Frequent Amplification of Chromosomal Region 20q12-q13 in Ovarian Cancer[1]

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ABSTRACT

DNA amplification at chromosomal region 20q12-q13, which is common in breast cancer, has recently been described also in ovarian tumors. We studied the amplification of the recently identified candidate oncogenes in this region in 24 sporadic, 3 familial and 4 hereditary ovarian carcinomas, and in 8 ovarian cancer cell lines. High-level amplification of at least one of the five nonsyntenic regions at 20q12-q13.2 was found in 13 sporadic (54%) and in all four hereditary tumors. Typically, two or more distinct amplicons (separated by nonamplified DNA) were found coamplified in various combinations. The regions defined by the *AIB1* and *PTPN1* genes (at 20q12 and 20q13.1, respectively) were amplified in 25% and 29% of the sporadic tumors, also without simultaneous coamplification of other regions. Amplification of *AIB1* (a steroid receptor coactivator gene) was associated with estrogen receptor positivity in sporadic ovarian carcinomas ($P = 0.01$) and showed a tendency to correlate with poor survival of patients. Of the genes amplified in breast cancer, the *BTAK* gene was amplified in 21%, the *MYBL2* gene in 17%, and the *ZNF217* gene in 12.5% of the sporadic tumors. The high frequency of gene amplification at 20q12-q13.2 suggests that the genes amplified therein may play a central role in the pathogenesis of sporadic and hereditary ovarian carcinoma.

INTRODUCTION

Ovarian cancer is thought to arise because of somatic alterations in genes involved in regulating cell proliferation, apoptosis, and genomic integrity. Alterations in several proto-oncogenes and tumor suppressor genes have been described, many of which are the same as in breast cancer. The *ERBB2* oncogene is amplified and subsequently overexpressed in 9–30% of ovarian cancers (1, reviewed in 2). In most studies, overexpression of *ERBB2* correlates with poor survival of patients (reviewed in ref. 2). Amplification of the *MYC* oncogene has been found in approximately 10–30% of ovarian cancers, more frequently in serous than in mucinous cancers (2). Other oncogenes amplified in ovarian cancer include *KRAS*, *INT2*, *FMS*, *MDM2*, and *AKT2*, but these amplifications appear to be relatively uncommon (3–5%; 1, reviewed in Ref. 2). However, cytogenetic studies indicate that homogeneously staining regions and double minute chromosomes are much more common, suggesting that ovarian cancers contain currently unknown gene amplifications (2).

CGH has revealed that chromosome arm 20q shows frequently copy number gains in ovarian cancer, similar to what has been observed in breast cancer (3, 4). The frequency of the copy number gain at 20q ranges from 20 to 48% (5–8). Studies in breast cancer have revealed that the amplification consists of at least three smaller subamplicons, at 20q11, 20q12–20q13.1, and 20q13.2 (9). These regions are frequently coamplified and separated with large regions of nonamplified DNA (9). The region 20q13.2 is the most commonly amplified region in breast cancer (9). Recent studies have identified several putative candidate oncogenes from the amplified regions. Chromosome microdissection and hybrid selection studies have recovered three overexpressed and amplified candidate target genes originating from 20q: *AIB3* and *AIB4* mapping to 20q11, and *AIB1* gene at 20q12. The latter is a member of SRC-1 family of nuclear receptor coactivators (10, 11). Amplification of the *AIB1* gene also has been found in an ovarian cancer cell line (11). The *MYBL2* gene, a member of v-*MYB*–related oncogenes, and the phosphotyrosine-phosphatase 1 gene (*PTPN1*; both mapping to 20q13.1) are independently amplified in a small fraction of breast cancers (9, 12). Two candidate genes for the 20q13.2 amplicon include *ZNF217* and *BTAK* (13, 14). The former encodes a putative C2H2 Kruppel-like transcription factor, and the latter a centrosome-associated kinase involved in chromosome replication (13, 14).

The frequency of 20q amplification and the involvement of the candidate genes are not known in ovarian carcinoma. Here, we studied the amplification of the chromosomal region 20q in sporadic and hereditary ovarian cancers and cell lines with CGH[3] and FISH using probes detecting the candidate genes found to be amplified in breast cancer.

