Tumour suppressor genes in sporadic epithelial ovarian cancer

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Ovarian cancer is the most frequent cause of death from gynaecological malignancies in the western world, and sporadic epithelial ovarian cancer is its most predominant form. The aetiology of sporadic ovarian cancer remains unknown. Genetic studies have enabled a better understanding of the evolution of tumour progression. A major focus of research has been to identify tumour suppressor genes implicated in sporadic ovarian cancer over the past decade. Several tumour suppressor genes have been identified by strategies such as positional cloning and differential expression display. Further research is warranted to understand fully their contribution to the pathogenesis of sporadic ovarian cancer.

The ovary is the seventh most common site of cancer in women, after cancer of the breast, cervix, colon/rectum, stomach, corpus uteri, and lung. Incidence rates for ovarian cancer show a wide geographic variation. The highest incidence rates are observed in north-western Europe, the USA and Canada, whereas the lowest rates are observed in Asia, Africa and Latin America (Chen *et al.*, 1992; Lynch *et al.*, 1993). In the USA, it was estimated that in 1999, 25 200 new cases would be diagnosed with ovarian cancer and that 14 500 women would die of this disease (Partridge and Barnes, 1999). In the UK, the incidence of ovarian cancer is over 5000 new cases every year and it accounts for 4275 deaths per year (Chang *et al.*, 1994). Patients with early-stage ovarian cancer, in whom the disease is confined to one or both ovaries (stage I), have good prognoses with a 5 year survival rate > 80%. The prognosis of patients with advanced disease, in whom the tumour has spread within or beyond the abdominal cavity (stage III and stage IV) have a significantly worse prognosis, with only 20% of patients surviving 5 years (Friedlander, 1998). The overall survival rate in ovarian cancer is as low as 30% as most (70–80%) patients present with advanced disease (Pecorelli *et al.*, 1999). Any method that enables the early detection of ovarian cancer would lead to a significant decrease in mortality.

Ovarian cancer encompasses a broad range of lesions: from localized benign tumours and tumours of borderline malignant potential, to invasive malignant adenocarcinomas. Histologically, most (85–90%) malignant ovarian tumours are of the epithelial type, which can be further grouped into histological types as: serous cystadenocarcinoma (42%), mucinous cystadenocarcinoma (12%), endometrioid carcinoma (15%), undifferentiated carcinoma (17%) and clear cell carcinoma (6%). Approximately 5–10% of ovarian cancer

is hereditary. It is clear now that most hereditary ovarian carcinoma cases (breast–ovarian cancer syndrome and site-specific ovarian cancer) occur in women with germline mutations in the *BRCA1* (for ‘breast cancer 1’) gene (Miki *et al.*, 1994) or the *BRCA2* (for ‘breast cancer 2’) gene (Wooster *et al.*, 1995). Women harbouring such mutations are at a 27–60% risk of developing this disease in their life (Ford *et al.*, 1998). Ovarian cancer associated with the hereditary nonpolyposis colon cancer syndrome (Lynch syndrome type II) is the result of mutations in one of the four known DNA mismatch repair genes: *hMSH2* (chromosome 2p), *hMLH1* (chromosome 3p), *hPMS1* (chromosome 2q) and *hPMS2* (chromosome 7p) (Aaltonen and Peltomaki, 1994). It was initially anticipated that somatic mutations in *BRCA1*, *BRCA2* and mismatch repair genes would be as important in the development of sporadic epithelial ovarian cancer as in the case of the *Rb1* gene, which contributes to the tumorigenesis of both hereditary and sporadic retinoblastoma. Surprisingly, very few mutations in *BRCA1* (Takahashi *et al.*, 1995), *BRCA2* (Lancaster *et al.*, 1996) and mismatch repair genes have been identified in sporadic cases, indicating a genetic difference between hereditary and sporadic ovarian cancer.

Epithelial ovarian cancer has a clonal origin (for a review, see Shelling *et al.*, 1995), and multiple genetic alterations must occur during the malignant transformation of a single ovarian surface epithelial cell (Godwin *et al.*, 1992). The genes that may contribute to the tumorigenesis of sporadic ovarian cancer fall into two categories: oncogenes and tumour suppressors. Oncogenes are dominant transforming genes as their activation can be caused by an alteration in a single allele. Activation of oncogenes can occur through a variety of mechanisms such as point mutation, amplification, overexpression and translocation. The normal counterpart of oncogenes, proto-oncogenes, are involved in the control of cell proliferation and differentiation. More than 60 oncogenes have been discovered and several, such as *cKRAS*, *cERB2*, *cFMS*, *cMYC* and *AKT2*, have been studied

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in ovarian cancer (Godwin *et al.*, 1997). Although oncogenes may influence ovarian cancer, little is known about how and at what level the oncogenes participate in the malignant transformation of the ovarian surface epithelium (Auersperg *et al.*, 1998). Tumour suppressor genes, unlike oncogenes, act recessively. That is, it is the loss or inactivation of both copies of a tumour suppressor gene that remove normal constraints to cell proliferation (Fig. 1a). Proof that a gene is a tumour suppressor is usually derived from transfection experiments by introduction of its wild type to malignant cells, and analysis of colony formation with a selection marker (Haber and Harlow, 1997). The ultimate proof comes from mouse knockout experiments. Two or more different suppressor genes may be inactivated in the same tumours, and the same suppressors, like the *TP53* gene, may be inactivated in different tumour types. Since tumour suppressor genes play an essential role in maintaining normal cell growth, effort has been put into isolation of tumour suppressors involved in the pathogenesis of ovarian cancer.

Positional cloning has been the main strategy applied to localization and identification of tumour suppressor genes. This strategy has been very successful as is evident from the genes identified in the past decade. The main approaches involved in this strategy are: (i) cytogenetic studies to identify chromosomal alterations in patients with cancer; (ii) DNA linkage techniques to localize genes involved in inherited predisposition to cancer; and (iii) examination for loss of heterozygosity (LOH) or allele loss studies in sporadic tumours. In the ideal case, a region with a homozygous deletion is a hallmark for the location of a tumour suppressor gene, as both of the alleles are lost, such as in the case of the *BRCA2* gene. This review focuses primarily on identified or potential tumour suppressor genes implicated in sporadic ovarian cancer.

Cytogenetic changes in sporadic ovarian cancer

Techniques applied in cytogenetics commonly include chromosome banding, microdissection and fluorescent *in situ* hybridization (FISH). In addition to these traditional methods, new methods have been developed to allow easier identification of genomic amplifications or deletions. Comparative genomic *in situ* hybridization (CGH) allows fluorescent identification of chromosome gain and loss in human cancers within a single experiment. This method does not require mitotic cells, cell culture, or prior knowledge of regions of abnormality, and can be performed with small amounts of DNA (Iwabuchi *et al.*, 1995). For example, studies using CGH have revealed the number of copies of one region at 3q26, where *PIK3CA* (PI3-kinase) that encodes the p110 α catalytic subunit of phosphatidylinositol 3-kinase is located, as having increased in approximately 40% of ovarian and other cancers. The association between the number of copies of *PIK3CA* and PI3-kinase activity makes *PIK3CA* a candidate oncogene, because a broad range of normal cellular functions have been associated with PI3-kinase-mediated signalling. Further study has indeed proved that *PIK3CA* is an oncogene and plays an important role in

ovarian cancer (Shayesteh *et al.*, 1999). Spectral karyotyping (SKY) is another FISH-related method in which all the chromosomes on a metaphase spread are hybridized in a single FISH reaction with a mixture of differentially labelled DNA from each human chromosome using a combination of five fluorochromes in different ratios (Chang and Mark, 1997). The FISH signals obtained can be integrated and transformed into a different colour for each chromosome using a fluorescent image system (Schrock *et al.*, 1996). This method has been very successful in the diagnosis of complicated translocations in cancer cell lines (Veldman *et al.*, 1997).

Cytogenetic analysis of ovarian carcinomas indicates that karyotypes in this disease are often very complex. A wide range of numerical and structural abnormalities such as highly fragmented chromosomes, quadriradials, telomeric fusion, and complexly rearranged chromosomes has been described (Heim and Mitelman, 1995). However, non-random chromosome changes have been reported, including deletions in the region of 6q15–21 and the translocation of chromosome 6 with chromosome 14, t(6;14)(q21;q24) (Wake *et al.*, 1980). Although deletion or translocation of chromosome 6 has not been described as the sole primary change, the loss of 6q remains the most common abnormality described in this tumour to date. The frequent chromosomal abnormalities in ovarian cancers are loss of genetic material in several regions, including 3p, 6q, 11p, 17q, and 17p13 (Mitelman, 1995), indicating that putative tumour suppressor genes residing in these regions play an important role in the pathogenesis of ovarian cancer.

