Genetic and Molecular Changes in Serous Gynecological Carcinomas

– comparison with other histological types, and clinical associations

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Academic Dissertation

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List of original publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals.

I Pere H, Tapper J, Wahlström T, Knuutila S, Butzow R: Distinct chromosomal imbalances in uterine serous and endometrioid carcinomas. Cancer Res 58: 892-895, 1998

II Pere H*, Tapper J*, Seppälä M, Knuutila S, Butzow R: Genomic alterations in fallopian tube carcinoma: comparison to serous uterine and ovarian carcinomas reveals similarity suggesting likeness in molecular pathogenesis. Cancer Res 58: 4274-4276, 1998

III Lassus H, Laitinen MP, Anttonen M, Heikinheimo M, Aaltonen LA, Ritvos O, Butzow R: Comparison of serous and mucinous ovarian carcinomas: distinct pattern of allelic loss at distal 8p and expression of transcription factor GATA-4. Lab Invest 81: 517-526, 2001

IV Lassus H, Salovaara R, Aaltonen LA, Butzow R: Allelic analysis of serous ovarian carcinoma reveals two putative tumor suppressor loci at 18q22-q23 distal to *SMAD4*, *SMAD2* and *DCC*. Am J Pathol 159: 35-42, 2001

V Lassus H, Leminen A, Lundin J, Lehtovirta P, Butzow R: P53 expression status – a useful prognostic marker in serous ovarian carcinoma. Submitted.

* These authors contributed equally to the study.

Abbreviations

AIB1	amplified in breast cancer 1 gene
AKT2	v-akt murine thymoma viral oncogene homolog 2
BRCA1	breast cancer gene 1
BRCA2	breast cancer gene 2
CA125	ovarian carcinoma antigen 125
cDNA	complementary deoxyribonucleic acid
CGH	comparative genomic hybridization
CMET	hepatocyte growth factor receptor gene
CMYC	avian myelocytomatosis viral oncogene homolog
DAPI	4', 6'-diamidino-2-phenylindole
dCTP	deoxycytidine triphosphate
DCC	deleted in colon cancer gene
dUTP	deoxyuridine triphosphate
EIC	endometrial intraepithelial carcinoma
EIF-5A2	eukaryotic initiation factor 5A2 gene
ERBB2	avian erythroblastic leukemia viral oncogene homolog 2 (alias: *HER2/ NEU*)
FIGO	International Federation of Obstetrics and Gynecology
FITC	fluorescein isothiocyanate
FISH	fluorescence in situ hybridization
GATA4	GATA-binding protein 4 gene
HNPCC	hereditary non-polyposis colorectal cancer
INT2	fibroblast growth factor 3, murine mammary tumor virus integration site (v-int-2) oncogene homolog (alias: *FGF3*)
KRAS	Kirsten rat sarcoma 2 viral oncogene homolog
LOH	loss of heterozygosity
Mb	megabase
MLH1	mutL (E. coli) homolog 1 gene
MIS	Müllerian inhibiting substance gene (alias: *AMH*)
mRNA	messenger ribonucleic acid
MTS1	cyclin-dependent kinase inhibitor 2A gene (alias: *CDKN2A/ p16*)
MSI	microsatellite instability
p	short arm of the chromosome
P53	gene for tumor protein p53 (alias: *TP53*)
PCR	polymerase chain reaction
PIK3CA	phosphatidylinositol 3-kinase gene
PTEN	phosphatase and tensin homolog gene (alias: *MMAC1*)
q	long arm of the chromosome
RB	retinoblastoma 1 gene
RFLP	restriction fragment length polymorphism
SMAD2	MAD (mothers against decapentaplegic) homolog 2 gene (alias: *MADH2*)
SMAD4	MAD (mothers against decapentaplegic) homolog 4 gene (alias: *MADH4*)
SNP	single-nucleotide polymorphism
TGF	transforming growth factor
TRITC	tetrarhodamine isothiocyanate
WT1	Wilms tumor 1 gene

Abstract

The aim of the present study was to identify chromosomal and molecular changes in endometrial, fallopian tube and ovarian carcinomas, with emphasis on the serous histological type. More detailed mapping of chromosomal regions that showed frequent losses in comparative genomic hybridization was performed using allelic analysis. The expression of known and potential tumor suppressor genes was examined by Northern blotting and immunohistochemical staining of ovarian carcinoma tissue microarrays. To understand the relationship between genetic and molecular changes and the biological and clinical behavior of the tumors, associations of the changes with clinicopathological characteristics and outcome of the patients were evaluated.

Comparative genomic hybridization analyses revealed distinct chromosomal changes in serous and endometrioid endometrial carcinomas. The changes were frequent and complex in serous carcinoma, which showed recurrent copy number gains at 3q, 8q, 5p, 6p and 1q. In the endometrioid type, the changes were less common and the most frequent aberration was gain at chromosome arm 1q. In the serous type, the number of alterations was associated with patient survival. These findings are in line with the aggressive behavior of serous carcinoma, and suggest distinct genetic backgrounds for these two histological types of endometrial carcinoma.

In serous fallopian tube carcinoma, recurrent and complex chromosomal alterations were identified, the most common regions of increased copy number being at 3q, 8q, 1q, 5p, 7q and 12p, and decreased copy number at 8p and 18q. The changes found were compared with those detected in serous carcinomas of the endometrium and ovary. The patterns of genomic alterations found in these serous carcinomas were very similar, suggesting that their molecular pathogeneses may be alike.

Allelotype analyses of distal 8p and distal 18q revealed more frequent and extensive allelic losses in serous than in mucinous ovarian carcinomas, which is in keeping with distinct molecular backgrounds of these carcinomas. Both LOH at 8p and 18q were associated with the grade of serous carcinomas, and LOH at 18q also with patient survival. In serous carcinoma, minimal common regions of loss, potential locations of tumor suppressor genes, were defined: three at 8p21.1-p23.1 and two at 18q22-q23. Expression of a transcription factor gene, *GATA4*, located at 8p23.1, was found to be lost in most serous carcinomas, but retained in the majority of mucinous carcinomas. The expression of each of three candidate tumor suppressor genes, *SMAD4*, *SMAD2* and *DCC*, located at 18q21.1, was reduced or lost in approximately 30% of serous carcinomas. An association between allelic loss at 18q21.1 and expression status of SMAD4, SMAD2 and DCC was found, but there was still a proportion of tumors showing LOH without loss of expression of these genes, supporting the existence of other tumor suppressor genes more distally at 18q.

Immunohistochemical staining of P53 protein in tissue microarrays showed weak immunopositivity in a proportion of normal epithelial cells and a similar pattern of staining in 41% of serous ovarian carcinomas. Two distinct patterns of aberrant P53 staining were identified in the carcinomas: excessive staining in 43% and completely negative staining in 16%. Both of these aberrant patterns of P53 staining were as-

sociated with aggressive clinicopathological characteristics of the tumors and poor overall survival. In multivariate analysis, P53 expression status was identified as an independent prognostic factor for overall survival. In addition, aberrant P53 expression was associated with a poor response to therapy and a shorter disease-free survival period. Both in stage I and stage III serous ovarian carcinomas, P53 expression status showed a potential to serve as a useful prognostic marker.

Gynecological carcinomas are heterogeneous diseases, and understanding of their molecular pathogenesis is needed for development of more individual cancer therapies. The similarity of changes detected in serous carcinomas of various gynecological organs and distinctiveness versus changes found in other histological types provides better understanding of the biological behavior and underlines the importance of histological type in classification of these carcinomas. In addition to understanding the biology of the disease, molecular markers are needed for predicting the outcome of individual patients and making treatment decisions. In the future, knowledge of the genomic sequence and high-throughput expression analyses will aid in discovery of the underlying genes located in the regions defined in the present study.

Introduction

Uterine and ovarian cancers are the third and the fourth most common cancers among women in Finland, whereas fallopian tube cancer is a relatively rare disease. Most cancers of the ovary, the fallopian tube and the uterus are of epithelial origin, i.e. carcinomas. The epithelia of these three organs have a common embryological background and contain cells that have the potential to differentiate along the same Müllerian pathways (Kaufman, 1992; Salazar et al., 1995). Thus, similar histological types of carcinoma, including serous, endometrioid and mucinous carcinomas, are found in these organs. Serous carcinoma is the predominant histological type in the ovary and the fallopian tube, whereas in the endometrium it is the second most common type (Kurman, 1994). The overall outcome in cases of endometrial carcinoma is relatively good due to early detection of the disease (Creasman et al., 2001). In contrast, ovarian and fallopian tube carcinomas carry poor prognosis, which is related to delay in detection, leading to advanced stages at diagnosis (Heintz et al., 2001a; Heintz et al., 2001b). Traditionally, classification and treatment of gynecological carcinomas has been based on the organ of origin. However, various histological types of carcinoma in these organs differ in respect to their associated risk factors and biological behavior (Bokhman, 1983; Omura et al., 1991; Risch et al., 1996).

Knowledge of the genetic and molecular alterations in gynecological carcinomas is needed for better understanding of the biology of the diseases and improvement of classification and treatment modalities. Evidence of distinct molecular backgrounds exists for different histological types of carcinoma in these organs, but most of the previous literature has covered various histological types together. In recent years, introduction of genome-wide screening techniques and array-based methods has facilitated identification of chromosomal and molecular alterations in solid tumors. The aim of this thesis was to characterize chromosomal and molecular changes in endometrial, fallopian tube and ovarian carcinomas, with emphasis on the serous histological type, and to evaluate associations between genetic changes, clinicopathological parameters and patient outcome.

Review of the literature

1. Clinical and histopathological characteristics

1.1. Embryological origin

There is a common embryological background for the ovarian surface epithelium and the epithelial lining of fallopian tubes, endometrium and endocervix. During embryonic development coelomic epithelium invaginates lateral to the gonadal ridge to form the Müllerian duct system. Müllerian ducts differentiate later to the fallopian tubes, the uterus and the upper part of the vagina. The ovary is covered by coelomic mesothelium which overlies the gonadal ridge (Salazar et al., 1995). In the mouse, it has been shown that in addition to this coelomic covering, the ovaries are enveloped later by Müllerian ducts (Kaufman, 1992). Thus, ovarian surface epithelium is of common origin with epithelia of the fallopian tubes and endometrium, due to a common coelomic or Müllerian background. In adult women, the epithelia of these organs contain cells that have the potential to differentiate along distinct Müllerian pathways and to develop into serous, endometrioid and mucinous tumors resembling epithelia of the fallopian tube, uterus and endocervix (Salazar et al., 1995).

1.2. Ovarian carcinoma

In Finland, 580 new cases of ovarian cancer were diagnosed in 1998. The age-standardized incidence was 13.3 per 100 000 person-years (adjusted for age to the "world standard population") (The Finnish Cancer Registry; http://www.cancerregistry.fi). The mean age at diagnosis is 62 years (Dickman et al., 1999). Epithelial ovarian cancer accounts for approximately 90% of ovarian malignancies. The most common histological type of ovarian carcinoma is serous, comprising over 50% of the cases. Mucinous and endometrioid types account for approximately 15% each. Less frequent histological types of ovarian carcinoma include clear cell carcinoma, undifferentiated carcinoma, malignant mixed epithelial tumor and malignant Brenner tumor (Kurman, 1994; Heintz et al., 2001b).

Multiparity, lactation, use of oral contraceptives, tubal ligation and hysterectomy are associated with a decreased risk of ovarian cancer (Whittemore et al., 1992; Hankinson et al., 1993). The risk factors have been reported to differ between histological subtypes: for example, the protective effects of parity and oral contraceptives appear not to involve mucinous carcinoma (Kvale et al., 1988; Risch et al., 1996). It has been estimated that about 5–10% of ovarian carcinomas are related to inherited predisposition. Known cancer-predisposing syndromes that are linked to ovarian carcinoma include breast and ovarian cancer syndrome (*BRCA1*/*BRCA2* genes) and hereditary non-polyposis colorectal cancer (HNPCC) syndrome (Boyd and Rubin, 1997).

The prognosis of ovarian carcinoma is poor, reflecting the frequent finding of advanced disease at diagnosis. The five-year overall survival rate is 48%, varying from 85% at stage I to 17% at stage IV (Heintz et al., 2001b). Mucinous carcinoma is associated with the best five-year survival rate (69%), whereas for serous and endometrioid carcinomas the rates are 40% and 60%, respectively (Heintz et al., 2001b). Compared with other histological types, it is typical of mucinous carcinoma to be associated with

a better prognosis at a low stage, but a worse prognosis in high stage disease (Omura et al., 1991; Vergote et al., 1993; Makar et al., 1995). Clear cell carcinoma is associated with the worst prognosis at all stages. In addition to FIGO stage and histological type, prognostic factors in ovarian carcinoma include histological grade, residual disease, performance status and age (Friedlander, 1998).

1.3. Endometrial carcinoma

In 1998, 738 new cases of uterine cancer were diagnosed in Finland. The age-standardized incidence was 15.5 per 100 000 person-years (adjusted for age to the "world standard population") (The Finnish Cancer Registry). Most patients are postmenopausal and the mean age at diagnosis is 66 years (Dickman et al., 1999). Epithelial malignancies represent over 90% of uterine cancers. Almost all of these are adenocarcinomas, and the most frequent histological type is endometrioid, seen in over 80% of cases. Serous carcinoma accounts for 5–10% of endometrial carcinomas. Other histological types include clear cell and mucinous. Foci of squamous differentation are found in the endometrioid, but not in the serous type (Kurman, 1994; Creasman et al., 2001).

Based on clinicopathological observations, two different categories of endometrial carcinoma have been described: type I (estrogen-dependent) and type II (estrogen-independent) (Bokhman, 1983; Deligdisch and Holinka, 1987). Type I tumors correspond to the endometrioid type of endometrial carcinoma, whereas type II tumors include serous carcinomas. Most of the risk factors for type I carcinomas are associated with excessive estrogen, which leads to continued stimulation of the endometrium. Risk factors for this type include obesity, unopposed exogenous estrogen, early menarche and late menopause, nulliparity, chronic anovulation, estrogen-producing tumors, diabetes and hypertension (Smith et al., 1975; Kelsey et al., 1982; Schwartz et al., 1985). No clear risk factors have been identified for type II carcinoma, which occurs in an older age group than type I carcinoma. It is frequently adjacent to atrophic endometrium and is not associated with hyperestrogenism (Bokhman, 1983; Deligdisch and Holinka, 1987). The known cancer-predisposing syndrome related to endometrioid endometrial carcinoma is HNPCC syndrome, which is linked to germline DNA mismatch repair gene mutations (Aarnio et al., 1995).

The majority of endometrial carcinomas are diagnosed at an early stage and the overall prognosis is good. The five-year overall survival rate is 77%, varying from 87% at stage I to 18% at stage IV (Creasman et al., 2001). Serous carcinomas are more advanced at the time of diagnosis and their prognosis tends to be worse at all stages compared with endometrioid carcinomas (Hendrickson et al., 1982; Creasman et al., 2001). The overall five-year survival rates are 54% and 80% for serous and endometrioid carcinomas, respectively (Creasman et al., 2001). In addition to stage and histological type of tumor, the histological grade, lymphovascular space involvement and patient age are of prognostic value in endometrial carcinoma (Connelly et al., 1982; Abeler and Kjorstad, 1991).

1.4. Fallopian tube carcinoma

Fallopian tube carcinoma is a relatively rare malignancy, with approximately 35 to 40 new cases diagnosed annually in Finland. In 1993–1997, the age-standardized incidence was 5.4 per 1 000 000 person-years (adjusted for age to the "world standard population") (The Finnish Cancer Registry). The mean age at diagnosis is approximately 62 years (Rosen et al., 1998; Baekelandt et al., 2000). The majority of fallopian tube carcinomas are of serous histology (Rosen et al., 1998; Baekelandt et al., 2000). Other histological types include endometrioid,

clear cell, mucinous, transitional cell and undifferentiated carcinomas (Alvarado-Cabrero et al., 1999; Baekelandt et al., 2000).

The prognosis of patients with fallopian tube carcinoma is poor. The five-year overall survival rate is approximately 45% (Rosen et al., 1998; Wolfson et al., 1998; Baekelandt et al., 2000; Heintz et al., 2001a), varying from 73% at stage I to 12% at stage IV (Baekelandt et al., 2000). Due to the relative rarity of the disease, most studies have included only limited numbers of cases. Findings concerning prognostic factors have varied, but FIGO stage, residual tumor size, histological grade and closure of the fimbriated end of the tube have shown independent prognostic value (Rosen et al., 1998; Alvarado-Cabrero et al., 1999; Baekelandt et al., 2000).

2. Cytogenetic and molecular genetic aberrations

2.1. Ovarian carcinoma

2.1.1. Cytogenetic findings

Most of the previous studies on cytogenetic and molecular changes in ovarian carcinoma have involved all histological types of carcinoma as a single disease entity. Cytogenetic analyses have revealed abnormal karyotypes in approximately 50–90% of ovarian carcinomas (Pejovic et al., 1992a; Pejovic et al., 1992b; Jenkins et al., 1993; Thompson et al., 1994a; Taetle et al., 1999b). The findings have varied in different studies, but most ovarian carcinomas show complex karyotypic changes with multiple numerical and structural aberrations. Simple changes, i.e. numerical changes only and/or a single structural change, are seen only in a minority of cases. The most common simple numerical aberration has been trisomy 12, which has been detected as a sole abnormality in some cases (Yang-Feng et al., 1991; Pejovic et al., 1992a; Pejovic et al., 1992b; Jenkins et al., 1993; Thompson et al., 1994b). Karyotypes with complex aberrations frequently show chromosome losses, deletions and unbalanced translocations, leading to loss of chromosomal material, especially at X, 6, 8, 13, 17 and 22 (Tanaka et al., 1989; Pejovic et al., 1992a; Jenkins et al., 1993; Thompson et al., 1994a; Tibiletti et al., 1996). Double minutes and homogeneously staining regions are also detected, indicating amplification of DNA sequences (Tanaka et al., 1989; McGill et al., 1993; Taetle et al., 1999b). The chromosomes most frequently involved as regards structural changes are 1, 3, 6, 7, 11, 12 and 19 (Tanaka et al., 1989; Pejovic et al., 1992a; Jenkins et al., 1993; Thompson et al., 1994a; Tibiletti et al., 1996; Taetle et al., 1999b). Cytogenetic abnormalities and their complexity are correlated with the grade of ovarian carcinomas (Pejovic et al., 1992b; Taetle et al., 1999b). In addition, cytogenetic alterations have been found more often in the serous histological type (Pejovic et al., 1992b). Patients with tumors showing abnormal karyotypes have showed reduced survival times (Pejovic et al., 1992b), and breakpoints at 1p and 3p have been shown to be independent predictors of poor prognosis (Taetle et al., 1999a).

2.1.2. Molecular genetic changes

Aberration of the tumor suppressor gene *P53* is the most frequent molecular alteration detected in ovarian carcinomas. *P53* mutation and/or overexpression of P53, which results from sequestration of mutated protein in the nucleus, are identified in about half of the cases of ovarian carcinoma (Marks et al., 1991; Milner et al., 1993; Klemi et al., 1995). P53 alterations have been associated with serous histology (Milner et al., 1993; Klemi et al., 1995; Eltabbakh et al., 1997; Rohlke et al., 1997; Anttila et al., 1999; Geisler et al., 2000),

high tumor grade (Hartmann et al., 1994; Henriksen et al., 1994; Klemi et al., 1995; Eltabbakh et al., 1997; Rohlke et al., 1997; Anttila et al., 1999; Baekelandt et al., 1999; Levesque et al., 2000; Reles et al., 2001) and high tumor stage (Henriksen et al., 1994; Eltabbakh et al., 1997; Anttila et al., 1999; Geisler et al., 2000; Levesque et al., 2000; Fallows et al., 2001). P53-defective ovarian carcinomas have shown resistance to platinum-based chemotherapy (Righetti et al., 1996; Buttitta et al., 1997; Reles et al., 2001), but seem to respond to paclitaxel/platinum-based therapy (Lavarino et al., 2000). Findings concerning the prognostic value of P53 status in ovarian carcinoma have been inconsistent: several investigators have reported P53 alterations to confer poor prognosis (Hartmann et al., 1994; Henriksen et al., 1994; Klemi et al., 1995; Eltabbakh et al., 1997; Rohlke et al., 1997; Anttila et al., 1999; Baekelandt et al., 1999; Geisler et al., 2000; Levesque et al., 2000; Reles et al., 2001), whereas others have not found such an association (Marks et al., 1991; Silvestrini et al., 1998; Gadducci et al., 2000; Fallows et al., 2001).

Lost expression of MTS1 has been identified in 20% of ovarian carcinomas, mainly in mucinous and endometrioid tumors (Milde-Langosch et al., 1998). Mutations of *PTEN* occur in about 20% of endometrioid ovarian carcinomas, but are rare in the serous histological type (Tashiro et al., 1997a; Obata et al., 1998). Frequent LOH at the *RB* locus (13q14) has been detected in ovarian carcinomas, but no changes in the expression of RB protein (Dodson et al., 1994). Mutations of *BRCA1* and *BRCA2* are rarely seen in sporadic ovarian carcinomas (Merajver et al., 1995; Takahashi et al., 1995; Takahashi et al., 1996).

