Combined Array Comparative Genomic Hybridization and Tissue Microarray Analysis Suggest *PAK1* at 11q13.5-q14 as a Critical Oncogene Target in Ovarian Carcinoma

Peter Schraml,* Georg Schwerdtfeger,* Felix Burkhalter,* Anna Raggi,* Dietmar Schmidt,[†] Teresa Ruffalo,[‡] Walter King,[‡] Kim Wilber,[‡] Michael J. Mihatsch,* and Holger Moch*

From the Institute of Pathology,* University of Basel, Basel, Switzerland; the Institute of Pathology,[†] Mannheim, Germany; and Vysis Incorporated,[‡] Downers Grove, Illinois

Amplification of chromosomal regions leads to an increase of DNA copy numbers and expression of oncogenes in many human tumors. The identification of tumor-specific oncogene targets has potential diagnostic and therapeutic implications. To identify distinct spectra of oncogenic alterations in ovarian carcinoma, metaphase comparative genomic hybridization (mCGH), array CGH (aCGH), and ovarian tumor tissue microarrays were used in this study. Twenty-six primary ovarian carcinomas and three ovarian carcinoma cell lines were analyzed by mCGH. Frequent chromosomal overrepresentation was observed on 2q (31%), 3q (38%), 5p (38%), 8q (52%), 11q (21%), 12p (21%), 17q (21%), and 20q (52%). The role of oncogenes residing in gained chromosomal loci was determined by aCGH with 59 genetic loci commonly amplified in human tumors. DNA copy number gains were most frequently observed for *PIK3CA* on 3q (66%), *PAK1* on 11q (59%), *KRAS2* on 12p (55%), and *STK15* on 20q (55%). The 11q13-q14 amplicon, represented by six oncogenes (*CCND1*, *FGF4*, *FGF3*, *EMS1*, *GARP*, and *PAK1*) revealed preferential gene copy number gains of *PAK1*, which is located at 11q13.5-q14. Amplification and protein expression status of both *PAK1* and *CCND1* were further examined by fluorescence *in situ* hybridization and immunohistochemistry using a tissue microarray consisting of 268 primary ovarian tumors. *PAK1* copy number gains were observed in 30% of the ovarian carcinomas and *PAK1* protein was expressed in 85% of the tumors. *PAK1* gains were associated with high grade ($P < 0.05$). In contrast, *CCND1* gene alterations and protein expression were less frequent (10.6% and 25%, respectively), suggesting that the critical oncogene target of amplicon 11q13-14 lies distal to *CCND1*. This study demonstrates that aCGH facilitates

further characterization of oncogene candidates residing in amplicons defined by mCGH. (*Am J Pathol* 2003, 163:985-992)

Ovarian carcinoma is the second most common gynecological tumor type in industrial countries. Asymptomatic clinical features and the lack of suitable screening tools account for its high mortality rate.¹ Recent studies characterizing genetic aberrations in ovarian cancer have implicated a number of oncogenes and tumor suppressor genes in ovarian tumor development.² The details of the genetic changes in ovarian tumorigenesis, however, are not well understood.

One of the major challenges in cancer research aims at generating molecular profiles of tumors to establish correlations between genetic changes and clinical parameters by screening technologies. Metaphase comparative genomic hybridization (mCGH) allows the detection of chromosomal aberrations across the entire genome with a maximal resolution of >10 Mb.³ The application of new DNA-microarray technologies and the structural information being available on all human genes permit a more detailed and simultaneous quantification of gene transcripts or genomic DNA copy numbers of many DNA sequences. Arrays with selected clones of genomic DNA sequences are used for array CGH (aCGH). These microchips may be applied for comprehensive, tumor type-specific amplicon profiling of many oncogenes^{4,5} as well as for quantitative mapping of amplicon structures.^{6,7}

Previous mCGH studies showed that chromosomal copy number gains are common in ovarian cancer. High-level amplifications were mainly observed in late-stage tumors,⁸ suggesting an important role of oncogenes for ovarian tumor progression. A limited number of known oncogenes residing in some of these chromosomal regions were found amplified such as *MYC* at 8q24,⁹⁻¹¹ *KRAS* at 12p12.1,^{12,13} *CCND2* at 12p13,¹⁴ *HER2* at

Supported by the Krebsforschung Schweiz grant KFS 1090-09-2000.