Allele loss studies in sporadic ovarian cancer

The analysis for LOH is the common approach used for identifying tumour suppressor genes involved in the aetiology of sporadic tumours. The basis for this strategy is the 'two hit' hypothesis by which the recessively acting genes are rendered inactive (Knudson, 1993). The first hit is usually a mutation in one of the alleles of the gene, and the second hit may occur by a variety of mechanisms including mutation or deletion. Deletion appears to be the most common mechanism associated with failure to detect heterozygosity of a polymorphic marker in tumour cells, which is, by contrast, present in the individual's normal DNA at that locus, hence the LOH (Fig. 1b). When a series of polymorphic markers that cover sites throughout the chromosomes are used on DNA from a panel of ovarian tumours and matching normal samples, the frequency of LOH in the specific region of the genome is determined. Southern blot analysis to detect restriction fragment length polymorphisms (RFLPs) was used initially to evaluate LOH, but amplification of polymorphic microsatellite repeat markers by polymerase chain reaction (PCR) has become the method of choice. Conventional autoradiographic procedures for analysing amplified microsatellites are prone to reading errors as a result of polymerase slippage. More recently, microsatellite repeat PCR products amplified by fluorescently labelled primers can be analysed on 373

Fig. 1. (a) Schematic illustration of Knudson's 'two hit' genetic model for sporadic and hereditary cancer (Knudson, 1993). (b) Schematic illustration of the principle behind analysis for loss of heterozygosity. A1/A2 and B1/B2 represent two polymorphic alleles at two different loci. Loss of one allele (B2) is demonstrated by the two techniques shown. LOH: loss of heterozygosity; RFLP: restriction fragment length polymorphism.

sequencer (PE Applied Biosystems) with the accompanying GENESCAN software. This fluorescence-based genotyping is as accurate as the standard radiolabelling technique (Schwengel *et al.*, 1994) and has been widely applied to assess allele loss in tumours (Cooke *et al.*, 1996).

Over 70 papers reporting LOH results in sporadic ovarian tumours have been published to date. However, only two of

the studies (termed 'allelotype studies') have examined the LOH rates on all the chromosome arms in the same panel of ovarian tumours independently (Sato *et al.*, 1991; Cliby *et al.*, 1993). The first study examined 37 ovarian tumours with 46 polymorphic markers (29 were variable numbers tandem repeat markers, and the remainder were RFLP markers) and reported that chromosomal arms 4p, 6p, 7p, 8q, 12q, 16p,

16q, 17p, 17q and 19q had LOH rates > 30% (Sato *et al.*, 1991). Owing to the poor density of polymorphic markers on each chromosome arm and the relatively low sensitivity of the detection method (Southern analysis), the results from this study are not completely accurate. In the second allelotyping study (Cliby *et al.*, 1993), 70 polymorphic markers were tested on the same number of tumours, and PCR was used to detect microsatellite markers. In summarizing the LOH rates from each chromosome arm, the authors did a one-side Student's *t* test to calculate the background rate of random chromosomal arm loss, and this was $\geq 35\%$, which accounted for the high background rate of LOH present in the tumours studied. From this study, frequent allele losses (> 35%) were seen in chromosomal arms 5q, 6q, 7p, 8p, 9q, 13q, 14q, 15q, 17p, 17q, 18q, 21q and 22q.

Most LOH studies on ovarian tumours were focused on one or several chromosome arms and the results were variable, making it difficult to locate clearly a region of interest. These differences may be the result of insufficient numbers of tumours being tested, uninformative loci on the particular chromosome arm, or the varying sensitivities of the methods used. Other causes may be more significant, such as inherent genetic differences in the population studied or the differences in the tumour subtype, stage and grade. In an attempt to adjust for some of these variables, the results of chromosome arm loss from a number of LOH studies have been pooled (Table 1, Fig. 2). Attempts have been made to avoid duplicating data from different studies, and only results from malignant tumours have been included. Chromosomal regions where potential tumour suppressor genes have been studied are discussed in detail below.

Chromosome 1

High rates of LOH have been recorded on chromosomal arm 1p (1p31 and 1p36) (Imyanitov *et al.*, 1999; Peng *et al.*, 2000). The *p73* gene, the homologue of *TP53*, has been isolated and mapped to 1p36, making it a candidate tumour suppressor gene. However, RT-PCR and western blot analyses revealed strong expression of *p73* in ovarian adenocarcinoma cell lines but very low or undetectable expression in normal ovarian surface epithelial cells. There was no association between 1p36 LOH and *p73* expression in ovarian tumours, nor between *p73* and *p53* expression (Imyanitov *et al.*, 1999). These findings indicate that *p73* is not the target of allele loss at 1p36. The *ARHI* (*NOEY2*) gene, located at 1p31 (Yu *et al.*, 1999; Peng *et al.*, 2000) is a maternally imprinted putative tumour-suppressor gene in ovarian and breast cancers with high homology to *ras* and *rap* (Yu *et al.*, 1999) identified by differential display PCR. In most of the tumour samples with loss of heterozygosity at this region, the non-imprinted functional allele was deleted. Re-expression of *ARHI* through transfection suppresses clonogenic growth of breast and ovarian cancer cell lines. The growth suppression was associated with downregulation of the cyclin D1 promoter activity and induction of *p21^{WAF1/CIP1}* (Yu *et al.*, 1999). Therefore, *ARHI* appears to

be an imprinted tumour suppressor gene but its role in the pathogenesis of ovarian cancer remains to be determined.

Chromosome 5

The search for a tumour suppressor gene on chromosome 5 was stimulated by studies showing a high percentage loss on 5q (46%) (Cliby *et al.*, 1993; Allan *et al.*, 1994; Tavassoli *et al.*, 1996). Because the adenomatous polyposis coli (*APC*) gene is located at 5q21–22, mutation analysis was performed on those tumours demonstrating LOH. However, no mutation was detected in these samples, leading to the conclusion that another tumour suppressor gene of relevance to ovarian cancer is located on 5q, probably at 5q13.1–21 (Tavassoli *et al.*, 1996).

There is a relatively low frequency of LOH on chromosome 5p. Nevertheless, the tumour suppressor gene *Dab2/DOC2* (for 'disabled-2/differentially expressed in ovarian carcinoma 2') was isolated independently by two groups using the differential expression display method (chromosome 5p13) (Xu *et al.*, 1995a; Mok *et al.*, 1998). *Dab2* functions in the mitogenic signal transduction pathway and is frequently inactivated by homozygous gene deletion in tumours (Xu *et al.*, 1998). The expression of *Dab2* was absent in all ovarian carcinoma cell lines and tissues examined, but was present in normal ovarian epithelial cell lines and tissues (Mok *et al.*, 1994). It has also been demonstrated that loss of *Dab2* expression is an early event in ovarian malignancies (Fazili *et al.*, 1999). Furthermore, when *DOC-2* was transfected into the ovarian carcinoma cell line SKOV3, the stable transfectants showed significantly reduced growth rate and ability to form tumours in nude mice (Mok *et al.*, 1998). These data indicate that downregulation of *Dab2/DOC-2* may play an important role in ovarian pathogenesis.

Chromosome 6

Allele loss of up to 62% (Cooke *et al.*, 1996) has been reported frequently (average 48%; Table 1) on the long arm of chromosome 6. The allele loss at 6q was observed in all types of epithelial ovarian cancer. Three common allele loss regions at 6q21–23, 6q25 and 6q27 have been identified (for a review, see Shelling *et al.*, 1995). Chromosomal regions at 6q14–21 (Sandhu *et al.*, 1996) and 6q24–25 (Wan *et al.*, 1999) suppress tumorigenicity in the ovarian cell lines.

The human homologue of the rat *Lot1* gene, *LOT1* (localized to 6q25) was identified through analysis of differential gene expression in normal and malignant rat ovarian surface epithelial cells. Both the human and rat ovarian carcinoma cell lines exhibited loss or decreased expression of this gene. Furthermore, upon transfection into ovarian cancer cell lines, it suppressed the growth of the cells (Abdollahi *et al.*, 1999). *LOT1* may be a novel target of growth factor signalling pathways and it may regulate the growth promoting signals as a zinc-finger motif-containing nuclear transcription factor (Abdollahi *et al.*, 1999).