Amplification or overexpression of the *ERBB2* oncogene is identified in approximately 30% of ovarian carcinomas (Slamon et al., 1989; Berchuck et al., 1990; Zheng et al., 1991; Singleton et al., 1994). It has been suggested that *ERBB2* activation in ovarian carcinoma is associated with tumor progression (Hellstrom et al., 2001). Findings concerning the clinical impact of *ERBB2* activation are conflicting: some investigators have found a significant correlation with prognosis, whereas others have not confirmed this association (Slamon et al., 1989; Berchuck et al., 1990; Singleton et al., 1994; Medl et al., 1995). Mutations of *KRAS* are detected more frequently in mucinous (46–75%) than in serous (5–20%) ovarian carcinomas (Enomoto et al., 1991b; Ichikawa et al., 1994; Suzuki et al., 2000a).

Amplification and/or overexpression of other oncogenes observed in ovarian carcinoma involve *CMYC* (29–37%) (Baker et al., 1990; Tashiro et al., 1992), *CMET* (28%) (Di Renzo et al., 1994), *INT2* (19%) (Medl et al., 1995) and *AIB1* (25%) (Tanner et al., 2000). Elevated levels of AKT2 activity have been detected in over 30% of ovarian carcinomas (Yuan et al., 2000), especially in serous tumors, and mutations of ß-catenin have been identified in 16% of endometrioid ovarian carcinomas (Wright et al., 1999).

Microsatellite instability (MSI), a characteristic feature of deficient mismatch repair, is observed in a subset of ovarian carcinomas (12%–17%) (Fujita et al., 1995; King et al., 1995; Sood et al., 2001). Some investigators have reported low frequencies of MSI, especially in serous ovarian carcinomas (0%–8%) (Fujita et al., 1995; King et al., 1995; Haas et al., 1999), whereas in endometrioid carcinomas instability has been seen more frequently (50%) (Fujita et al., 1995).

Differences between various histological types of ovarian carcinoma are also detected as regards e.g. structural proteins. The main cytokeratins expressed in ovarian surface epithelium and ovarian carcinomas are 7, 8, 18 and 19 (Moll et al., 1983). In distinction to ovarian surface epithelium and serous carcinoma, mucinous carcinomas express cytokeratin 20 (Moll et al., 1992). The

ovarian surface epithelium and serous carcinoma express WT1, whereas it is rare in the mucinous and endometrioid types (Shimizu et al., 2000). On the other hand, CA125 expression is typical of serous and endometrioid carcinomas, but it is usually not found in mucinous carcinomas (de la Cuesta et al., 1999).

2.2. Endometrial carcinoma

2.2.1. Cytogenetic findings

Cytogenetic studies, involving mostly endometrioid endometrial carcinomas, have revealed relatively simple numerical and structural aberrations, and the modal chromosome number has been near diploid. The most consistent finding is gain of 1q chromosomal material (Fujita et al., 1985; Couturier et al., 1986; Couturier et al., 1988; Milatovich et al., 1990; Shah et al., 1994; Bardi et al., 1995). Most of the chromosome 1 imbalances are rearrangements involving centromeric or paracentromeric break-points and some cases have shown isochromosome 1q formation (Fujita et al., 1985; Couturier et al., 1986; Shah et al., 1994). Other frequent findings include trisomy of chromosomes 10, 7 and 12 (Couturier et al., 1986; Couturier et al., 1988; Simon et al., 1990; Shah et al., 1994; Bardi et al., 1995). One study showed deletion of distal 6q as the most common finding (Tibiletti et al., 1997). Four cases of serous endometrial carcinomas have been included in cytogenetic analyses. One showed no changes, one was not analyzable and two presented with multiple complex changes and intratumor heterogeneity distinct from changes in endometrioid carcinomas (Bardi et al., 1995; Tibiletti et al., 1997).

2.2.2. Molecular genetic changes

During the years when this study was performed, new information about the molecular genetic background of endometrioid endometrial carcinoma has emerged. Frequent allelic loss at 10q23-q26 was detected in endometrial carcinomas (Peiffer et al., 1995). Subsequently, a putative tumor suppressor gene *PTEN* was identified at 10q23.3 (Li et al., 1997; Steck et al., 1997) and frequent mutations of this gene (34%–50%) were found in endometrioid endometrial carcinomas (Kong et al., 1997; Risinger et al., 1997; Tashiro et al., 1997a). Mutations were also described in about 20%–30% of endometrial hyperplasias, the putative precursor lesions of endometrioid carcinoma (Levine et al., 1998; Maxwell et al., 1998). Furthermore, histologically normal premenopausal endometria were found to contain occasional glands that failed to express PTEN protein because of mutation and/or deletion (Mutter et al., 2001). Thus, loss of PTEN expression seems to occur early in the pathogenesis of endometrial adenocarcinoma.

Microsatellite instability (MSI) is frequent in endometrial tumors associated with HNPCC (Risinger et al., 1993) and is due to germline mutations in mismatch repair genes. MSI is detected in approximately 20% of sporadic endometrioid endometrial carcinomas (Risinger et al., 1993; Burks et al., 1994; Duggan et al., 1994a; Kobayashi et al., 1995; Peiffer et al., 1995; Caduff et al., 1996), but mutations of the known mismatch repair genes are rarely observed (Katabuchi et al., 1995; Kowalski et al., 1997; Gurin et al., 1999). Recent studies have suggested that deficient mismatch repair in sporadic endometrial carcinomas may result from inactivation of *MLH1* due to promoter hypermethylation of the gene (Esteller et al., 1998; Gurin et al., 1999; Simpkins et al., 1999; Salvesen et al., 2000). Both MSI and *MLH1* promoter hypermethylation have been detected in complex hyperplasias with coexisting endometrial adenocarcinoma, but not in normal endometrium (Mutter et al., 1996; Esteller et al., 1999).

Mutations of the *KRAS* oncogene are

identified in approximately 20% of endometrioid endometrial carcinomas (Enomoto et al., 1991a; Sasaki et al., 1993; Duggan et al., 1994b; Caduff et al., 1995; Lax et al., 2000). *KRAS* mutations are also found in cases of endometrial hyperplasia, and mutations are not associated with grade or stage of endometrial carcinomas, suggesting that *KRAS* mutation may represent an early event in a subset of endometrial carcinomas. Overexpression and mutations of *P53* are detected in about 20% of cases of endometrioid endometrial carcinoma (Kohler et al., 1992; Zheng et al., 1996; Lax et al., 2000). Alterations of *P53* are associated with high tumor grade and stage and they are not seen in endometrial hyperplasia (Kohler et al., 1992; Zheng et al., 1996; Lax et al., 2000), suggesting that *P53* mutations in endometrioid carcinoma are related to progression rather than tumor initiation. Amplification and overexpression of the *ERBB2* oncogene has been detected in a subset of endometrial carcinomas and it has been associated with high tumor grade and poor overall survival (Saffari et al., 1995; Rolitsky et al., 1999).

In contrast to the endometrioid histological type, serous endometrial carcinoma presents with frequent *P53* alterations (90%), which are observed with similar frequency in cases of endometrial intraepithelial carcinoma (EIC), the putative precursor of serous carcinoma (Sherman et al., 1995; Moll et al., 1996; Zheng et al., 1996; Tashiro et al., 1997b; Lax et al., 2000). However, *PTEN* and *KRAS* mutations and MSI are rarely identified in serous endometrial carcinomas (Duggan et al., 1994a; Duggan et al., 1994b; Caduff et al., 1995; Tashiro et al., 1997a; Tashiro et al., 1997c; Lax et al., 2000). An association between *ERBB2* amplification and serous rather than endometrioid histological type has also been reported (Rolitsky et al., 1999). Most serous endometrial carcinomas are negative for estrogen and progesterone receptors (Umpierre et al., 1994; Moll et al., 1996), in contrast to endometrioid endometrial carcinomas, particularly those of low grade, which show hormone receptor positivity (Nyholm et al., 1992).

2.3. Fallopian tube carcinoma

Few investigations have been carried out on the genetic background of fallopian tube carcinoma, and its pathogenesis is poorly understood. Complex karyotypic abnormalities were reported in cytogenetic analysis of one case of fallopian tube carcinoma (Bardi et al., 1994). Overexpression and mutations of *P53* are detected in approximately 60% of cases of fallopian tube carcinoma (Lacy et al., 1995; Runnebaum et al., 1996; Zheng et al., 1997; Chung et al., 2000). Alterations of *P53* are seen at all stages of tumors with similar frequency, including in situ carcinomas (Zheng et al., 1997; Demopoulos et al., 2001), and the frequency of *P53* alterations is higher in serous than in other histological types (Zheng et al., 1997). One group reported an association between *P53* alterations and poor clinical outcome (Zheng et al., 1997), but others have not found correlations with clinicopathological parameters (Lacy et al., 1995; Chung et al., 2000; Demopoulos et al., 2001). Mutations of the *KRAS* oncogene and overexpression of ERBB2 protein, but no amplification of the gene, have been observed in fallopian tube carcinomas (Lacy et al., 1995; Mizuuchi et al., 1995; Stuhlinger et al., 1995; Chung et al., 2000).

3. Overview of comparative genomic hybridization (CGH)

3.1. Methodology

Comparative genomic hybridization, introduced in 1992, is based on simultaneous hybridization of differentially labeled tumor and normal DNAs on normal metaphase chromosomes (Kallioniemi et al., 1992; Kallioniemi et al., 1994). Analysis of the

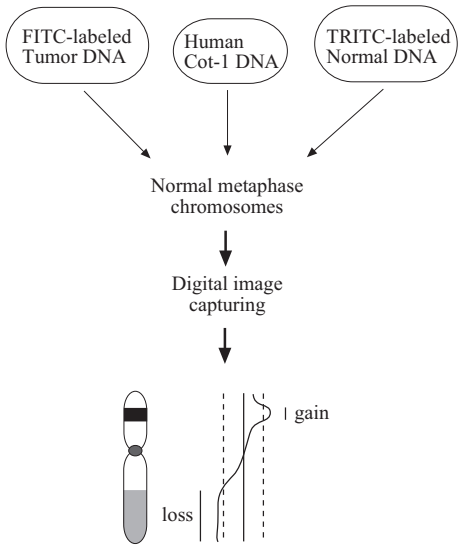

Figure 1. The principle of comparative genomic hybridization (CGH). Differentially labeled tumor and normal DNAs are hybridized together with Cot-1 DNA to normal metaphase chromosomes. Separate images are captured for counterstain (DAPI), tumor DNA (FITC, green) and normal DNA (TRITC, red). Differences in the tumor to normal fluorescence intensity ratio on the chromosomes reflect DNA copy number changes in the tumor sample. The ratio is calculated as CGH profile.

ratio of the test and control fluorescence intensities provides an indication of the DNA sequence copy number changes throughout the tumor genome in a single experimental setting (Figure 1). The fluorescence intensity ratios are measured using a digital image analysis system. Ratios that are increased or decreased compared with the normal ratio reveal gains and losses of DNA sequences in the test sample. Gained or amplified regions of the tumor genome are thought to contain oncogenes, whereas losses are thought indicate locations of tumor suppressor genes.

The main advantage of CGH compared with traditional cytogenetics is that no culturing of the tumor sample is needed. This makes CGH especially suitable for analysis of copy number changes in solid tumors, where high quality metaphase preparations are often difficult to make. Furthermore, solid tumors often show complex karyotypes, which are laborious and sometimes impossible to interpret. However, CGH cannot detect balanced translocations, inversions or ploidy changes. The sensitivity of the method depends on the size and the magnitude of the copy number aberration (Kallioniemi et al., 1994). If the sequence is highly amplified (5–10-fold), copy number increases as small as 1 Mb can be detected, whereas deletions of less than 10 Mb are unlikely to be seen (Forozan et al., 1997; Bentz et al., 1998). Some genomic areas, such as pericentromeric and heterochromatic regions, contain highly repetitive sequences and are blocked by unlabeled Cot-1 DNA and thus cannot be reliably analyzed. Ratio changes in the telomeric regions should be interpreted with caution because fluorescence intensities decrease towards the telomeres, approaching the background fluorescence, and therefore unreliable results may be obtained (Kallioniemi et al., 1994). Direct fluorochrome-conjugated nucleotides have replaced the indirect labeling system, which has improved the sensitivity of the method (Kallioniemi et al., 1994). Ratio artefacts, which may occur in CG-rich genomic areas, can be minimized by using a mixture of dCTP and dUTP nucleotides in the labeling procedure (El-Rifai et al., 1997). Degenerate oligonucleotide-primed PCR has enabled the use of very small amounts of DNA (Speicher et al., 1993; Kuukasjarvi et al., 1997).

3.2. CGH studies

3.2.1. Ovarian carcinoma

So far, at least 13 studies, covering over 400 cases of primary ovarian carcinoma have been published (Iwabuchi et al., 1995; Arnold et al., 1996; Sonoda et al., 1997b; Tapper et al., 1997; Wolff et al., 1997; Tapper et al., 1998; Kudoh et al., 1999; Pejovic

Table 1. The most frequent copy number changes detected by CGH in 405 ovarian carcinomas (Iwabuchi et al., 1995; Arnold et al., 1996; Sonoda et al., 1997b; Tapper et al., 1997; Wolff et al., 1997; Tapper et al., 1998; Kudoh et al., 1999; Pejovic et al., 1999; Blegen et al., 2000; Patael-Karasik et al., 2000; Kiechle et al., 2001; Shridhar et al., 2001) and 86 endometrial carcinomas (Sonoda et al., 1997a; Suzuki et al., 1997; Suehiro et al., 2000; Baloglu et al., 2001).

Ovarian carcinoma				Endometrial carcinoma			
Gains	Frequency	Losses	Frequency	Gains	Frequency	Losses	Frequency
+ 8q	58%	- 4q	31%	+ 1q	36%	- 4q	13%
+ 3q	52%	- 18q	30%	+ 8q	31%	- 13q	12%
+ 1q	44%	- 13q	30%	+ 10q	19%	- 8p	10%
+ 20q	41%	- 8p	27%	+ 3q	16%		
+ 12p	31%	- 5q	27%	+ 10p	16%		
+ 7q	31%	- 6q	22%	+ 20p	13%		
+ 1p	27%	- 16q	21%	+ 2p	12%		
+ 5p	26%	- 17p	19%				
+ 6p	26%	- 9p	18%				
+ 2q	25%	- 17q	17%				

et al., 1999; Blegen et al., 2000; Patael-Karasik et al., 2000; Suzuki et al., 2000b; Kiechle et al., 2001; Shridhar et al., 2001). In these studies approximately 60% of the carcinomas were of serous histology. Chromosomal changes observed in ovarian carcinomas were generally frequent and complex (Table 1). Copy number alterations were found in approximately 95% of ovarian carcinomas and the average number of aberrations per tumor varied from 4.0 to 20.

In a previous study by our group, serous, mucinous and endometrioid ovarian carcinomas were analyzed separately, and distinct genomic aberrations in the different histological types were found (Tapper et al., 1997). Serous carcinomas showed more chromosomal alterations than mucinous and endometrioid carcinomas, the average number of changes being 7.5 for serous, 4.4 for mucinous and 4.5 for endometrioid carcinomas. Gains at 1q occurred only in serous and endometrioid carcinomas, whereas an increased copy number of 17q was mostly seen in mucinous tumors. Overrepresentation of 11q was typical of serous carcinoma and gain at 10q was typical of mucinous carcinoma.

3.2.2. Endometrial carcinoma

Since the introduction of CGH, 86 cases of endometrial carcinoma have been analyzed by this method fu (Sonoda et al., 1997a; Suzuki et al., 1997; Suehiro et al., 2000; Baloglu et al., 2001) (Table 1). In these studies over 90% of the cases have been of endometrioid histological type. Chromosomal aberrations were seen in 73% of the tumors and the average number of chromosomal changes detected per tumor varied from 3.4 to 5.7.

3.2.3. Fallopian tube carcinoma

Previously, a single CGH study of fallopian tube carcinoma has been published (Heselmeyer et al., 1998). It showed copy number alterations in all 12 carcinomas and the average number of aberrations per tumor was 19.7. Gains at chromosome arms 3q and 1q were seen in 11 of the 12 tumors. Other frequent overrepresentations were located at 2q, 7q, 8q, 5p, 6p, 12p and 14q (>50% of the cases). The most recurrent regions of underrepresentation were at 16q, 22q, 6q, 8p, 18q and Xq (>50% of the cases).

4. Overview of allelic analysis

4.1. Loss of heterozygosity (LOH)

According to the classical two-hit model, inactivation of both alleles of a tumor suppressor gene is needed for cancer formation (Knudson, 1971). One allele is usually inactivated by mutation, either somatic or inherited (Figure 2). The other allele can be inactivated by various mechanisms, such as loss of the whole or part of a chromosome, loss of the normal chromosome and reduplication of the mutated one, gene conversion, mitotic recombination, point mutation, deletion or epigenetic mechanism, such as promoter hypermethylation (Knudson, 1971; Cavenee et al., 1983; Esteller et al., 2000). In LOH analysis, also called allele analysis or allelotyping, the loss of one allele of a tumor suppressor gene can be observed as loss of heterozygosity of intragenic or nearby polymorphic markers in tumor tissue compared with normal tissue from the same individual. Thus, regions of the genome showing frequent LOH are thought to contain tumor suppressor genes.

To analyze LOH, restriction fragment length polymorphisms (RFLPs) and Southern blotting were used initially. Introduction of polymorphic microsatellite markers and PCR-based amplification facilitated allelic analyses by consuming less time and DNA, and by increasing resolution (Weber and May, 1989). Further improvement was made by way of fluorescence-labeled primers and computer-based measurement of sizes and intensities of alleles (Ziegle et al., 1992; Reed et al., 1994). Comparisons of radiographic and fluorescence-based methods have shown high concordance between the findings (Schwengel et al., 1994; Canzian et al., 1996). The main advantages of semiautomated fluorescence-based allelotyping are possibility of multiplexing loci and objective scoring of alleles.

4.2. LOH in ovarian carcinoma

4.2.1. Genome-wide analyses

In ovarian carcinoma several LOH studies have been performed, and allelic loss has been found in all chromosomes at varying frequencies. Studies in which the whole genome has been screened, with one or a few loci per chromosome arm, showed frequent losses at 5q, 6p, 6q, 9q, 13q, 17p, 17q, 18q, 19p, 22q and Xp (Sato et al., 1991; Cliby et al., 1993; Dodson et al., 1993; Yang-Feng et al., 1993; Osborne and Leech, 1994). These regions showed allelic loss in over 30% of informative cases and

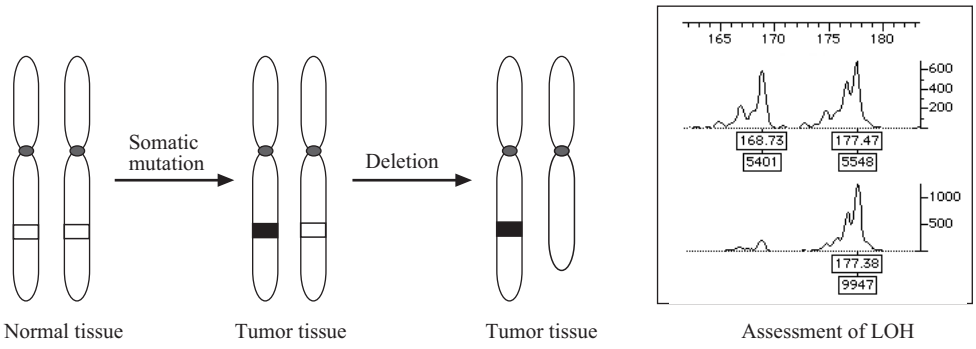

Figure 2. The principle of loss of heterozygosity (LOH) in a sporadic tumor. One allele of the gene is inactivated by mutation and the other allele by deletion. In allelic analysis the deletion is seen as loss of one allele of the microsatellite marker. *Upper lane*, amplification from normal DNA. *Lower lane*, amplification from tumor DNA.

the highest frequency of LOH, over 50% of informative cases, was observed at chromosome 17. In addition to these regions, studies concentrating on specific chromosomes have shown frequent allelic losses at 1p, 2q, 3p, 7q, 8p, 9p, 11p, 11q, 14q and 16q (Zheng et al., 1991; Weitzel et al., 1994; Gabra et al., 1996; Bandera et al., 1997; Edelson et al., 1997; Lu et al., 1997; Saretzki et al., 1997; Lounis et al., 1998; Wright et al., 1998; Fullwood et al., 1999; Huang et al., 1999; Imyanitov et al., 1999; Launonen et al., 2000).

Differences in the frequency and pattern of LOH have been observed in different histological types of ovarian carcinoma. Serous carcinomas display a higher overall frequency of allelic loss than non-serous histological types (Sato et al., 1991; Cliby et al., 1993; Saretzki et al., 1997). Specific chromosomal arms that show a higher frequency of LOH in serous than in non-serous tumors, especially mucinous carcinomas, include 6q, 13q, 11p, 11q, 17p, 17q, 19q and 22q (Sato et al., 1991; Saito et al., 1992; Foulkes et al., 1993; Orphanos et al., 1995; Pieretti et al., 1995; Papp et al., 1996; Lu et al., 1997; Bryan et al., 2000; Garcia et al., 2000; Launonen et al., 2000; Suzuki et al., 2000a). On the other hand, losses at 9p have been seen more frequently in mucinous than in serous carcinomas (Watson et al., 1998).

