Review

The Mutational Profile of Sporadic Epithelial Ovarian Carcinoma

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Abstract. *Mutations occurring in sporadic epithelial ovarian carcinomas are reviewed and their functional significance in terms of prognosis and prediction of anticancer drug activity are discussed. Alterations in the BRCA1/2 genes, TP53, PTEN, PI3Kinase, KRAS/BRAF and CTNNB1 are described. TP53 is likely to be a driver in high grade serous tumours, but is less useful than BRCA status in prediction of response to the platinum or PARPi agents. It is expected that mutation profiling will become integrated into current morphological/ immunohistochemical primary diagnostic assessment of tumours once the cost and quality control issues of the technology are addressed.*

Current estimates suggest there are between 30 and 80 mutations involved in every tumour, of which 6-8 are in driver genes, and a greater number of copy number variations, splice variants and methylations. The full significance of these in the prediction of clinical outcome has yet to be determined. Around 10% of mutations are found in oncogenes, and can be directly targeted for therapy, while the remainder are in tumour suppressor genes and are less easily targeted, although related pathways and gene therapy are promising options. While the volume of data generated over the next 5 years, including gene sequencing and expression profiling from The Cancer Genome Atlas (TCGA), will increase dramatically, the tools for interpretation require further development. This paper reviews the major published studies on mutations in epithelial ovarian cancer (EOC) subtypes and their clinical implications.

Current clinical management of EOC is based on histological subtype at primary diagnosis, assessed largely by morphology combined with limited histochemistry. Serous ovarian cancer accounts for about two-thirds of EOC cases and usually presents at an advanced stage (1, 2). A recent analysis of 8704 women with stage III/IV ovarian cancer from 7 randomized trials has demonstrated clear cell and mucinous histology are independent predictors of poor survival (3). Endometrioid tumours have been less extensively studied, but one review has suggested an adverse prognosis compared to serous tumours (4). 'BRCAness' has been established as a more recent clinical concept by comparing the clinical features of familial cases with those of high grade serous tumours (5-9). With the recent publication of *BRCA* sequencing in large series of sporadic cases, *BRCA*1/2 alterations are beginning to emerge not only as a prognostic factor, but also as being predictive of response to platinum compounds and the Poly ADP ribose polymerase (PARP) inhibitors.

BRCA1 and BRCA2 Genes

The *BRCA1* and *BRCA2* genes comprise a family whose inactivation by mutation or methylation in breast or ovarian cancer leads to defects in homologous repair, which can be exploited by the PARP inhibitors (10). Promising phase 2 results have been shown in ovarian cancer. Mutations are not hot-spot specific and inactivating mutations cover the entire open reading frame. Following some initial clinical studies of platinum-sensitive serous tumours, it has become recognised that as many as 23% of cases of this subtype of ovarian cancer demonstrate *BRCA* alterations. Secondly, reversion of the *BRCA* mutation after treatment is associated with the development of platinum resistance. Patients with *BRCA*

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mutated tumours have a better prognosis compared to those with wild-type genes (11), and both patients with tumours harbouring mutations and those with gene alterations show improved progression-free survival following platinum-based chemotherapy compared to those with a normal profile.

Data on the occurrence of mutations in multiple genes is sparse, and whether these represent true driver mutations or reflect genetic instability is far from clear. Most cases of *BRCA* alteration co-exist with *TP53* mutations. There is also a rationale for targeting the phosphoinositide 3-kinase (PI3K) pathway together with *BRCA*-related DNA repair defects (10). In contrast, the clinical categories of relapsed disease are defined in functional terms as platinum-sensitive, partially sensitive, resistant and refractory (12). Integration of molecular data with these categories is one of the major unmet needs in treating EOC. Acquired resistance to platinum based chemotherapy has been shown to be associated with reversion of *BRCA* gene mutations (12, 13), but data on secondary mutations, as demonstrated in gastrointestinal stromal tumours, for example, is limited.