Table 1. Allele loss in sporadic ovarian cancer

Chromosome arm	Allele loss (%)	Minimal allele loss region	Gene(s) studied
5q	41/114(36)	D5S424–D5S644	<i>APC, Dab2/DOC2</i>
6p	60/171(35)		
6q	218/453 (48)	6q14–16, 6q21–23, 6q25–27	<i>LOT1</i>
9p	70/206 (34)	9p21	<i>CDKN2A</i>
10q	19/121 (16)	10q23	<i>PTEN</i>
11p	188/542 (35)	11p15	<i>WT1, TSG101</i>
11q	108/245 (43)	11q23–qter	<i>ATM</i>
13q	105/260 (40)	13q14, 13q12–13	<i>RB1, BRCA2</i>
14q	67/188 (36)	14q12–13, 14q32	
17p	407/667 (61)	17p13	<i>TP53, OVCA1, OVCA2, HIC1</i>
17q	499/860 (58)	17q25, or whole arm loss	<i>NF1, BRCA1, septin</i>
18q	75/179 (42)	18q21, 18q23	<i>SMAD4, DCC</i>
19p	53/181 (30)	19p13.3	<i>LKB1</i>
22q	111/224 (49)	22q12, D22S284–CYP2D	<i>NF2</i>
Xp	30/78 (38)	Xp21.1–p11.4	
Xq	38/116 (34)	Xq11.2–12	<i>GPC3</i>

Data summarized from: Shelling *et al.* (1995); Bryan *et al.* (1996); Cooke *et al.* (1996); Davis *et al.* (1996); Kerr *et al.* (1996); Phillips *et al.* (1996); Saito *et al.* (1996); Tangir *et al.* (1996); Tavassoli *et al.* (1996); Tibiletti *et al.* (1996, 1998); Wertheim *et al.* (1996); Bandera *et al.* (1997); Choi *et al.* (1997); Koike *et al.* (1997); Roy *et al.* (1997); Shih *et al.* (1997, 1998); Colitti *et al.* (1998); Edelson *et al.* (1998); Launonen *et al.* (1998); Suzuki *et al.* (1998, 2000); Watson *et al.* (1998); Brown *et al.* (1999); Fullwood *et al.* (1999); Huang *et al.* (1999); Imyaninov *et al.* (1999); Lin *et al.* (1999); Niederacher *et al.* (1999); Nishioka *et al.* (1999); Shridhar *et al.* (1999); Dion *et al.* (2000); Peng *et al.* (2000); Veugeliers *et al.* (2000).

Fig. 2. Frequency of allele loss on each chromosome arm (taken as total number of markers lost in an individual chromosome arm per total number of tumours analysed). □: short (p) arm; ■: long (q) arm. Horizontal line indicates the average loss of heterozygosity (35%) in all the chromosome arms analysed.

Therefore, *LOT1*, is a putative tumour suppressor gene and further research is required to characterize this gene.

Among the several common allele loss regions on the long arm of chromosome 6, 6q27 has been reported most frequently (Saito *et al.*, 1992, 1996; Cooke *et al.*, 1996; Tibiletti *et al.*, 1996, 1998; Suzuki *et al.*, 1998). The

D6S149–D6S193 region at 6q27 was altered in all samples analysed by FISH studies using yeast artificial chromosomes (YACs) on direct metaphase spreads from fresh ovarian tumours, indicating that this change might be important even in early ovarian tumours (Tibiletti *et al.*, 1996, 1998). The analysis of 70 malignant ovarian tumours using cosmids

mapping to chromosomal arm 6q initially defined a minimal region of allele loss between D6S149 and D6S193 (1.9 cM) in one tumour (Saito *et al.*, 1992, 1996). Subsequent studies have shown increased frequency of allele loss on 6q around the same region, although a minimal region was not defined (Wan *et al.*, 1994; Orphanos *et al.*, 1995). On the basis of analysis of 56 malignant ovarian tumours, the minimum region of allele loss on 6q27 is between D6S297 and D6S264 (3 cM) (Cooke *et al.*, 1996; Chenevix-Trench *et al.*, 1997). The maximum frequency of allele loss occurred at D6S193 (62%) and D6S297 (52%). Three tumours showed loss of D6S193 only, while retaining flanking markers, thus indicating that the putative tumour suppressor gene is close to D6S193. Taking into account the two previous allele loss studies, we suggest that the putative gene lies within the region between D6S264 and D6S149 (approximately 4.9 cM). A positional cloning approach was undertaken involving isolation of YACs, cosmids, P1-derived artificial chromosomes (PACs), bacterial artificial chromosomes (BACs) and construction of a physical and transcript map around the key polymorphic markers. Seven genes have been identified in the interval between D6S264 and D6S149 (Liu *et al.*, 2002). Expression and mutation analysis of these genes are being carried out to identify the putative tumour suppressor gene.

Chromosome 9

The overall allele loss in chromosome 9p is 34% (Table 1). The tumour suppressor gene *CDKN2A* (p16, MTS1, CDK4I mapped to 9p 21), which encodes a cell cycle regulatory protein, was initially found to be homozygously deleted in gliomas (Kamb *et al.*, 1994) or, alternatively, hypermethylated in the promoter region in some gliomas with an intact *CDKN2A* gene (Herman *et al.*, 1995). Both of these events led to the loss of expression of this gene. In ovarian tumours, although 21% of tumours did not express p21, methylation deletions or mutations were not found (Shih *et al.*, 1997).

Chromosome 10

PTEN/MMAC1 (for 'phosphatase and tensin homologue/mutated in multiple advanced cancers') on chromosome 10q23 is a recently identified tumour suppressor gene, which is frequently mutated in a wide range of tumours including glioma, prostate cancer, renal cancer, melanoma and endometrial cancer (Li *et al.*, 1997). In ovarian cancer, mutations of *PTEN* have been found in only 20% of endometrioid type, but not in serous or mucinous types, of epithelial ovarian tumours (Obata *et al.*, 1998). When immunohistochemistry and mutation analysis are combined, *PTEN* can be inactivated in other subtypes of ovarian cancer (Kurose *et al.*, 2001).

Chromosome 11

Somatic mutations have not been identified in the coding region of the *WT1* (maps to 11p13) gene in a panel of ovarian

tumours in which LOH at 11p13 has been observed (Bruening *et al.*, 1993; Viel *et al.*, 1994; Shimizu *et al.*, 2000). A tumour suppressor gene termed *TSG101* (for 'tumour susceptibility gene 101') is located at 11p15, where 40–50% ovarian cancers have deletions (Lu *et al.*, 1997). In examining three ovarian cancer cell lines and 27 primary advanced stage epithelial ovarian cancers, the full length *TSG101* transcript was detected in all the samples by RT-PCR and northern blot analysis.

On the long arm of chromosome 11, two regions of allele loss (11q22–23.3 and 11q23.3–24.3) have been defined by LOH studies. Allele loss at the 11q23.3–24.3 region is significantly associated with poor survival (Gabra *et al.*, 1996). The ataxia-telangiectasia mutated (*ATM*) gene, which is responsible for a multisystem recessive disease ataxia-telangiectasia, is located at 11q23 (Lavin, 1999). LOH in the *ATM* gene occurred in 44% of informative cases, but no somatic alterations of the *ATM* gene were found in these ovarian cancer samples, including those with LOH present in the *ATM* gene (Koike *et al.*, 1999). *BARX2*, a gene encoding a transcription factor that is homologous with the *Drosophila* homeobox-domain-containing proteins, was identified and mapped to 11q25, and shown to be a tumour suppressor gene by functional analysis (Sellar *et al.*, 2001). However, it is unclear if *BARX2* is the cause of the high LOH rate at 11q23.3–24.3.

Chromosome 13

The chromosomal arm 13q shows frequent LOH in ovarian cancer, in particular in high grade tumours (Sato *et al.*, 1991; Kim *et al.*, 1994). Initially, the retinoblastoma gene (*Rb1*), located at 13q14, was examined and functional RB protein was shown to be present in ovarian carcinomas despite LOH at the RB locus (Dodson *et al.*, 1994; Kim *et al.*, 1994). *BRCA2*, a gene predisposing for familial breast cancer (Wooster *et al.*, 1995), has been mapped to 13q12–13. *BRCA2* is rarely mutated in sporadic ovarian tumours despite the relatively high frequency of LOH detected in sporadic cases (Lancaster *et al.*, 1996), indicating that a tumour suppressor gene or genes other than *RB1* or *BRCA2* on chromosome 13q must be involved in the progression of ovarian cancer.

Chromosome 16

Although the overall LOH rates on chromosome 16 arms are beneath the threshold of 34%, a 700 kb region at chromosome 16q23.2 was shown to be homozygously deleted in an ovarian tumour. The *WWOX* (WW domain containing oxidoreductase) gene, encoding a protein with two WW domains and a short-chain dehydrogenase domain, has been mapped to this region. Homozygous deletions of *WWOX* exons have been reported in one ovarian cancer cell line and one primary ovarian tumour (Paige *et al.*, 2000, 2001).