The total number of allelic losses in ovarian carcinoma has been associated with tumor grade and patient survival (Zheng et al., 1991; Cliby et al., 1993; Dodson et al., 1993; Saretzki et al., 1997). Losses at chromosomes 3 and 11 and chromosome arms 6q, 13q and 15q have been associated with high tumor grade (Zheng et al., 1991; Dodson et al., 1993; Foulkes et al., 1993; Kim et al., 1994), whereas losses at 3p and 16q have been correlated with high tumor stage (Fullwood et al., 1999; Launonen et al., 2000). Poor patient survival has been observed in association with tumors showing LOH at chromosomes 11 (11p15.5 and 11q23.3-q24.3) and 17 (Gabra et al., 1996; Chenevix-Trench et al., 1997; Launonen et al., 2000).

4.2.2. Chromosome arm 8p

In LOH studies involving all chromosomal arms in ovarian carcinoma, allelic loss at 8p was found in 23% to 40% of the cases (Cliby et al., 1993; Dodson et al., 1993; Yang-Feng et al., 1993; Osborne and Leech, 1994). Studies in which mapping of 8p was performed with several markers showed LOH at a frequency of 50–78% (Wright et al., 1998; Brown et al., 1999; Pribill et al., 2001). Allelic loss at this chromosomal arm has been associated with high tumor grade (Dodson et al., 1993; Pribill et al., 2001) and high tumor stage (Wright et al., 1998; Brown et al., 1999; Pribill et al., 2001). In these studies no association between LOH at 8p and histological type of tumor was observed. Wright et al. defined three regions of overlap, two at 8p23 and one at 8p22 (Wright et al., 1998). Brown et al. found the highest frequency of allelic loss at marker D8S136 (8p21) (Brown et al., 1999). Pribill et al. found three smallest regions of overlap: one at 8p22, one at 8p21 and one at 8p12-21 (Pribill et al., 2001). The minimal common regions of LOH defined in these three studies (Wright et al., 1998; Brown et al., 1999; Pribill et al., 2001) are discussed in more detail in the Discussion.

4.2.3. Chromosome arm 18q

Studies of ovarian carcinoma in which the whole genome was screened, with one or a few loci per chromosome arm, the long arm of chromosome 18 showed allelic loss at a frequency varying from 0% to 47% of cases (Sato et al., 1991; Cliby et al., 1993; Dodson et al., 1993; Yang-Feng et al., 1993; Osborne and Leech, 1994). However, investigators using several microsatellite markers at 18q have observed higher fre-

quencies of LOH, ranging from 41% to 60% (Chenevix-Trench et al., 1992; Takakura et al., 1999). The highest frequencies of allelic loss have been detected distal to 18q21 (Chenevix-Trench et al., 1992; Zborovskaya et al., 1999). LOH at this chromosomal arm has been found to be associated with high stage ovarian carcinomas (Chenevix-Trench et al., 1992; Zborovskaya et al., 1999).

Aims of the study

The aims of the present study were:

1. to identify copy number changes in endometrial and fallopian tube carcinomas (I, II)

2. to compare the copy number karyotypes of serous and endometrioid endometrial carcinomas (I)

3. to compare the copy number karyotypes of serous carcinomas of the fallopian tube, endometrium and ovary (II)

4. to compare the allelotypes of serous and mucinous ovarian carcinomas at chromosome arms 8p and 18q (III, IV)

5. to define the putative tumor suppressor locus/loci more precisely at 8p21-p23 and 18q12.3-q23 by allelic analysis in serous ovarian carcinoma (III, IV)

6. to compare genomic and molecular aberrations with histopathological parameters and clinical outcome (I, III, IV, V)

Materials and methods

1. Clinical material (I–V)

Tumor samples were obtained from patients undergoing primary surgery for gynecological carcinomas at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital (Table 2). The studies were approved by the Ethics Committee of the Department of Obstetrics and Gynecology. Informed consent was obtained from the patients in regard to blood samples and fresh tumor material.

All the tumor specimens in a particular study were reviewed by the same investigator as regards histological subtype and grade (I: Torsten Wahlström; II–V: Ralf Bützow). Tumor stage and other clinical information on the patients was extracted from the medical records of the Department of Obstetrics and Gynecology (I–V). Additional survival information was obtained from the Population Register Center of Finland. In study I, cases of endometrioid endometrial carcinomas were selected to match the stage of the serous endometrial carcinomas.

2. Methods

2.1. Comparative genomic hybridization (I, II)

Genomic DNA from frozen tissues and leucocytes of healthy women, which was used as normal reference DNA in the hybridizations and for negative control experiments, was extracted by using standard methods. DNA from paraffin-embedded tissues was extracted according to the protocol described by Isola et al. (Isola et al., 1994). Metaphase slides were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes from healthy individuals, according to standard protocols.

Table 2. Samples and methods.

Samples	Sample type[a]	Used in study (no. of tumor samples)	Method
24 serous EC	22 paraffin, 2 frozen	I (24), II (24)	CGH
24 endometrioid EC	paraffin	I (24)	CGH
20 serous FTC	13 paraffin, 7 frozen	II (20)	CGH
75 serous OC and blood samples	frozen	III (62), IV (64)	LOH
14 mucinous OC and blood samples	frozen	III (14), IV (9)	LOH, MD
33 serous OC	frozen	III (33)	NB
26 mucinous OC	frozen	III (26)	NB
Tissue microarray			
545 serous OC	paraffin	III (528), IV (60), V (522)	ICH
75 mucinous OC	paraffin	III (75)	ICH
34 normal ovarian samples	paraffin	III, IV, V	ICH
23 normal fallopian tube samples	paraffin	V	ICH

EC = endometrial carcinoma; FTC = fallopian tube carcinoma; OC = ovarian carcinoma; [a] paraffin = paraffin embedded sample; frozen = fresh frozen sample; CGH = comparative genomic hybridization; LOH = allelic analysis; MD = microdissection; NB = Northern blot; ICH = immunohistochemistry

Comparative genomic hybridization was performed as described previously (Kallioniemi et al., 1992; Kallioniemi et al., 1994) and a protocol involving directly fluorochrome-conjugated nucleotides was followed, with some modifications (El-Rifai et al., 1997). Tumor DNA was labeled with FITC-12-dUTP or a mixture of FITC-12-dUTP and FITC-12-dCTP (1:1; DuPont, Boston, MA, USA). The reference DNA was conjugated to Texas Red-5-dUTP or a mixture of Texas Red-5-dUTP and Texas Red-5-dCTP (1:1; DuPont). DNA was labeled using a standard nick-translation reaction, and the reaction was optimized to produce DNA fragments of 600 to 2000 bp in length. One μg of labeled tumor and normal female DNA, as well as 20 μg of unlabeled human Cot-1 DNA (Gibco BRL, Gaithersburg, MD), were precipitated in 1/10 volume of 3 M sodium acetate (pH 6.0) and 3 volumes of absolute ethanol at -20 °C overnight and dissolved in 10 μl of hybridization buffer (50% formamide/ 10% dextran sulfate/ 2× SSC, pH 7.0) at 37 °C. Metaphase preparations were pretreated in 2× SSC at 40 °C for 30 min, and dehydrated in a series of 70%, 85% and 100% ethanol. The preparations were then denatured in formamide solution (70% formamide/ 2× SSC, pH 7.0) at 62–66 °C for 2 min, dehydrated in an ethanol series on ice, treated with proteinase K (0.1–0.2 μg/ml in 20 mM Tris-HCl, 2 mM $CaCl_2$, pH 7.6) and dehydrated in an ethanol series. The DNA probe mixture was denatured at 75 °C for 5 min just before application to the metaphase preparation. Hybridization was carried out in a moist chamber at 37 °C for 2–3 days. After hybridization, the preparations were washed to remove unbound DNA: three times in 50% formamide/ 2× SSC, pH 7.0, twice in 2× SSC and once in 0.1× SSC at 45 °C for 10 min each, followed by washes in 2× SSC, PN buffer (0.1 M Na_2HPO_4, 0.1 M NaH_2PO_4, 0.1% Nonidet P-40, pH 8.0) and distilled water at room temperature for 10 min each. The preparations were subsequently stained with 4,6-diamino-2-phenylindole (DAPI) and covered with antifade solution (Vectashield™, Vector Laboratories, Burlingame, CA, USA).

Analysis was performed using a Leitz or an Olympus fluorescence microscope connected to a non-cooled CCD camera and an ISIS digital image analysis system (MetaSystems GmbH, Altlussheim, Germany). Three-color images were captured, green (FITC) and red (Texas Red) for the tumor and reference DNA, respectively, and blue (DAPI) for the counterstain on the chromosomes. Several metaphase images were captured, after which approximately 10 were karyotyped on the basis of the chromosome banding pattern obtained by means of the DAPI staining. Signal intensity ratios of green to red along all chromosomes were calculated for the karyotyped metaphases. Data from individual chromosome homologues were combined and the mean green to red ratio profile for each chromosome was displayed adjacent to chromosome ideograms. Cut-off values were set at 0.85 and 1.17, and all the findings were confirmed using a confidence interval of 99%. The chromosomal regions with a green to red ratio under 0.85 were considered to be underrepresented (showing loss), whereas the regions with a ratio above 1.17 were considered to be overrepresented (showing gain). The cut-off values were set on the basis of negative control experiments where two differently labeled normal DNAs were hybridized together. Tumor DNA with known copy number alterations was used in positive control experiments. The cut-off value for high-level amplification was 1.5. Telomeric and heterochromatic regions were excluded from the analysis. In study I, reverse labeling CGH was performed on three samples, which confirmed the alterations detected by the standard technique.

2.2. Laser microdissection (III, IV)

Laser microdissection was performed as de-

scribed previously (Schutze and Lahr, 1998), using a Robot-MicroBeam (PALM, Wolfratshausen, Germany). Five-µm frozen sections of mucinous ovarian carcinomas were mounted onto slides covered with polyethylene membrane (PALM) and poly-L-lysine. The Robot-MicroBeam consists of a pulsed, low-energy nitrogen laser and a computer-controlled microscope. Selected carcinoma cell areas were circumscribed with the laser in order to isolate them from surrounding normal cells. In cases in which the selected area contained non-tumor cells, these were eliminated by directed laser shots. The isolated target specimens were collected with forceps into tubes containing proteinase K buffer and DNA was extracted using a proteinase K-phenol-chloroform method.

2.3. Loss of heterozygosity analysis (III, IV)

In mucinous carcinomas, as a rule the amount of non-neoplastic cells was high and the laser microbeam microdissection technique was used to separate carcinoma cells before DNA extraction. In serous carcinoma, only tissue samples with more than 40–50% of cells representing tumor cells were included in the studies (range 40–95%; median 70%), and no microdissection was needed. Tumor DNA was extracted from fresh frozen tumor samples and normal DNA from blood lymphocytes of these patients. A standard proteinase K-phenol-chloroform method was used for DNA extraction.

In order to study LOH at 8p and 18q, sets of 18 and 27 highly polymorphic microsatellite markers at 8p21-p23 and 18q12.3-q23, respectively, were used. Primer sequences and reaction conditions for dinucleotide markers were obtained from the Genethon human linkage map (http://ftp.genethon.fr), and for tri- and tetranucleotide markers, from Genome Database (http://gdbwww.gdb.org). The genetic order of the markers was based on the Genethon map, the Genome Database and GeneMap'99 (http://www.ncbi.nlm.nih.gov/genemap/). The oligonucleotides were labeled fluorescently with one of three dyes (6-FAM, TET or HEX; Institute of Biotechnology, University of Helsinki, Finland). A fourth dye (TAMRA; Perkin-Elmer, Foster City, CA) was reserved for the size standard.

The PCR reactions for genotyping were carried out in a volume of 10 µl and included GeneAmp 1× PCR buffer (Perkin-Elmer), each dNTP at 50 µmol/l, 60 ng DNA (5–10 ng DNA from the microdissected samples), 0.5 U AmpliTaq Gold polymerase (Perkin-Elmer) and 5 pmol of each primer (one of them fluorescently labeled). The reaction mixtures were given 30–35 cycles of 5 s at 96 °C, 59 s at 92 °C, 1 min 15 s at 55 °C (60 °C for D18S474, D18S815, D18S844 and D18S845) and 45 s at 72 °C, preceded by a 10-min hot start at 96 °C for enzyme activation and followed by final extension at 72 °C for 30 min.

The products were pooled in groups for electrophoresis. Each group consisted of nine markers and the mix included 1 µl of each PCR product. One µl of this mixture was added to 12.5 µl formamide and 0.5 µl TAMRA 500 size standard and it was denatured at 96 °C for 3 min before loading the samples into an ABI Prism 310 Genetic Analyzer (Perkin-Elmer), which uses polymer-filled capillary for electrophoresis. Analysis of raw data and assessment of LOH were performed with GeneScan and Genotyper software (Perkin-Elmer). The peaks of the normal DNA sample were used to determine whether the sample was homozygous (one peak only) or heterozygous (two peaks). If the normal DNA sample was heterozygous as regards a given marker, the marker was informative for LOH analysis. The sizes of the allele peaks were assigned according to the area under the highest peak. When two alleles were present in normal tissue and one was absent in the tumor, the result was determined to be LOH. In cases where the assessment was not clear-cut, the

ratio of alleles was calculated for each normal and tumor sample, and the tumor ratio was divided by the normal ratio, i.e. T2:T1/N2:N1 (T1 and N1 are the area values for the shorter length alleles and T2 and N2 are the values for the longer length alleles, for tumor and normal tissue respectively). If the ratio was <0.6 or >1.67, the result was determined to be LOH (Canzian et al., 1996). In ambiguous cases, the PCR was repeated and electrophoresis was performed without pooling.

2.4. Tumor tissue microarrays (III–V)

The tissue microarrays were constructed as described previously (Kononen et al., 1998). A representative tumor area was selected from hematoxylin-eosin-stained sections of each tumor. Core tissue biopsy specimens (diameter 0.8 mm) were taken from these areas of individual donor blocks and precisely arrayed into a new recipient paraffin block with a custom-built instrument (Beecher Instruments, Silver Spring, MD). Four core tissue biopsies were obtained from each carcinoma specimen. After the block construction was completed, 5-μm sections were cut with a microtome. The presence of tumor tissue in the arrayed samples was verified on hematoxylin-eosin-stained sections.

2.5. Immunohistochemistry (III–V)

Primary antibodies used for immunohistochemistry were: goat polyclonal anti-mouse GATA-4 IgG (final concentration 1 μg/ml; sc-1237, Santa Cruz Biotechnology Inc., Santa Cruz, CA), mouse monoclonal anti-human SMAD4 (2 μg/ml; sc-7966, Santa Cruz Biotechnology Inc.), goat polyclonal anti-human SMAD2 IgG (6 μg/ml; sc-6200, Santa Cruz Biotechnology Inc.), mouse monoclonal anti-human DCC (5 μg/ml; clone G97-499, Pharmingen, San Diego, CA) and mouse monoclonal anti-human P53 (1:100 dilution, clone DO-7, Dako, Glostrup, Denmark). The sections were pretreated in a microwave oven in buffered sodium citrate prior to SMAD4, DCC and P53 immunohistochemistry. An avidin-biotin immunoperoxidase system was used to visualize the bound antibody. For SMAD4 and P53, the procedure was run in a Techmate automated machine (Peroxidase DAB detection kit; DAKO ChemMate, Denmark). For GATA-4, SMAD2 and DCC, the procedure was performed manually (Vectastain Elite ABC kits, Vector Laboratories, Burlingame, CA) and 3-amino-9-ethylcarbazole was used as the chromogen. The sections were counterstained with Mayer's hematoxylin. Nonimmune goat IgG (for GATA-4 analysis) (III), blocking of the antibody by peptide preincubation (for SMAD2 analysis) (IV) or omission of the primary antibody were used for negative controls. Normal ovarian samples were used as positive controls for GATA-4, SMAD2 and DCC. For SMAD4, colon carcinoma cell lines shown to express SMAD4 were used as positive controls. The staining patterns of each antigen in normal epithelial cells of ovaries and fallopian tubes were used as references of normal expression, and staining diverging from these in tumor cells was considered aberrant.

2.6. Northern blot analysis (III)

RNA from ovarian carcinoma samples was extracted and Northern blotting was performed as previously described (Laitinen et al., 2000). As probes for filter hybridization we used human GATA-4 cDNA (White et al., 1995) and rat glyceraldehyde-6-phosphate dehydrogenase (GAPDH) cDNA (Laitinen et al., 1997). The cDNAs were labeled with [^{32}P]-α-deoxy-CTP using Prime-a-gene kits (Promega, Madison, WI).

2.7. Statistical analyses (I, III–V)

Differences in chromosomal changes (I),

LOH and lost expression (III, IV) were tested by using Fisher's exact test, and differences in total number of changes (I) and allelic loss of informative markers (III, IV) by using the nonparametric Mann-Whitney U test. Associations between P53 status and clinicopathological parameters (V) were analyzed by using the Fisher's exact and χ^2 tests. The product-limit method was used to construct survival curves and statistical significance was tested by log-rank analysis (I, IV, V). Multivariate survival analysis was carried out by using the Cox proportional hazards model (I, IV, V). P-values were two-tailed and values <0.05 were considered significant.

RESULTS

1. DNA copy number changes detected by CGH (I, II)

1.1. Serous endometrial carcinoma (I)

DNA sequence copy number changes were detected in 71% (17/24) of serous endometrial carcinomas, with gains predominating over losses (2.8:1). The most common copy number increases were at 3q (50%), 8q (33%), 1q (29%), 5p (29%), 6p (29%), 2q (25%), 7q (21%), 11q (21%) and 19q (21%). The minimal common regions of gain at 3q and 8q were 3q26.1-qter and 8q23. High-level amplification was detected in 25% of the tumors, and the minimal common regions were 2q31 (two cases), 3q24-q26.3, 6p, 8q22-q24.1 (two cases), 15q25-qter, 18p11.2, 18q11.2-q12 and 20q13.1-qter. The most common copy number losses were at 4q32-qter, 15qcen-q15 and 18q22-qter (17% each).

1.2. Endometrioid endometrial carcinoma (I)

Copy number changes were observed in 50% (12/24) of endometrioid endometrial carcinomas, with gains being more frequent than losses (3.6:1). The most frequent copy number gains were identified at 1q (29%), 2q24-q31 (13%) and 8q (13%). High-level amplification was observed in 13% of the cases (one region/sample): at 1q, 1q31 and 6p21-p23. Losses were rarely seen: two cases showed loss at 15qcen-q15 and two cases at 16qcen-q13, whereas other losses were detected at separate regions.

1.3. Comparison of serous and endometrioid endometrial carcinomas (I)

Serous endometrial carcinomas showed more copy number alterations than endometrioid carcinomas (5.7 and 1.5 alterations/tumor, respectively). Half of the endometrioid carcinomas presented with a normal copy number karyotype, whereas less than a third of the serous carcinomas showed no changes according to CGH. High-level amplification was more common in serous than in endometrioid carcinomas (10 and 3 amplifications, respectively). In serous carcinoma, frequent gains were de-

Table 3. The most common chromosomal changes detected by CGH in serous (n=24) and endometrioid (n=24) endometrial carcinomas.

Gains[a]	Serous	%	Endometrioid	%	Losses[b]	Serous	%	Endometrioid	%
+ 1q	7/24	29	7/24	29	- 4q	4/24	17	0/24	0
+ 2q	6/24	25	3/24	13	- 9p	3/24	13	0/24	0
+ 3q	12/24	50	2/24	8	- 15q	4/24	17	2/24	8
+ 5p	7/24	29	0/24	0	- 18q	4/24	17	0/24	0
+ 6p	7/24	29	2/24	8					
+ 7q	5/24	21	0/24	0					
+ 8q	8/24	33	3/24	13					
+ 11q	5/24	21	1/24	4					
+ 19q	5/24	21	0/24	0					

[a] chromosome arms showing gain in over 20% of serous or endometrioid carcinomas
[b] chromosome arms showing loss in over 10% of serous or endometrioid carcinomas

tected at several chromosomal regions, including 3q, 8q, 1q, 5p, 6p and 2q, whereas in endometrioid carcinoma the only recurrent copy number increase was the gain of the whole or large part of chromosome arm 1q. In both subtypes losses were relatively rare, particularly in endometrioid carcinoma (Table 3).

1.4. Clinicopathological associations in endometrial carcinoma (I)

High-stage (stage III-IV) tumors showed more copy number abnormalities than low-stage (stage I-II) tumors in both serous and endometrioid endometrial carcinomas (mean numbers of changes: 6.9 and 3.7 for serous tumors, and 1.7 and 1.1 for endometrioid tumors, respectively). In endometrioid carcinomas the number of changes correlated with tumor grade (mean numbers of changes: grade 1, 0.73, grade 2, 2.2 and grade 3, 2.3), but in serous carcinomas no such association was found.

Patients with serous endometrial carcinomas showing copy number changes had poor overall survival when compared with patients with serous carcinomas showing no changes (RR=10.8; 95% CI 1.38–85.2). In multivariate analysis neither stage nor number of changes were independent prognostic factors. In endometrioid carcinoma, no association between copy number changes and survival was found.