TP53 and p53

The *TP53* tumour suppressor gene encodes the tumour suppressor protein p53, a transcription factor that plays a vital role in regulating the cell cycle, DNA repair and cell death through apoptosis. It is considered the most frequently altered gene in human cancers and loss of functional p53 protein has been observed in a majority of epithelial ovarian cancers, with the exception of the clear cell sub-type. In *BRCA*-deficient tumours *TP53* mutations are almost universal (11), and this holds also for all high-grade serous tumours (14). Alterations of the p53 protein caused by missense mutations and loss of the p53 protein by nonsense or frameshift mutations play important roles in clonal expansion of neoplastic cells (15). Other histological subtypes of EOC that more commonly present at an early stage (endometrioid, clear cell, mucinous) have a much lower incidence of *TP53* mutations (16). It is thought that somatic mutations of *TP53* may be involved in drug resistance (17), however studies investigating this issue have given variable results, and gene alterations have not been developed as clinical predictive biomarkers (18, 19). Mutations and/or loss of heterozygosity of *TP53* have been identified in early carcinomas and epithelial inclusions of the ovary (20, 21), suggesting *TP53* mutation may be an early event in the pathogenesis of high-grade serous carcinoma (21, 22).

KRAS and BRAF Protein Kinase Pathways

KRAS and its effector *BRAF* are part of the ras oncogene family, and act as the initiators of the *RAS/RAF/MEK/* extracellular signal regulated kinase (ERK)/mitogen-activated protein kinase (*MAPK*) pathway (Figure 2) that mediates cellular responses such as proliferation, differentiation and cell survival (23). Most activating KRAS mutations are present on codons 12, 13 or 61, resulting in constitutive activation of the encoded GTPase which leads to stimulation of growth (24). The majority of human epithelial malignancies that have sustained RAS mutations, including gynaecologic cancers, are affected at *KRAS* codon 12 (25). Mutations of *KRAS* lead to constitutive phosphorylation of its downstream target, *MAPK*, often referred to as *ERK* (23, 26).

KRAS mutations have previously been described in mucinous ovarian tumours and serous borderline tumours, and rarely in high-grade tumours, and often correlate with overexpression of activated *ERK1/2* (27-31). The most common molecular genetic alteration in mucinous borderline tumours and mucinous carcinomas is a point mutation in *KRAS* (31, 32). Other studies (30, 33) have reported that *KRAS* mutations at codons 12 and 13 occur in one third of invasive low-grade micropapillary serous carcinomas and another one-third of serous borderline tumours. *KRAS* mutations have also been observed in approximately 10% of endometrioid ovarian carcinomas (30-32, 34).

RAF is a serine and threonine protein kinase that mediates *MAPK* pathway and is one of the many direct downstream cascades of RAS (35). *BRAF* is an oncoprotein and one of the direct downstream effectors of *RAS*. Somatic mutations of *BRAF* occur in 8% of human carcinomas, and one frequently observed somatic mutation is found on codon 600 in several tumour types, such as melanoma, papillary thyroid carcinoma, colon cancer and ovarian cancer (36). In colorectal cancer, the presence of *KRAS* mutations is associated with resistance to epidermal growth factor receptor (*EGFR*) targeted therapy with the antibodies cetuximab and panitumumab. Furthermore, some of the patients with wild-type *KRAS* also have the *BRAF* V600E mutation, which may be a contributory factor in resistance to these agents (37, 38).

