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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