1.5. Serous fallopian tube carcinoma (II)

DNA copy number changes were detected in all 20 serous fallopian tube carcinomas, with a mean of 7.0 changes per tumor (Figure 3). Gains were identified more often than losses (1.6:1). The most frequent copy number gains were observed at 8q (75%), 3q (70%), 1q (40%), 12p (40%), 7q (35%), 5p (30%), 20q (30%), 6p (25%), 11q (25%), and 20p (25%). The minimal com-

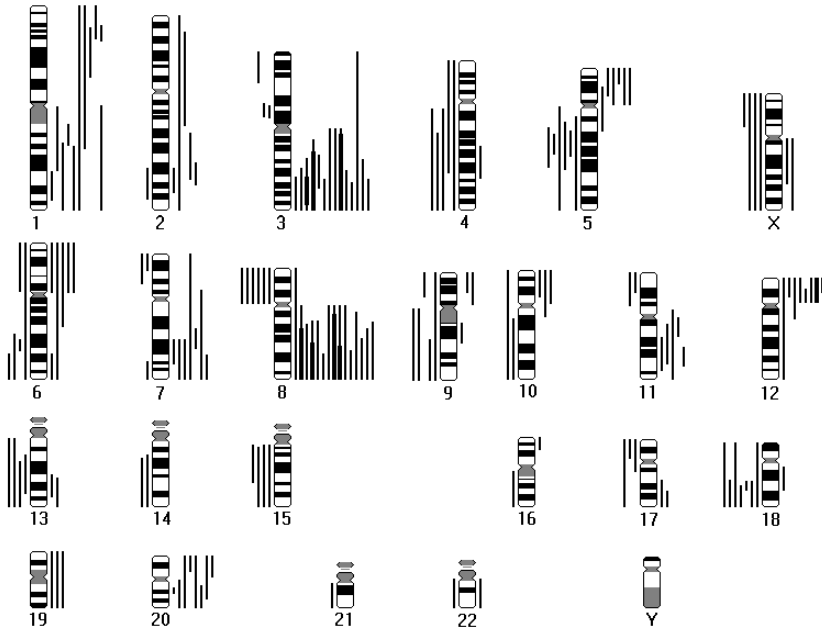

Figure 3. Summary of gains and losses detected by CGH in 20 serous fallopian tube carcinomas. Gains are shown on the *right* and losses on the *left* side of each chromosome. Each line represents an aberration detected in one tumor sample. High-level amplifications are displayed in bold.

mon regions of gain at 3q and 8q were 3q25-qter and 8q22-qter. High-level amplification was detected in 30% of the tumors, and the minimal common regions were 8q22-qter (four tumors), 3q25-q28 (three tumors) and 12p (one tumor). The most common regions of decreased copy number were at 18q (35%), 5q (30%), 8p (30%) and 4q (25%).

1.6. Comparison of serous carcinomas of the fallopian tube, endometrium and ovary (II)

The genetic aberrations detected in fallopian tube carcinomas were compared with the changes detected in 24 serous endometrial carcinomas (I) and 20 serous ovarian carcinomas (Tapper et al., 1998). The pattern of chromosomal alterations detected by CGH was very similar in serous carcinomas of the fallopian tube, endometrium and ovary (Table 4). The most frequently gained regions, including those at 3q, 8q, 1q, 5p, 6p, 7q, 12p and 20q, and the most commonly lost regions, including those at 4q, 8p and 18q, were alike in these three carcinomas. Some differences were observed: gain at 19q and loss at 15q were found only in fallopian tube (in 15% and 20% of cases, respectively) and endometrial (in 21% and 17% of cases, respectively) carcinoma, whereas loss at 17p occurred only in fallopian tube and ovarian carcinomas (in 15% and 25% of cases, respectively). In all three types, gains were more frequent than losses.

Table 4. Chromosomal changes detected by CGH in serous fallopian tube (n=20), endometrial (n=24) and ovarian (n=20) carcinomas.

Change[a]	Fallopian tube	Endometrium	Ovary
+ 1q	40%	29%	30%
+ 3q	70%	50%	40%
+ 5p	30%	29%	30%
+ 6p	25%	29%	30%
+ 7q	35%	21%	35%
+ 8q	75%	33%	70%
+ 12p	40%	17%	30%
+ 20q	30%	17%	20%
- 4q	25%	17%	20%
- 5q	30%	4%	15%
- 8p	30%	8%	25%
- 17p	15%	0%	25%
- 18q	35%	17%	20%
Number of tumors with changes	20/20	17/24	17/20
Mean number of changes/ tumor	7.0	5.7	7.5
Gains:losses	1.6:1	2.8:1	2.3:1

[a] chromosome arms showing gain in at least 30% and loss in at least 25% of serous fallopian tube, endometrial or ovarian carcinomas

2. Allelic analysis of 8p21-p23 and 18q12.3-q23 in ovarian carcinoma (III, IV)

The samples were informative on average at 13 of 18 loci studied at 8p (range 9–17), and at 18 of 27 loci studied at 18q (range 13–25). One serous tumor (sample 210) showed microsatellite instability of several markers at 8p and it was excluded from the analysis because of a possible mismatch-repair-system deficiency. Instability of one to three markers was seen in six serous tumors: samples 852, 223 and 412, one marker each at 8p, samples 852, 810 and 1106, one marker each at 18q, and sample 1097, three markers at 18q. In mucinous carcinomas, one tumor (sample 783) showed instability of one locus at 8p.

2.1. Comparison of serous and mucinous ovarian carcinomas

Allelic loss at 8p and 18q regions was more frequent in serous than in mucinous ovarian carcinoma. In serous carcinoma, allelic loss was detected at 8p in 67% (41/61) and loss at 18q in 59% (38/64) of tumors, whereas in mucinous carcinoma LOH was found in 21% (3/14) and 11%

(1/9) of tumors, respectively (p=0.0025 and p=0.018). In addition, at 8p the deletions were larger in serous than in mucinous tumors: the average number of markers with LOH in tumors showing allelic loss was 9.6 in serous carcinomas and 1.3 in mucinous carcinomas. The only mucinous tumor presenting with allelic loss at 18q showed LOH at 12 of 15 informative markers, and in serous carcinoma, LOH at 18q was found on average at 12.8 markers. The mean degrees of LOH of informative alleles in serous carcinoma were 50% at 8p and 42% at 18q, whereas in mucinous carcinoma the figures were 1.9% and 8.3%, respectively (p=0.0008 and p=0.0013). Grades and stages of mucinous tumors were lower than those of serous tumors. However, when taking into account only grade 1-2 or stage I-II tumors, the difference in the degree of LOH remained significant.

2.2. Comparison of allelic loss at 8p and 18q in serous carcinomas

A larger number of serous than mucinous tumors were analyzed to define the putative tumor suppressor locus/loci more precisely at 8p and 18q (62 tumors as regards 8p and 64 tumors as regards 18q). LOH was seen in approximately 60% of serous carcinomas at both of these regions (67% showed loss at 8p and 59% at 18q) (p=0.46). Allelic loss at all informative markers was seen in 51% (21/41) of tumors showing LOH at 8p and in 39% (15/38) of tumors showing LOH at 18q (p=0.37). Several tumors presented with a complex pattern of allelic loss at both regions, showing multiple interstitial losses and retained alleles.

In 51 cases, allelic analysis was performed at both 8p and 18q. In 65% of the cases (33/51) LOH status was similar (either LOH at both 8p and 18q, or no LOH at either 8p or 18q), and in 35% of the cases (18/51) LOH status was different at 8p and 18q. The association between LOH at these regions was not significant (p=0.13).

2.3. Clinicopathological characteristics

In serous carcinoma, LOH was associated with tumor grade at both 8p and 18q. At 8p LOH was detected in 36% of grade 1 tumors, in 68% of grade 2 tumors and in 77% of grade 3 tumors (p=0.029), and at 18q in 7.1%, 72% and 77% of tumors, respectively (grade 1 vs. grades 2 and 3, p<0.001). No association was found between LOH and stage of serous tumors. In mucinous carcinoma, there was no correlation between LOH and clinicopathological parameters.

Patients with serous carcinomas showing LOH at 18q had poor overall survival when compared with patients with serous carcinomas showing no LOH (p=0.044). Tumor grade was also associated with survival (p=0.0009), but the association between tumor stage and survival did not reach statistical significance (p=0.058). In multivariate analysis, only grade was an independent prognostic factor.

2.4. Minimal common regions of loss in serous carcinoma

2.4.1. 8p21-p23 (III)

Twenty serous carcinomas showing partial losses at 8p21-p23 were used to construct a deletion map. There were three markers that showed LOH in ≥60% of informative alleles: D8S499 (8p21.1), D8S552 (8p22) and D8S1721 (8p23.1). Based on the deletion map, three distinct minimal common regions of loss could be defined around these markers: R1 between D8S1810 and D8S1771 at 8p21.1 (size approximately 7 cM), R2 between D8S1731 and D8S640 at 8p22-8p23.1 (approximately 4 cM) and R3 between D8S520 and D8S277 at 8p23.1 (approximately 11 cM).

2.4.2. 18q12.3-q23 (IV)

Twenty-three serous tumors presenting with partial losses at 18q12.3-q23 were used for deletion mapping. The highest frequencies of LOH were seen in the distal part of the chromosome arm, 18q22-q23. Three markers showed LOH in >75% of informative cases: D18S483 (18q22), D18S979 (18q22) and D18S871 (18q23). Two minimal common regions of loss could be defined around these markers: MCRL1 between markers D18S465 and D18S61 at 18q22 (size approximately 4 cM), and MCRL2 between markers D18S462 and D18S70 at 18q23 (approximately 5 cM).

3. Expression analysis of candidate genes located at 8p21-p23 and 18q12.3-q23

3.1. GATA-4 (III)

The *GATA4* gene is located at one of the minimal common regions of loss detected in this study (R3 at 8p23.1). In Northern blotting, GATA-4 mRNA expression was detected in 62% (16/26) of mucinous and 12% (4/33) of serous ovarian carcinomas.

Immunohistochemistry of normal ovarian samples showed GATA-4 in the nuclei of surface epithelial cells, particularly in the metaplastic cuboidal and columnar cells on the surface and in the inclusion cysts. Positive immunostaining was noted in the nuclei of stromal cells in some of the samples (normal ovary, serous and mucinous carcinomas). Positive nuclear staining in carcinoma cells was detected in 66% (49/75) of mucinous carcinomas, but only in 2.3% (12/528) of serous carcinomas ($p<0.0001$). The difference remained significant when only grade 1-2 ($p<0.0001$) or stage I-II ($p<0.0001$) tumors were taken into account.

In mucinous carcinomas, GATA-4 staining correlated negatively with the grade and stage of the tumors ($p=0.016$ for grade, $p<0.0001$ for stage). In serous carcinomas, there was no such association.

Forty-one serous and 10 mucinous tumors were analyzed for both LOH at 8p and GATA-4 immunostaining. All the serous cases showing allelic loss of the whole of distal 8p had lost GATA-4 protein expression and only 1 of 7 cases showing partial deletions including the GATA-4 region presented with positive GATA-4 staining. Of the 19 serous cases showing no LOH at distal 8p, only two were positive for GATA-4 staining. In mucinous carcinomas two cases showed allelic loss at one marker located at R3 (249 at D8S1140, 783 at D8S1721) and both of these cases were positive for GATA-4 immunostaining. Seven of the 8 mucinous carcinomas showing no LOH at distal 8p had positive GATA-4 staining and in the one remaining sample the staining was not interpretable.

3.2. SMAD4, SMAD2 and DCC (IV)

SMAD4, *SMAD2* and *DCC* genes are located at 18q21.1. In normal ovarian tissue positive immunoreactivity of SMAD4 (moderate to strong), SMAD2 (weak) and DCC (focally weak to moderate) was observed in surface epithelial cells and a proportion of stromal cells. Lost or very weak expression of SMAD4, SMAD2 and DCC was found in 28% (17/60), 28% (17/60) and 30% (18/60) of serous carcinomas, respectively. There was a tendency towards a higher amount of lost expression of SMAD4, SMAD2 and DCC in tumors with LOH at 18q21.1 compared with the tumors with no LOH at 18q21.1 (42–46% and 17–20%, respectively). When analyzing the additive effect of all three factors, a total of 83% of the tumors with LOH at 18q21.1 had lost SMAD4, SMAD2 and/or DCC expression, whereas 40% of the tumors with no LOH at 18q21.1 had lost expression of one or more of these proteins.

4. P53 immunostaining and clinical correlates in serous ovarian carcinomas (V)

4.1. P53 immunohistochemistry

Weak P53 immunopositivity was detected in a small proportion of normal ovarian surface and fallopian tube epithelial cells. P53 staining was interpretable in 505 of 522 serous ovarian carcinomas. Forty-one percent of the tumors showed weak immunostaining similar to that seen in normal epithelium and were regarded as showing "normal P53 staining". Two patterns of aberrant P53 expression were identified: in "excessive staining", the majority of tumor cells (>50%) showed homogeneous moderate or strong immunopositivity, and in "negative staining" all tumor cells were completely devoid of staining. Excessive P53 staining was seen in 43% and negative staining in 16% of the tumor samples.

4.2. Association with clinicopathological characteristics

Both excessive and negative P53 staining were associated with advanced stage ($p<0.0001$ for both), high grade ($p<0.0001$ for both), large residual tumor size ($p<0.0001$ for both), presence of ascites ($p<0.0001$, $p=0.0088$) and greater patient age ($p<0.0001$, $p=0.0009$) compared with tumors showing normal P53 staining.

4.3. Association with overall survival

Both excessive and negative P53 staining were associated with poor overall survival compared with tumors showing normal P53 staining ($p<0.0001$ for both comparisons). There was no significant difference in overall survival between tumors showing excessive and negative P53 staining ($p=0.21$), and they were combined as one group, called "aberrant P53" for further analyses. Univariate analyses of clinicopathological characteristics and P53 status in the whole cohort and distinct subgroups are shown in Table 5. Multivariate analysis showed independent prognostic value for residual tumor size, FIGO stage, patient age, tumor grade and P53 status (Table 6). When stage I and stage III carcinomas were analyzed separately, P53 status was still an independent prognostic factor of overall survival (Table 6).

Table 5. Prognostic significance of different factors for overall survival according to univariate analyses by log-rank test in serous ovarian carcinoma.

Variable	All tumors (n = 522)	Stage I (n = 109)	Stage III (n = 283)
FIGO Stage	< 0.0001	*	*
Grade	< 0.0001	0.0013	< 0.0001
Residual tumor	< 0.0001	0.5613	< 0.0001
Age	< 0.0001	0.4681	< 0.0001
Tumor size	0.0333	0.0127	0.1124
Ascites	< 0.0001	0.5287	0.0869
P53	< 0.0001	< 0.0001	< 0.0001

* = not analyzable

4.4. Association with response to therapy and disease-free survival

Carcinomas with aberrant P53 staining more frequently showed no response to therapy compared with tumors with normal P53 expression ($p<0.0001$). This association was also found when stage III ($p=0.0016$) carcinomas were analyzed separately.

The disease-free survival time of patients with tumors showing aberrant P53 expression was shorter than that of those with normal P53 expression ($p<0.0001$). This association was also seen in stage I ($p<0.0001$) and stage III ($p<0.0001$) carcinomas analyzed separately.

4.5. Patients treated with platinum-based combination chemotherapy

In patients treated with platinum-based combination therapy (n=347) similar as-

Table 6. Cox proportional hazards models of independent prognostic factors for overall survival in serous ovarian carcinoma.

		All carcinomas (n=446)		Stage I (n=106)		Stage III (n=259)	
Variable		HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
FIGO Stage	I	ref		*	*	*	*
	II	2.4 (1.2- 4.7)	0.0112				
	III	3.3 (1.8- 6.1)	0.0001				
	IV	4.6 (2.3- 9.2)	< 0.0001				
Residual tumor > 1 cm		2.2 (1.6- 3.1)	< 0.0001	ns	ns	2.8 (1.9-4.2)	< 0.0001
Grade	1	ref		ns	ns	ns	ns
	2	1.8 (1.2- 2.9)	0.0107				
	3	2.1 (1.3- 3.4)	0.0026				
Age >57 years		1.6 (1.2- 2.1)	0.0006	ns	ns	1.8 (1.3-2.4)	.0006
Aberrant P53		1.8 (1.2- 2.8)	0.0054	10.0 (3.6-27.8)	< 0.0001	2.9 (1.8-4.5)	< 0.0001

HR = hazard ratio; CI = confidence interval; ref = reference category; * = not analyzable; ns = non-significant

sociations were found as for the whole cohort: aberrant P53 expression was associated with poor overall survival, both in univariate ($p<0.0001$) and multivariate ($p=0.042$) analyses, poor response to therapy ($p<0.0001$) and shorter disease-free survival ($p<0.0001$).

Patients who received platinum compounds with cyclophosphamide or cyclophosphamide and epirubicin (group 1: normal P53, n=95; aberrant P53, n=143) and patients receiving platinum in combination with paclitaxel (group 2: normal P53, n=31; aberrant P53, n=66) were compared (median follow-up times 115 and 23 months, respectively). In group 1, the 2-year and 5-year overall survival rates were 94% and 84% for those with normal P53, and 50% and 28% for those with aberrant P53 ($p<0.0001$). In group 2, the 2-year and 5-year overall survival rates were 83% and 75% for those with normal P53, and 71% and 33% for those with aberrant P53 ($p=0.075$). In group 1, 33% of carcinomas with aberrant P53, compared with 61% of carcinomas with normal P53, showed a complete response to therapy ($p<0.0001$). In group 2, a complete response was seen in 44% of tumors with aberrant P53 and 63% of tumors with normal P53 ($p=0.052$). In cases of tumors with aberrant P53, patients treated with paclitaxel/platinum (n=66) tended to show better overall survival compared with those on platinum/cyclophosphamide treatment (n=143) ($p=0.056$), but no such association was seen as regards tumors with normal P53.

Discussion

1. Evaluation of the methods

Comparative genomic hybridization is a powerful method of screening for gains and losses in tumor genomes and pinpointing locations of potential oncogenes and tumor suppressor genes (amplifications and losses) (Kallioniemi et al., 1992). It has produced large amount of new knowledge of recurrent chromosomal changes in cancers (Knuutila et al., 1998; Knuutila et al., 1999), especially in solid tumors, and led to the identification of genes that play important roles in cancer development and progression (Visakorpi et al., 1995a; Anzick et al., 1997; Hemminki et al., 1998). In addition to pinpointing locations of important genes, the pattern of changes detected by CGH can also be used as a "fingerprint" when comparing different tumor types. Both of these qualities were utilized in this study.

For more detailed mapping of lost or amplified chromosomal regions detected by CGH, several methods can be used, including LOH and FISH analyses as well as new array-based CGH techniques (Pinkel et al., 1998; Pollack et al., 1999). In this study, allelic analysis was used for fine mapping of recurrent regions of loss. Both in CGH and LOH analyses a sufficient proportion of tumor cells in the samples is important to avoid underestimation of changes. For this reason, only samples containing over 50% of tumor cells were used for CGH in this study, and for LOH, microdissection was used when the proportion of tumor cells was considered to be too low.

High overall concordance of LOH and CGH results has been reported, ranging from 76% to 92% (Iwabuchi et al., 1995; Joos et al., 1995; Visakorpi et al., 1995b). In ovarian carcinoma, the concordance has varied from 56% to 100%, depending on the locus, and being 84% overall (Iwabuchi et al., 1995). Various reasons may cause discrepant results between the methods at certain loci: The resolution of CGH allows detection of physical deletions of over 10–20 Mb in length. If loss is small and discontinuous, or if LOH is due to mitotic recombination, it cannot be detected by CGH (Kallioniemi et al., 1994). Areas of homozygous loss may or may not be seen by CGH, depending on the size of the deletion. In LOH analysis, because of PCR-based method, homozygous loss may be seen as retention of heterozygosity due to amplification of a small amount of contaminating DNA from non-neoplastic cells. Thus, in this study, the loci showing heterozygosity in the deletion maps were checked for sizes of allele peaks, and no reduction of intensity suggesting homozygous loss was detected. When one allele is lost and the other allele is duplicated (uniparental disomy), CGH shows no copy number change, but LOH is observed. Gain or amplification of one allele may also appear as LOH, because scoring for LOH is based on quantitative analysis of the intensity of the two alleles (Orsetti et al., 1999; Rodriguez et al., 2000). If LOH is suspected to represent gain of one allele rather than loss of the other allele, the term allelic imbalance has been used instead of allelic loss. Because our analyses were based on previous results showing frequent loss detected by CGH in ovarian carcinomas at the studied regions, LOH was unlikely to be due to gain of chromosomal material in this study. Comparison of these results, however, was not possible, because most cases analyzed for LOH in this study had not been analyzed by

CGH. In conclusion, advantages of CGH and allelic analysis complement each other; whereas allelic analysis has higher resolution, CGH is able to detect the direction of the change (loss or gain).

