evidence that DNA copy number at the ch19p13.2 subchromosomal region is increased in approximately one-fifth of high-grade serous carcinomas. This finding is based on two large independent cohorts, using two independent techniques including SNP arrays and FISH. The frequency of ch19p13.2 amplification is comparable to that at the *CCNE1*, *NOTCH3*, *RSF1*, *AKT2*, and *PIK3CA* loci, which were found to be amplified in 36, 32, 16, 14, and 11% of high-grade serous carcinomas, respectively.[4] However, it is uncertain whether ch19p13.2 represents a discrete amplicon or a continuum of a larger region of DNA copy number gain in ch19p, perhaps involving the whole arm. The significant co-amplification event of ch19p13.2 and the *NOTCH3* locus (ch19p13.12) suggests such a possibility given the proximal location of both loci. Nevertheless, based on our FISH and immunohistochemistry analysis of the same tumor samples, NAC1 expression likely

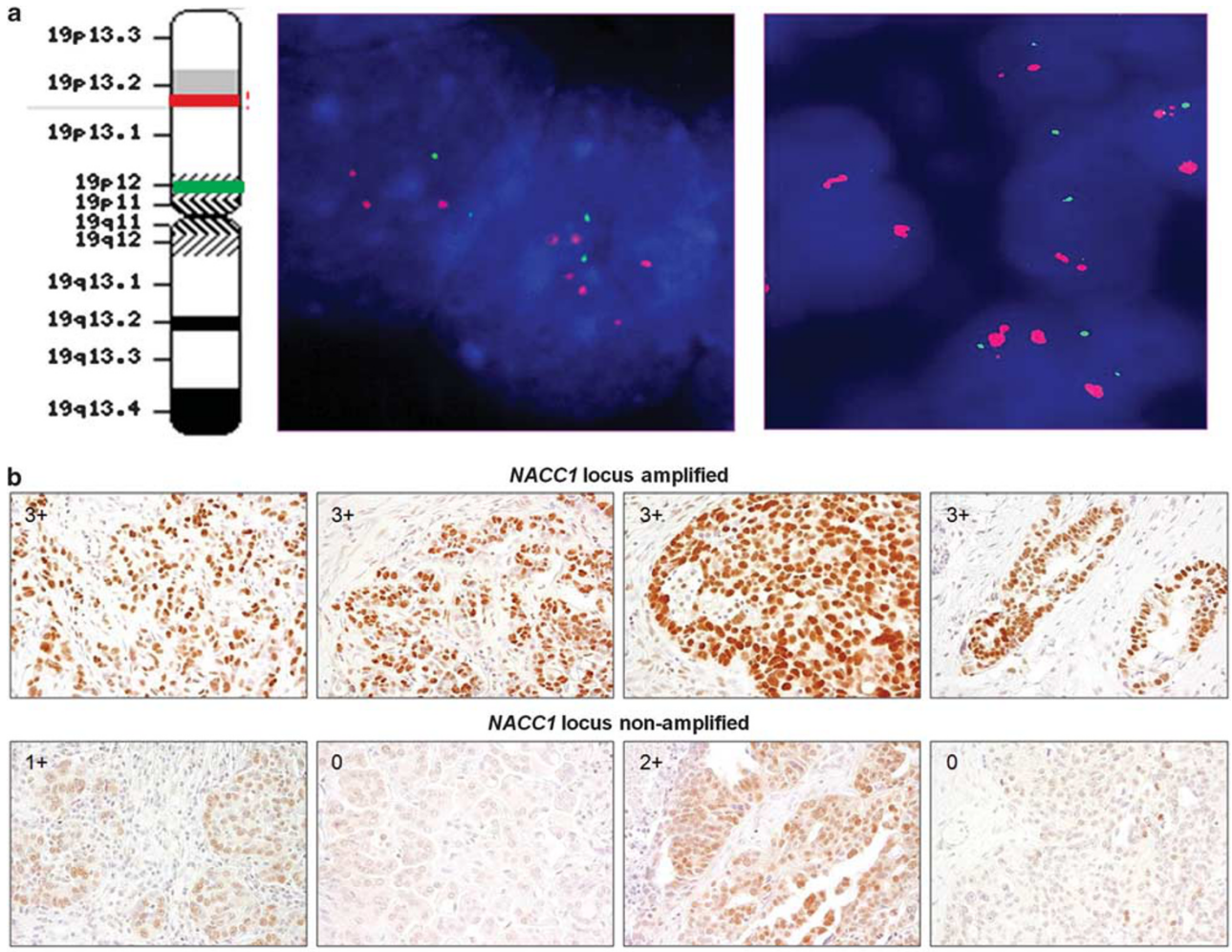

Figure 2 DNA copy number at the *NACC1* locus and NAC1 immunoreactivity from representative high-grade serous carcinomas. (**a**) Two-color fluorescence *in situ* hybridization in two high-grade serous carcinomas shows increased signal of the ch19p13.2 probe (red fluorescence) whereas the control probe (green fluorescence) that hybridizes to a region near the ch19p12 does not shown any gain of intensity. The case on the left panel shows discrete red probe signals whereas the case on the right shows the homogeneously staining regions. The ideogram on the left illustrates the locations, which hybridize to the ch19p13.2 probe (red bar) and to the ch19p12 control probe (green bar). (**b**) Immunohistochemistry of NAC1. High-grade ovarian serous carcinomas with high levels of ch19p13.2 amplification show intense immunostaining (3+) of NAC1 in the nuclei (top panels). High-grade ovarian serous carcinomas without detectable alteration of DNA copy number at the ch19p13.2 show variable immunostaining intensity with staining scores ranging from 0 to 2+ (bottom panel). Immunostaining scores are indicated in each photomicrograph.

Table 2 Correlation of *NACC1* DNA copy number and staining intensity in 175 high-grade serous carcinomas

	3+	*2+*	*1+*	*0*	*Total*
NACC1 amplification (copy number > 3.5)	5	1	2	0	8
NACC1 gain (3.5 > copy number > 2.5)	7	6	9	5	27
Non-amplified	18	45	33	45	140
Total	30	52	44	50	175

Fisher's exact test $P = 0.0047$.