Chromosome 17

Allele loss occurs frequently on both arms of chromosome 17 (17p, 53%; 17q, 57%) (Table 1). On the short arm, the tumour suppressor gene TP53 is located on chromosome 17p13.1 (McBride *et al.*, 1986). Mutant p53 protein has been the most frequently encountered genetic abnormality in human malignancy (Harris and Hollstein, 1993). TP53 mutation is not a common feature of benign or borderline tumours. Furthermore, the TP53 mutation appears to be less common in localized tumours than in advanced stage tumours (Shelling *et al.*, 1995). Patients with tumours with a mutant p53 have a poor survival rate. It remains unclear whether mutation of the TP53 gene is an independent prognostic factor, and studies with larger numbers of cases are required (Katso *et al.*, 1997). It is possible to suppress the growth of an ovarian cancer cell line using recombinant adenovirus carrying a wild-type TP53 gene (AxCap53) in the presence of cisplatin (Kigawa and Terakawa, 2000). Another recombinant adenovirus carrying a wild-type TP53 (Avp53) effectively suppressed the growth of peritoneal tumours *in vivo* in nude mice and prolonged the survival of the treated group, especially when the tumour burden was less (Kim *et al.*, 1999).

Up to 80% (39 of 49) of allelic loss has been observed at 17p13.3 (Phillips *et al.*, 1996). Chromosome 17p13.3 loss may precede TP53 region deletion, because allele loss was observed in early stage ovarian cancers at this region and the alterations in TP53 have been found only in advanced stages (Phillips *et al.*, 1996; Wiper *et al.*, 1998). Three tumour suppressor genes, *HIC-1* (for 'hypermethylated in cancer'), *OVCA1* and *OVCA2* (for 'ovarian cancer genes' 1 and 2), have been identified in this region. *HIC-1* was identified by analysis of a DNA site hypermethylated in tumour DNA (Wales *et al.*, 1995) and is a new zinc-finger transcription factor gene that is ubiquitously expressed in normal tissues, but absent in different tumour cells, in which it is hypermethylated. The suppression of the neomycin selectability of cultured brain, breast and colon cancer cells after transfection of *HIC-1* make *HIC-1* a strong candidate for a tumour suppressor (Wales *et al.*, 1995). *OVCA1* and *OVCA2* were identified at 17p13.3 by positional cloning (Schrock *et al.*, 1996), are ubiquitously expressed and encode proteins of 443 and 227 amino acids, respectively, with no known functional motifs. Northern blot analysis revealed that *OVCA1* and *OVCA2* mRNA was expressed in the normal surface epithelial cells of the ovary, but that expression of this transcript was significantly reduced or was undetectable in 92% (11/12) of the ovarian tumours and tumour cell lines analysed (Schultz *et al.*, 1996). Western blot analysis of extracts prepared from breast and ovarian carcinomas revealed reduced expression of *OVCA1* compared with extracts from normal epithelial cells from these tissues (Bruening *et al.*, 1999). Reduction of colony formation was also demonstrated in ovarian cancer cell lines transfected with plasmids expressing *OVCA1* compared with appropriate controls. As no mutation was

identified in the tumour and cell lines that have LOH at 17q13.3, haploinsufficiency, which was observed in the mouse gene *p27/kip* and the *PTEN* genes when they lost their tumour suppression ability, may be the mechanism silencing the gene (Di Cristofano *et al.*, 1998; Fero *et al.*, 1998). However, as the methylation status of *OVCA1* in samples that have allele loss at 17q13.3 has not been studied, the mechanism of inactivation of this gene needs further research.

On the long arm of chromosome 17, at least three distinct commonly deleted regions at 17q12.2 (the *NF1* locus), 17q21 (including the *BRCA1* locus), and from 17q25.1 to 17qter have been identified in ovarian cancers. No mutation has been identified in the *NF1* gene (Foulkes *et al.*, 1994; Iyengar *et al.*, 1999) and very few mutations have been found in the *BRCA1* gene in sporadic ovarian cancers (Takahashi *et al.*, 1995). However, expression of *BRCA1* may be silenced by hypermethylation in its promoter region (Mancini *et al.*, 1998). As the region between 17q25.1 and 17qter has a much higher frequency of LOH (Eccles *et al.*, 1990; Russell *et al.*, 1990), effort has been put into mapping this region and a human septin gene has been identified at 17q25 (Russell *et al.*, 2000). Given that the septins are a family of genes involved in cytokinesis and cell cycle control, and that this human septin gene is expressed widely in human adult and fetal tissues (including the ovary) (Kalikin *et al.*, 2000), the human septin gene merits further investigation as an attractive candidate tumour suppressor in sporadic ovarian cancer.

Chromosome 18

Overall, 42% of tumours showed LOH on 18q (Table 1). The allele loss between D18S5 and D18S11 at 18q23 was detected more frequently in stage III and IV tumours than in stage I and II tumours, implying that this allele loss may represent a late event in the progression of ovarian cancers (Chenevix-Trench *et al.*, 1997). *SMAD4* (*DPC4*), deleted in colorectal carcinoma, is located at 18q21. Allele loss was detected at the *D18S46* (31%) and *D18S474* (36%) loci, which are adjacent to the *SMAD4* gene. Missense mutations were detected in 2/42 ovarian tumours and 3/8 ovarian cancers cell lines, whereas a silent mutation was detected in 1/42 primary ovarian cancers (Takakura *et al.*, 1999).

Chromosome 19

LOH has been reported in about 30% of ovarian cancers on 19p13.3 (Table 1). Germline mutations in the *LKB1* (*STK11*) gene (chromosome 19p13.3) cause characteristic hamartomas and pigmentation in patients with Peutz-Jeghers syndrome. Peutz-Jeghers syndrome carries an overall risk of cancer up to 20 times that of the general population. Patients with Peutz-Jeghers syndrome are also at increased risk of benign and malignant ovarian tumours, particularly granulosa cell tumours. However, like those of the *BRCA1* and *BRCA2* genes, *LKB1* mutations can cause

Table 2. Tumour suppressor genes implicated in the pathogenesis of only sporadic epithelial ovarian cancer

Gene	Position	Protein	Function	Location	Strategy	Mechanism
<i>TP53</i>	17q13.1	Nuclear protein P53	Multiple (for example, transcription factor, DNA damage response, apoptosis, angiogenesis)	Nucleus	Virus transformation study	Mutation
<i>PTEN</i>	10q23	Phosphatase with homology to tensin and auxilin	Negative regulation of PI3'K/PKB/Akt signalling pathway	Cytoplasm	Positional cloning	Mutation
<i>ARHI/NOEY2</i>	1p31	Ras family protein	May truncate Ras/mitogen signalling	Cell membrane Cytoplasm	Differential display	Imprinting
<i>Dab2/DOC2</i>	5p13	Phosphoprotein	Mitogen signal transduction pathway	Cytoplasm	Differential display	Homozygous deletion
<i>LOT1</i>	6q25	Nuclear protein	Transcription factor	Nucleus	Differential display	Unknown
<i>CDKN2A</i>	9p21	P16	Binds to the cyclin-CDK4 complex	Nucleus	Positional cloning	Homozygous deletion, hypermethylation in the promoter region
<i>BRAX2</i>	Homeobox-domain-containing protein	Transcription factor that mediates ras/raf dependent transcription of the calcitonin gene	Nucleus	11q25	DNA affinity cloning	Unknown
<i>WWOX</i>	16q23.2	WW domain containing oxidoreductase	Mediator of apoptosis	Unknown	Differential display	Deletion and mutation
<i>HIC-1</i>	17p13	Nuclear zinc-finger protein	Transcription factor	Nucleus	Positional cloning	Hypermethylation in the promoter region
<i>OVCA1</i>	17p13	Protein with unknown functional motifs	Unknown	Throughout cell	Positional cloning	Halopinsufficiency or hypermethylation?
<i>SMAD4/DPC4</i>	18q21	Protein homologous to <i>mad</i> family	TGF- β signalling	Cytoplasm	Positional cloning	Mutation
<i>GPC3</i>	Xq26	Glypican integral membrane protein	Regulate interactions between growth factors and their receptors	Cell membrane	Differential display	Hypermethylation in the promoter region
<i>BRCA1</i>	17q25	Transcription factor	DNA repair	Nucleus	Positional cloning	Methylation

ovarian tumours when present in the germline, but occur rarely in the somatic tissue (Nishioka *et al.*, 1999; Wang *et al.*, 1999). Therefore, the *LKB1* gene may play a limited role in the development of ovarian carcinomas.

Chromosome X

High allele loss rate has been reported on both chromosomal arm Xp (38%) and Xq (34%) (Table 1). A region located at Xq26 (Xq11.2–12) is frequently deleted in advanced ovarian cancer (Choi *et al.*, 1997). The *GPC3* gene, located at Xq26, encodes a glypican integral membrane protein and is mutated in patients with Simpson–Golabi–Behmel syndrome (Pilia *et al.*, 1996). No mutations were found in *GPC3*, but its expression was lost in four (31%) of the cell lines analysed (Lin *et al.*, 1999). In all of the cases in which *GPC3* expression was lost, the *GPC3* promoter was hypermethylated. Expression of *GPC3* was restored by treatment of the cells with the demethylating agent 5-aza-2'-deoxycytidine. A colony-forming assay confirmed that ectopic *GPC3* expression inhibited the growth of ovarian cancer cell lines. These results show that *GPC3*, a gene involved in the control of organ growth, is frequently inactivated in a subset of ovarian cancers, indicating that it may function as a tumour suppressor in the ovary.