2. Chromosomal changes in endometrial carcinoma – comparison of serous and endometrioid histological types (I)

Most of the endometrial carcinomas previously analyzed cytogenetically or by CGH have been of the endometrioid histological type, since it accounts for the majority of endometrial carcinomas. The finding of relatively simple chromosomal changes in the endometrioid type, in the present study, is in agreement with previous cytogenetic analyses. As in this study, the most frequent aberration found in cytogenetic and CGH studies (Table 1) has been the gain at 1q (Fujita et al., 1985; Couturier et al., 1986; Couturier et al., 1988; Milatovich et al., 1990; Shah et al., 1994; Bardi et al., 1995; Sonoda et al., 1997a; Suzuki et al., 1997; Suehiro et al., 2000; Baloglu et al., 2001). Chromosome arm 8q is the second most common region showing increased copy number in CGH in the present and other studies. In contrast to the present results, other CGH studies have revealed a higher number of chromosomal changes, particularly losses, in endometrial carcinoma (Sonoda et al., 1997a; Suzuki et al., 1997; Suehiro et al., 2000; Baloglu et al., 2001). The explanation for this discrepancy is unknown. It is not likely to be due to less aggressive pathological characteristics of the tumors, since our material was selected to match the serous endometrial carcinomas and thus it was of higher stage and grade than endometrioid carcinomas in general.

Karyotypic or CGH analyses have been performed only on a few cases of serous endometrial carcinoma (Bardi et al., 1995; Sonoda et al., 1997a; Tibiletti et al., 1997). In accordance with the literature, the present results showed extensive and complex aberrations in serous endometrial carcinomas (Bardi et al., 1995; Tibiletti et al., 1997). The most common aberration found in the present study was gain at chromosome arm 3q, which was seen in half of the serous samples. Gain and amplification at 3q has also been seen frequently in ovarian carcinoma as well as in cancers of the uterine cervix and lung (Knuutila et al., 1998). Several putative oncogenes have been proposed at the distal part of 3q. *PIK3CA*, which encodes a catalytic subunit of phosphatidylinositol 3-kinase and is located at 3q26, has been found to be amplified in ovarian cancers. Amplification was found to be associated with increased PIK3CA transcription, protein expression and PI3-kinase activity (Shayesteh et al., 1999). Recently, another candidate oncogene, *EIF-5A2*, was isolated at 3q26. It was amplified and overexpressed in primary ovarian cancers and ovarian cancer cell lines (Guan et al., 2001). *PIK3CA* and *EIF-5A2* might be affected in serous endometrial carcinoma, but copy number and expression of these genes has not been studied in endometrial carcinoma. The gains at 3q were usually large and in addition to the above-mentioned genes, the affected region contains many other genes that might be involved in serous endometrial carcinoma.

In the present study, serous and endometrioid endometrial carcinomas were found to be distinct in respect to their DNA copy number karyotypes. Chromosomal imbalances were more common and complex in serous than in endometrioid carcinomas. Several regions, including 3q, 5p, 6p, 7q, 8q, 11q and 19q, showed copy number gains more frequently in the serous than in the endometrioid type. Losses were less frequent than gains in both histological types, but in particular they were rare in the endometrioid type. Serous carcinoma has poor prognosis (Hendrickson et al., 1982; Bokhman, 1983), and according to the present findings aggressive biological

behavior associated with the extent of genomic change. Recently, molecular evidence has emerged supporting distinct pathways of pathogenesis for these two types of endometrial carcinoma. Mutations of *PTEN* are frequent in endometrioid carcinoma and endometrial hyperplasia, but they are not seen in serous carcinoma (Kong et al., 1997; Risinger et al., 1997; Tashiro et al., 1997a; Levine et al., 1998; Maxwell et al., 1998). In contrast, mutations of *P53* and protein overexpression are typical of serous carcinoma and its putative precursor EIC, but they are infrequently found in endometrioid carcinoma and not seen in endometrial hyperplasia (Kohler et al., 1992; Sherman et al., 1995; Moll et al., 1996; Zheng et al., 1996; Tashiro et al., 1997b; Lax et al., 2000). Microsatellite instability and *KRAS* mutations have been identified in subsets of endometrioid, but not of serous carcinoma (Risinger et al., 1993; Duggan et al., 1994a; Caduff et al., 1995; Tashiro et al., 1997c; Lax et al., 2000). To summarize, the present and previous findings suggest distinct pathogenetic pathways for serous and endometrioid endometrial carcinomas, and support the clinicopathological model of two types of endometrial carcinoma (Bokhman, 1983).

3. Chromosomal changes in serous fallopian tube carcinoma – comparison with serous endometrial and ovarian carcinomas (II)

Interestingly, the pattern of chromosomal changes found in serous endometrial carcinoma (I) resembled the pattern found in serous ovarian carcinoma (Tapper et al., 1998), which prompted us to analyze another serous carcinoma of Müllerian origin, fallopian tube carcinoma. Serous fallopian tube carcinoma presented with frequent and complex chromosomal aberrations, which is in agreement with previous data (Bardi et al., 1994; Heselmeyer et al., 1998). The regions of the most common copy number changes in the present study showed similarity with those detected by Heselmeyer et al. (Heselmeyer et al., 1998). In particular, the most frequently gained regions were very similar, with common copy number increases at 3q, 8q, 1q, 5p, 7q and 12p in both studies. Both studies also revealed frequent copy number decreases at 18q and 8p. However, in the study by Heselmayer et al. there were common losses at 16q, 22q and 1p, which were not detected in the present study. One reason for this discrepancy may be sensitivity of these regions to artefacts (Kallioniemi et al., 1994; Bjorkqvist et al., 1998).

The pattern of chromosomal changes detected in fallopian tube carcinoma by CGH was very similar to those found in serous endometrial (I) and serous ovarian (Tapper et al., 1998) carcinomas. All these carcinomas showed complex and extensive aberrations, with gains predominating over losses, and the most frequent copy number gains and losses showed similarity. Furthermore, the pattern of changes in serous carcinomas was different from those detected in other histological types of endometrial and ovarian carcinoma (endometrioid and mucinous) (I) (Tapper et al., 1997). The epithelia of the uterus, fallopian tube and ovary share a common embryological background, and serous carcinomas derived from these organs present with a similar appearance in histopathological examination. Mutations of *P53* and overexpression of the protein are frequently observed in all these carcinomas (Milner et al., 1993; Klemi et al., 1995; Lacy et al., 1995; Sherman et al., 1995; Tashiro et al., 1997b; Zheng et al., 1997). Clinically, they all exhibit invasive behavior, early dissemination and poor prognosis (Kurman, 1994). These findings suggest that serous carcinomas of the endometrium, fallopian tube and ovary share common genetic events in tumor development and progression.

4. Allelic analysis of ovarian carcinoma at chromosome arms 8p and 18q – comparison of serous and mucinous histological types (III, IV)

Chromosomal regions that showed frequent losses in CGH analyses, distal parts of chromosome arms 8p and 18q, were mapped further using allelic analysis. The microsatellite markers chosen for allelic analyses at these regions showed a high level of informativeness: on average 70% of the loci were informative in each sample. Replication error of several microsatellites, indicating MSI phenotype (Boland et al., 1998), was seen only in one serous tumor. The frequency of MSI was lower than reported for ovarian carcinoma in general (Fujita et al., 1995; King et al., 1995; Sood et al., 2001). However, consistent with the present findings, a lower prevalence of MSI has been found in the serous histological type (Fujita et al., 1995; King et al., 1995; Haas et al., 1999).

In the present study, at both 8p and 18q allelic losses were more frequent and extensive in serous than in mucinous ovarian carcinomas. Previously, a higher frequency of LOH at other chromosomal arms, including 6q, 13q, 11p, 11q, 17p, 17q, 19q and 22q, has been reported for serous versus non-serous carcinomas, especially mucinous ones (Sato et al., 1991; Pieretti et al., 1995; Papp et al., 1996; Lu et al., 1997; Bryan et al., 2000; Launonen et al., 2000). These regions may contain tumor suppressor genes relevant to serous ovarian carcinoma, but the high frequency of changes may also reflect general genomic instability of serous carcinomas. Many of the previous LOH studies on ovarian carcinoma have not revealed differences between the histological types, including those that have concentrated on chromosome arms 8p and 18q (Chenevix-Trench et al., 1992; Dodson et al., 1993; Yang-Feng et al., 1993; Osborne and Leech, 1994; Wright et al., 1998; Brown et al., 1999; Takakura et al., 1999; Pribill et al., 2001). This may be partly due to the small number of non-serous tumors, especially mucinous tumors, included in these studies. On a molecular level, differences between serous and mucinous ovarian carcinomas have been identified in alterations of *KRAS* and *P53*. *KRAS* mutations are typical of mucinous carcinomas, but rare in other histological types, whereas *P53* mutations are frequent in serous carcinomas and uncommon in mucinous ovarian carcinomas (Enomoto et al., 1991b; Milner et al., 1993; Ichikawa et al., 1994; Klemi et al., 1995; Suzuki et al., 2000a). In addition, distinct chromosomal aberrations have been identified in serous and mucinous ovarian carcinomas in cytogenetic analysis and CGH (Pejovic et al., 1992b; Diebold et al., 1996; Diebold et al., 1997; Tapper et al., 1997). Consistent with these findings, the present results suggest a different molecular pathogenesis for serous and mucinous ovarian carcinomas.

5. Fine allelotype mapping and expression of candidate genes (III, IV)

5.1. LOH at 8p21-p23 and 18q12.3-q23 in serous ovarian carcinoma

Because LOH was more frequent and extensive in serous carcinomas than in mucinous carcinomas, a larger number of serous carcinomas were analyzed at both 8p and 18q to define the putative tumor suppressor locus/loci more precisely. Similar overall patterns of allelic loss were found at 8p21-p23 and 18q12.3-q23 in serous ovarian carcinomas. At both regions LOH was seen in approximately 60% of tumors and about half of these tumors showed LOH at all informative markers. Complex patterns of LOH showing multiple interstitial losses and retained alleles between them were detected in several tumors at both regions. This phenomenon has also been identified at other chromosomal regions in ovarian carcinoma (Lu et al., 1997; Fullwood et al., 1999). LOH at 8p and 18q were not sig-

nificantly associated with each other. This suggests that the events are not linked and the frequent LOH seen at these sites is not a random consequence of genomic instability.

In the present study, three minimal common regions of loss were defined at 8p21-p23 (Figure 4). Other allelotype studies of ovarian carcinoma have each revealed one to three smallest regions of loss at 8p (Wright et al., 1998; Brown et al., 1999; Pribill et al., 2001). However, these studies involved analysis of various histological types of ovarian carcinoma as a single disease entity and used different selections of microsatellite markers, therefore making direct comparison of the results difficult. Only the analysis by Wright et al. included several markers distal to D8S261 (8p22). The distal and middle regions found by them overlap with the R3 and R2 regions defined in the present study. The most distal region found by Pribill et al. was around marker D8S261. The telomeric breakpoint of this region was difficult to define, because the more distal marker D8S1992 showed a higher frequency of LOH than D8S261. This region overlaps with the R2 region of the present study. The studies by Pribill et al. and Brown et al. revealed the highest frequencies of allelic loss at marker D8S136, which was not included in the present study. However, the centromeric breakpoint of this region defined by Pribill et al. extends more proximally and this region overlaps with the R1 region of the present study. The most proximal region defined by Pribill et al. was located around markers that were not included in the present study. Despite the differences in tumor material and markers used in these studies, the findings suggest the presence of more than one tumor suppressor gene at 8p involved in ovarian carcinoma, especially its serous histological type.

The highest frequency of allelic loss at 18q was found distal to 18q21 in the

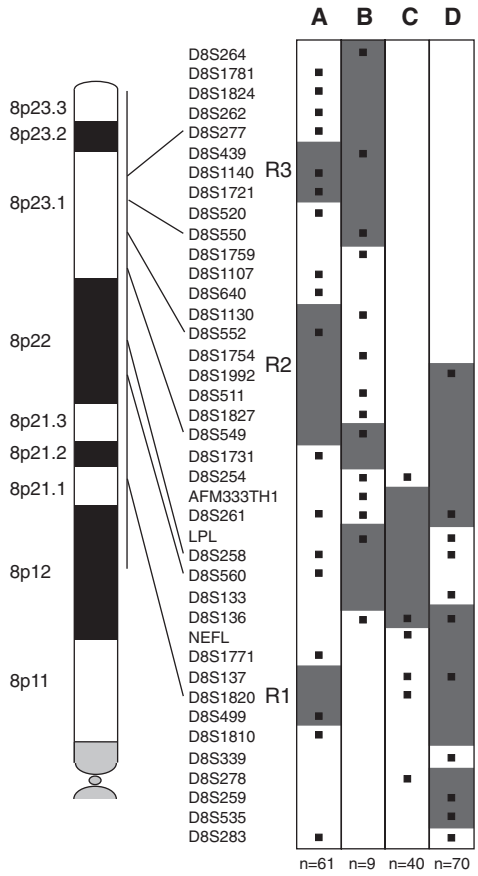

Figure 4. Integration map of minimal common regions of LOH at 8p12-p23 in ovarian carcinoma: **A** the present study (III), **B** study by Wright et al., **C** by Brown et al. and **D** by Pribill et al. (Wright et al., 1998; Brown et al., 1999; Pribill et al., 2001). The approximate loci of anchor markers from Genemap'99 are shown on the right side of chromosome 8p figure. ■, markers used in each study. *Shaded areas*, minimal common regions of loss (extend to the flanking markers retaining heterozygosity). R1, R2 and R3, minimal common regions of loss in the present study. Numbers of tumor samples analyzed in each study are shown under the corresponding column.

present study. Two minimal common regions of loss were identified: one between D18S465 and D18S61 at 18q22 and the other between D18S462 and D18S70 at 18q23. To our knowledge, the present work represents the first detailed allelotype mapping of distal 18q in ovarian carcinoma. In

one study, including six markers at 18q21, the highest frequency of allelic loss was found at marker D18S474 (Takakura et al., 1999). The marker showed LOH in 36% of the cases, a frequency which is in agreement with the present findings concerning that marker. However, the study by Takakura and colleagues was limited to markers located at 18q21. Consistent with the present results, in another study, including five markers at 18q, the highest frequency of losses was found distal to 18q21 (Chenevix-Trench et al., 1992). In that study only two markers were located at 18q22-q23 and a more detailed analysis of the distal region was not possible. The findings in the present study suggest the presence of as yet unknown tumor suppressor genes at 18q22-q23 in serous ovarian carcinoma.

5.2. GATA-4

GATA4 is located at 8p23.1 in the R3 minimal common region of loss in the present study. GATA-4 belongs to a family of zinc finger transcription factors, which by binding to a consensus GATA motif present in the promoter of target genes regulate cell differentiation and proliferation in a variety of tissues including the ovary. GATA-4 is expressed in the normal surface epithelium of human and mouse ovary (Heikinheimo et al., 1997; Laitinen et al., 2000). GATA binding sites have been identified in the promoters of Müllerian inhibiting substance (MIS) and inhibin-α genes, and GATA-4 has been found to regulate the expression of these genes (Viger et al., 1998; Ketola et al., 1999; Tremblay and Viger, 1999; Watanabe et al., 2000). Both MIS and inhibin-α belong to TGF-ß superfamily, which is known to have tumor suppressing activities. In the present study, expression of GATA-4 was lost in most serous ovarian carcinomas, but retained in the majority of mucinous carcinomas. Loss of GATA-4 expression has also been reported in gastric carcinoma cell lines (Bai et al., 2000). In contrast, an amplicon of 8p22-p23 containing GATA4 and cathepsin B has been identified in a subset of esophageal and gastric cardia adenocarcinomas (13%) (Lin et al., 2000), and adrenocortical carcinomas have been shown to express GATA-4 (Kiiveri et al., 1999). Overall, little is so far known about the possible role of GATA-4 in cancer development and progression.

5.3. SMAD4, SMAD2 and DCC

The putative tumor suppressor genes *SMAD4*, *SMAD2* and *DCC* are located at 18q21.1 (Fearon et al., 1990; Eppert et al., 1996; Hahn et al., 1996). SMAD4 and SMAD2 are part of the transforming growth factor-ß signaling pathway. *DCC* was found as a gene frequently deleted in colon carcinoma (Fearon et al., 1990), and it has been shown to code a receptor for the axonal chemoattractant netrin-1 (Fazeli et al., 1997). Mutations of *SMAD4* have been detected in under 5% of primary ovarian carcinomas studied (Schutte et al., 1996; Takakura et al., 1999; Wang et al., 2000). We found lost or reduced expression of SMAD4 in 28% of serous ovarian carcinomas, in accordance with reduced SMAD4 mRNA expression found in ovarian cancer cell lines (Hu et al., 2000). Previously, deletion in one intron of *SMAD2* has been identified in a subset of ovarian carcinomas, but no abnormal expression of SMAD2 was found by Western blotting in those samples (Wang et al., 2000). This is in contrast to our finding that 28% of serous ovarian carcinomas showed lost or reduced expression of SMAD2. One explanation for the discrepancy could be possible normal cell contamination in samples used for Western blotting. In agreement with the present results, decreased expression of DCC has been previously reported in a subset of ovarian carcinomas (Enomoto et al., 1995; Saegusa et al., 2000).

5.4. Association of LOH with expression of candidate genes

Loss of expression of GATA-4, SMAD4, SMAD2 and DCC was associated with LOH at the locations of these genes. However, almost 90% of serous carcinomas without LOH at 8p23.1 presented with negative GATA-4 immunostaining, and about 20% of serous carcinomas that had no LOH at 18q21.1 showed loss of expression of SMAD4, SMAD2 or DCC. These tumors may contain small deletions that reside between the markers and could not be detected by the LOH analysis. Other mechanisms including biallelic mutations, regulation at the transcriptional level or epigenetic events such as hypermethylation may also cause down-regulation of expression. On the other hand, more than 50% of serous carcinomas with LOH at 18q21.1 presented with positive SMAD4, SMAD2 or DCC immunostaining. If one allele of the gene is deleted, as indicated by LOH analysis, the other allele of the gene must still be expressed in these cases and the gene may be functionally active. These findings suggest the existence of other tumor suppressor gene(s) as additional and maybe prime targets of frequent allelic loss at distal 18q.

6. Clinical associations and prognostic value of chromosomal and molecular changes in serous carcinomas (I, III–V)

A serous histological type is regarded as a poor prognostic factor in endometrial carcinoma (Hendrickson et al., 1982), but little is known of the prognostic factors within serous endometrial carcinoma. In the present study, patients with serous endometrial carcinomas that showed changes detected by CGH had poor overall survival, indicating heterogeneity in this disease, which may be related to the level of genomic imbalance. An association between genomic aberrations detected by CGH and patient outcome has also been reported in endometrioid endometrial carcinoma as well as in ovarian and breast cancers (Isola et al., 1995; Iwabuchi et al., 1995; Suehiro et al., 2000; Suzuki et al., 2000b).

Allelic loss at 8p has been associated with the tumor grade of ovarian carcinomas (Dodson et al., 1993; Pribill et al., 2001). In addition to LOH at 8p, we found an association between tumor grade and LOH at 18q in serous ovarian carcinomas. When all histological types have been analyzed in combination, allelic losses at 8p and 18q have been associated with advanced stage (Chenevix-Trench et al., 1992; Wright et al., 1998; Brown et al., 1999; Zborovskaya et al., 1999; Pribill et al., 2001), but no such association was seen in our series of serous ovarian carcinomas. We found an association between LOH at distal 18q and poor overall survival. The present finding is in agreement with the results of a genome-wide study of copy number changes in ovarian carcinoma showing an association between loss at 18q and poor survival (Suzuki et al., 2000b). Also in accordance with our findings, Suzuki et al. found that the number of chromosomal changes and alterations in specific regions were associated with grade, but not with stage, suggesting that tumor grade is a better measure of genome evolution than tumor stage in ovarian carcinoma (Suzuki et al., 2000b).

The biological behavior, response to treatment and prognosis of apparently similar cases of ovarian carcinoma are variable. At the moment, decisions on the use of adjuvant therapy are mainly based on the spread of the disease (stage) and histological differentiation (grade). Surgery alone is regarded as adequate in patients with well differentiated, stage Ia and Ib ovarian carcinomas; others (including all patients with clear cell carcinomas) receive chemotherapy (NIH consensus, 1995). In advanced carcinoma, residual tumor size has proved to be an important prognostic indicator. Patient age and performance status are closely related and both have been shown to have in-

dependent prognostic significance in ovarian carcinoma. The importance of standard clinicopathological prognostic factors (FIGO stage, residual tumor size, histologic grade and patient age), which have previously been established for all ovarian carcinoma subtypes together (Makar et al., 1995; Friedlander, 1998; Chi et al., 2001; Vergote et al., 2001), was verified for serous ovarian carcinomas in the present study.