Accepted for publication May 30, 2003.

Address reprint requests to Peter Schraml, Ph.D., Institute of Pathology, University Hospital, Schönbeinstrasse 40, 4031 Basel, Switzerland. E-mail: peter.schraml@unibas.ch.

17q21,^{12,15} and *AIB1* at 20q12-13.¹⁶ However, the target genes of most amplifications are still unknown. The aims of this study were to screen ovarian tumors for potential oncogene candidates by mCGH and aCGH. Using a tissue microarray (TMA) containing 268 ovarian tumors, we specifically examined amplification and protein expression of two oncogenes and their clinicopathological impact for ovarian cancer.

Materials and Methods

Tumor Samples and Cell Lines

Frozen tumor specimens were obtained from the archives of the Institute of Pathology, Mannheim, Germany. All ovarian tumor samples were histologically reviewed. Twenty-six ovarian tumor specimens with a minimum of 75% tumor cells in the sample and without necrosis were selected for the study on the basis of hematoxylin and eosin-stained tissue sections. Tumors were staged according to the International Federation of Gynecology and Obstetrics criteria for ovarian cancer.¹⁷ The histological subtype was defined according to the World Health Organization.¹⁸ There were 14 serous, 3 clear cell, 3 endometrioid, 3 mucinous carcinomas, 1 müllerian-mixed tumor, and 2 undifferentiated ovarian tumors. One patient had stage I, 1 stage II, 16 stage III, and 3 patients stage IV disease. There were 8 grade 1, 13 grade 2, and 5 grade 3 tumors. The International Federation of Gynecology and Obstetrics stage of five tumors could not be defined retrospectively because of missing clinical information. Cell lines CRL-1978, CRL-10303, and HTB-161 (American Type Culture Collection, Manassas, VA) were cultured with OPTI-MEM (Invitrogen AG, Basel, Switzerland) supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (BioConcept, Allschwil, Switzerland).

mCGH

Tissue preparation and extraction of high-molecular weight DNA from the primary tumors and the cancer cell lines for mCGH analysis was as previously described.¹⁹ One μg of tumor DNA was nick translated by using a commercial kit (BioNick kit; Life Technologies, Gaithersburg, MD) and Spectrum Green-dUTPs (Vysis Inc., Downers Grove, IL) for direct labeling of tumor DNA. Spectrum Red-labeled normal reference DNA (Vysis) was used for co-hybridization. The hybridization mixture consisted of 200 ng of Spectrum Green-labeled tumor DNA, 200 ng of Spectrum Red-labeled normal reference DNA, and 20 μg of Cot-1 DNA (Invitrogen AG) dissolved in 10 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2 $\times$ standard saline citrate, pH 7.0). Hybridization, image acquisition, image analysis, and control experiments were exactly as described.¹⁹ Thresholds used for definition of DNA sequence copy number gains and losses were >1.2 and <0.8 , respectively. Overrepresentations were considered amplifications when the fluorescence ratio values exceeded 1.5 in a subregion of

a chromosome arm according to previous recommendations.²⁰

aCGH

DNA for aCGH was prepared using the Gentra Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). DNA quality was verified on a 1% agarose gel and DNA concentration was determined spectrophotometrically. aCGH was performed using the AmpliOnc I microarray kit (Vysis). Briefly, tumor and cell line DNA were labeled by nick translation with Alexa Fluor 488-5-dUTP (Molecular Probes, Leiden, The Netherlands) and the normal reference DNA was labeled with Alexa Fluor 594-5-dUTP (Molecular Probes). Labeled test-DNA and normal reference DNA (0.5 μg each) were mixed in 30 μl of hybridization buffer (50% formamide, 10% dextran sulfate, 2 $\times$ standard saline citrate, 0.1 mg Cot-1 DNA, pH 7.0) and hybridized to AmpliOnc I microarray (Vysis). The chip contained 59 different DNA sequences deriving from P1, PAC, or BAC. Each clone was represented by three target spots. Arrays were hybridized for 16 hours, washed three times for 10 minutes in 50% formamide, 2 $\times$ standard saline citrate at 40°C, followed by four times 5 minutes in 1 $\times$ standard saline citrate at room temperature, and counterstained with DAPI IV mounting solution (Vysis). Imaging and data analysis of the arrays was implemented with the GenoSensor Reader System (Vysis). The software automatically captured three images of each chip, specific for the blue, the green, and the red color planes. The test/reference ratio was defined as the ratio of the sum of test intensity pixel values to the sum of reference intensity pixel values (mass ratio) after pixel intensity analysis within each spot and local background subtraction. The values were further normalized by dividing test/reference mass ratios by the test/reference ratio obtained from modal DNA copy numbers.