Table 3 Co-amplification of *NACC1* and *NOTCH3* loci

	NOTCH3 amplified	*NOTCH3 non-amplified*	*Total*
NACC1 amplified	15	10	25
NACC1 non-amplified	34	87	121
Total	49	97	146

Fisher's exact test $P = 0.0044$.

depends on amplification within the ch19p13.2 amplicon. The above results have several important implications with respect to molecular genetic changes and pathogenesis of tumor recurrence in ovarian cancer.

To determine the significance of amplified genes in human cancer, we applied an approach based on the rationale that a tumor-driving gene, when amplified, is almost always overexpressed, which activates the oncogenic pathway, while co-amplified

Table 4 Correlation of *NACC1* locus amplification and the time to first recurrence

	Recur within 6 months	*Recur after 6 months*	*Total*
NACC1 amplified	6	10	16
NACC1 non-amplified	5	31	36
Total	11	41	52

Fisher's exact test $P = 0.013$.

'passenger' genes that are unrelated to tumor pathogenesis may or may not be overexpressed.[24] Therefore, we analyzed all genes within all amplified regions detected by the TCGA ovarian cancer data set to correlate DNA and transcript copy numbers within the same tumor samples. As a result, we have identified 100 genes with the most significant correlation between DNA copy numbers and RNA expression levels and have found that several ovarian cancer-associated oncogenes were on the list, including *CCNE1* (Farley *et al*[17]), *AKT2* (Cheng *et al*[18]), *Pak1* (Schraml *et al*[5]), *Rsf-1* (Choi *et al*[19]), and *Paf1* (Chaudhary *et al*[20]). This finding, together with the data showing *CCNE1* as the most frequently amplified region (copy number >3.5) in ovarian serous carcinoma, supports the robustness of this approach in identifying potential cancer driver genes within amplicons. From this perspective, we found that *NACC1* was listed with an *R* value as high as 0.558. This finding suggests that *NACC1* is one of the genes that contribute to tumor progression in ovarian cancer in which ch19p13.2 amplification is found. Of note, analysis of the 100 top gene list reveals that the majority of the amplified ovarian cancer driver genes are located on three chromosomes, 8, 11, and 19, a finding consistent with our previous FISH study that profiled all amplicons in ovarian serous carcinoma.[4] This information may provide a potential roadmap to study the pathogenesis of ovarian serous carcinoma in the future.