Other strategies

The sequencing of the human genome (<http://genome.cse.ucsc.edu/>) is likely to accelerate the identification of important genes in the pathogenesis of cancer. One possibility is to search the database of proteins predicted from the draft human genome for paralogues of known tumour suppressor genes, although no novel genes have been identified by this method to date (Futreal *et al.*, 2001). Another impact of the sequencing of the human genome has been the identification of 1.42 million single nucleotide polymorphisms (SNPs), which provides an average density on the available sequence of one SNP for every 1.9 kilobases (kb) (International SNP Map Working Group, 2001). With this information, a more precise analysis of LOH and delineation of minimal intervals of allele loss can be performed. The LOH status of a single gene in tumour samples can also be evaluated, as 60 000 SNPs fall within exons (coding and untranslated regions) and 85% of exons are within 5 kb of the nearest SNP (International SNP Map Working Group, 2001). In addition, when a positional cloning investigation points to a genomic segment, any gene located in that region becomes a candidate gene. Therefore, in future, the 'positional candidate gene' approach will be the method used to identify tumour suppressor genes. The availability of the human genome sequence accelerates the determination of whether a candidate gene is mutated in sporadic ovarian tumours.

The Human Genome Project has also answered questions about genetic evolution. Only 94 of 1278 protein families in the human genome appear to be specific to

vertebrates (Baltimore, 2001) and the number of genes contained in the human genome (30 000–40 000) is only about twice the number needed to make a fruitfly, worm or plant (Rubin, 2001). In the past decade, 50 tumour suppressor genes have been discovered in *Drosophila* and at least nine show clear homology to mammalian genes (Watson *et al.*, 1994), for example, the *LATS1* gene, the human homologue of *lats* (for 'large tumour suppressor') in *Drosophila*. The deletions in *lats* lead to over-proliferation and tumours in multiple tissues in flies (Xu *et al.*, 1995b) and the human homologue protein Lats1 binds to the crucial cell-cycle regulator CDC2 in a cell-cycle-dependent manner (Tao *et al.*, 1999). Furthermore, re-expression of human *LATS1* in flies completely suppressed tumour growth and rescued the 'aberrant' phenotype (Tao *et al.*, 1999). In the meantime, *Lats1*^{-/-} mice were prone to soft-tissue sarcomas, ovarian tumours and pituitary dysfunction (St John *et al.*, 1999). Although it has yet to be determined whether *LATS1* is a human tumour suppressor gene, it is clear that the use of model organisms, even invertebrates, is a valid approach that may lead to the identification of novel human cancer genes (Kemp, 1999).

The transgenic mouse has been a very good genetic model in characterizing known genes as tumour suppressors. For example in the study of inhibin-deficient mice, both male and female inhibin-deficient mice generated by embryo stem cell (ES) technology were found to be viable, but developed sex cord stromal tumours with almost complete penetrance (Matzuk *et al.*, 1994). However, the heterozygous mice developed normally, without any tumour formation. Since inhibins are secreted proteins, this observation is not surprising, as heterozygous cells would continue to receive the protein from the surrounding cells, thereby preventing any tumour formation. The above findings have resulted in the identification of the first secreted tumour suppressor protein. This approach can also be used to identify novel tumour suppressor genes. By deleting the region of a mouse chromosome that is syntenic to the human chromosome region with high allele loss, the tumour susceptibility of the mouse carrying the deletion can be observed (Zhang *et al.*, 1994).

Future developments

The advances in isolating and characterizing putative tumour suppressor genes have been impressive during the past 5 years, and the current focus is on the functional analysis of the candidate genes. Besides the traditional approaches of positional cloning and knockout mouse strategies, methods such as differential expression display by means of the PCR have been successful in identifying tumour suppressors, for example the *NOEY2*, *LOT1*, *Dab/DOC2* and *GPC3* genes (Table 2). Nevertheless, allele loss studies have provided information about where to look for tumour suppressors. The completion of sequencing genomes in humans and other species will be of great benefit to the search for tumour suppressor genes.

The mechanisms involved in silencing a tumour suppressor gene are mutation, deletion, rearrangement in its coding region, or hypermethylation in its promoter region. Methylation, as an 'epigenetic' gene silencing mechanism, is increasingly recognized as a common mechanism by which cancer-related genes are inactivated (Baylin *et al.*, 1998; Jones and Laird, 1999). One example is the abnormal methylation of the promoter of the *P14^{ARF}* gene, which results in the downregulation of the gene and the degradation of p53, disabling the p53-mediated suicide pathway (Robertson and Jones, 1998). The results of a study of melanomas showed that the *Apaf-1* gene is switched off, rather than deleted or mutated, by methylation, and can be activated again by treatment with 5-aza-2'-deoxycytidine (Soengas *et al.*, 2001). This finding has clinical implications in that the search for ways to reactivate the genes subject to this epigenetic downregulation may be much more straightforward than previously thought. The mechanism of inactivation of potential tumour suppressor genes involved in sporadic ovarian cancer (Table 2) indicates that hypermethylation of the promoter region may be particularly significant in ovarian cancer.

An array of tumour suppressor genes has been implicated in sporadic ovarian cancer and the current knowledge is summarized (Table 2). With the exception of *TP53* and *PTEN*, most of these genes have not been analysed in large numbers of ovarian tumours or cell lines, and their biochemical properties and exact role in the cause of sporadic ovarian cancer need further research. Taken as a group, the many presumed functions for the other known tumour suppressor genes support the notion that these genes control critical points in a wide range of cellular pathways that regulate proliferation, differentiation, apoptosis and response to genetic damage (Haber and Harlow, 1997) (Table 2). It is possible that delineation of the genetic and functional characteristics of tumour suppressor genes will enable us to diagnose and manage patients with ovarian cancer more precisely and effectively.