Validation of aCGH

Variations of the green and red ratios on the arrayed oncogenes were defined by three comparative hybridizations using normal male DNA as test and reference DNA (Vysis). The green/red fluorescence ratios for each spotted genomic clone ranged from 0.92 to 1.12 indicating consistent and reproducible hybridization for all 59 DNA targets on the AmpliOnc I microarray. Normal male DNA as reference was used as an additional control to confirm the 1:1 ratio of chromosome X copies in tumors with chromosome X loss and the 1:2 ratio of chromosome X copies in tumors without chromosome X loss. As expected, 11 ovarian tumors with complete Xq loss showed averaged fluorescence mean ratios of the *AR* gene (*AR* 3' and *AR* 5') ranging from 1.00 to 1.19 confirming loss of one *AR* copy in these tumors. The green/red ratio of *AR* ranged from 1.26 to 1.65 in four tumors with partial Xq deletions without affecting the *AR* locus Xq11-q12, in 13 tumors that retained two chromosome X copies, and in 1 tumor with increased copy number in the telomeric region of Xq. Based on these findings aCGH fluorescence ratio

Figure 1. A: The ovarian carcinoma TMA (overview) and two array elements ($\times 40$ -fold, H&E staining) with a serous (B) and a mucinous (C) adenocarcinoma.

thresholds used for definition of DNA sequence copy number gains was >1.20 . Ratios >2.0 were regarded as amplifications according to the recommendations of the standardized protocols (Vysis). A loss of DNA sequences was presumed at chromosomal regions in which the tumor-to-normal ratio was <0.80 . Similar values were used in two previous studies.^{4,5}

TMA

The ovarian carcinoma TMA was constructed as previously described.²¹ It contained 120 serous, 68 endometrioid, 40 mucinous, 24 clear cell, and 16 undifferentiated ovarian tumors (Figure 1). All tumors were reviewed by one pathologist (HM). There were 86 grade 1, 87 grade 2, and 89 grade 3 tumors. Fifty-two tumors were stage pT1, 28 pT2, 117 pT3, and 11 were pT4. Histological tumor grading and TNM staging was done as described in Tumor Samples and Cell Lines. Tumor-specific survival data were obtained by reviewing the hospital records, by direct communication with the attending physicians, and from the Cancer Registry of Basel.

Fluorescence in Situ Hybridization (FISH)

The TMA sections were treated according to the Paraffin Pretreatment Reagent Kit protocol (Vysis) before hybridization. FISH was performed with Spectrum Orange-labeled *CCND1* and *PAK1* probes. Spectrum Green-labeled chromosome 11 centromeric probe CEP11 was used as a reference (Vysis). Hybridization and posthybridization washes were according to the LSI procedure (Vysis). Slides were counterstained with 125 ng/ml of 4', 6-diamino-2-phenylindole in anti-fade solution. FISH signals were scored with a Zeiss fluorescence microscope equipped with double-band pass filters for simultaneous visualization of Spectrum Green and Spectrum Orange signals (Vysis). Gain was assumed in tumors in which there were more *CCND1* or *PAK1* signals than centromere 11 signals in at least 50% of the cells. Amplification

was defined as presence of more than 10 gene signals, or tight clusters of at least five gene signals, or more than three times as many *CCND1* or *PAK1* than centromere 11 signals in $\geq 5\%$ of tumor cells.