New clinically useful prognostic factors are needed for ovarian carcinoma. Several factors are under investigation, but so far, no molecular marker has shown strong enough prognostic value for clinical purposes (Friedlander, 1998; Eisenhauer et al., 1999). The possible prognostic role of P53 alterations in ovarian carcinomas has been assessed in many studies, with inconsistent findings (Marks et al., 1991; Hartmann et al., 1994; Henriksen et al., 1994; Klemi et al., 1995; Eltabbakh et al., 1997; Rohlke et al., 1997; Silvestrini et al., 1998; Anttila et al., 1999; Baekelandt et al., 1999; Gadducci et al., 2000; Geisler et al., 2000; Levesque et al., 2000; Fallows et al., 2001). Several investigators have reported overexpression in immunohistochemistry to confer poor outcome, but only four groups to date have shown an independent prognostic value of P53 immunostaining status (Klemi et al., 1995; Rohlke et al., 1997; Baekelandt et al., 1999; Geisler et al., 2000). In our cohort, P53 expression status was an independent prognostic factor for overall survival both at an early stage as well as in advanced carcinoma, and aberrant P53 predicted a poor response to therapy and a shorter disease-free survival time. The present study, indicating a strong prognostic role for P53 status, was distinct from previous studies in at least three aspects. Firstly, we analyzed only serous carcinoma, the type that has previously shown the highest frequency of P53 alterations (Milner et al., 1993; Klemi et al., 1995; Eltabbakh et al., 1997; Rohlke et al., 1997; Anttila et al., 1999; Geisler et al., 2000). On the basis of the results of the present (III, IV) and previous studies, the molecular background and biological behavior of various histological types of ovarian carcinoma are different (Enomoto et al., 1991b; Omura et al., 1991; Sato et al., 1991; Milner et al., 1993; Klemi et al., 1995; Makar et al., 1995; Diebold et al., 1997; Tapper et al., 1997; Obata et al., 1998). Thus, the prognostic value of a given marker in various subtypes may not be similar. Secondly, the use of a tissue microarray technique (Kononen et al., 1998) enabled evaluation of a large number of samples, providing power to statistical analyses. Thirdly, a negative P53 staining result has previously been interpreted as wild-type P53. However, only missense mutations have been associated with increased P53 protein (Casey et al., 1996; Skilling et al., 1996; Shahin et al., 2000; Reles et al., 2001), and normal tissues have shown P53 immunopositivity in a small proportion of cells (V) (Wen et al., 1999). In the present series, tumor samples with completely negative P53, distinct from those showing a normal (wild-type) expression pattern, were associated with as poor an outcome as those with excessive P53 staining.

In stage I serous ovarian carcinomas, where histological differentiation has previously been identified as the most powerful prognostic factor (Vergote et al., 2001), we found P53 expression status to be a stronger predictor of overall survival than tumor grade. Based on the present findings, adjuvant therapy should be considered for stage I serous carcinomas with aberrant P53, even if the tumor is well differentiated. In stage III serous carcinomas, P53 status could aid in predicting the response to chemotherapy and identifying patients with particularly poor prognosis. The value of P53 expression status in clinical decision-making needs to be evaluated in a prospective setting.

Future prospects

Knowledge of the pathogeneses of gynecological carcinomas is essential in order to develop strategies for early diagnosis and optimal treatment of individual patients. Until now most research on gynecological carcinomas has been based on the organ of origin. However, the different histological types of endometrial and ovarian carcinoma in this study presented with distinct genetic changes, and serous carcinomas from different organs showed similar chromosomal aberrations. These findings emphasize the importance of histology in classification of gynecological carcinomas. Appropriate classification of cancers into biologically meaningful entities is becoming increasingly important, as more specific and effective treatment modalities are being developed.

Comparative genomic hybridization revealed several regions of the genome that are likely to contain genes involved in the development and progression of gynecological carcinomas. In the future, recently developed methods such as CGH and cDNA microarrays will facilitate investigation of the affected regions and discovery of the underlying genes (Schena et al., 1995; Pinkel et al., 1998; Pollack et al., 1999; Monni et al., 2001). For comparison of mRNA expression, e.g. in cDNA microarray analyses, use of appropriate reference material is essential. However, in the ovary, normal surface epithelium is scarce and represents modified mesothelial rather than epithelial differentiation (Kurman, 1994). On the basis of the present findings, serous fallopian tube carcinoma and normal tubal epithelium might be used as a model to discover differentially expressed genes that could be involved in serous ovarian carcinoma as well.

In the present study, allelic analysis was used for further mapping of two recurrently lost regions in serous ovarian carcinoma. The critical regions were reduced to approximately 30% at 8p and to 10–15% at 18q, of the size defined in CGH. The definitive locations of the markers and genes at the minimal common regions of loss will be revealed on completion of the sequence of the human genome (Lander et al., 2001; Venter et al., 2001). Data on the genomic sequence will allow mutation and epigenetic analyses of the candidate genes. Based on the currently available information, the sizes of the minimal common regions defined in the present study range from 1.6 Mb to 5.4 Mb. Additional polymorphic microsatellite markers as well as single-nucleotide polymorphisms (SNPs) (Wang et al., 1998; Lindblad-Toh et al., 2000) can be used for further mapping of the regions of interest. Increasing knowledge of the functions of genes will aid in selecting candidate genes for structural analyses as well as mRNA and protein expression analyses.

Tissue microarrays (Kononen et al., 1998) proved to be efficient for evaluating the expression of candidate genes and for linking this information to clinicopathological characteristics of tumors and patient outcome. In the future, tissue arrays constructed from gynecological carcinomas can be used for both expression analyses and DNA copy number detection of candidate genes. The collection of clinical, pathological and molecular data in the same database will enable analysis of associations between the different factors and evaluation of possible independent predictive and prognostic value of molecular markers.

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References

Aarnio M, Mecklin JP, Aaltonen LA, Nystrom-Lahti M, Jarvinen HJ. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Int J Cancer 64: 430-3, 1995

Abeler VM, Kjorstad KE. Endometrial adenocarcinoma in Norway. A study of a total population. Cancer 67: 3093-103, 1991

Alvarado-Cabrero I, Young RH, Vamvakas EC, Scully RE. Carcinoma of the fallopian tube: a clinicopathological study of 105 cases with observations on staging and prognostic factors. Gynecol Oncol 72: 367-79, 1999

Anttila MA, Ji H, Juhola MT, Saarikoski SV, Syrjanen KJ. The prognostic significance of p53 expression quantitated by computerized image analysis in epithelial ovarian cancer. Int J Gynecol Pathol 18: 42-51, 1999

Anzick SL, Kononen J, Walker RL, Azorsa DO, Tanner MM, Guan XY, Sauter G, Kallioniemi OP, et al. AIB1, a steroid receptor coactivator amplified in breast and ovarian cancer. Science 277: 965-8, 1997

Arnold N, Hagele L, Walz L, Schempp W, Pfisterer J, Bauknecht T, Kiechle M. Overrepresentation of 3q and 8q material and loss of 18q material are recurrent findings in advanced human ovarian cancer. Genes Chromosomes Cancer 16: 46-54, 1996

Baekelandt M, Kristensen GB, Nesland JM, Trope CG, Holm R. Clinical significance of apoptosis-related factors p53, Mdm2, and Bcl-2 in advanced ovarian cancer. J Clin Oncol 17: 2061-5, 1999

Baekelandt M, Jorunn Nesbakken A, Kristensen GB, Trope CG, Abeler VM. Carcinoma of the fallopian tube. Cancer 89: 2076-84, 2000

Bai Y, Akiyama Y, Nagasaki H, Yagi OK, Kikuchi Y, Saito N, Takeshita K, Iwai T, Yuasa Y. Distinct expression of CDX2 and GATA4/5, development-related genes, in human gastric cancer cell lines. Mol Carcinog 28: 184-8, 2000

Baker VV, Borst MP, Dixon D, Hatch KD, Shingleton HM, Miller D. c-myc amplification in ovarian cancer. Gynecol Oncol 38: 340-2, 1990

Baloglu H, Cannizzaro LA, Jones J, Koss LG. Atypical endometrial hyperplasia shares genomic abnormalities with endometrioid carcinoma by comparative genomic hybridization. Hum Pathol 32: 615-22, 2001

Bandera CA, Takahashi H, Behbakht K, Liu PC, LiVolsi VA, Benjamin I, Morgan MA, King SA, et al. Deletion mapping of two potential chromosome 14 tumor suppressor gene loci in ovarian carcinoma. Cancer Res 57: 513-5, 1997

Bardi G, Sukhikh T, Pandis N, Holund B, Heim S. Complex karyotypic abnormalities in a primary carcinoma of the fallopian tube. Genes Chromosomes Cancer 10: 207-9, 1994

Bardi G, Pandis N, Schousboe K, Holund B, Heim S. Neardiploid karyotypes with recurrent chromosome abnormalities characterize early-stage endometrial cancer. Cancer Genet Cytogenet 80: 110-4, 1995

Bentz M, Plesch A, Stilgenbauer S, Dohner H, Lichter P. Minimal sizes of deletions detected by comparative genomic hybridization. Genes Chromosomes Cancer 21: 172-5, 1998

Berchuck A, Kamel A, Whitaker R, Kerns B, Olt G, Kinney R, Soper JT, Dodge R, et al. Overexpression of HER-2/neu is associated with poor survival in advanced epithelial ovarian cancer. Cancer Res 50: 4087-91, 1990

Bjorkqvist AM, Tammilehto L, Nordling S, Nurminen M, Anttila S, Mattson K, Knuutila S. Comparison of DNA copy number changes in malignant mesothelioma, adenocarcinoma and large-cell anaplastic carcinoma of the lung. Br J Cancer 77: 260-9, 1998

Blegen H, Einhorn N, Sjovall K, Roschke A, Ghadimi BM, McShane LM, Nilsson B, Shah K, et al. Prognostic significance of cell cycle proteins and genomic instability in borderline, early and advanced stage ovarian carcinomas. Int J Gynecol Cancer 10: 477-487, 2000

Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol 15: 10-7, 1983

Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 58: 5248-57, 1998

Boyd J, Rubin SC. Hereditary ovarian cancer: molecular genetics and clinical implications. Gynecol Oncol 64: 196-206, 1997

Brown MR, Chuaqui R, Vocke CD, Berchuck A, Middleton LP, Emmert-Buck MR, Kohn EC. Allelic loss on chromosome arm 8p: analysis of sporadic epithelial ovarian tumors. Gynecol Oncol 74: 98-102, 1999

Bryan EJ, Thomas NA, Palmer K, Dawson E, Englefield P, Campbell IG. Refinement of an ovarian cancer tumour suppressor gene locus on chromosome arm 22q and mutation analysis of CYP2D6, SREBP2 and NAGA. Int J Cancer 87: 798-802, 2000

Burks RT, Kessis TD, Cho KR, Hedrick L. Microsatellite instability in endometrial carcinoma. Oncogene 9: 1163-6, 1994

Buttitta F, Marchetti A, Gadducci A, Pellegrini S, Morganti M, Carnicelli V, Cosio S, Gagetti O, et al. p53 alterations are predictive of chemoresistance and aggressiveness in ovarian carcinomas: a molecular and immunohistochemical study. Br J Cancer 75: 230-5, 1997

Caduff RF, Johnston CM, Frank TS. Mutations of the Ki-ras oncogene in carcinoma of the endometrium. Am J Pathol 146: 182-8, 1995

Caduff RF, Johnston CM, Svoboda-Newman SM, Poy EL, Merajver SD, Frank TS. Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol 148: 1671-8, 1996

Canzian F, Salovaara R, Hemminki A, Kristo P, Chadwick RB, Aaltonen LA, de la Chapelle A. Semiautomated as-

sessment of loss of heterozygosity and replication error in tumors. Cancer Res 56: 3331-7, 1996

Casey G, Lopez ME, Ramos JC, Plummer SJ, Arboleda MJ, Shaughnessy M, Karlan B, Slamon DJ. DNA sequence analysis of exons 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. Oncogene 13: 1971-81, 1996

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, Strong LC, White RL. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305: 779-84, 1983

Chenevix-Trench G, Leary J, Kerr J, Michel J, Kefford R, Hurst T, Parsons PG, Friedlander M, Khoo SK. Frequent loss of heterozygosity on chromosome 18 in ovarian adenocarcinoma which does not always include the DCC locus. Oncogene 7: 1059-65, 1992

Chenevix-Trench G, Kerr J, Hurst T, Shih YC, Purdie D, Bergman L, Friedlander M, Sanderson B, et al. Analysis of loss of heterozygosity and KRAS2 mutations in ovarian neoplasms: clinicopathological correlations. Genes Chromosomes Cancer 18: 75-83, 1997

Chi DS, Liao JB, Leon LF, Venkatraman ES, Hensley ML, Bhaskaran D, Hoskins WJ. Identification of prognostic factors in advanced epithelial ovarian carcinoma. Gynecol Oncol 82: 532-7, 2001

Chung TK, Cheung TH, To KF, Wong YF. Overexpression of p53 and HER-2/neu and c-myc in primary fallopian tube carcinoma. Gynecol Obstet Invest 49: 47-51, 2000

Cliby W, Ritland S, Hartmann L, Dodson M, Halling KC, Keeney G, Podratz KC, Jenkins RB. Human epithelial ovarian cancer allelotype. Cancer Res 53: 2393-8, 1993

Connelly PJ, Alberhasky RC, Christopherson WM. Carcinoma of the endometrium. III. Analysis of 865 cases of adenocarcinoma and adenoacanthoma. Obstet Gynecol 59: 569-75, 1982

Couturier J, Vielh P, Salmon R, Dutrillaux B. Trisomy and tetrasomy for long arm of chromosome 1 in near-diploid human endometrial adenocarcinomas. Int J Cancer 38: 17-9, 1986

Couturier J, Vielh P, Salmon RJ, Lombard M, Dutrillaux B. Chromosome imbalance in endometrial adenocarcinoma. Cancer Genet Cytogenet 33: 67-76, 1988

Creasman WT, Odicino F, Maisonneuve P, Beller U, Benedet JL, Heintz AP, Ngan HY, Sideri M, Pecorelli S. Carcinoma of the corpus uteri. J Epidemiol Biostat 6: 47-86, 2001

de la Cuesta R, Maestro ML, Solana J, Vidart JA, Escudero M, Iglesias E, Valor R. Tissue quantification of CA 125 in epithelial ovarian cancer. Int J Biol Markers 14: 106-14, 1999

Deligdisch L, Holinka CF. Endometrial carcinoma: two diseases? Cancer Detect Prev 10: 237-46, 1987

Demopoulos RI, Aronov R, Mesia A. Clues to the pathogenesis of fallopian tube carcinoma: a morphological and immunohistochemical case control study. Int J Gynecol Pathol 20: 128-32, 2001

Di Renzo MF, Olivero M, Katsaros D, Crepaldi T, Gaglia P, Zola P, Sismondi P, Comoglio PM. Overexpression of the Met/HGF receptor in ovarian cancer. Int J Cancer 58: 658-62, 1994

Dickman PW, Hakulinen T, Luostarinen T, Pukkala E, Sankila R, Soderman B, Teppo L. Survival of cancer patients in Finland 1955-1994. Acta Oncol 38 Suppl 12: 1-103, 1999

Diebold J, Deisenhofer I, Baretton GB, Blasenbreu S, Suchy B, Schneiderbanger K, Meier W, Haas CJ, Lohrs U. Interphase cytogenetic analysis of serous ovarian tumors of low malignant potential: comparison with serous cystadenomas and invasive serous carcinomas. Lab Invest 75: 473-85, 1996

Diebold J, Siegert S, Baretton GB, Suchy B, Meier W, Haas CJ, Lohrs U. Interphase cytogenetic analysis of mucinous ovarian neoplasms. Lab Invest 76: 661-70, 1997

Dodson MK, Hartmann LC, Cliby WA, DeLacey KA, Keeney GL, Ritland SR, Su JQ, Podratz KC, Jenkins RB. Comparison of loss of heterozygosity patterns in invasive low-grade and high-grade epithelial ovarian carcinomas. Cancer Res 53: 4456-60, 1993

Dodson MK, Cliby WA, Xu HJ, DeLacey KA, Hu SX, Keeney GL, Li J, Podratz KC, et al. Evidence of functional RB protein in epithelial ovarian carcinomas despite loss of heterozygosity at the RB locus. Cancer Res 54: 610-3, 1994

Duggan BD, Felix JC, Muderspach LI, Tourgeman D, Zheng J, Shibata D. Microsatellite instability in sporadic endometrial carcinoma. J Natl Cancer Inst 86: 1216-21, 1994a

Duggan BD, Felix JC, Muderspach LI, Tsao JL, Shibata DK. Early mutational activation of the c-Ki-ras oncogene in endometrial carcinoma. Cancer Res 54: 1604-7, 1994b

Edelson MI, Scherer SW, Tsui LC, Welch WR, Bell DA, Berkowitz RS, Mok SC. Identification of a 1300 kilobase deletion unit on chromosome 7q31.3 in invasive epithelial ovarian carcinomas. Oncogene 14: 2979-84, 1997

Eisenhauer EA, Gore M, Neijt JP. Ovarian cancer: should we be managing patients with good and bad prognostic factors in the same manner? Ann Oncol 10 Suppl 1: 9-15, 1999

El-Rifai W, Larramendy ML, Bjorkqvist AM, Hemmer S, Knuutila S. Optimization of comparative genomic hybridization using fluorochrome conjugated to dCTP and dUTP nucleotides. Lab Invest 77: 699-700, 1997

Eltabbakh GH, Belinson JL, Kennedy AW, Biscotti CV, Casey G, Tubbs RR, Blumenson LE. p53 overexpression is not an independent prognostic factor for patients with primary ovarian epithelial cancer. Cancer 80: 892-8, 1997

Enomoto T, Inoue M, Perantoni AO, Buzard GS, Miki H, Tanizawa O, Rice JM. K-ras activation in premalignant and malignant epithelial lesions of the human uterus. Cancer Res 51: 5308-14, 1991a

Enomoto T, Weghorst CM, Inoue M, Tanizawa O, Rice JM. K-ras activation occurs frequently in mucinous adenocarcinomas and rarely in other common epithelial tumors of the human ovary. Am J Pathol 139: 777-85, 1991b

Enomoto T, Fujita M, Cheng C, Nakashima R, Ozaki M, Inoue M, Nomura T. Loss of expression and loss of heterozygosity in the DCC gene in neoplasms of the human female reproductive tract. Br J Cancer 71: 462-7, 1995

Eppert K, Scherer SW, Ozcelik H, Pirone R, Hoodless P, Kim H, Tsui LC, Bapat B, et al. MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 86: 543-52, 1996

Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG. MLH1 promoter hypermethylation is associated with the

microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene 17: 2413-7, 1998

Esteller M, Catasus L, Matias-Guiu X, Mutter GL, Prat J, Baylin SB, Herman JG. hMLH1 promoter hypermethylation is an early event in human endometrial tumorigenesis. Am J Pathol 155: 1767-72, 1999

Esteller M, Silva JM, Dominguez G, Bonilla F, Matias-Guiu X, Lerma E, Bussaglia E, Prat J, et al. Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst 92: 564-9, 2000

Fallows S, Price J, Atkinson RJ, Johnston PG, Hickey I, Russell SE. P53 mutation does not affect prognosis in ovarian epithelial malignancies. J Pathol 194: 68-75, 2001

Fazeli A, Dickinson SL, Hermiston ML, Tighe RV, Steen RG, Small CG, Stoeckli ET, Keino-Masu K, et al. Phenotype of mice lacking functional Deleted in colorectal cancer (Dcc) gene. Nature 386: 796-804, 1997

Fearon ER, Cho KR, Nigro JM, Kern SE, Simons JW, Ruppert JM, Hamilton SR, Preisinger AC, et al. Identification of a chromosome 18q gene that is altered in colorectal cancers. Science 247: 49-56, 1990

Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi OP. Genome screening by comparative genomic hybridization. Trends Genet 13: 405-9, 1997

Foulkes WD, Ragoussis J, Stamp GW, Allan GJ, Trowsdale J. Frequent loss of heterozygosity on chromosome 6 in human ovarian carcinoma. Br J Cancer 67: 551-9, 1993

Friedlander ML. Prognostic factors in ovarian cancer. Semin Oncol 25: 305-14, 1998

Fujita H, Wake N, Kutsuzawa T, Ichinoe K, Hreshchyshyn MM, Sandberg AA. Marker chromosomes of the long arm of chromosome 1 in endometrial carcinoma. Cancer Genet Cytogenet 18: 283-93, 1985

Fujita M, Enomoto T, Yoshino K, Nomura T, Buzard GS, Inoue M, Okudaira Y. Microsatellite instability and alterations in the hMSH2 gene in human ovarian cancer. Int J Cancer 64: 361-6, 1995

Fullwood P, Marchini S, Rader JS, Martinez A, Macartney D, Broggini M, Morelli C, Barbanti-Brodano G, et al. Detailed genetic and physical mapping of tumor suppressor loci on chromosome 3p in ovarian cancer. Cancer Res 59: 4662-7, 1999

Gabra H, Watson JE, Taylor KJ, Mackay J, Leonard RC, Steel CM, Porteous DJ, Smyth JF. Definition and refinement of a region of loss of heterozygosity at 11q23.3-q24.3 in epithelial ovarian cancer associated with poor prognosis. Cancer Res 56: 950-4, 1996

Gadducci A, Cianci C, Cosio S, Carnino F, Fanucchi A, Buttitta F, Conte PF, Genazzani AR. p53 status is neither a predictive nor a prognostic variable in patients with advanced ovarian cancer treated with a paclitaxel-based regimen. Anticancer Res 20: 4793-9, 2000