The PI3K/AKT Pathway

The *PI3K/AKT* signalling pathway controls many cellular processes, such as cell proliferation, apoptosis and motility (39). Several components of this pathway, including proteins encoded by *PTEN, PIK3CA* and *AKT* genes, are deregulated by loss- or gain-of-function in various cancer types (39). The *PIK3CA* gene encoding the catalytic subunit (p110α) of *PI3K* protein is a transforming oncogene which phosphorylates *PIP2* to *PIP3*, leading to activation of *AKT*. In turn, this encourages proliferation and inhibits apoptosis. The 3q26 region containing *PIK3CA* is amplified in various tumours, including cervical (70%), head and neck (37%) and gastric cancers (36.4%) (41-43). *PI3K/AKT* is activated in multiple cancers leading to oncogenic transformation and may result from mutations of the catalytic subunit of p110α

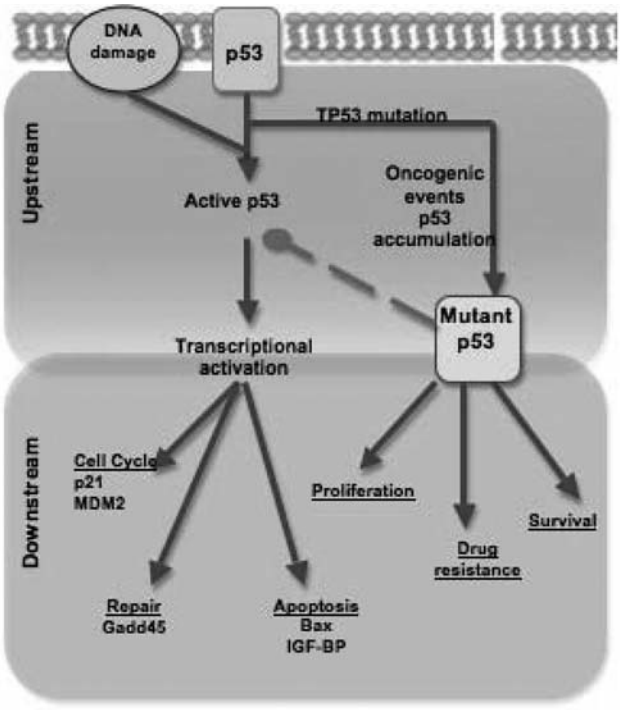

Figure 1. *The effects of TP53 mutations on cell cycle progression, DNA repair and apoptosis.*

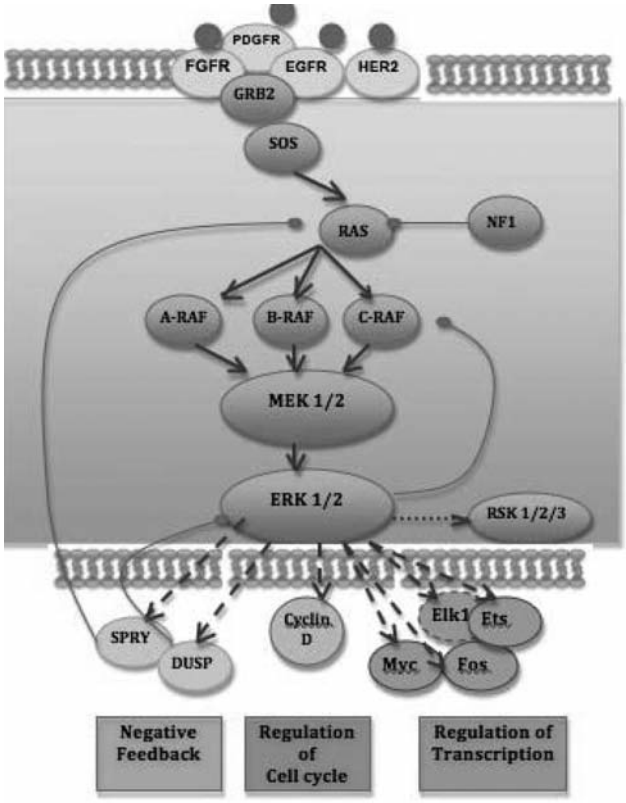

Figure 2. *The RAS-RAF-MEK-ERK signalling pathway is activated in human tumours by several mechanisms including mutations in RAS, BRAF, and MEK1. Phosphorylation and activation of ERK regulates transcription of target genes that promote cell cycle progression and tumour survival. In tumours with V600E BRAF expression, the pathway output is enhanced by impaired upstream feedback regulation (adapted from (72)).*

of the *PIK* gene (*PIK3CA*), amplification of *PIK3CA* or as a result of an inactivating mutation in the tumour suppressor gene *PTEN* (44-45).