Immunohistochemistry

Standard indirect immunoperoxidase procedures were used for immunohistochemistry (ABC-Elite; Vector Laboratories, Burlingame, CA). A monoclonal antibody to human *CCND1* (clone P2D11F11, microwave oven, pronase III; Ventana, Tucson, AZ) and a polyclonal antibody to *PAK1* (1:50 diluted, microwave oven; Cell Signaling Technology, Beverly, MA) were used for detection of *CCND1* and *PAK1* expression. Diaminobenzidine was used as a chromogen. The primary antibody was omitted for negative controls. Tumors were considered positive if cytoplasmic (*PAK1*) or nuclear (*CCND1*) expression was found.

Statistics

Contingency table analysis was used to analyze the association between *CCND1* or *PAK1* alterations and tumor grade, stage, and allelic loss. Survival analyses were performed using the Kaplan-Meier method. Statistical differences between groups were determined with log-rank test. A Cox proportional hazard analysis was used to test for independent prognostic information.

Results

mCGH

mCGH showed chromosomal aberrations in all 29 ovarian tumors. DNA sequence copy number gains (mean 4.2 ± 3.6 /tumor) were slightly more frequent than deletions (3.8 ± 3.8 /tumor). The most common overrepresentations were seen at 8q (52%), 20q (52%), 5p (38%), 3q (38%), 2q (31%), 11q (21%), and 12p (21%). Chromosomal deletions were most prevalent at Xq (52%); Xp (45%); 4q (41%); 5q, 9p, and 18q (35%); and 17p (31%).

aCGH

A detailed oncogene profiling was obtained from all primary tumors and cell lines. An image of a hybridized microarray is shown in Figure 2. Fluorescent ratios of green-labeled tumor and normal red-labeled DNA ranged from 1.21 to 5.54 for overrepresentations and from 0.79 to 0.53 for deletions. Highest frequency of allelic gains was seen for *PIK3CA* (66%), *PAK1*, *STK15*, *KRAS2* (55% each), *CCND2* (41%), *EGFR* (38%), and *MYC*, *AIB1*, *JUNB* (35% each). Amplifications were observed on *AIB1* and *PTPN1* in one tumor; *AIB1* and *ZNF217* in one tumor; *MYC* in one tumor and CRL-10303; *ERBB2* and *MET* in one tumor each; *CCND1*, *FGF4/FGF3*, and *EMS1* in CRL-1987; *GARP* and *PAK1* in HTB-161. The result of aCGH analysis is shown in Figure 3.

Figure 2. Image of an AmpliOnc I microarray after hybridization of 59 genomic loci. Oncogenes *CCND1*, *FGF4/FGF3*, and *EMS1* located on 11q13 are amplified.

Comparing mCGH with aCGH data revealed high concordance between increased copy numbers of chromosomal regions and gains/amplifications of oncogenes located in these amplicons. Overall, we were able to compare 134 of 198 overrepresentations detected by mCGH in 29 ovarian tumors with data obtained by aCGH. Eighty of 134 (60%) gains identified by mCGH were confirmed by aCGH. Interestingly, chromosomal deletions observed by mCGH correlated well with corresponding DNA sequence copy number losses seen by aCGH. Gained and lost copies of oncogenes and the corresponding mCGH profiles yielded from one ovarian tumor are shown in Figure 4.

Whereas most ovarian carcinomas showed gains of large chromosomal areas, regionary high-level amplifications were rare. Oncogenes located within one amplicon were further analyzed to determine the presence of co-

gains/co-amplifications. Overrepresentations of at least two adjacent genes were seen at 3q26.3, 11q13-q14, 12p12-p13, and 20q12-q13. There were six oncogenes at 11q13-q14. *PAK1* showed preferential copy number gains emphasizing the differing relevance of neighboring oncogenes for ovarian cancer (Figure 3). Gains and amplifications of oncogenes occurred in all stages and subtypes of ovarian cancer. There were no associations of specific oncogenes with tumor grade, tumor stage, and histological subtypes.

FISH

High-quality hybridization signals for centromeric and gene-specific probes were obtained in 77 (*PAK1*) and 160 (*CCND1*) ovarian carcinomas, respectively. Twenty-three ovarian carcinomas (30%) showed *PAK1* gains, but only seven (10.6%) of the tumors had increased copy number of *CCND1*. *PAK1* was found amplified in one serous ovarian adenocarcinoma. None of the tumors revealed a *CCND1* amplification. Examples of tumors with *PAK1* amplification and *CCND1* gain are shown in Figure 5, A and B. *PAK1* gains were associated with the differentiation grade ($P < 0.05$). Most undifferentiated ovarian carcinomas (83%) displayed *PAK1* gain (Table 1).