The association of the ch19p13.2 amplification and shorter disease relapse time may be related to NAC1 overexpression. NAC1, encoded by *NACC1*, belongs to the BTB/POZ domain gene family and contains the BTB/POZ domain, which is responsible for homodimerization and heterodimerization with other BTB/POZ proteins as well as the BEN domain that mediate protein–DNA and protein–protein interactions during chromatin organization and transcription.[25] The role of NAC1 in the development of human cancer has recently emerged. NAC1 is significantly overexpressed in several types of human cancers including ovarian high-grade serous carcinoma,[16,22,26,27] endometrial carcinoma,[28] and cervical carcinoma.[29] Like several BTB/POZ family members, NAC1 proteins homo-dimerize through the BTB domain. Induced expression of a NAC1 deletion mutant (N130) containing exclusively the BTB domain attenuates the tumor-promoting functions of NAC1.[16] On the other hand, overexpression of full-length NAC1 is sufficient to enhance tumorigenicity of ovarian surface epithelial cells and NIH3T3 cells in athymic *nu/nu* mice.[16] More recently, we observed that enforced expression of NAC1 conferred drug resistance, and NAC1 knockdown by shRNA sensitized paclitaxel cytotoxicity in ovarian cancer cells *in vitro*.[22] NAC1 contributed to the development of drug resistance through multiple mechanisms including upregulating fatty acid synthase[30] and negatively regulating the components of the Gadd45 tumor suppressor pathway including Gadd45α and its binding protein, Gadd45-gip1 (Jinawath *et al*[22]; Nakayama *et al*[26]). The above findings may explain the clinical observation that upregulation of NAC1 immunoreactivity in primary ovarian tumors is associated with aggressive clinical behavior and tumor recurrence in ovarian cancer patients.[16,27] We did not attempt to analyze the clinical correlation of *NACC1* amplification using the TCGA data set because of concerns about the difference in treatment regimens and patient populations from the various institutions that contributed to the data set.

The frequent co-amplification of the *NACC1* and *NOTCH3* is of interest. Our previous studies showed amplification of the *NOTCH3* locus in 32% of ovarian high-grade serous carcinomas,[12] and Notch3 overexpression is related to the recurrence of ovarian cancer and confers drug resistance.[31] Ovarian cancer cells that had amplified and overexpressed Notch3 were dependent on Notch3 signaling for cellular survival and growth. Thus, it is likely that increased DNA copy number in both genes may contribute to the resistance of carboplatin and paclitaxel that are routinely used in treating advanced stage ovarian cancer patients. Interestingly, we have recently demonstrated that inactivation of the Notch3 pathway led to inhibition of NAC1 expression, indicating that Notch3 signaling may regulate the expression of NAC1.[31] Further studies are required to confirm the molecular crosstalk between these pathways in the development of chemoresistance.

In summary, based on analysis of the TCGA ovarian cancer data set and our FISH result, we were able to demonstrate that amplification at the *NACC1* locus was one of the frequent molecular genetic alterations in ovarian high-grade serous carcinomas. NAC1 overexpression may be, in part, attributed to the increase in DNA copy number, explaining why amplification at the *NACC1* locus is related to early tumor recurrence in ovarian cancer. Future studies should aim at fine mapping the ch19p13.2 amplified region and assessing the potential of other genes at the ch19p13.2 locus to contribute to the aggressive behavior of ovarian serous carcinomas that harbor this amplification.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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