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[3] The abbreviations used are: CGH, comparative genomic hybridization; FISH, fluorescence *in situ* hybridization.

Table 1 Clinical and pathobiological characteristics of 31 ovarian tumors[a]

Tumor ID	Histotype	Stage	ER status	PR status	*ERBB2* status	Survival
Sporadic						
1	Serous	I	Neg	Neg	Neg	Alive at 108 mo
2	Undifferentiated	III	Pos	Neg	Neg	Dead at 10 mo
3	Undifferentiated	III	Pos	Pos	Neg	Dead at 29 mo
4	Serous	—	Neg	Neg	Neg	N.A.[b]
5	Serous	III	Pos	Pos	Neg	Alive at 119 mo
6	Serous	III	Neg	Neg	Neg	Dead at 11 mo
7	Endometrioid	III	Pos	Pos	Neg	Dead at 19 mo
8	Undifferentiated	III	Pos	Neg	Neg	Dead at 11 mo
9	Endometrioid	II	Neg	Neg	Neg	Dead at 34 mo
10	Serous	III	Pos	Neg	Neg	Dead at 35 mo
11	Endometrioid	III	Neg	Neg	Neg	Dead at 25 mo
12	Undifferentiated	IV	Neg	Neg	Neg	Dead at 51 mo
13	Serous	III	Neg	Neg	Neg	Dead at 102 mo
14	Serous	I	Neg	Neg	Neg	Dead at 59 mo
15	Serous	I	Pos	Pos	Neg	Alive at 96 mo
16	Serous	II	Neg	Neg	Neg	Dead at 56 mo
17	Serous	III	Pos	Pos	Neg	Dead at 72 mo
18	Serous	II	Neg	Neg	Pos	Dead at 3 mo
19	Undifferentiated	III	Neg	Neg	Neg	Alive at 109 mo
20	Serous	III	Neg	Neg	Pos	Dead at 5 mo
21	Undifferentiated	III	Neg	Neg	Neg	Dead at 35 mo
22	Mucinous	–	Neg	Neg	Neg	N.A.
23	Mucinous	–	Neg	Neg	Neg	N.A.
24	Borderline	–	Pos	Pos	Neg	N.A.
Familial						
1	Endometrioid	I	Neg	Neg	Neg	Alive at 40 mo
2	Mucinous	III	Neg	Neg	Neg	Dead at 11 mo
3	Endometrioid	II	Pos	Pos	Neg	Alive at 43 mo
Hereditary						
1	Serous (*BRCA1*)	III	Pos	Pos	Neg	N.A.
2	Serous (*BRCA1*)	III	Pos	Pos	Neg	N.A.
3	Serous (*BRCA2*)	III	Pos	Neg	Neg	N.A.
4	Serous (*BRCA2*)	III	Neg	Neg	Neg	N.A.

[a] The bolded tumor IDs are from 20q amplified tumors and are identical to Fig. 2 to allow cross-reference with 20q amplifications.
[b] N.A., not available.

MATERIALS AND METHODS

Ovarian Cancer Cell Lines and Primary Tumors. Eight ovarian cancer cell lines (UT-OV-1, UT-OV2, UT-OV3, UTOV4, UT-OV5, UT-OV7, UT-OV8, UT-OV10) were derived from primary or metastatic epithelial ovarian carcinomas. Before the experiments the cells were cultured in complete Eagle's minimal essential medium supplemented with 2 mM L-glutamine, 1% nonessential amino acids, 100 IU/ml penicillin, 100 IU/ml streptomycin, and 10% fetal bovine serum. The cells were kept in logarithmic growth with regular passages. The cells were harvested at confluency to obtain interphase nuclei from cells that were predominantly in the G_1-phase of the cell cycle (9). Trypsinized cells were cytocentrifuged and air-dried at room temperature until fixed and processed for FISH. Thirty-one primary untreated sporadic ovarian carcinomas were obtained from the University Lund and the University Tampere. These included 24 patients without a family history of breast or ovarian cancer, and 7 patients with a family history of breast or ovarian cancer. Five of the 7 familial ovarian cancer cases have been analyzed for *BRCA1* and *BRCA2* mutations. Two were found to be carriers of *BRCA1* germline mutations 3172ins5 (termination at codon 1025) and 4744C→G (Ser1542Cys), respectively. Two patients were identified as carriers of *BRCA2* germline mutations 6174delT (ter2003) and 7829C→T (affecting the splice donor site of exon 15), whereas no mutation was identified in the fifth patient. The two remaining familial cases had not been analyzed for *BRCA1* and *BRCA2* mutations. Thus, 4 of 31 patients were considered as having a hereditary disease, 3 of 31 as familial ovarian cancer, and 24 of 31 as having sporadic ovarian carcinoma. Estrogen and progesterone status was determined immunohistochemically (sporadic tumors) or with EIA (hereditary tumors; Refs. 15, 16). The clinical and pathobiological characteristics of 31 primary tumors are shown in Table 1. The primary tumors were freshly frozen and stored at −70°C. Imprint touch preparations were made for FISH analysis by lightly pressing a semithawed frozen tumor onto Superfrost Plus microscope slides (Menzel, Germany) microscope slides and air-dried. Before hybridization, slides were stained with May–Grunwald–Giemsa staining to ensure that imprint touch preparations were cytologically representative of tumor tissue.