Immunohistochemistry

Strong cytoplasmic *PAK1* protein expression was observed in 172 of 199 ovarian cancers. *PAK1* protein was most frequent in grade 3 tumors and was present in all undifferentiated ovarian carcinomas, but this trend was not significant. Nuclear *CCND1* expression was seen in

Figure 3. aCGH results of 26 primary ovarian carcinomas and three ovarian carcinoma cell lines.

Figure 4. Comparison of aCGH and mCGH data obtained from tumor 4. aCGH (left): Amplified genes are represented in dark green, genes with gained copy numbers in green, and deleted genes in red columns. mCGH (right): Green and red bars indicate deleted or gained chromosomal regions (mean ratio + SD obtained from at least four chromosomes). Asterisk: Gene loss or gain not corresponding with mCGH profile.

65 of 260 (25%) tumors (Table 1). Examples of positive cases are shown in Figure 5, C and D. The association between *PAK1* overexpression and gene gain/amplification could be analyzed in 72 ovarian cancers with interpretable immunohistochemistry and FISH results on consecutive sections. This analysis revealed no significant association between *PAK1* copy number increase and overexpression.

Discussion

aCGH revealed frequent gene copy number gains of *PIK3CA* at 3q26.3, *PAK1* at 11q13.5-q14, *KRAS2* at 12p12.1, and *STK15* at 20q13 in ovarian carcinomas. Gene amplifications were detected for *MET* on 7q; *MYC* on 8q; *CCND1*, *FGF4/FGF3*, *EMS1*, *GARP*, and *PAK1* on 11q; *ERBB2* on 17q; and *AIB1*, *PTPN1*, *ZNF217* on 20q. These results correlated well with the mCGH showing frequent overrepresentations on chromosome 3q, 8q, 11q, 12p, 17q, and 20q. Previous mCGH studies of ovar-

ian tumors have reported similar findings.⁸ Our mCGH data further suggested that gains and low-level amplifications of different chromosomal regions are a common phenomenon in the pathogenesis of ovarian cancer, but that specific oncogenes are the targets of such large chromosomal copy number gains. Examples of oncogenes located in large amplicons, which were preferentially gained include *PIK3CA* at 3q26.3, *PAK1* at 11q13.5-14, *JUNB* at 19p13.2, *AIB1* and *STK15* at 20q12-13. The dominating role of *AIB1* within the 20q12-13 amplicon has recently been shown in ovarian cancer.²² One might therefore speculate that the remaining oncogenes in these amplicons, eg, *TERC* on 3q26.3; *CCND1*, *FGF4/FGF3*, *EMS1*, and *GARP* on 11q13; *INSR* on 19p13; *MYBL2*, *PTPN1*, and *ZNF217* on 20q13 are of minor biological relevance for ovarian cancer.

On the basis of our aCGH data, *PAK1* seems to be an important genetic target of the 11q13.5-14 amplicon. Whereas *CCND1* on 11q13.3 is a well-described oncogene in this region, which has been implicated in many human cancers, little is known about *PAK1* aberrations in ovarian cancer. The striking difference of *PAK1* and *CCND1* gain frequencies was decisive to further examine and validate *PAK1* and *CCND1* gene copy number alterations and protein expression on an ovarian carcinoma TMA.

11q13.5-q14 amplifications involving the *PAK1* locus have been recently reported in bladder²³ and breast cancer.²⁴ Our FISH analysis demonstrated a high percentage of tumors with *PAK1* gains including one tumor with a high-level amplification. This result was consistent with the data obtained from the aCGH. The high number of *PAK1* protein expression in ovarian carcinoma further supports the theory that *PAK1* is an important oncogene for ovarian cancer.