Garcia A, Bussaglia E, Machin P, Matias-Guiu X, Prat J. Loss of heterozygosity on chromosome 17q in epithelial ovarian tumors: association with carcinomas with serous differentiation. Int J Gynecol Pathol 19: 152-7, 2000

Geisler JP, Geisler HE, Miller GA, Wiemann MC, Zhou Z, Crabtree W. p53 and bcl-2 in epithelial ovarian carcinoma: their value as prognostic indicators at a median follow-up of 60 months. Gynecol Oncol 77: 278-82, 2000

Guan XY, Sham JS, Tang TC, Fang Y, Huo KK, Yang JM. Isolation of a novel candidate oncogene within a frequently amplified region at 3q26 in ovarian cancer. Cancer Res 61: 3806-9, 2001

Gurin CC, Federici MG, Kang L, Boyd J. Causes and consequences of microsatellite instability in endometrial carcinoma. Cancer Res 59: 462-6, 1999

Haas CJ, Diebold J, Hirschmann A, Rohrbach H, Schmid S, Lohrs U. Microsatellite analysis in serous tumors of the ovary. Int J Gynecol Pathol 18: 158-62, 1999

Hahn SA, Schutte M, Hoque AT, Moskaluk CA, da Costa LT, Rozenblum E, Weinstein CL, Fischer A, et al. DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science 271: 350-3, 1996

Hankinson SE, Hunter DJ, Colditz GA, Willett WC, Stampfer MJ, Rosner B, Hennekens CH, Speizer FE. Tubal ligation, hysterectomy, and risk of ovarian cancer. A prospective study. Jama 270: 2813-8, 1993

Hartmann LC, Podratz KC, Keeney GL, Kamel NA, Edmonson JH, Grill JP, Su JQ, Katzmann JA, Roche PC. Prognostic significance of p53 immunostaining in epithelial ovarian cancer. J Clin Oncol 12: 64-9, 1994

Heikinheimo M, Ermolaeva M, Bielinska M, Rahman NA, Narita N, Huhtaniemi IT, Tapanainen JS, Wilson DB. Expression and hormonal regulation of transcription factors GATA-4 and GATA-6 in the mouse ovary. Endocrinology 138: 3505-14, 1997

Heintz AP, Odicino F, Maisonneuve P, Beller U, Benedet JL, Creasman WT, Ngan HY, Sideri M, Pecorelli S. Carcinoma of the Fallopian tube. J Epidemiol Biostat 6: 89-103, 2001a

Heintz AP, Odicino F, Maisonneuve P, Beller U, Benedet JL, Creasman WT, Ngan HY, Sideri M, Pecorelli S. Carcinoma of the ovary. J Epidemiol Biostat 6: 107-38, 2001b

Hellstrom I, Goodman G, Pullman J, Yang Y, Hellstrom KE. Overexpression of HER-2 in ovarian carcinomas. Cancer Res 61: 2420-3, 2001

Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 391: 184-7, 1998

Hendrickson M, Ross J, Eifel PJ, Cox RS, Martinez A, Kempson R. Adenocarcinoma of the endometrium: analysis of 256 cases with carcinoma limited to the uterine corpus. Pathology review and analysis of prognostic variables. Gynecol Oncol 13: 373-92, 1982

Henriksen R, Strang P, Wilander E, Backstrom T, Tribukait B, Oberg K. p53 expression in epithelial ovarian neoplasms: relationship to clinical and pathological parameters, Ki-67 expression and flow cytometry. Gynecol Oncol 53: 301-6, 1994

Heselmeyer K, Hellstrom AC, Blegen H, Schrock E, Silfversward C, Shah K, Auer G, Ried T. Primary carcinoma of the fallopian tube: comparative genomic hybridization reveals high genetic instability and a specific, recurring pattern of chromosomal aberrations. Int J Gynecol Pathol 17: 245-54, 1998

Hu W, Wu W, Nash MA, Freedman RS, Kavanagh JJ, Verschraegen CF. Anomalies of the TGF-beta postreceptor signaling pathway in ovarian cancer cell lines. Anticancer Res 20: 729-33, 2000

Huang H, Reed CP, Mordi A, Lomberk G, Wang L, Shridhar V, Hartmann L, Jenkins R, Smith DI. Frequent deletions within FRA7G at 7q31.2 in invasive epithelial ovarian cancer. Genes Chromosomes Cancer 24: 48-55, 1999

Ichikawa Y, Nishida M, Suzuki H, Yoshida S, Tsunoda H, Kubo T, Uchida K, Miwa M. Mutation of K-ras protooncogene is associated with histological subtypes in human mucinous ovarian tumors. Cancer Res 54: 33-5, 1994

Imyanitov EN, Birrell GW, Filippovich I, Sorokina N, Arnold J, Mould MA, Wright K, Walsh M, et al. Frequent loss of heterozygosity at 1p36 in ovarian adenocarcinomas but the gene encoding p73 is unlikely to be the target. Oncogene 18: 4640-2, 1999

Isola J, DeVries S, Chu L, Ghazvini S, Waldman F. Analysis of changes in DNA sequence copy number by comparative genomic hybridization in archival paraffin-embedded tumor samples. Am J Pathol 145: 1301-8, 1994

Isola JJ, Kallioniemi OP, Chu LW, Fuqua SA, Hilsenbeck SG, Osborne CK, Waldman FM. Genetic aberrations detected by comparative genomic hybridization predict outcome in node-negative breast cancer. Am J Pathol 147: 905-11, 1995

Iwabuchi H, Sakamoto M, Sakunaga H, Ma YY, Carcangiu ML, Pinkel D, Yang-Feng TL, Gray JW. Genetic analysis of benign, low-grade, and high-grade ovarian tumors. Cancer Res 55: 6172-80, 1995

Jenkins RB, Bartelt D, Jr., Stalboerger P, Persons D, Dahl RJ, Podratz K, Keeney G, Hartmann L. Cytogenetic studies of epithelial ovarian carcinoma. Cancer Genet Cytogenet 71: 76-86, 1993

Joos S, Bergerheim US, Pan Y, Matsuyama H, Bentz M, du Manoir S, Lichter P. Mapping of chromosomal gains and losses in prostate cancer by comparative genomic hybridization. Genes Chromosomes Cancer 14: 267-76, 1995

Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258: 818-21, 1992

Kallioniemi OP, Kallioniemi A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Genes Chromosomes Cancer 10: 231-43, 1994

Katabuchi H, van Rees B, Lambers AR, Ronnett BM, Blazes MS, Leach FS, Cho KR, Hedrick L. Mutations in DNA mismatch repair genes are not responsible for microsatellite instability in most sporadic endometrial carcinomas. Cancer Res 55: 5556-60, 1995

Kaufman MH. The atlas of mouse development. London: Academic Press; 453-64, 1992

Kelsey JL, LiVolsi VA, Holford TR, Fischer DB, Mostow ED, Schwartz PE, O'Connor T, White C. A case-control study of cancer of the endometrium. Am J Epidemiol 116: 333-42, 1982

Ketola I, Rahman N, Toppari J, Bielinska M, Porter-Tinge SB, Tapanainen JS, Huhtaniemi IT, Wilson DB, Heikinheimo M. Expression and regulation of transcription factors GATA-4 and GATA-6 in developing mouse testis. Endocrinology 140: 1470-80, 1999

Kiechle M, Jacobsen A, Schwarz-Boeger U, Hedderich J, Pfisterer J, Arnold N. Comparative genomic hybridization detects genetic imbalances in primary ovarian carcinomas as correlated with grade of differentiation. Cancer 91: 534-40, 2001

Kiiveri S, Siltanen S, Rahman N, Bielinska M, Lehto VP, Huhtaniemi IT, Muglia LJ, Wilson DB, Heikinheimo M. Reciprocal changes in the expression of transcription factors GATA-4 and GATA-6 accompany adrenocortical tumorigenesis in mice and humans. Mol Med 5: 490-501, 1999

Kim TM, Benedict WF, Xu HJ, Hu SX, Gosewehr J, Velicescu M, Yin E, Zheng J, et al. Loss of heterozygosity on chromosome 13 is common only in the biologically more aggressive subtypes of ovarian epithelial tumors and is associated with normal retinoblastoma gene expression. Cancer Res 54: 605-9, 1994

King BL, Carcangiu ML, Carter D, Kiechle M, Pfisterer J, Pfleiderer A, Kacinski BM. Microsatellite instability in ovarian neoplasms. Br J Cancer 72: 376-82, 1995

Klemi PJ, Pylkkanen L, Kiilholma P, Kurvinen K, Joensuu H. p53 protein detected by immunohistochemistry as a prognostic factor in patients with epithelial ovarian carcinoma. Cancer 76: 1201-8, 1995

Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68: 820-3, 1971

Knuutila S, Aalto Y, Autio K, Bjorkqvist AM, El-Rifai W, Hemmer S, Huhta T, Kettunen E, et al. DNA copy number losses in human neoplasms. Am J Pathol 155: 683-94, 1999

Knuutila S, Bjorkqvist AM, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, et al. DNA copy number amplifications in human neoplasms: review of comparative genomic hybridization studies. Am J Pathol 152: 1107-23, 1998

Kobayashi K, Sagae S, Kudo R, Saito H, Koi S, Nakamura Y. Microsatellite instability in endometrial carcinomas: frequent replication errors in tumors of early onset and/or of poorly differentiated type. Genes Chromosomes Cancer 14: 128-32, 1995

Kohler MF, Berchuck A, Davidoff AM, Humphrey PA, Dodge RK, Iglehart JD, Soper JT, Clarke-Pearson DL, et al. Overexpression and mutation of p53 in endometrial carcinoma. Cancer Res 52: 1622-7, 1992

Kong D, Suzuki A, Zou TT, Sakurada A, Kemp LW, Wakatsuki S, Yokoyama T, Yamakawa H, et al. PTEN1 is frequently mutated in primary endometrial carcinomas. Nat Genet 17: 143-4, 1997

Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4: 844-7, 1998

Kowalski LD, Mutch DG, Herzog TJ, Rader JS, Goodfellow PJ. Mutational analysis of MLH1 and MSH2 in 25 prospectively-acquired RER+ endometrial cancers. Genes Chromosomes Cancer 18: 219-27, 1997

Kudoh K, Takano M, Koshikawa T, Hirai M, Yoshida S, Mano Y, Yamamoto K, Ishii K, et al. Gains of 1q21-q22 and 13q12-q14 are potential indicators for resistance to cisplatin-based chemotherapy in ovarian cancer patients. Clin Cancer Res 5: 2526-31, 1999

Kurman RJ. Blaustein's Pathology of Female Genital Tract. 4. ed. New York: Springer Verlag, 1994

Kuukasjarvi T, Tanner M, Pennanen S, Karhu R, Visakorpi T, Isola J. Optimizing DOP-PCR for universal amplification of small DNA samples in comparative genomic hybridization. Genes Chromosomes Cancer 18: 94-101, 1997

Kvale G, Heuch I, Nilssen S, Beral V. Reproductive factors and risk of ovarian cancer: a prospective study. Int J Cancer 42: 246-51, 1988

Lacy MQ, Hartmann LC, Keeney GL, Cha SC, Wieand HS, Podratz KC, Roche PC. c-erbB-2 and p53 expression in fallopian tube carcinoma. Cancer 75: 2891-6, 1995

Laitinen M, Ristimaki A, Honkasalo M, Narko K, Paavonen K, Ritvos O. Differential hormonal regulation of vascular endothelial growth factors VEGF, VEGF-B, and VEGF-C messenger ribonucleic acid levels in cultured human granulosa-luteal cells. Endocrinology 138: 4748-56, 1997

Laitinen MP, Anttonen M, Ketola I, Wilson DB, Ritvos O, Butzow R, Heikinheimo M. Transcription factors GATA-4 and GATA-6 and a GATA family cofactor, FOG-2, are expressed in human ovary and sex cord-derived ovarian tumors. J Clin Endocrinol Metab 85: 3476-83, 2000

Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, et al. Initial sequencing and analysis of the human genome. Nature 409: 860-921, 2001

Launonen V, Mannermaa A, Stenback F, Kosma VM, Puistola U, Huusko P, Anttila M, Bloigu R, et al. Loss of heterozygosity at chromosomes 3, 6, 8, 11, 16, and 17 in ovarian cancer: correlation to clinicopathological variables. Cancer Genet Cytogenet 122: 49-54, 2000

Lavarino C, Pilotti S, Oggionni M, Gatti L, Perego P, Bresciani G, Pierotti MA, Scambia G, et al. p53 gene status and response to platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma. J Clin Oncol 18: 3936-45, 2000

Lax SF, Kendall B, Tashiro H, Slebos RJ, Hedrick L. The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. Cancer 88: 814-24, 2000

Levesque MA, Katsaros D, Massobrio M, Genta F, Yu H, Richiardi G, Fracchioli S, Durando A, et al. Evidence for a dose-response effect between p53 (but not p21WAF1/Cip1) protein concentrations, survival, and responsiveness in patients with epithelial ovarian cancer treated with platinum-based chemotherapy. Clin Cancer Res 6: 3260-70, 2000

Levine RL, Cargile CB, Blazes MS, van Rees B, Kurman RJ, Ellenson LH. PTEN mutations and microsatellite instability in complex atypical hyperplasia, a precursor lesion to uterine endometrioid carcinoma. Cancer Res 58: 3254-8, 1998

Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275: 1943-7, 1997

Lin L, Aggarwal S, Glover TW, Orringer MB, Hanash S, Beer DG. A minimal critical region of the 8p22-23 amplicon in esophageal adenocarcinomas defined using sequence tagged site-amplification mapping and quantitative polymerase chain reaction includes the GATA-4 gene. Cancer Res 60: 1341-7, 2000

Lindblad-Toh K, Tanenbaum DM, Daly MJ, Winchester E, Lui WO, Villapakkam A, Stanton SE, Larsson C, et al. Loss-of-heterozygosity analysis of small-cell lung carcinomas using single-nucleotide polymorphism arrays. Nat Biotechnol 18: 1001-5, 2000

Lounis H, Mes-Masson AM, Dion F, Bradley WE, Seymour RJ, Provencher D, Tonin PN. Mapping of chromosome 3p deletions in human epithelial ovarian tumors. Oncogene 17: 2359-65, 1998

Lu KH, Weitzel JN, Kodali S, Welch WR, Berkowitz RS, Mok SC. A novel 4-cM minimally deleted region on chromosome 11p15.1 associated with high grade nonmucinous epithelial ovarian carcinomas. Cancer Res 57: 387-90, 1997

Makar AP, Baekelandt M, Trope CG, Kristensen GB. The prognostic significance of residual disease, FIGO substage, tumor histology, and grade in patients with FIGO stage III ovarian cancer. Gynecol Oncol 56: 175-80, 1995

Marks JR, Davidoff AM, Kerns BJ, Humphrey PA, Pence JC, Dodge RK, Clarke-Pearson DL, Iglehart JD, et al. Overexpression and mutation of p53 in epithelial ovarian cancer. Cancer Res 51: 2979-84, 1991

Maxwell GL, Risinger JI, Gumbs C, Shaw H, Bentley RC, Barrett JC, Berchuck A, Futreal PA. Mutation of the PTEN tumor suppressor gene in endometrial hyperplasias. Cancer Res 58: 2500-3, 1998

McGill JR, Beitzel BF, Nielsen JL, Walsh JT, Drabek SM, Meador RJ, Von Hoff DD. Double minutes are frequently found in ovarian carcinomas. Cancer Genet Cytogenet 71: 125-31, 1993

Medl M, Sevelda P, Czerwenka K, Dobianer K, Hanak H, Hruza C, Klein M, Leodolter S, et al. DNA amplification of HER-2/neu and INT-2 oncogenes in epithelial ovarian cancer. Gynecol Oncol 59: 321-6, 1995

Merajver SD, Pham TM, Caduff RF, Chen M, Poy EL, Cooney KA, Weber BL, Collins FS, et al. Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. Nat Genet 9: 439-43, 1995

Milatovich A, Heerema NA, Palmer CG. Cytogenetic studies of endometrial malignancies. Cancer Genet Cytogenet 46: 41-53, 1990

Milde-Langosch K, Ocon E, Becker G, Loning T. p16/MTS1 inactivation in ovarian carcinomas: high frequency of reduced protein expression associated with hyper-methylation or mutation in endometrioid and mucinous tumors. Int J Cancer 79: 61-5, 1998

Milner BJ, Allan LA, Eccles DM, Kitchener HC, Leonard RC, Kelly KF, Parkin DE, Haites NE. p53 mutation is a common genetic event in ovarian carcinoma. Cancer Res 53: 2128-32, 1993

Mizuuchi H, Mori Y, Sato K, Kamiya H, Okamura N, Nasim S, Garrett CT, Kudo R. High incidence of point mutation in K-ras codon 12 in carcinoma of the fallopian tube. Cancer 76: 86-90, 1995

Moll R, Levy R, Czernobilsky B, Hohlweg-Majert P, Dallenbach-Hellweg G, Franke WW. Cytokeratins of normal epithelia and some neoplasms of the female genital tract. Lab Invest 49: 599-610, 1983

Moll R, Lowe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol 140: 427-47, 1992

Moll UM, Chalas E, Auguste M, Meaney D, Chumas J. Uterine papillary serous carcinoma evolves via a p53-driven pathway. Hum Pathol 27: 1295-300, 1996

Monni O, Barlund M, Mousses S, Kononen J, Sauter G, Heiskanen M, Paavola P, Avela K, et al. Comprehensive copy number and gene expression profiling of the 17q23 amplicon in human breast cancer. Proc Natl Acad Sci U S A 98: 5711-6, 2001

Mutter GL, Boynton KA, Faquin WC, Ruiz RE, Jovanovic AS. Allelotype mapping of unstable microsatellites establishes direct lineage continuity between endometrial

precancers and cancer. Cancer Res 56: 4483-6, 1996
Mutter GL, Ince TA, Baak JP, Kust GA, Zhou XP, Eng C. Molecular identification of latent precancers in histologically normal endometrium. Cancer Res 61: 4311-4, 2001
NIH consensus. Ovarian cancer. Screening, treatment, and follow-up. NIH Consensus Development Panel on Ovarian Cancer. Jama 273: 491-7, 1995
Nyholm HC, Nielsen AL, Lyndrup J, Norup P, Thorpe SM. Biochemical and immunohistochemical estrogen and progesterone receptors in adenomatous hyperplasia and endometrial carcinoma: correlations with stage and other clinicopathologic features. Am J Obstet Gynecol 167: 1334-42, 1992
Obata K, Morland SJ, Watson RH, Hitchcock A, Chenevix-Trench G, Thomas EJ, Campbell IG. Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors. Cancer Res 58: 2095-7, 1998
Omura GA, Brady MF, Homesley HD, Yordan E, Major FJ, Buchsbaum HJ, Park RC. Long-term follow-up and prognostic factor analysis in advanced ovarian carcinoma: the Gynecologic Oncology Group experience. J Clin Oncol 9: 1138-50, 1991
Orphanos V, McGown G, Hey Y, Thorncroft M, Santibanez-Koref M, Russell SE, Hickey I, Atkinson RJ, Boyle JM. Allelic imbalance of chromosome 6q in ovarian tumours. Br J Cancer 71: 666-9, 1995
Orsetti B, Courjal F, Cuny M, Rodriguez C, Theillet C. 17q21-q25 aberrations in breast cancer: combined allelotyping and CGH analysis reveals 5 regions of allelic imbalance among which two correspond to DNA amplification. Oncogene 18: 6262-70, 1999
Osborne RJ, Leech V. Polymerase chain reaction allelotyping of human ovarian cancer. Br J Cancer 69: 429-38, 1994
Papp J, Csokay B, Bosze P, Zalay Z, Toth J, Ponder B, Olah E. Allele loss from large regions of chromosome 17 is common only in certain histological subtypes of ovarian carcinomas. Br J Cancer 74: 1592-7, 1996
Patael-Karasik Y, Daniely M, Gotlieb WH, Ben-Baruch G, Schiby J, Barakai G, Goldman B, Aviram A, Friedman E. Comparative genomic hybridization in inherited and sporadic ovarian tumors in Israel. Cancer Genet Cytogenet 121: 26-32, 2000
Peiffer SL, Herzog TJ, Tribune DJ, Mutch DG, Gersell DJ, Goodfellow PJ. Allelic loss of sequences from the long arm of chromosome 10 and replication errors in endometrial cancers. Cancer Res 55: 1922-6, 1995
Pejovic T, Heim S, Mandahl N, Baldetorp B, Elmfors B, Floderus UM, Furgyik S, Helm G, et al. Chromosome aberrations in 35 primary ovarian carcinomas. Genes Chromosomes Cancer 4: 58-68, 1992a
Pejovic T, Himmelmann A, Heim S, Mandahl N, Floderus UM, Furgyik S, Elmfors B, Helm G, et al. Prognostic impact of chromosome aberrations in ovarian cancer. Br J Cancer 65: 282-6, 1992b
Pejovic T, Burki N, Odunsi K, Fiedler P, Achong N, Schwartz PE, Ward DC. Well-differentiated mucinous carcinoma of the ovary and a coexisting Brenner tumor both exhibit amplification of 12q14-21 by comparative genomic hybridization. Gynecol Oncol 74: 134-7, 1999
Pieretti M, Cavalieri C, Conway PS, Gallion HH, Powell DE, Turker MS. Genetic alterations distinguish different types of ovarian tumors. Int J Cancer 64: 434-40, 1995
Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet 20: 207-11, 1998
Pollack JR, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. Nat Genet 23: 41-6, 1999
Pribill I, Speiser P, Leary J, Leodolter S, Hacker NF, Friedlander ML, Birnbaum D, Zeillinger R, Krainer M. High frequency of allelic imbalance at regions of chromosome arm 8p in ovarian carcinoma. Cancer Genet Cytogenet 129: 23-9, 2001
Reed PW, Davies JL, Copeman JB, Bennett ST, Palmer SM, Pritchard LE, Gough SC, Kawaguchi Y, et al. Chromosome-specific microsatellite sets for fluorescence-based, semi-automated genome mapping. Nat Genet 7: 390-5, 1994
Reles A, Wen WH, Schmider A, Gee C, Runnebaum IB, Kilian U, Jones LA, El-Naggar A, et al. Correlation of p53 Mutations with Resistance to Platinum-based Chemotherapy and Shortened Survival in Ovarian Cancer. Clin Cancer Res 7: 2984-97, 2001
Righetti SC, Della Torre G, Pilotti S, Menard S, Ottone F, Colnaghi MI, Pierotti MA, Lavarino C, et al. A comparative study of p53 gene mutations, protein accumulation, and response to cisplatin-based chemotherapy in advanced ovarian carcinoma. Cancer Res 56: 689-93, 1996
Risch HA, Marrett LD, Jain M, Howe GR. Differences in risk factors for epithelial ovarian cancer by histologic type. Results of a case-control study. Am J Epidemiol 144: 363-72, 1996
Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT, Boyd J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res 53: 5100-3, 1993
Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res 57: 4736-8, 1997
Rodriguez C, Causse A, Ursule E, Theillet C. At least five regions of imbalance on 6q in breast tumors, combining losses and gains. Genes Chromosomes Cancer 27: 76-84, 2000
Rohlke P, Milde-Langosch K, Weyland C, Pichlmeier U, Jonat W, Loning T. p53 is a persistent and predictive marker in advanced ovarian carcinomas: multivariate analysis including comparison with Ki67 immunoreactivity. J Cancer Res Clin Oncol 123: 496-501, 1997
Rolitsky CD, Theil KS, McGaughy VR, Copeland LJ, Niemann TH. HER-2/neu amplification and overexpression in endometrial carcinoma. Int J Gynecol Pathol 18: 138-43, 1999
Rosen AC, Ausch C, Hafner E, Klein M, Lahousen M, Graf AH, Reiner A. A 15-year overview of management and prognosis in primary fallopian tube carcinoma. Austrian Cooperative Study Group for Fallopian Tube Carcinoma. Eur J Cancer 34: 1725-9, 1998
Runnebaum IB, Kohler T, Stickeler E, Kieback HR, Kreienberg R. p53 mutation is associated with high S-phase fraction in primary fallopian tube adenocarcinoma. Br J Cancer 74: 1157-60, 1996
Saegusa M, Machida D, Okayasu I. Loss of DCC gene ex-

pression during ovarian tumorigenesis: relation to tumour differentiation and progression. Br J Cancer 82: 571-8, 2000