CGH. CGH was performed according to a published protocol (17). Briefly, genomic DNA from ovarian cancer cell lines and normal female reference DNA were extracted using a standard protocol and labeled with FITC–dUTP and Texas Red–dUTP (DuPont, Boston, MA) using nick translation. Labeled DNAs (400 ng each) and 10 μg of unlabeled Cot-1 DNA

Table 2 Characteristics of the probes used in FISH

Clone identification	Type of clone	Flpter[a]	Reference	Function
E2F	P1	0.541	18	Cellular transcription factor
AIB3/AIB4	P1	0.58	10	Not known
SRC	P1	0.60	18	Homolog of v-*src* oncogene
AIB1	P1	0.67	11	Steroid receptor coactivator
MYBL2	BAC	0.69	12	Transcription factor
PTPN1/PTP1B	P1	0.78	18	Protein tyrosine phosphatase
RMC20P4001	P1	0.82	13	Genes unknown
ZNF217	BAC	0.83	13	Putative transcription factor
BTAK/STK15/Aurora2	PAC	0.86	14	Centrosome-associated kinase

[a] Fractional length from p-telomere.

(Life Technologies, Gaithersburg, MD) were hybridized onto commercially available normal metaphase chromosomes (Vysis Inc., Downers Grove, IL). The hybridizations were evaluated using the QUIPS digital image analysis system (Vysis Inc.).

Probes for FISH. Nine P1, PAC, or BAC probes defining genes and loci along 20q (10–13, 18) were used in the study. The probes were physically mapped by fractional length from p-telomere (FLpter) measurements as described (18). In case of closely spaced probes, pairwise interphase mapping and data from contiguous assembly of the 20q13 region was used (13). The probes and their physical chromosomal localizations are listed in Table 2. The probes were used in our previous studies, except for *BTAK*, which was screened from the human PAC library for this study (18). A probe recognizing the pericentromeric region of chromosome 20 (RMC20L116) was used as reference probe to determine the overall copy number of chromosome 20. The probe cocktail containing the *ERBB2* oncogene and the chromosome 17 centromere probe was obtained from Vysis Inc. (Chicago, IL).

FISH. Samples were fixed with 50 and 70%, and twice in 100% Carnoy's solution (3:1 methanol:acetic acid, 10 min each). Two-color FISH was carried out as previously described (9) with minor modifications. In brief, the slides were denatured in 70% formamide–2× SSC at 72°C for 3 min. Five nanograms of differentially labeled pericentromeric probe, 20 ng locus- or gene-specific probe (when appropriate), and 10 μg of human placental DNA were hybridized overnight at +37°C as described (9). FISH of *ERBB2* was done according to the manufacturer's instructions (Vysis Inc., Chicago, IL). After posthybridization washes, bound probes were detected immunohistochemically with avidin–FITC (Vector Laboratories, Burlingame, CA) and anti-digoxigenin rhodamine (Boehringer; Ref. 9). Slides were counterstained with 0.2 μM 4,6 diamino-2-phenylindole in an antifade solution (Vectashield; Vector Laboratories). Hybridization signals were scored using Zeiss Axioplan 2 epifluorescence microscope equipped with dual band-pass fluorescence filter (Chromatechnology, Brattleboro, NV), which enables simultaneous detection of both fluorescein and Texas Red fluorescence. Hybridization signals from at least 300 nuclei were scored to assess the average copy number status per cell. The scoring results were expressed as the number of signals relative to the centromere signals (level of amplification). Digital images were taken with a Hamamatsu 9585 camera (Hamamatsu Inc, Hamamatsu City, Japan) operated via ISIS image analysis software (MetaSystems GmbH, Altslussheim, Germany). Control hybridizations to normal fibroblasts were done to ascertain that the hybridization efficiencies of the test and reference probes were similar.