A number of functional properties being characteristic for an oncogene have recently been reported for *PAK1* in breast cancer. *PAK1* is a member of a family of serine/threonine kinases and regulates anchorage-independent growth, invasiveness, and abnormal organization of mitotic spindles of human epithelial breast cancer cells.²⁵ *PAK1* promotes hyperplasia in mammary epithelium by phosphorylating and transactivating estrogen receptor- α .²⁶ It is also involved in angiogenesis by controlling vascular endothelial growth factor expression, vascular endothelial growth factor secretion, and function.²⁷

The TMA approach used in this study allowed us to evaluate the associations between *PAK1* alterations and clinicopathological parameters because the number of tumors was large enough to perform statistical analyses. Importantly, *PAK1* expression and copy number gains were seen in all undifferentiated ovarian carcinomas and in many high-grade tumors suggesting that *PAK1* activation is responsible for the progression of ovarian cancer.

Interestingly, copy number increases of *PAK1* at 11q13.5-q14 were often associated with *MLL* loss at 11q23. The combination of 11q gain and 11q loss is suggestive of isochromosome formation and raises the question of whether the loss of *MLL* or the gain of *PAK1* is the more important alteration. Allelic loss at 11q23 was previously described in cervical neoplasia, breast, ovarian, and head and neck carcinomas.²⁸⁻³¹ Tumor growth

Figure 5. **A:** *PAK1* amplification: tumor cells with clusters of red *PAK1* signals and two green centromere 11 signals. **B:** *CCND1* gain with four to six red *CCND1* signals and two to four centromere 11 signals (green) in an ovarian carcinoma on the TMA. **C:** Cytoplasmic *PAK1* protein expression in an ovarian carcinoma. **D:** Nuclear *CCND1* protein expression in an ovarian carcinoma.

suppression by transfecting murine fibrosarcoma cells with 11q23.1-containing YACs indicates a tumor suppressor gene locus in close vicinity to *MLL*.³¹ Because some *PAK1* gains were not associated with *MLL* losses, increased copy numbers of *PAK1* may be more significant for ovarian cancer than *MLL* loss. On the basis of its known function, it is highly unlikely that *MLL* is the target gene of the 11q deletion.

Our aCGH and FISH results for *CCND1* on 11q13 are consistent with recent studies showing that *CCND1* amplification is rare in ovarian cancer.^{14,32} Although *CCND1* amplification is uncommon in ovarian cancer, nuclear *CCND1* overexpression has been reported in 15 to 30% of ovarian tumors^{33,34} indicating that other molecular mechanisms are responsible for gene up-regulation. Our results support this finding because 14 of the 65 ovarian tumors with strong *CCND1* protein expression had elevated gene copy numbers. Interestingly a co-gain of

CCND1 and *PAK1* was observed in 13 of 17 tumors. We hypothesize that *CCND1* gain seems to play a minor role in ovarian cancer.

In summary, the aCGH technique permits to precisely characterize the amplification status of specific oncogenes and to perform detailed regional amplicon mapping. This study demonstrates that combined application of tumor-related gene chips and TMAs facilitates the identification of new genetic targets in human tumors and will lead to a better understanding of the key events in the pathogenesis of cancer.

Acknowledgments

We thank Rita Epper, Susanne Griesshaber, Yvonne Knecht, Martina Mirlacher, Hedvika Novotny, and Sandra Schneider for their excellent technical support.

Table 1. PAK1/CCND1 Gain and Expression and Tumor Phenotype

	PAK1 gain			CCND1 gain			PAK1 protein expression			CCND1 protein expression		
	<i>n</i>	%	<i>P</i> value	<i>n</i>	%	<i>P</i> value	<i>n</i>	%	<i>P</i> value	<i>n</i>	%	<i>P</i> value
Stage												
pT1	10	25		38	5		40	82		51	22	
pT2	12	20		26	8		20	75		27	22	
pT3	41	52		60	10		89	89		112	29	
pT4	4	0	n.s.	3	0	n.s.	11	100	n.s.	11	27	n.s.
Grade												
G1	16	19		60	7		67	76		83	29	
G2	25	12		67	4		66	83		85	20	
G3	34	44	<0.02	65	14	n.s.	66	92	n.s.	87	26	n.s.
Histologic type												
Serous	38	32		71	11		84	90		115	32	
Mucinous	6	17		25	4		31	71		40	13	
Endometrioid	19	21		41	7		59	83		67	21	
Clear cell	8	14		14	7		12	71		23	22	
Undifferentiated	6	83	<0.05	9	44	n.s.	13	100	n.s.	15	27	n.s.