Saffari B, Jones LA, el-Naggar A, Felix JC, George J, Press MF. Amplification and overexpression of HER-2/neu (c-erbB2) in endometrial cancers: correlation with overall survival. Cancer Res 55: 5693-8, 1995

Saito S, Saito H, Koi S, Sagae S, Kudo R, Saito J, Noda K, Nakamura Y. Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers. Cancer Res 52: 5815-7, 1992

Salazar H, Godwin AK, Getts LA, Testa JR, Daly M, Rosenblum N, Hogan M, Ozols RF, C. HT. Spontaneous transformation of the ovarian surface epithelium and the biology of ovarian cancer. In: Sharp F, Mason P, Blackett T, Berek J, eds. Ovarian Cancer 3. London: Chapman & Hall; 145-56, 1995

Salvesen HB, MacDonald N, Ryan A, Iversen OE, Jacobs IJ, Akslen LA, Das S. Methylation of hMLH1 in a population-based series of endometrial carcinomas. Clin Cancer Res 6: 3607-13, 2000

Saretzki G, Hoffmann U, Rohlke P, Psille R, Gaigal T, Keller G, Hofler H, Loning T, et al. Identification of allelic losses in benign, borderline, and invasive epithelial ovarian tumors and correlation with clinical outcome. Cancer 80: 1241-9, 1997

Sasaki H, Nishii H, Takahashi H, Tada A, Furusato M, Terashima Y, Siegal GP, Parker SL, et al. Mutation of the Ki-ras protooncogene in human endometrial hyperplasia and carcinoma. Cancer Res 53: 1906-10, 1993

Sato T, Saito H, Morita R, Koi S, Lee JH, Nakamura Y. Allelotype of human ovarian cancer. Cancer Res 51: 5118-22, 1991

Schena M, Shalon D, Davis RW, Brown PO. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270: 467-70, 1995

Schutte M, Hruban RH, Hedrick L, Cho KR, Nadasdy GM, Weinstein CL, Bova GS, Isaacs WB, et al. DPC4 gene in various tumor types. Cancer Res 56: 2527-30, 1996

Schutze K, Lahr G. Identification of expressed genes by laser-mediated manipulation of single cells. Nat Biotechnol 16: 737-42, 1998

Schwartz Z, Dgani R, Flugelman MY, Lancet M, Gelerenter I. A novel approach to the analysis of risk factors in endometrial carcinoma. Gynecol Oncol 21: 228-34, 1985

Schwengel DA, Jedlicka AE, Nanthakumar EJ, Weber JL, Levitt RC. Comparison of fluorescence-based semi-automated genotyping of multiple microsatellite loci with autoradiographic techniques. Genomics 22: 46-54, 1994

Shah NK, Currie JL, Rosenshein N, Campbell J, Long P, Abbas F, Griffin CA. Cytogenetic and FISH analysis of endometrial carcinoma. Cancer Genet Cytogenet 73: 142-6, 1994

Shahin MS, Hughes JH, Sood AK, Buller RE. The prognostic significance of p53 tumor suppressor gene alterations in ovarian carcinoma. Cancer 89: 2006-17, 2000

Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, Collins C, Pinkel D, Powell B, et al. PIK3CA is implicated as an oncogene in ovarian cancer. Nat Genet 21: 99-102, 1999

Sherman ME, Bur ME, Kurman RJ. p53 in endometrial cancer and its putative precursors: evidence for diverse pathways of tumorigenesis. Hum Pathol 26: 1268-74, 1995

Shimizu M, Toki T, Takagi Y, Konishi I, Fujii S. Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors. Int J Gynecol Pathol 19: 158-63, 2000

Shridhar V, Lee J, Pandita A, Iturria S, Avula R, Staub J, Morrissey M, Calhoun E, et al. Genetic analysis of early- versus late-stage ovarian tumors. Cancer Res 61: 5895-904, 2001

Silvestrini R, Daidone MG, Veneroni S, Benini E, Scarfone G, Zanaboni F, Villa A, Presti M, et al. The clinical predictivity of biomarkers of stage III-IV epithelial ovarian cancer in a prospective randomized treatment protocol. Cancer 82: 159-67, 1998

Simon D, Heyner S, Satyaswaroop PG, Farber M, Noumoff JS. Is chromosome 10 a primary chromosomal abnormality in endometrial adenocarcinoma? Cancer Genet Cytogenet 47: 155-62, 1990

Simpkins SB, Bocker T, Swisher EM, Mutch DG, Gersell DJ, Kovatich AJ, Palazzo JP, Fishel R, Goodfellow PJ. MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. Hum Mol Genet 8: 661-6, 1999

Singleton TP, Perrone T, Oakley G, Niehans GA, Carson L, Cha SS, Strickler JG. Activation of c-erbB-2 and prognosis in ovarian carcinoma. Comparison with histologic type, grade, and stage. Cancer 73: 1460-6, 1994

Skilling JS, Sood A, Niemann T, Lager DJ, Buller RE. An abundance of p53 null mutations in ovarian carcinoma. Oncogene 13: 117-23, 1996

Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244: 707-12, 1989

Smith DC, Prentice R, Thompson DJ, Herrmann WL. Association of exogenous estrogen and endometrial carcinoma. N Engl J Med 293: 1164-7, 1975

Sonoda G, du Manoir S, Godwin AK, Bell DW, Liu Z, Hogan M, Yakushiji M, Testa JR. Detection of DNA gains and losses in primary endometrial carcinomas by comparative genomic hybridization. Genes Chromosomes Cancer 18: 115-25, 1997a

Sonoda G, Palazzo J, du Manoir S, Godwin AK, Feder M, Yakushiji M, Testa JR. Comparative genomic hybridization detects frequent overrepresentation of chromosomal material from 3q26, 8q24, and 20q13 in human ovarian carcinomas. Genes Chromosomes Cancer 20: 320-8, 1997b

Sood AK, Holmes R, Hendrix MJ, Buller RE. Application of the National Cancer Institute international criteria for determination of microsatellite instability in ovarian cancer. Cancer Res 61: 4371-4, 2001

Speicher MR, du Manoir S, Schrock E, Holtgreve-Grez H, Schoell B, Lengauer C, Cremer T, Ried T. Molecular cytogenetic analysis of formalin-fixed, paraffin-embedded solid tumors by comparative genomic hybridization after universal DNA-amplification. Hum Mol Genet 2: 1907-14, 1993

Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 15: 356-62, 1997

Stuhlinger M, Rosen AC, Dobianer K, Hruza C, Helmer H, Kein M, Koukal T, Reiner A, et al. HER-2 oncogene is not amplified in primary carcinoma of the fallopian

tube. Austrian Cooperative Study Group for Fallopian Tube Carcinoma. Oncology 52: 397-9, 1995

Suehiro Y, Umayahara K, Ogata H, Numa F, Yamashita Y, Oga A, Morioka H, Ito T, et al. Genetic aberrations detected by comparative genomic hybridization predict outcome in patients with endometrioid carcinoma. Genes Chromosomes Cancer 29: 75-82, 2000

Suzuki A, Fukushige S, Nagase S, Ohuchi N, Satomi S, Horii A. Frequent gains on chromosome arms 1q and/or 8q in human endometrial cancer. Hum Genet 100: 629-36, 1997

Suzuki M, Saito S, Saga Y, Ohwada M, Sato I. Mutation of K-RAS protooncogene and loss of heterozygosity on 6q27 in serous and mucinous ovarian carcinomas. Cancer Genet Cytogenet 118: 132-5, 2000a

Suzuki S, Moore DH, 2nd, Ginzinger DG, Godfrey TE, Barclay J, Powell B, Pinkel D, Zaloudek C, et al. An approach to analysis of large-scale correlations between genome changes and clinical endpoints in ovarian cancer. Cancer Res 60: 5382-5, 2000b

Taetle R, Aickin M, Panda L, Emerson J, Roe D, Thompson F, Davis J, Trent J, Alberts D. Chromosome abnormalities in ovarian adenocarcinoma: II. Prognostic impact of nonrandom chromosome abnormalities in 244 cases. Genes Chromosomes Cancer 25: 46-52, 1999a

Taetle R, Aickin M, Yang JM, Panda L, Emerson J, Roe D, Adair L, Thompson F, et al. Chromosome abnormalities in ovarian adenocarcinoma: I. Nonrandom chromosome abnormalities from 244 cases. Genes Chromosomes Cancer 25: 290-300, 1999b

Takahashi H, Behbakht K, McGovern PE, Chiu HC, Couch FJ, Weber BL, Friedman LS, King MC, et al. Mutation analysis of the BRCA1 gene in ovarian cancers. Cancer Res 55: 2998-3002, 1995

Takahashi H, Chiu HC, Bandera CA, Behbakht K, Liu PC, Couch FJ, Weber BL, LiVolsi VA, et al. Mutations of the BRCA2 gene in ovarian carcinomas. Cancer Res 56: 2738-41, 1996

Takakura S, Okamoto A, Saito M, Yasuhara T, Shinozaki H, Isonishi S, Yoshimura T, Ohtake Y, et al. Allelic imbalance in chromosome band 18q21 and SMAD4 mutations in ovarian cancers. Genes Chromosomes Cancer 24: 264-71, 1999

Tanaka K, Boice CR, Testa JR. Chromosome aberrations in nine patients with ovarian cancer. Cancer Genet Cytogenet 43: 1-14, 1989

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 6: 1833-9, 2000

Tapper J, Butzow R, Wahlstrom T, Seppala M, Knuutila S. Evidence for divergence of DNA copy number changes in serous, mucinous and endometrioid ovarian carcinomas. Br J Cancer 75: 1782-7, 1997

Tapper J, Sarantaus L, Vahteristo P, Nevanlinna H, Hemmer S, Seppala M, Knuutila S, Butzow R. Genetic changes in inherited and sporadic ovarian carcinomas by comparative genomic hybridization: extensive similarity except for a difference at chromosome 2q24-q32. Cancer Res 58: 2715-9, 1998

Tashiro H, Miyazaki K, Okamura H, Iwai A, Fukumoto M. c-myc over-expression in human primary ovarian tumours: its relevance to tumour progression. Int J Cancer 50: 828-33, 1992

Tashiro H, Blazes MS, Wu R, Cho KR, Bose S, Wang SI, Li J, Parsons R, Ellenson LH. Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 57: 3935-40, 1997a

Tashiro H, Isacson C, Levine R, Kurman RJ, Cho KR, Hedrick L. p53 gene mutations are common in uterine serous carcinoma and occur early in their pathogenesis. Am J Pathol 150: 177-85, 1997b

Tashiro H, Lax SF, Gaudin PB, Isacson C, Cho KR, Hedrick L. Microsatellite instability is uncommon in uterine serous carcinoma. Am J Pathol 150: 75-9, 1997c

Thompson FH, Emerson J, Alberts D, Liu Y, Guan XY, Burgess A, Fox S, Taetle R, et al. Clonal chromosome abnormalities in 54 cases of ovarian carcinoma. Cancer Genet Cytogenet 73: 33-45, 1994a

Thompson FH, Liu Y, Emerson J, Weinstein R, Makar R, Trent JM, Taetle R, Alberts DS. Simple numeric abnormalities as primary karyotype changes in ovarian carcinoma. Genes Chromosomes Cancer 10: 262-6, 1994b

Tibiletti MG, Bernasconi B, Furlan D, Riva C, Trubia M, Buraggi G, Franchi M, Bolis P, et al. Early involvement of 6q in surface epithelial ovarian tumors. Cancer Res 56: 4493-8, 1996

Tibiletti MG, Bernasconi B, Taborelli M, Furlan D, Fabbri A, Franchi M, Taramelli R, Trubia M, Capella C. Involvement of chromosome 6 in endometrial cancer. Br J Cancer 75: 1831-5, 1997

Tremblay JJ, Viger RS. Transcription factor GATA-4 enhances Mullerian inhibiting substance gene transcription through a direct interaction with the nuclear receptor SF-1. Mol Endocrinol 13: 1388-401, 1999

Umpierre SA, Burke TW, Tornos C, Ordonez N, Levenback C, Morris M. Immunocytochemical analysis of uterine papillary serous carcinomas for estrogen and progesterone receptors. Int J Gynecol Pathol 13: 127-30, 1994

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, et al. The sequence of the human genome. Science 291: 1304-51, 2001

Vergote IB, Kaern J, Abeler VM, Pettersen EO, De Vos LN, Trope CG. Analysis of prognostic factors in stage I epithelial ovarian carcinoma: importance of degree of differentiation and deoxyribonucleic acid ploidy in predicting relapse. Am J Obstet Gynecol 169: 40-52, 1993

Vergote I, De Brabanter J, Fyles A, Bertelsen K, Einhorn N, Sevelda P, Gore ME, Kaern J, et al. Prognostic importance of degree of differentiation and cyst rupture in stage I invasive epithelial ovarian carcinoma. Lancet 357: 176-82, 2001

Viger RS, Mertineit C, Trasler JM, Nemer M. Transcription factor GATA-4 is expressed in a sexually dimorphic pattern during mouse gonadal development and is a potent activator of the Mullerian inhibiting substance promoter. Development 125: 2665-75, 1998

Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9: 401-6, 1995a

Visakorpi T, Kallioniemi AH, Syvanen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi OP. Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 55: 342-7, 1995b

Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R,

Ghandour G, Perkins N, et al. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science 280: 1077-82, 1998

Wang D, Kanuma T, Mizunuma H, Takama F, Ibuki Y, Wake N, Mogi A, Shitara Y, Takenoshita S. Analysis of specific gene mutations in the transforming growth factor-beta signal transduction pathway in human ovarian cancer. Cancer Res 60: 4507-12, 2000

Watanabe K, Clarke TR, Lane AH, Wang X, Donahoe PK. Endogenous expression of Mullerian inhibiting substance in early postnatal rat sertoli cells requires multiple steroidogenic factor-1 and GATA-4-binding sites. Proc Natl Acad Sci U S A 97: 1624-9, 2000

Watson RH, Neville PJ, Roy WJ, Jr., Hitchcock A, Campbell IG. Loss of heterozygosity on chromosomes 7p, 7q, 9p and 11q is an early event in ovarian tumorigenesis. Oncogene 17: 207-12, 1998

Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44: 388-96, 1989

Weitzel JN, Patel J, Smith DM, Goodman A, Safaii H, Ball HG. Molecular genetic changes associated with ovarian cancer. Gynecol Oncol 55: 245-52, 1994

Wen WH, Reles A, Runnebaum IB, Sullivan-Halley J, Bernstein L, Jones LA, Felix JC, Kreienberg R, et al. p53 mutations and expression in ovarian cancers: correlation with overall survival. Int J Gynecol Pathol 18: 29-41, 1999

White RA, Dowler LL, Pasztor LM, Gatson LL, Adkison LR, Angeloni SV, Wilson DB. Assignment of the transcription factor GATA4 gene to human chromosome 8 and mouse chromosome 14: Gata4 is a candidate gene for Ds (disorganization). Genomics 27: 20-6, 1995

Whittemore AS, Harris R, Itnyre J. Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group. Am J Epidemiol 136: 1184-203, 1992

Wolff E, Liehr T, Vorderwülbecke U, Tulusan AH, Husslein EM, Gebhart E. Frequent gains and losses of specific chromosome segments in human ovarian carcinomas shown by comparative genomic hybridization. Int J Oncol 11: 19-23, 1997

Wolfson AH, Tralins KS, Greven KM, Kim RY, Corn BW, Kuettel MR, Philippart C, Raub WA, Jr., Randall ME. Adenocarcinoma of the fallopian tube: results of a multi-institutional retrospective analysis of 72 patients. Int J Radiat Oncol Biol Phys 40: 71-6, 1998

Wright K, Wilson PJ, Kerr J, Do K, Hurst T, Khoo SK, Ward B, Chenevix-Trench G. Frequent loss of heterozygosity and three critical regions on the short arm of chromosome 8 in ovarian adenocarcinomas. Oncogene 17: 1185-8, 1998

Wright K, Wilson P, Morland S, Campbell I, Walsh M, Hurst T, Ward B, Cummings M, Chenevix-Trench G. beta-catenin mutation and expression analysis in ovarian cancer: exon 3 mutations and nuclear translocation in 16% of endometrioid tumours. Int J Cancer 82: 625-9, 1999

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 48: 678-81, 1991

Yang-Feng TL, Han H, Chen KC, Li SB, Claus EB, Carcangiu ML, Chambers SK, Chambers JT, Schwartz PE. Allelic loss in ovarian cancer. Int J Cancer 54: 546-51, 1993

Yuan ZQ, Sun M, Feldman RI, Wang G, Ma X, Jiang C, Coppola D, Nicosia SV, Cheng JQ. Frequent activation of AKT2 and induction of apoptosis by inhibition of phosphoinositide-3-OH kinase/Akt pathway in human ovarian cancer. Oncogene 19: 2324-30, 2000

Zborovskaya I, Gasparian A, Karseladze A, Elcheva I, Trofimova E, Driouch K, Trassard M, Tatosyan A, Lidereau R. Somatic genetic alterations (LOH) in benign, borderline and invasive ovarian tumours: intratumoral molecular heterogeneity. Int J Cancer 82: 822-6, 1999

Zheng JP, Robinson WR, Ehlen T, Yu MC, Dubeau L. Distinction of low grade from high grade human ovarian carcinomas on the basis of losses of heterozygosity on chromosomes 3, 6, and 11 and HER-2/neu gene amplification. Cancer Res 51: 4045-51, 1991

Zheng W, Cao P, Zheng M, Kramer EE, Godwin TA. p53 overexpression and bcl-2 persistence in endometrial carcinoma: comparison of papillary serous and endometrioid subtypes. Gynecol Oncol 61: 167-74, 1996

Zheng W, Sung CJ, Cao P, Zhang ZF, Cai R, Godwin TA, Kramer EE, Lauchlan SC. Early occurrence and prognostic significance of p53 alteration in primary carcinoma of the fallopian tube. Gynecol Oncol 64: 38-48, 1997

Ziegle JS, Su Y, Corcoran KP, Nie L, Mayrand PE, Hoff LB, McBride LJ, Kronick MN, Diehl SR. Application of automated DNA sizing technology for genotyping microsatellite loci. Genomics 14: 1026-31, 1992