RESULTS

Copy Number Gains by CGH. Three of eight ovarian cancer cell lines showed a copy number gain at chromosome 20q (Fig. 1). In the cell line UT-OV1 the entire 20q was gained, without evidence of highly amplified regions within 20q. In UT-OV5, a copy number gain was found at 20q11-q13.2. A regional copy number gain was found at 20q12-qter in UT-OV-8. (Fig. 1). The copy number profiles were in the normal range in the remaining five cell lines.

Amplification at 20q in Ovarian Carcinoma Cell Lines by FISH. Analysis of gene amplification by FISH confirmed the findings obtained by CGH (Figs. 1 and 2). As expected, CGH failed to show difference between copy number gain and amplification (Fig. 1). In UT-OV1 all studied genes and loci between *AIB1* and *BTAK* showed either a copy number gain (ratio to 20 centromere copy number, 1.5–3) or a high-level amplification (ratio to 20 centromere copy number, >3). In UT-OV5 the low-level amplification extended from 20q11 (*AIB3/AIB4*) to 20q13.2 (*BTAK*). High-level amplification of *PTPN1* and *ZNF217* was found both in UT-OV1 and UT-OV8. The amplification pattern was discontinuous in UT-OV8, with a nonamplified region between *PTPN1* and *ZNF217* (defined by RMC20P4001).

Amplification at 20q in Primary Ovarian Carcinomas. Gene amplification for at least one region along 20q arm was found in 13 of 24 (54%) primary sporadic ovarian carcinomas, in 1 of the 3 familial tumors, and in all 4 hereditary tumors (Fig. 2, examples of FISH in Fig. 3). The mean level of amplification ranged from 2- to 12-fold, and individual tumor cells showed up to 15-fold amplification (compared with 20 centromere count). All hereditary, 1 familial (33%), and 12 sporadic tumors (50%) showed high-level amplification (>3-fold) with one or more of the following genes/loci tested: *AIB1* at 20q12, *MYBL2* and *PTPN1* at 20q13.1, *ZNF217* and *BTAK* at 20q13.2 (Fig. 2). Other genes/loci did not show high-level amplification (Fig. 2).

When the prevalence of high-level amplification was evaluated (Table 3), amplicons defined by *PTPN1* and *AIB1* were the most common in sporadic ovarian cancer, but because of coamplifications, regions defined by *BTAK*, *MYBL2*, and *ZNF217* also were amplified almost as often (Table 3). No