References

- Boring CC, Squires TS, Tong T, Montgomery S: Cancer statistics, 1994. *CA Cancer J Clin* 1994, 44:7-26
- Shridhar V, Lee J, Pandita A, Iturria S, Avula R, Staub J, Morrissey M, Calhoun E, Sen A, Kalli K, Keeney G, Roche P, Cliby W, Lu K, Schmandt R, Mills GB, Bast Jr RC, James CD, Couch FJ, Hartmann LC, Lillie J, Smith DI: Genetic analysis of early- versus late-stage ovarian tumors. *Cancer Res* 2001, 61:5895-5904
- Isola J, Visakorpi T, Holli K, Kallioniemi OP: Association of overexpression of tumor suppressor protein p53 with rapid cell proliferation and poor prognosis in node-negative breast cancer patients. *J Natl Cancer Inst* 1992, 84:1109-1114
- Daigo Y, Chin SF, Gorringer KL, Bobrow LG, Ponder BA, Pharoah PD, Caldas C: Degenerate oligonucleotide primed-polymerase chain reaction-based array comparative genomic hybridization for extensive amplicon profiling of breast cancers: a new approach for the molecular analysis of paraffin-embedded cancer tissue. *Am J Pathol* 2001, 158:1623-1631
- Hui AB, Lo KW, Yin XL, Poon WS, Ng HK: Detection of multiple gene amplifications in glioblastoma multiforme using array-based comparative genomic hybridization. *Lab Invest* 2001, 81:717-723
- Albertson DG, Ylstra B, Segreaves R, Collins C, Dairkee SH, Kowbel D, Kuo WL, Gray JW, Pinkel D: Quantitative mapping of amplicon structure by array CGH identifies CYP24 as a candidate oncogene. *Nat Genet* 2000, 25:144-146
- Pinkel D, Segreaves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG: High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 1998, 20:207-211
- Knuutila S, Autio K, Aalto Y: Online access to CGH data of DNA sequence copy number changes. *Am J Pathol* 2000, 157:689
- Abeyasinghe HR, Cedrone E, Tyan T, Xu J, Wang N: Amplification of C-MYC as the origin of the homogeneous staining region in ovarian carcinoma detected by micro-FISH. *Cancer Genet Cytogenet* 1999, 114:136-143
- Wang ZR, Liu W, Smith ST, Parrish RS, Young SR: c-myc and chromosome 8 centromere studies of ovarian cancer by interphase FISH. *Exp Mol Pathol* 1999, 66:140-148
- Diebold J, Suchy B, Baretton GB, Blasenbren S, Meier W, Schmidt M, Rabes H, Lohrs U: DNA ploidy and MYC DNA amplification in ovarian carcinomas. Correlation with p53 and bcl-2 expression, proliferative activity and prognosis. *Virchows Arch* 1996, 429:221-227
- Bian M, Fan Q, Huang S, Ma J, Lang J: Amplifications of proto-oncogenes in ovarian carcinoma. *Chin Med J* 1995, 108:844-848
- Yang-Feng TL, Li SB, Leung WY, Carcangiu ML, Schwartz PE: Trisomy 12 and K-ras-2 amplification in human ovarian tumors. *Int J Cancer* 1991, 48:678-681
- Courjal F, Louason G, Speiser P, Katsaros D, Zeillinger R, Theillet C: Cyclin gene amplification and overexpression in breast and ovarian cancers: evidence for the selection of cyclin D1 in breast and cyclin E in ovarian tumors. *Int J Cancer* 1996, 69:247-253
- Seki A, Yoshinouchi M, Seki N, Kodama J, Miyagi Y, Kudo T: Detection of c-erbB-2 and FGF-3 (INT-2) gene amplification in epithelial ovarian cancer. *Int J Oncol* 2000, 17:103-106
- Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, Trent JM, Meltzer PS: AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. *Science* 1997, 277:965-968
- Sobin LH, Wittekind C: TNM Classification of Malignant Tumours. New York, Wiley-Liss, 2002
- Scully RE, Sobin LH: Histological Typing of Ovarian Tumours. Berlin, Springer, 1999
- Jiang F, Richter J, Schraml P, Bubendorf L, Gasser T, Sauter G, Mihatsch MJ, Moch H: Chromosomal imbalances in papillary renal cell carcinoma: genetic differences between different subtypes. *Am J Pathol* 1998, 153:1467-1473
- Kallioniemi A, Kallioniemi OP, Piper J, Tanner M, Stokke T, Chen L, Smith HS, Pinkel D, Gray JW, Waldman FM: Detection and mapping of amplified DNA sequences in breast cancer by comparative genomic hybridization. *Proc Natl Acad Sci USA* 1994, 91:2156-2160
- Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998, 4:844-847
- Tanner MM, Grenman S, Koul A, Johannsson O, Meltzer P, Pejovic T, Borg A, Isola JJ: Frequent amplification of chromosomal region 20q12-q13 in ovarian cancer. *Clin Cancer Res* 2000, 6:1833-1839
- Simon R, Nocito A, Hubscher T, Bucher C, Torhorst J, Schraml P, Bubendorf L, Mihatsch MM, Moch H, Wilber K, Schotzau A, Kononen J, Sauter G: Patterns of her-2/neu amplification and overexpression in primary and metastatic breast cancer. *J Natl Cancer Inst* 2001, 93:1141-1146
- Bekri S, Adelaide J, Merscher S, Grosgeorge J, Caroli-Bosc F, Perucca-Lostanlen D, Kelley PM, Pebusque MJ, Theillet C, Birnbaum D, Gaudray P: Detailed map of a region commonly amplified at 11q13->q14 in human breast carcinoma. *Cytogenet Cell Genet* 1997, 79:125-131
- Vadlamudi RK, Adam L, Wang RA, Mandal M, Nguyen D, Sahin A, Chernoff J, Hung MC, Kumar R: Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. *J Biol Chem* 2000, 275:36238-36244
- Wang RA, Mazumdar A, Vadlamudi RK, Kumar R: P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. *EMBO J* 2002, 21:5437-5447
- Bagheri-Yarmand R, Vadlamudi RK, Wang RA, Mendelsohn J, Kumar