Studies on ovarian cancer cell lines and animal models revealed that activation of the *PI3K/AKT* pathway may lead to resistance to platinum and taxane resistance by suppression of apoptosis (46-49). *PIK3CA*, the gene encoding the p110a catalytic subunit of *PI3K*, was found to be mutated in breast (27%), endometrial (23%), colorectal (14%), urinary tract (17%) and ovarian (8%) cancers (http://www.sanger.ac.uk/genetics/CGP/cosmic) whereas amplification of *PI3KCA* was found in 50% of ovarian carcinomas (40). *PIK3CA* gene mutations in ovarian cancers are less commonly observed, except in endometrioid and clear cell sub-types, in which they are detected with an approximate frequency of 20% (50,51).

PTEN is an important tumour suppressor that regulates crucial cellular functions, including insulin and other growth factor signalling, lipid and glucose metabolism, and cell survival and apoptosis. *PTEN* switches off signalling through the *PI3K-AKT* axis and by doing so, controls growth factor signalling, thereby acting as a potent tumour suppressor and is mutated or deleted in many human tumours (51). Partial inhibition of its activity is sufficient to promote carcinogenesis (52). Among gynaecological malignancies, *PTEN* has been extensively evaluated in endometrial cancer but only a few studies have investigated *PTEN* expression or mutation in ovarian cancer (53).

Common genes showing sequence alteration include those in the *TP53, PTEN, PI3K* and *BRCA* pathways. Mutations may be inherited or caused by somatic changes such as deletions, duplications, inversions, or translocations. In low-grade tumours, *BRAF* and *KRAS* alterations may also be seen in these groups, as well as in mucinous tumours, and β-catenin is mutated in the endometrioid subgroups. Of these genes, the ones most clearly related to chemosensitivity both to the platinum agents and *PARP* inhibitors are the *BRCA* 1/2 genes, where the mechanism appears to be related to homologous DNA repair (10).

Table I shows mutation data extracted from recent studies. There was variation in the methods both of sample collection and analysis in these studies which limits the interpretation of the data. Figure 3 focuses on the *KRAS* and *BRAF* genes, demonstrating that mutations are concentrated in low-grade tumours. The true incidence of mutations may be distorted

Table I. *Mutational frequency of KRAS, BRAF, PTEN, CTNNB1, TP53, BRCA and ARID1A in different histological subtypes of ovarian carcinoma.*

Gene	Subtype	Sample number	Mutation detected	%	Overall mutation rate (%)	Mutational analysis
KRAS (55-59)	Serous	386	34	9	11	Codons 12, 13, 61
	Mucinous	88	30	34		
	Endometrioid	209	15	7		
	Clear cell	118	12	10		
BRAF (55, 58, 59)	Serous	47	3	6	4	Codons 600, 1796
	Mucinous	19	3	16		
	Endometrioid	148	4	3		
	Clear cell	97	1	1		
PTEN (56, 58, 61)	Serous	56	22	39	17	Exons 1-4, 6-9
	Mixed	20	7	35		
	Endometrioid	42	3	7		
	Clear cell	97	5	5		
CTNNB1 (56)	Endometrioid	29	10	34	10	GSK-3β
	Clear cell	116	4	3		
PI3KCA (51, 58)	Endometrioid	15	4	27	20	Codons 546, 542,
	Clear cell	126	42	33		1047, 545, 3q26,
	Serous	84	0	0		exons 9, 20
TP53 (1, 14, 51,	Serous	311	244	78	62	Codons 199, 273,
55, 56, 59)	Epithelial	154	50	33		241, 220, exons 4-8
	Mixed	20	15	75		
	Endometrioid	21	17	81		
	Clear Cell	22	3	14		
BRCA (11, 63)	Epithelial	235	44	23	23	
ARID1A (71)	Clear Cell	19	11	59	59	6018-6020delGCT, 404delC, 5518delG, C4201T, C5164T, 3948delG, 5541insG, T5953C, C1680A

by publication bias in smaller series, and may vary with ethnic backgrounds of the population.