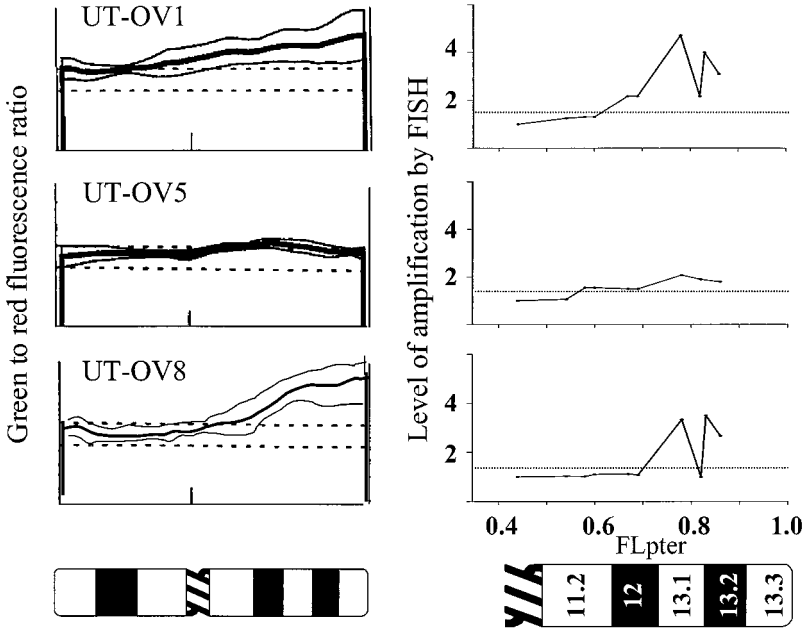

Fig. 1 Amplification at chromosome arm 20q in ovarian carcinoma cell lines UT-OV1, UT-OV5, and UT-OV8 by CGH and FISH. The CGH profiles demonstrate large regional copy number gains (entire 20q in UT-OV1, 20q11-q13.2 in UT-OV5, and 20q12-qter in UT-OV8) The *dotted lines* show the cutoff values of *green-to-red* fluorescence ratios (0.8 and 1.2). The copy numbers by FISH (relative to the copy number of the reference probe) show discontinuous amplicon structure for cell lines UT-OV1 and UT-OV8, whereas cell line UT-OV5 shows low-level copy number gain from 20q11 to 20q13.2. The *dotted horizontal line* illustrates cutoff level (1.5-fold) for gene amplification.

constant patterns of coamplification were found in these tumors (Fig. 2). Two or more separate amplicons were found together in various combinations. Amplified regions were in most tumors interspersed with nonamplified regions (Fig. 2), indicating discontinuous amplicons along the 20q. The *PTPN1* gene was amplified alone at 20q in three tumors, *AIB1* in two tumors, whereas *MYBL2*, *ZNF217,* and *BTAK* were never amplified alone at high level (Fig. 2). In hereditary tumors, *BTAK* was amplified in all samples and was the only highly amplified gene in one case (Fig. 2, Table 3).

Clinical Correlations of Different Amplicons. Five of six sporadic tumors with high-level *AIB1* amplification were estrogen receptor positive, compared with 4 of 18 tumors without *AIB1* amplification ($P = 0.0147$, Fischer's exact test, two-sided *P* value). No significant correlation was found between progesterone receptor and *AIB1* amplification. Other highly amplified genes showed no correlation with estrogen or progesterone receptor status. In sporadic tumors, all three endometrioid tumors, four of six undifferentiated and 6 of 12 serous adenocarcinomas showed 20q amplification. These differences were not statistically significant. No 20q amplifications were found in mucinous tumors or borderline tumors. None of the 20q amplifications correlated with tumor staging. When cumulative survival of the patients was studied, only tumors with *AIB1* amplification showed a tendency to correlate with poor patient survival ($P = 0.066$, Mantel-Cox test, Fig. 4). Tumors in Fig. 2 and Table 1 are presented with same numbers to allow cross-reference.

Amplification of *ERBB2* in Ovarian Tumors. To further characterize the tumors with 20q amplification, we analyzed amplification of *ERBB2* in the same set of tumors. Three cell lines (UT-OV-1, UT-OV4, and UT-OV-5), two sporadic tumors (8.3%), and none of the familial or hereditary tumors showed amplification for *ERBB2*. *ERBB2* and 20q were not amplified in the same primary tumors (Table 1).

DISCUSSION

On the basis of the high frequency of amplification, genes located at chromosome 20q are, together with *ERBB2* and *MYC*, the most common targets of gene amplification in ovarian cancer. In the present tumor material, *ERBB2* and genes at 20q were not amplified in the same primary tumors. Thus, gene amplification at 20q may identify a new subgroup of sporadic ovarian cancers. Amplification at 20q was found also in four of four hereditary ovarian tumors, suggesting that carcinogenesis involving inactivation of susceptibility genes *BRCA1* and *BRCA2* may specifically lead to a tumor type that includes amplification at 20q. *ERBB2* amplification was not found in the hereditary tumors, which is in agreement with recent observations of hereditary breast and ovarian tumors (19, 20).