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References

- Key references are identified by asterisks.
- Aaltonen LA and Peltomaki P (1994) Genes involved in hereditary nonpolyposis colorectal carcinoma *Anticancer Research* **14** 1657–1660
- Abdollahi A, Bao R and Hamilton TC (1999) LOT1 is a growth suppressor gene downregulated by the epidermal growth factor receptor ligands and encodes a nuclear zinc-finger protein *Oncogene* **18** 6477–6487
- Allan GJ, Cottrell S, Trowsdale J and Foulkes WD (1994) Loss of heterozygosity on chromosome 5 in sporadic ovarian carcinoma is a late event and is not associated with mutations in APC at 5q21–22 *Human Mutation* **3** 283–291
- Auersperg N, Edelson M, Mok S, Johnson S and Hamilton T (1998) The biology of ovarian cancer *Seminars in Oncology* **25** 281–304
- Baltimore D (2001) Our genome unveiled *Nature* **409** 814–816
- Bandera CA, Takahashi H, Behbakht K *et al.* (1997) Deletion mapping of two potential chromosome 14 tumor suppressor gene loci in ovarian carcinoma *Cancer Research* **57** 513–515
- Baylin SB, Herman JG, Graff JR, Vertino PM and Issa JP (1998) Alterations in DNA methylation: a fundamental aspect of neoplasia *Advances in Cancer Research* **72** 141–196
- Brown MR, Chuaqui R, Vocke CD, Berchuck A, Middleton LP, Emmert-Buck MR and Kohn EC (1999) Allelic loss on chromosome arm 8p: analysis of sporadic epithelial ovarian tumors *Gynecologic Oncology* **74** 98–102
- Bruening W, Gros P, Sato T, Stanimir J, Nakamura Y, Housman D and Pelletier J (1993) Analysis of the 11p13 Wilms' tumor suppressor gene (WT1) in ovarian tumors *Cancer Investigation* **11** 393–399
- Bruening W, Prowse AH, Schultz DC, Holgado-Madruga M, Wong A and Godwin AK (1999) Expression of OVCA1, a candidate tumor suppressor, is reduced in tumors and inhibits growth of ovarian cancer cells *Cancer Research* **59** 4973–4983
- Bryan EJ, Watson RH, Davis M, Hitchcock A, Foulkes WD and Campbell IG (1996) Localization of an ovarian cancer tumor suppressor gene to a 0.5-cM region between D22S284 and CYP2D, on chromosome 22q *Cancer Research* **56** 719–721
- Chang J, Bridgewater J, Gore M, Fisher C, Schofield J, Kelland and Harap K (1994) Non-surgical aspects of ovarian cancer *Lancet* **343** 335–340
- Chang SS and Mark HF (1997) Emerging molecular cytogenetic technologies *Cytobios* **90** 7–22
- Chen Y, Wu PC, Lang JH, Ge WJ, Hartge P and Brinton LA (1992) Risk factors for epithelial ovarian cancer in Beijing, China *International Journal of Epidemiology* **21** 23–29
- Chenevix-Trench G, Kerr J, Hurst T *et al.* (1997) Analysis of loss of heterozygosity and KRAS2 mutations in ovarian neoplasms: clinico-pathological correlations *Genes, Chromosomes and Cancer* **18** 75–83
- Choi C, Cho S, Horikawa I *et al.* (1997) Loss of heterozygosity at chromosome segment Xq25–26.1 in advanced human ovarian carcinomas *Genes, Chromosomes and Cancer* **20** 234–242
- Cliby W, Ritland S, Hartmann L, Dodson M, Halling KC, Keeney G, Podratz KC and Jenkins RB (1993) Human epithelial ovarian cancer allelotyping *Cancer Research* **53** 2393–2398
- Colitti CV, Rodabaugh KJ, Welch WR, Berkowitz RS and Mok SC (1998) A novel 4 cM minimal deletion unit on chromosome 6q25.1–q25.2 associated with high grade invasive epithelial ovarian carcinomas *Oncogene* **16** 555–559
- Cooke IE, Shelling AN, Le Meuth VG, Charnock ML and Ganesan TS (1996) Allele loss on chromosome arm 6q and fine mapping of the region at 6q27 in epithelial ovarian cancer *Genes, Chromosomes and Cancer* **15** 223–233
- Davis M, Hitchcock A, Foulkes WD and Campbell IG (1996) Refinement of two chromosome 11q regions of loss of heterozygosity in ovarian cancer *Cancer Research* **56** 741–744
- Di Cristofano A, Pesce B, Cordon-Cardo C and Pandolfi PP (1998) Pten is essential for embryonic development and tumour suppression *Nature Genetics* **19** 348–355
- Dion F, Mes-Masson AM, Seymour RJ, Provencher D and Tonin PN (2000) Allelotyping defines minimal imbalance at chromosomal region 17q25 in non-serous epithelial ovarian cancers *Oncogene* **19** 1466–1472
- Dodson MK, Cliby WA, Xu HJ *et al.* (1994) Evidence of functional RB protein in epithelial ovarian carcinomas despite loss of heterozygosity at the RB locus *Cancer Research* **54** 610–613
- Eccles DM, Cranston G, Steel CM, Nakamura Y and Leonard RC (1990) Allele losses on chromosome 17 in human epithelial ovarian carcinoma *Oncogene* **5** 1599–1601
- Edelson MI, Lau CC, Colitti CV, Welch WR, Bell DA, Berkowitz RS and Mok SC (1998) A one centimorgan deletion unit on chromosome Xq12 is commonly lost in borderline and invasive epithelial ovarian tumors *Oncogene* **16** 197–202
- Fazili Z, Sun W, Mittelstaedt S, Cohen C and Xu XX (1999) Disabled-2 inactivation is an early step in ovarian tumorigenicity *Oncogene* **18** 3104–3113
- Fero ML, Randel E, Gurley KE, Roberts JM and Kemp CJ (1998) The murine gene p27Kip1 is haplo-insufficient for tumour suppression *Nature* **396** 177–180

- Ford D, Easton DF, Stratton M *et al.* (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium *American Journal of Human Genetics* **62** 676–689
- Foulkes WD, Englefield P and Campbell IG (1994) Mutation analysis of RASK and the 'FLR' exon' of NF1 in sporadic ovarian carcinoma *European Journal of Cancer* **30A** 528–530
- Friedlander M (1998) Prognostic factors. In *Ovarian Cancer* 5 pp 143–152 Eds F Sharp *et al.* Isis Medical Media, Oxford
- Fullwood P, Marchini S, Rader JS *et al.* (1999) Detailed genetic and physical mapping of tumor suppressor loci on chromosome 3p in ovarian cancer *Cancer Research* **59** 4662–4667
- Futreal P, Kasprzyk A, Birney E, Mullikin J, Wooster R and Stratton M (2001) Cancer and genomics *Nature* **409** 850–852
- Gabra H, Watson JE, Taylor KJ, Mackay J, Leonard RC, Steel CM, Porteous DJ and Smyth JF (1996) Definition and refinement of a region of loss of heterozygosity at 11q23.3–q24.3 in epithelial ovarian cancer associated with poor prognosis *Cancer Research* **56** 950–954
- Godwin AK, Testa JR, Handel LM, Liu Z, Vanderveer LA, Tracey PA and Hamilton TC (1992) Spontaneous transformation of rat ovarian surface epithelial cells: association with cytogenetic changes and implications of repeated ovulation in the etiology of ovarian cancer *Journal of the National Cancer Institute* **84** 592–601
- Godwin A, Schultz D and Hamilton T (1997) Oncogenes and tumour suppressor genes. In *Principles and Practice of Gynecological Oncology* pp 107–148 Eds W Hoskins *et al.* Lippincott, Philadelphia, PA
- Haber D and Harlow E (1997) Tumour-suppressor genes: evolving definitions in the genomic age *Nature Genetics* **16** 320–322
- Harris CC and Hollstein M (1993) Clinical implications of the p53 tumor-suppressor gene *New England Journal of Medicine* **329** 1318–1327
- Heim S and Mitelman F (1995) *Cancer Cytogenetics: Chromosomal and Molecular Genetic Aberrations of Tumor Cells* 2nd Edn. Wiley-Liss, New York
- Herman JG, Merlo A, Mao L, Lapidus RG, Issa JP, Davidson NE, Sidransky D and Baylin SB (1995) Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers *Cancer Research* **55** 4525–4530
- Huang H, Reed CP, Mordì A, Lomberg G, Wang L, Shridhar V, Hartmann L, Jenkins R and Smith DI (1999) Frequent deletions within FRA7G at 7q31.2 in invasive epithelial ovarian cancer *Genes, Chromosomes and Cancer* **24** 48–55
- Ilyanov EN, Birrell GW, Filippovich I *et al.* (1999) Frequent loss of heterozygosity at 1p36 in ovarian adenocarcinomas but the gene encoding p73 is unlikely to be the target *Oncogene* **18** 4640–4642
- International SNP Map Working Group (2001) A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms *Nature* **409** 928–933
- Iwabuchi H, Sakamoto M, Sakunaga H, Ma YY, Carcangiu ML, Pintel D, Yang-Feng TL and Gray JW (1995) Genetic analysis of benign, low-grade, and high-grade ovarian tumors *Cancer Research* **55** 6172–6180
- Iyengar TD, Ng S, Lau CC, Welch WR, Bell DA, Berkowitz RS and Mok SC (1999) Differential expression of NF1 type I and type II isoforms in sporadic borderline and invasive epithelial ovarian tumors *Oncogene* **18** 257–262
- Jones PA and Laird PW (1999) Cancer epigenetics comes of age *Nature Genetics* **21** 163–167
- Kalikini LM, Sims HL and Petty EM (2000) Genomic and expression analyses of alternatively spliced transcripts of the MLL septin-like fusion gene (MSF) that map to a 17q25 region of loss in breast and ovarian tumors *Genomics* **63** 165–172
- Kamb A, Gruis NA, Weaver-Feldhaus J *et al.* (1994) A cell cycle regulator potentially involved in genesis of many tumor types *Science* **264** 436–440
- Katso RM, Manek S, O'Byrne K, Playford MP, Le Meuth V and Ganesan TS (1997) Molecular approaches to diagnosis and management of ovarian cancer *Cancer and Metastasis Reviews* **16** 81–107
- Kemp CJ (1999) You don't need a backbone to carry a tumour suppressor gene [news; comment] *Nature Genetics* **21** 147–148
- Kerr J, Leary JA, Hurst T *et al.* (1996) Allelic loss on chromosome 7q in ovarian adenocarcinomas: two critical regions and a rearrangement of the PLANH1 locus *Oncogene* **13** 1815–1818
- Kigawa J and Terakawa N (2000) Adenovirus-mediated transfer of a p53 gene in ovarian cancer *Advances in Experimental Medicine and Biology* **465** 207–214
- Kim J, Hwang ES, Kim JS, You EH, Lee SH and Lee JH (1999) Intraperitoneal gene therapy with adenoviral-mediated p53 tumor suppressor gene for ovarian cancer model in nude mouse *Cancer Gene Therapy* **6** 172–178
- Kim TM, Benedict WF, Xu HJ *et al.* (1994) 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 Research* **54** 605–609
- *Knudson AG (1993) Antioncogenes and human cancer *Proceedings of the National Academy of Sciences USA* **90** 10 914–10 921
- Koike M, Takeuchi S, Yokota J, Park S, Hatta Y, Miller CW, Tsuruoka N and Koeffler HP (1997) Frequent loss of heterozygosity in the region of the D7S523 locus in advanced ovarian cancer *Genes, Chromosomes and Cancer* **19** 1–5
- Koike M, Takeuchi S, Park S, Hatta Y, Yokota J, Tsuruoka N and Koeffler HP (1999) Ovarian cancer: loss of heterozygosity frequently occurs in the ATM gene, but structural alterations do not occur in this gene *Oncology* **56** 160–163
- Kurose K, Zhou XP, Araki T, Cannistra SA, Maher ER and Eng C (2001) Frequent loss of PTEN expression is linked to elevated phosphorylated Akt levels, but not associated with p27 and cyclin D1 expression, in primary epithelial ovarian carcinomas *American Journal of Pathology* **158** 2097–2106
- Lancaster JM, Wooster R, Mangion J *et al.* (1996) Brca2 mutations in primary breast and ovarian cancers *Nature Genetics* **13** 238–240
- Launonen V, Stenback F, Puistola U, Bloigu R, Huusko P, Kytola S, Kaupila A and Winqvist R (1998) Chromosome 11q22.3–q25 LOH in ovarian cancer: association with a more aggressive disease course and involved subregions *Gynecologic Oncology* **71** 299–304
- Lavin MF (1999) ATM: the product of the gene mutated in ataxia-telangiectasia *International Journal of Biochemistry and Cell Biology* **31** 735–740
- Li J, Yen C, Liaw D *et al.* (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer *Science* **275** 1943–1947
- Lin H, Huber R, Schlessinger D and Morin PJ (1999) Frequent silencing of the GPC3 gene in ovarian cancer cell lines *Cancer Research* **59** 807–810
- Liu Y, Emilion G, Mungall AJ, Dunham I, Beck S, Le Meuth-Metzinger VG, Shelling AN, Charnock FML and Ganesan TS (2002) Physical and transcript map of the region between D6S264 and D6S149 on chromosome 6q27, the minimal region of allele loss in sporadic epithelial ovarian cancer *Oncogene* **21** 387–399
- Lu KH, Weitzel JN, Kodali S, Welch WR, Berkowitz RS and Mok SC (1997) A novel 4-cM minimally deleted region on chromosome 11p15.1 associated with high grade nonmucinous epithelial ovarian carcinomas *Cancer Research* **57** 387–390
- Lynch HT, Watson P, Lynch JF, Conway TA and Fili M (1993) Hereditary ovarian cancer. Heterogeneity in age at onset *Cancer* **71** 573–581
- McBride OW, Merry D and Givol D (1986) The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13) *Proceedings of the National Academy of Sciences USA* **83** 130–134
- Mancini DN, Rodenhiser DI, Ainsworth PJ, O'Malley FP, Singh SM, Xing W and Archer TK (1998) CpG methylation within the 5' regulatory region of the BRCA1 gene is tumor specific and includes a putative CREB binding site *Oncogene* **16** 1161–1169
- Matzuk MM, Finegold MJ, Mather JP, Krummen L, Lu H and Bradley A (1994) Development of cancer cachexia-like syndrome and adrenal tumors in inhibin-deficient mice *Proceedings of the National Academy of Sciences USA* **91** 8817–8821
- Miki Y, Swensen J, Shattuck-Eidens D *et al.* (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1 *Science* **266** 66–71
- Mitelman F (1995) *Catalog of Chromosome Aberrations in Cancer* 5th Edn. Wiley-Liss, New York
- Mok SC, Wong KK, Chan RK, Lau CC, Tsao SW, Knapp RC and Berkowitz RS