- R: Vascular endothelial growth factor up-regulation via p21-activated kinase-1 signaling regulates heregulin-beta1-mediated angiogenesis. *J Biol Chem* 2000, 275:39451–39457
28. Evans MF, Koreth J, Bakkenist CJ, Herrington CS, McGee JO: Allelic deletion at 11q23.3-q25 is an early event in cervical neoplasia. *Oncogene* 1998, 16:2557–2564
29. Jin Y, Hoglund M, Jin C, Martins C, Wennerberg J, Akervall J, Mandahl N, Mitelman F, Mertens F: FISH characterization of head and neck carcinomas reveals that amplification of band 11q13 is associated with deletion of distal 11q. *Genes Chromosom Cancer* 1998, 22:312–320
30. Launonen V, Stenback F, Puistola U, Bloigu R, Huusko P, Kytola S, Kauppila A, Winqvist R: Chromosome 11q22.3-q25 LOH in ovarian cancer: association with a more aggressive disease course and involved subregions. *Gynecol Oncol* 1998, 71:299–304
31. Koreth J, Bakkenist CJ, Larin Z, Hunt NC, James MR, McGee JO: 11q23.1 and 11q25-qter YACs suppress tumour growth in vivo. *Oncogene* 1999, 18:1157–1164
32. Schraml P, Kononen J, Bubendorf L, Moch H, Bissig H, Nocito A, Mihatsch M, Kallioniemi O, Sauter G: Tissue microarrays for gene amplification surveys in many different tumor types. *Clin Cancer Res* 1999, 5:1966–1975
33. Kusume T, Tsuda H, Kawabata M, Inoue T, Umesaki N, Suzuki T, Yamamoto K: The p16-cyclin D1/CDK4-pRb pathway and clinical outcome in epithelial ovarian cancer. *Clin Cancer Res* 1999, 5:4152–4157
34. Dhar KK, Branigan K, Parkes J, Howells RE, Hand P, Musgrove C, Strange RC, Fryer AA, Redman CW, Hoban PR: Expression and subcellular localization of cyclin D1 protein in epithelial ovarian tumour cells. *Br J Cancer* 1999, 81:1174–1181