The main finding in sporadic ovarian carcinoma was that the amplification at 20q involves at least five separate regions. Similarly, as in breast cancer (9), these regions are distinct (*i.e.*, separated by nonamplified DNA), but consistently coamplified with each other in various combinations. Despite similar discontinuous amplicon structure, several differences between breast and ovarian cancer also were found. The overall frequency of amplification of any region was at least two times more common in ovarian than in breast cancer. In breast cancer, the *ZNF217* gene has been defined as the core of the 20q amplification (9), whereas in ovarian carcinomas it was amplified less frequently than the more proximally located genes *AIB1* and *PTPN1*. The steroid-receptor coactivator gene *AIB1* was amplified in a higher proportion of ovarian than in breast cancers. More importantly, *AIB1* also was amplified alone, suggesting that it also may be selected independently in ovarian cancer. Another striking difference was a lack of high-level amplification at 20q11, suggesting that *AIB3* and *AIB4* are unlikely to be the targets for amplification in ovarian cancer.

The high frequency of amplification at 20q by FISH is in

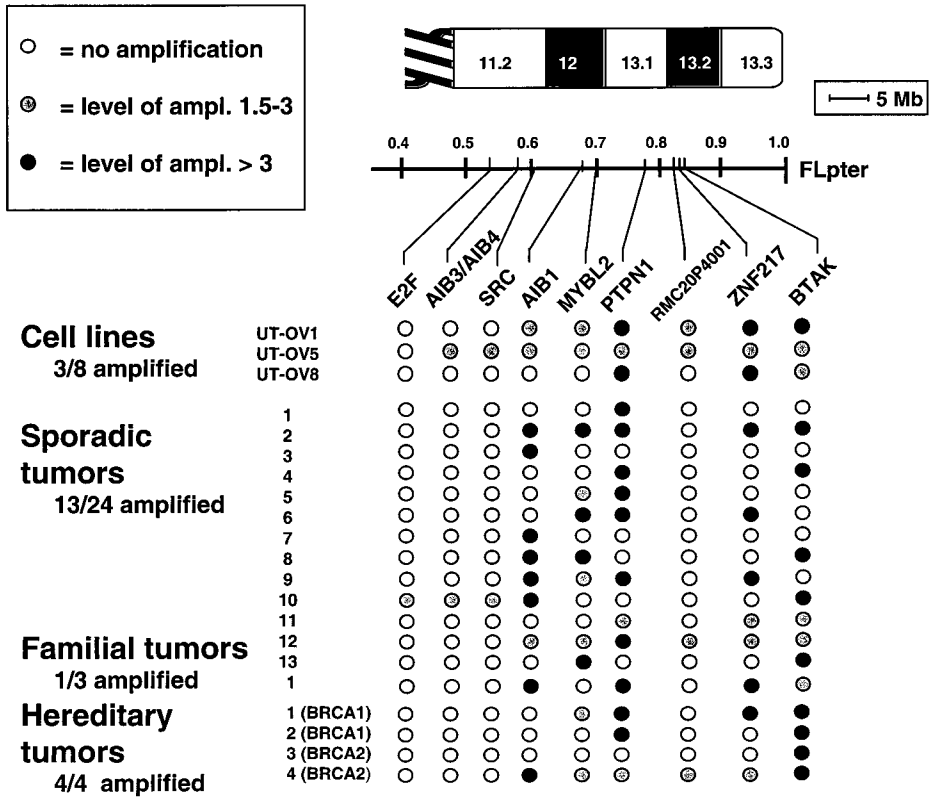

Fig. 2 Amplification of the nine different probes along 20q in ovarian cancer cell lines and primary ovarian (sporadic, familial, and hereditary) carcinomas. Cases showing >1.5-fold amplification with at least one of the probes are included in the plot. The chromosome ideogram and the FLpter scale are given as an approximate reference for the location of the probes.

accordance with studies by CGH (5–8). However, as also indicated in the present study, the resolution of CGH is too low to detect small amplification peaks and to distinguish high-level amplification from a low-level copy number gain of a larger region. Amplification at 20q has been studied in ovarian cancer in two previous studies (1, 21). The clone RMC20C001, which defines the minimal amplicon at 20q13.2 (including the *ZN217* gene), has previously shown amplification in ~3% of ovarian tumors by Southern analysis (1). This differs from our results (ZN217 amplified in 12.5% in our study), but can be explained by the lower sensitivity of Southern blot analysis to detect low-level amplification. Furthermore, the clone RMC20C001 may not detect all amplicons, which include *ZNF217* (13). The *ZNF217* gene, a candidate gene for 20q13.2 amplification, was recently cloned by positional cloning (13). It encodes a putative C2H2 Kruppel-like transcription factor, and it was the only gene constantly amplified and overexpressed in the ~250-kb minimal common region of amplification in breast cancer (13). In the current study *ZNF217* was not amplified alone, suggesting a less central role in ovarian cancer. The same holds for *MYBL2*, which is sometimes amplified without coamplification of the 20q13.2 region in breast cancer (12).