- (1994) Molecular cloning of differentially expressed genes in human epithelial ovarian cancer *Gynecologic Oncology* **52** 247–252
- *Mok SC, Chan WY, Wong KK *et al.* (1998) DOC-2, a candidate tumor suppressor gene in human epithelial ovarian cancer *Oncogene* **16** 2381–2387
- Niederacher D, Yan HY, An HX, Bender HG and Beckmann MW (1999) CDKN2A gene inactivation in epithelial sporadic ovarian cancer *British Journal of Cancer* **80** 1920–1926
- Nishioka Y, Kobayashi K, Sagae S, Sugimura M, Ishioka S, Nagata M, Terasawa K, Tokino T and Kudo R (1999) Mutational analysis of STK11 gene in ovarian carcinomas *Japanese Journal of Cancer Research* **90** 629–632
- *Obata K, Morland SJ, Watson RH, Hitchcock A, Chenevix-Trench G, Thomas EJ and Campbell IG (1998) Frequent PTEN/MMAC mutations in endometrioid but not serous or mucinous epithelial ovarian tumors *Cancer Research* **58** 2095–2097
- Orphanos V, McGown G, Hey Y, Thorncroft M, Santibanez-Koref M, Russell SE, Hickey I, Atkinson RJ and Boyle JM (1995) Allelic imbalance of chromosome 6q in ovarian tumours *British Journal of Cancer* **71** 666–669
- Paige AJ, Taylor KJ, Stewart A, Sgouros JG, Gabra H, Sellar GC, Smyth JF, Porteous DJ and Watson JE (2000) A 700-kb physical map of a region of 16q23.2 homozygously deleted in multiple cancers and spanning the common fragile site FRA16D *Cancer Research* **60** 1690–1697
- *Paige AJ, Taylor KJ, Taylor C *et al.* (2001) WWOX: a candidate tumor suppressor gene involved in multiple tumor types *Proceedings of the National Academy of Sciences USA* **98** 11 417–11 422
- Partridge EE and Barnes MN (1999) Epithelial ovarian cancer: prevention, diagnosis, and treatment *Ca-A Cancer Journal For Clinicians* **49** 297–320
- Pecorelli S, Benedet JL, Creasman WT and Shepherd JH (1999) FIGO staging of gynecologic cancer. 1994–1997 FIGO Committee on Gynecologic Oncology. International Federation of Gynecology and Obstetrics [comment] *International Journal of Gynaecology and Obstetrics* **65** 243–249
- Peng H, Xu F, Pershad R *et al.* (2000) ARHI is the center of allelic deletion on chromosome 1p31 in ovarian and breast cancers *International Journal of Cancer* **86** 690–694
- Phillips NJ, Ziegler MR, Radford DM, Fair KL, Steinbrueck T, Xynos FP and Donis-Keller H (1996) Allelic deletion on chromosome 17p13.3 in early ovarian cancer *Cancer Research* **56** 606–611
- Pilia G, Hughes-Benzie RM, MacKenzie A *et al.* (1996) Mutations in GPC3, a glypican gene, cause the Simpson–Golabi–Behmel overgrowth syndrome *Nature Genetics* **12** 241–247
- Robertson KD and Jones PA (1998) The human ARF cell cycle regulatory gene promoter is a CpG island which can be silenced by DNA methylation and downregulated by wild-type p53 *Molecular and Cellular Biology* **18** 6457–6473
- Roy WJ, Jr, Watson RH, Hitchcock A and Campbell IG (1997) Frequent loss of heterozygosity on chromosomes 7 and 9 in benign epithelial ovarian tumours *Oncogene* **15** 2031–2035
- Rubin G (2001) Comparing species *Nature* **409** 820–821
- Russell SE, Hickey GI, Lowry WS, White P and Atkinson RJ (1990) Allele loss from chromosome 17 in ovarian cancer *Oncogene* **5** 1581–1583
- Russell SE, McIlhatton MA, Burrows JF *et al.* (2000) Isolation and mapping of a human septin gene to a region on chromosome 17q, commonly deleted in sporadic epithelial ovarian tumors *Cancer Research* **60** 4729–4734
- Saito S, Saito H, Koi S, Sagae S, Kudo R, Saito J, Noda K and Nakamura Y (1992) Fine-scale deletion mapping of the distal long arm of chromosome 6 in 70 human ovarian cancers *Cancer Research* **52** 5815–5817
- Saito S, Sirahama S, Matsushima M, Suzuki M, Sagae S, Kudo R, Saito J, Noda K and Nakamura Y (1996) Definition of a commonly deleted region in ovarian cancers to a 300-kb segment of chromosome 6q27 *Cancer Research* **56** 5586–5589
- Sandhu AK, Kaur GP, Reddy DE, Rane NS and Athwal RS (1996) A gene on 6q 14–21 restores senescence to immortal ovarian tumor cells *Oncogene* **12** 247–252
- Sato T, Saito H, Morita R, Koi S, Lee JH and Nakamura Y (1991) Allelotype of human ovarian cancer *Cancer Research* **51** 5118–5122
- Schrock E, du Manoir S, Veldman T *et al.* (1996) Multicolor spectral karyotyping of human chromosomes *Science* **273** 494–497
- Schultz DC, Vanderveer L, Berman DB, Hamilton TC, Wong AJ and Godwin AK (1996) Identification of two candidate tumor suppressor genes on chromosome 17p13.3 *Cancer Research* **56** 1997–2002
- Schwengel DA, Jedlicka AE, Nanthakumar EJ, Weber JL and Levitt RC (1994) Comparison of fluorescence-based semi-automated genotyping of multiple microsatellite loci with autoradiographic techniques *Genomics* **22** 46–54
- Sellar GC, Li L, Watt KP *et al.* (2001) BAX2 induces cadherin 6 expression and is a functional suppressor of ovarian cancer progression *Cancer Research* **61** 6977–6981
- Shayesteh L, Lu Y, Kuo WL *et al.* (1999) PIK3CA is implicated as an oncogene in ovarian cancer *Nature Genetics* **21** 99–102
- Shelling AN, Cooke IE and Ganesan TS (1995) The genetic analysis of ovarian cancer *British Journal of Cancer* **71** 521–527
- Shih YC, Kerr J, Liu J, Hurst T, Khoo SK, Ward B, Wainwright B and Chenevix-Trench G (1997) Rare mutations and no hypermethylation at the CDKN2A locus in epithelial ovarian tumours *International Journal of Cancer* **70** 508–511
- Shih YC, Kerr J, Hurst TG, Khoo SK, Ward BG and Chenevix-Trench G (1998) No evidence for microsatellite instability from allelotype analysis of benign and low malignant potential ovarian neoplasms *Gynecologic Oncology* **69** 210–213
- Shimizu M, Toki T, Takagi Y, Konishi I and Fujii S (2000) Immunohistochemical detection of the Wilms' tumor gene (WT1) in epithelial ovarian tumors *International Journal of Gynecological Pathology* **19** 158–163
- Shridhar V, Staub J, Huntley B, Cliby W, Jenkins R, Pass HI, Hartmann L and Smith DI (1999) A novel region of deletion on chromosome 6q23.3 spanning less than 500 Kb in high grade invasive epithelial ovarian cancer *Oncogene* **18** 3913–3918
- Soengas M, Capodici P, Polsky D *et al.* (2001) Inactivation of the apoptosis effector Apaf-1 in malignant melanoma *Nature* **409** 207–211
- St John MA, Tao W, Fei X, Fukumoto R, Carcangiu ML, Brownstein DG, Parlow AF, McGrath J and Xu T (1999) Mice deficient of Lats1 develop soft-tissue sarcomas, ovarian tumours and pituitary dysfunction *Nature Genetics* **21** 182–186
- Suzuki M, Saito S, Saga Y, Ohwada M and Sato I (1998) Loss of heterozygosity on chromosome 6q27 and p53 mutations in epithelial ovarian cancer *Medical Oncology* **15** 119–123
- Suzuki M, Saito S, Saga Y, Ohwada M and Sato I (2000) Mutation of K-RAS proto-oncogene and loss of heterozygosity on 6q27 in serous and mucinous ovarian carcinomas *Cancer Genetics and Cytogenetics* **118** 132–135
- Takahashi H, Behbakht K, McGovern PE *et al.* (1995) Mutation analysis of the BRCA1 gene in ovarian cancers *Cancer Research* **55** 2998–3002
- Takakura S, Okamoto A, Saito M *et al.* (1999) Allelic imbalance in chromosome band 18q21 and SMAD4 mutations in ovarian cancers *Genes, Chromosomes and Cancer* **24** 264–271
- Tangir J, Muto MG, Berkowitz RS, Welch WR, Bell DA and Mok SC (1996) A 400 kb novel deletion unit centromeric to the BRCA1 gene in sporadic epithelial ovarian cancer *Oncogene* **12** 735–740
- Tao W, Zhang S, Turenchalk GS, Stewart RA, St John MA, Chen W and Xu T (1999) Human homologue of the *Drosophila melanogaster* lats tumour suppressor modulates CDC2 activity *Nature Genetics* **21** 177–181
- Tavassoli M, Steingrimsdottir H, Pierce E, Jiang X, Alagöz M, Farzaneh F and Campbell IG (1996) Loss of heterozygosity on chromosome 5q in ovarian cancer is frequently accompanied by TP53 mutation and identifies a tumour suppressor gene locus at 5q13.1–21 *British Journal of Cancer* **74** 115–119
- Tibiletti MG, Bernasconi B, Furlan D *et al.* (1996) Early involvement of 6q in surface epithelial ovarian tumors *Cancer Research* **56** 4493–4498
- Tibiletti MG, Trubia M, Ponti E *et al.* (1998) Physical map of the D6S149–D6S193 region on chromosome 6Q27 and its involvement in benign surface epithelial ovarian tumours *Oncogene* **16** 1639–1642
- Veldman T, Vignon C, Schrock E, Rowley JD and Ried T (1997) Hidden

- chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping *Nature Genetics* **15** 406–410
- Veugeliers M, Cat BD, Muyldermans SY et al.** (2000) Mutational analysis of the GPC3/GPC4 glypican gene cluster on Xq26 in patients with Simpson–Golabi–Behmel syndrome: identification of loss-of-function mutations in the GPC3 gene *Human Molecular Genetics* **9** 1321–1328
- Viel A, Giannini F, Capozzi E, Canzonieri V, Scarabelli C, Gloghini A and Boiocchi M** (1994) Molecular mechanisms possibly affecting WT1 function in human ovarian tumors *International Journal of Cancer* **57** 515–521
- Wake N, Hreshchyshyn M, Piver S, Matsui S and Sandberg A** (1980) Specific cytogenetic changes in ovarian cancer involving chromosomes 6 and 14 *Cancer Research* **50** 4512
- Wales MM, Biel MA, el Deiry W, Nelkin BD, Issa JP, Cavenee WK, Kuerbitz SJ and Baylin SB** (1995) p53 activates expression of HIC-1, a new candidate tumour suppressor gene on 17p13.3 *Nature Medicine* **1** 570–577
- Wan M, Zweizig S, D’Ablaing G, Zheng J, Velicescu M and Dubeau L** (1994) Three distinct regions of chromosome 6 are targets of loss of heterozygosity in human ovarian carcinomas *International Journal of Oncology* **5** 1043–1048
- Wan M, Sun T, Vyas R, Zheng J, Granada E and Dubeau L** (1999) Suppression of tumorigenicity in human ovarian cancer cell lines is controlled by a 2 cM fragment in chromosomal region 6q24–q25 *Oncogene* **18** 1545–1551
- Wang ZJ, Churchman M, Campbell IG, Xu WH, Yan ZY, McCluggage WG, Foulkes WD and Tomlinson IP** (1999) Allele loss and mutation screen at the Peutz–Jeghers (LKB1) locus (19p13.3) in sporadic ovarian tumours *British Journal of Cancer* **80** 70–72
- Watson KL, Justice RW and Bryant PJ** (1994) *Drosophila* in cancer research: the first fifty tumor suppressor genes *Journal of Cell Science – Supplement* **18** 19–33
- Watson RH, Neville PJ, Roy WJ, Jr, Hitchcock A and Campbell IG** (1998) Loss of heterozygosity on chromosomes 7p, 7q, 9p and 11q is an early event in ovarian tumorigenesis *Oncogene* **17** 207–212
- Wertheim I, Tangir J, Muto MG, Welch WR, Berkowitz RS, Chen WY and Mok SC** (1996) Loss of heterozygosity of chromosome 17 in human borderline and invasive epithelial ovarian tumors *Oncogene* **12** 2147–2153
- Wiper DW, Zanotti KM, Kennedy AW, Belinson JL and Casey G** (1998) Analysis of allelic imbalance on chromosome 17p13 in stage I and stage II epithelial ovarian cancers *Gynecologic Oncology* **71** 77–82
- Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C and Micklem G** (1995) Identification of the breast cancer susceptibility gene BRCA2 *Nature* **378** 789–792
- Xu XX, Yang W, Jackowski S and Rock CO** (1995a) Cloning of a novel phosphoprotein regulated by colony-stimulating factor 1 shares a domain with the *Drosophila* disabled gene product *Journal of Biological Chemistry* **270** 14 184–14 191
- Xu T, Wang W, Zhang S, Stewart RA and Yu W** (1995b) Identifying tumor suppressors in genetic mosaics: the *Drosophila* lats gene encodes a putative protein kinase *Development* **121** 1053–1063
- Xu XX, Yi T, Tang B and Lambeth JD** (1998) Disabled-2 (Dab2) is an SH3 domain-binding partner of Grb2 *Oncogene* **16** 1561–1569
- *Yu Y, Xu F, Peng H et al.** (1999) NOEY2 (ARHI), an imprinted putative tumor suppressor gene in ovarian and breast carcinomas *Proceedings of the National Academy of Sciences USA* **96** 214–219
- Zhang H, Hasty P and Bradley A** (1994) Targeting frequency for deletion vectors in embryonic stem cells *Molecular and Cellular Biology* **14** 2404–2410