Various studies have shown *KRAS* mutations to be most commonly found in low grade ovarian carcinomas and rarely in high grade (53-57) (Figure 3). The mutational frequency of *KRAS* is relatively low and previous studies have observed rates between 7-30% (median/mean of 11%). Different methods of sample collection were used in these studies but appeared to have no significant impact on mutation rates observed. Mutations of codon 12 of the *KRAS* gene (GGT to GAT) are the most common (55, 57). Mutations are common in mucinous tumours (34%) with an observed frequency of only 7-10% in serous, endometrioid and clear cell tumours (Figure 3).

As with *KRAS, BRAF* mutations are more common in low grade tumours (Figure 3) (55, 57, 59, 60). Two studies found no mutations in high grade tumours (57, 60). In all cases codon 600 was found to be mutated, and one case found exon 15 to be mutated (58). The occurrence of *KRAS* and BRAF mutations appears to be mutually exclusive (55, 59, 61) with one exception in a mucinous carcinoma (59). Overall, *BRAF* mutations were observed most commonly in mucinous tumours (14%) (Figure 1). *PTEN* mutations were observed in 5-39% of ovarian tumours, and most commonly occur in high grade serous and mixed serous and endometrioid tumours (58, 61) (Table I). *PTEN* mutations were only detected in paraffin embedded tissue samples.

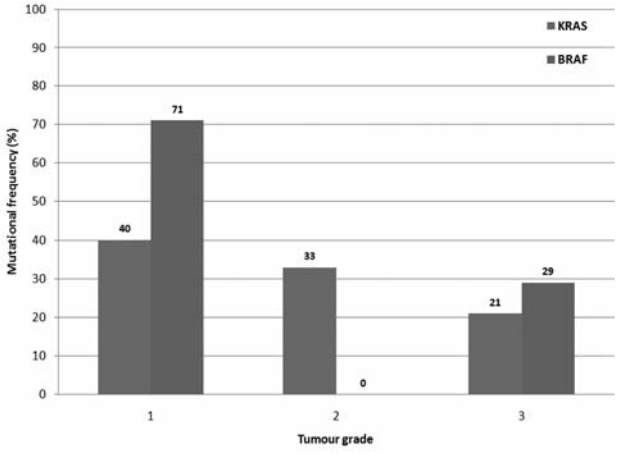

Figure 3. *Mutational frequency of KRAS and BRAF according to tumour grade.*

Expression of *CTNNB1* (β-catenin) appears to occur most frequently in low grade endometrioid tumours and to a lesser extent in low grade clear cell tumours (56, 58, 62) although overexpression was observed at a high frequency in higher grade tumours (56). Alterations of β-catenin have been associated with improved prognosis. Ninety-three percent of patients with alterations of β-catenin were alive and disease free compared to 63% who had no observable alterations in expression or mutation (56). However, interpretation of the studies of this gene are limited by the small numbers.

PIK3CA was frequently mutated in clear cell ovarian carcinoma and rarely in other histological subtypes (58). Most of the *PIK3CA* mutations were mapped to exons 9 and 20, resulting in kinase activation of p110α, which has been shown to result in increased cellular survival and invasion. Mutations were more commonly found in low-grade tumours and rarely in high-grade. Mutations in *PIK3CA* were mostly missense (62). Where amplification of *PI3KCA* was studied (60), it was found to be significantly amplified in 24% of tumour samples.