The *AIB1* gene, mapping to 20q12, has previously been shown amplified and overexpressed in one ovarian carcinoma cell line (11) and in 7.4% of primary tumors by Southern analysis (21). We found *AIB1* amplified in 25% of sporadic ovarian tumors, again indicating a higher sensitivity of FISH. Amplification was significantly associated with positive estrogen receptor status of the tumor ($P = 0.01$). Interestingly, *AIB1* has been shown to interact with the estrogen receptor and to enhance estrogen receptor–dependent transcription (11). The ovarian surface epithelium, from which ovarian carcinomas arise, has been described to express estrogen receptor protein (22). The ovarian surface epithelium undergoes rapid proliferation after ovulation (reviewed in Ref. 23), similar to that in mammary epithelium during the menstrual cycle. This may explain why this gene is selected for amplification in ovarian cancer, because *AIB1* amplification may activate an estrogen-dependent, growth-promoting pathway in transformed epithelial cells forming the malignant tumors. *AIB1* amplification also has been associated with estrogen and progesterone receptor status in breast tumors (21). Despite the small number of patients, *AIB1* amplification also showed nearly statistically significant correlation with poor patient survival, underlining the importance of this amplification in the tumor progression of ovarian cancer.

The *PTPN1* gene, a phosphotyrosine phosphatase gene mapping to 20q13.1, was highly amplified in 29% of ovarian tumors and 25% of cell lines. *PTPN1* is overexpressed in breast and ovarian (24, 25) cancer, but according to our previous results in breast cancer (9),[4] *PTPN1* is almost always constantly expressed without any clear correlation be-

[4] Unpublished results.

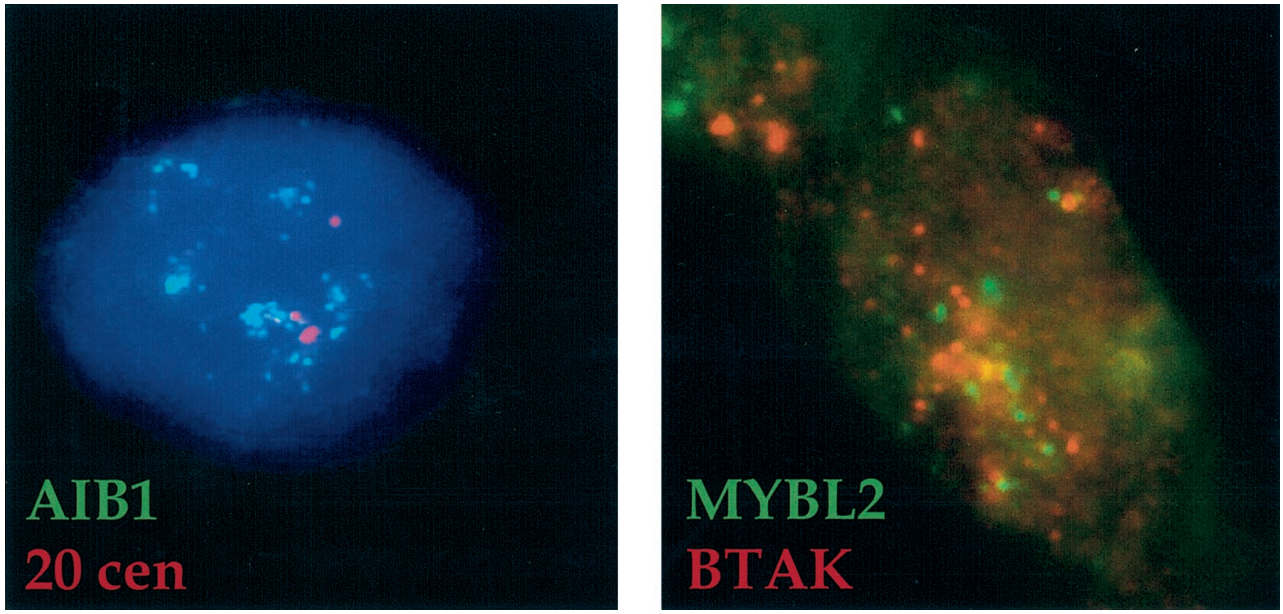

Fig. 3 Amplification of chromosome 20q12-q13 in ovarian cancer by FISH. Hybridized probes are visualized either in *green* or *red* fluorescence. *Left:* a sporadic tumor sample (no. 3 in Fig. 2) showing a high-level amplification (~40 signals, partly in tight clusters) of *AIB1* (*green*) compared with chromosome 20 centromere (*red,* six signals). Counterstained with DAPI (*blue*). *Right:* a sporadic ovarian carcinoma (no. 13) showing coamplification of *MYBL2* (*green,* ~15 signals) and *BTAK* (*red,* ~20 signals). Note that all of the signals are not in the same focal plane.

Table 3 Prevalence of high-level amplification of various candidate oncogenes in ovarian cancer

Tumor entity	*AIB1*	*MYBL2*	*PTPN1*	*ZNF217*	*BTAK*
Cell lines (n = 8)	0	0	25	25	12.5
Sporadic (n = 24)	25	17	29	12.5	21
Familial (n = 3)	33	0	33	33	0
Hereditary[a] (n = 4)	25	0	50	25	100

For high-level amplification, the copy number is more than three times higher than that of the reference probe. Values are percentages.

[a] Consisting of 2 tumors from *BRCA1* and *BRCA2* from *BRCA2* germline mutation carriers.

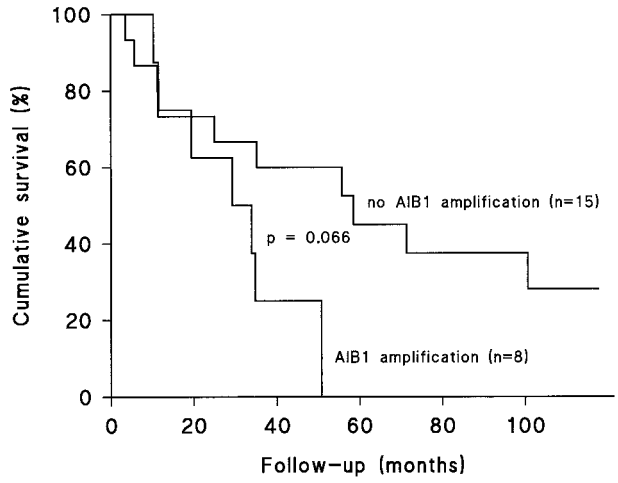

Fig. 4 Cumulative survival of 23 patients according to *AIB1* amplification. Patients having *AIB1* amplification have almost significantly shorter cumulative survival (P = 0.066, Mantel-Cox test) compared with those having no amplification.

tween amplification and expression. Therefore, it is possible that another gene in this region is the target for this amplification. The *BTAK* gene was amplified in 21% of sporadic tumors, and in four of four hereditary ovarian tumors with the *BRCA1* or *BRCA2* germline mutation. *BTAK* encodes a centrosome-associated kinase, which has recently been shown to be the target gene of the 20q13 amplification in breast and colon cancer (14). Up-regulation of *BTAK* leads to centrosome amplification, chromosomal instability, and aneuploidy (14). Interestingly, all sporadic and hereditary ovarian tumors analyzed here were aneuploid for chromosome 20 in this material (data not shown), suggesting that *BTAK* amplification may be specifically associated with aneuploidization in breast and ovarian cancers.

According to the present results and previous data from breast cancer, the coamplification of separate regions at 20q is likely to reflect the molecular mechanisms underlying gene amplification. Similar coamplifications of separate regions along the same chromosome arm have been described in 17q (*ERBB2* at 17q12 and in unidentified regions at 17q23–24) and 8q24.1 (*MYC*) and 8q22 regions (26, 27). The exact molecular mechanisms are unknown, although multiple chromosomal breaks and unbalanced translocations may be predisposing cytogenetic aberrations. Coamplifications also suggest synergism between genes up-regulated by gene amplification. The amplified genes may, for example, share a common pathway in growth regulation.

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