Various studies have observed a high frequency of mutations of the *TP53* gene encoding p53 in serous ovarian carcinomas but only low mutational frequency in low-grade tumours. The overall figures are 79% in high-grade and 34% in low-grade tumours. However, these are composite data and one recent study suggested *TP53* mutations are universal in high grade serous tumours (14) (Figure 1). This mutational frequency has also been observed in high grade endometrioid tumours (56). The site of mutation of the gene showed no significant effect on overall and progression free survival. In studies that stated type of mutation, it was found that missense mutations were most common in all histological subtypes, ranging from 60-90% (1, 57, 61), and as expected, correlated strongly with p53 overexpression, with 85% of tumours overexpressing p53 having a missense mutation (61). An analysis of 64 published studies of TP53 alterations found extensive heterogeneity in methodology and reporting but found that only 10% fulfilled the minimum criteria for the method of detecting mutations or assignment of clinical response (14, 62), and they were unable to form conclusions on prognostic value.

Studies investigating germline mutational frequency of *BRCA* genes (63, 64) found that *BRCA1* mutations were more frequent than *BRCA2* (19-39% compared to 7-21%). One study found that patients carrying *BRCA* mutations had a significantly higher survival rate than non-carriers (66% *vs.* 52%) (63). There was no difference between survival for patients with BRCA1 and those with *BRCA2* mutations. Overall *BRCA* mutations were observed in 23% of 235 unselected epithelial tumours (11) and *BRCA* mutations or alterations (defined as including expression changes) were also a favourable prognostic factor. Most cases of *BRCA* mutations also show mutations in *TP53*, and a model of sequential mutation, chromosomal instability and copy number alterations has recently been proposed to explain the diversity of high -grade serous carcinomas (65).

Overexpression of some genes may occur in the absence of mutation, and *AKT* has been found to be overexpressed in almost 60% of epithelial carcinomas (66, 67) with more frequent detection of *P-AKT*-positive cells in grade 3 tumours. Activating mutations in *PI3K* and *PTEN* are frequently seen in association with *BRCA* deficiency (67). A combination of a PARP inhibitor with a *PI3K* or *AKT* inhibitor may therefore be a logical approach in *PTEN* inactivated tumours (10). DNA copy number aberrations (CNA) alter the amount and organization of genomic material, which can increase or decrease the transcriptional activity of critical genes or regulatory RNAs. They may be small, altering the function of a single gene or potentially affect a large chromosomal region. High throughput technologies including comparative genomic hybridization, digital karyotyping, representational oligonucleotide microarray (ROMA), single nucleotide polymorphism arrays, molecular inversion probes, and next generation sequencing are now capable of rapidly and efficiently profiling genetic alterations across entire cancer genomes (68). One such study identified cyclin E copy number and protein expression as a marker of chemoresistance (69). However, there are problems caused by errors induced during amplification steps, and can be overcome by single molecule sequencing, currently an expensive technology. A mass spectroscopy-based approach can facilitate rapid, high-throughput and cost-effective detection of hot-spot gene mutations (*i.e.*, in *PIK3CA, AKT1, KRAS*) but is not applicable to genes targeted by non-hot-spot mutations in tumor suppressors such as *TP53* or *PTEN* (70).

Single-agent activity of targeted therapy to date has been promising but of limited effectiveness, and further progress is likely to require use of agents in combinations. However, concerns over unpredictable toxicity have slowed the development of these approaches. These approaches can also be applied to assess germline changes in metabolizing enzymes that could alter therapy efficacy (*e.g.* CYP-2D6). The introduction of trastuzumab was facilitated by the use of low-cost high-throughput immunohistochemistry, later supplemented by fluorescence *in situ* hybridization for borderline cases. More recently epidermal growth factor receptor 1 (*EGFR1*) sequencing has been required as a companion biomarker for the use of gefinitib in non-small cell lung cancer, and KRAS sequencing has been used to exclude mutant cases from cetuximab therapy in colorectal cancer (37). With the falling costs of exome-wide sequencing, a mutation profile including *BRCA1/2, TP53, KRAS, BRAF, PI3K* and *PTEN* may soon become comparable in cost to conventional imaging.

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